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ORIGINAL ARTICLE

CONTENTS

Effectiveness of thermal radiofrequency minimally invasive methods for the treatment of varicose veins: surgical experience in the Ukrainian population

Dmytro M. Ivashchenko[1], Mykola I. Kravtsiv[1], Kyrylo Y. Parkhomenko[2], Maksym O. Dudchenko[1], Oleh H. Krasnov[1], Mykola P. Shevchuk[1], Tamara V. Horodova-Andrieieva[1], Mykhailo S. Myroshnychenko[3]

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ABSTRACT

Aim: Evaluate the results of study of the effectiveness of radiofrequency ablation in patients with chronic venous insufficiency in the Ukrainian population.

Materials and Methods: Observation included the evaluation of the great saphenous vein occlusion and reflux at the patients undergone radiofrequency ablation on great saphenous vein. The condition was assessed according to the clinical severity scale of chronic venous disease and CEAP classification. Data were interpreted according to an analysis of the Kaplan-Meyer survival model.

Results: After radiofrequency ablation all the patients under observation had complete occlusion of the great saphenous vein when being discharged. By the one-year of the observation, Kaplan Meyer's analysis determined that over the course of 12 months, the probability of an outcome without recurrence of varicose after radiofrequency ablatio was 98,77%. Reflux of blood was determined in reopened perforating vein of the leg in one patient in three months according to the results of duplex ultrasound. According to the CEAP scale at the time of admission, there were 56.72% of the patients with C2, and 34.33% with C3, 8.96% with C4. The average clinical severity scale scores improved from 4.69 in the preoperative period to 2.90 on the first day after surgery and 2.52, 1.27, 0.92, 0.71, 0.64, 0.60 on the 7th day, 1, 3, 6, 9 and 12 months respectively.

Conclusion: Radiofrequency ablation showed its efficiency for endovenous treatment of incompetent great saphenous vein with sustained clinical and anatomical success for most patients.

KEY WORDS: venous insufficiency [c14.907.952], radiofrequency ablation [e04.014.760], endovascular procedures [e04.502.382], ultrasonography, doppler [e01.370.350.850.850]

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INTRODUCTION

Chronic venous insufficiency (CVI) is a common problem that affects an average number of women from 1% to 40%, and men from 1% to 17%. At the same time, more people suffer from varicose veins (VV): correspondingly from 1% to 73% of female and from 2% to 56% of male [1]. Chronic venous insufficiency may be manifested as a cosmetic skin irritation at early stages and eventually develop to the state of decompensation. Signs and symptoms of this condition include visible varicose veins, pain or swelling of legs, cramps or fatigue in lower extremities and heavy legs. Depigmentation of the skin, formation of sealing tissues and trophic ulcers may occur without treatment [2]. Very frequently incompetent valves in the great or small saphenous veins cause pathological venous reflux and contribute to the development of severity of the condition. Surgical treatment of great or small saphenous veins incompetence by its removal or elimination out of lower limb venous system either removes or reduces signs and symptoms of the disease. Since open legation the surgical treatment of vascular diseases of the lower extremities has significantly improved. Such minimally invasive procedures are efficiently used as: foam sclerotherapy, endothermal ablation technology, endovenous laser therapy (ELT), endovenous radiofrequency ablation (RFA) and the methods of mechano-chemical ablation (MOCA) having been introduced into practice recently as well. ELT and radiofrequency ablation (RFA) were recommended as primary surgical procedures in the treatment of venous disease according to the recommendations of the NICE 2013 and they showed their preference to open surgery

and foam sclerotherapy [3]. The EVRF® system (Endo Venous Radio Frequency) (F Care Systems, Belgium) has proved to be safe and efficient. However, there is the limited number of clinical evaluations of using of EVRF system and medium-term results of treatment in the literature [4]. Thus, in our investigation we have tried to fill a gap in current knowledge and we have assessed one-year results after using RFA by EVRF system in patients with VV of lower limbs.

AIM

The aim of the study was to evaluate the results of a two-year study of the effectiveness of radiofrequency ablation in patients with chronic venous insufficiency in the Ukrainian population; to analyze the short-term and long-term results of the impact of this surgical method on the clinical picture of varicose veins and the chances of recurrence.

MATERIALS AND METHODS

This prospective investigation was made to evaluate the medium-term effects RFA of great saphenous vein (GSV) with using EVRF system which includes a monopolar radiofrequency catheter with integrated heating element of the production F Care Systems (Belgium). There were taken patients examined in advance with signs and symptoms of venous insufficiency of the lower limbs in the GSV system with confirmed reflux for this investigation. The reflux was determined as the retrograde flow of blood more than 500 ms in Duplex ultrasound (DUS). It was the indication for endovenous ablative therapy. The inclusion criteria were as follows: age from 18 to 80 years old at the time of the examination, physical condition allowing active lifestyle after the procedure and the opportunity to be present at the control examinations during the investigation. Patients with vein thrombosis, pregnant women and the ones who had allergy to medicines used in the investigation were excluded. There were 134 patients taken part in the investigation with 174 incompetent GSV who received RFA therapy. All the investigators had experience to work with RFA by EVRF® system (F Care Systems, Belgium) and underwent the appropriate training for using this system before the start of the investigation. The control visit of the patient included clinical examination and diagnosing according to CEAP, filling clinical severity scale of chronic venous disease (VCSS) profiles, DUS of the lower extremities' veins in the horizontal position and the reverse Trendelenburg position. The key point of the examination was the reflux absence and complete occlusion of the RFA treated vein, which was interpreted as absence of recurrence of VV at the time of examination. According to the DUS examination of the GSV, which was treated by the RFA was assessed in all the length of the vein as a closed, not closed / re-open or de novo. Commission of the Ethics approved the research protocol with compliance. The investigation was conducted in compliance with the Declaration of Helsinki and subsequent additions. All the subjects of research provided written informed agreement. Control visits took place before the operation, on the 1st and 7th day after the operation, on the 1st, 3rd, 6th, 9th and 12th month after the procedure.

Intraoperative manipulation included the vein puncture, RFA of GSV trunk and perforating or communicating veins. RFA was made under the DUS guidance with the help of ultrasound machine ULTIMA PA Expert of RADMIR company (Ukraine) using of a linear probe. Performing RFA procedure had a number of additions without coming into conflict with NICE's 2013 recommendations. To perform the first stage of the procedure the CR45i guide wire was used. It was put into the great saphenous vein in its distal part next to the medial condyle using the Seldinger technique with 6F introducer up to 1.5 cm distal inferior to the sapheno-femoral junction (SFJ). We should mention that obliteration of GSV is performed only with distal part of 0.5 cm which is withdrawn little by little to close the vein completely. Fast entry and withdrawal of the catheter is provided by special polytetrafluoroethylene (PTFE) coating of its flexible part.

After the satisfactory positioning of the guide wire had been confirmed with ultrasound, tumescent anesthetic solution was injected into the soft tissue surrounding the target vein along its entire length. Tumescent anesthesia with standard Klein's solution was used by mixing 20 mL of 2% lidocaine with 1L of normal saline and adding 0.5 mL of epinephrine 1:1000 and 12.5 mL of 8.4% $NaHCO_3$.

CR12i and CR30i wires with special introducers were used for the RFA of varicose tributaries of the GSV with diameter from 1.5 to 5 mm and for the perforating veins. As the generator of radio frequent impulses was used system EVFR. Thus, for coagulation of GSV trunk there was used the power of 25 W and consequently for the perforating and collateral veins from 8 to 20 W based on the vein diameter. The metal wire tip produced the radio frequent signal with 4 MHz frequency. Under the influence of this radio frequency the wall of the surrounding vein was heated and coagulated.

After the procedure, compression stockings and bandages were applied to reduce postoperative bruising and tenderness. Within 7 postoperative days Nadroparin was administered subcutaneously for the

venous thromboembolism prevention. The first dose was injected in the hospital after that in an outpatient setting. There was also recommended intake of outpatient-based combination therapies by Diosmin and Hesperidin.

Patient's data as well as case histories, clinical symptoms of VV were summarized and analyzed in accordance with the descriptive statistics, which included mean, median, standard deviation at 95% confidence interval. The primary endpoint of the investigation was to identify recurrence of VV after RFA within 12 months. The secondary endpoint was to assess the clinical course of the investigated patient groups according to the classification scales CEAP and VCSS. Endpoints were assessed before the surgery and on the 1st and 7th day after the surgery and also in 1, 3, 6, 9 and 12 months. The investigation included patients who were examined on the 1st day after the surgery. The patients with VV on the two lower limbs were analyzed as a whole during analyzing the subject characteristics of the investigation. In assessing the VV in patients who have vein disease on both lower limbs, each leg was analyzed separately. Kaplan-Meier analysis was used to describe the presence of reflux or recurrence in the veins treated by RFA. Analysis of variance (ANOVA) and multiple comparison procedure Tukey-Cramer performed on all of control visits to protection between logical conclusions and save of conventional threshold for declaring statistical significance and the error rate of type 1. There was made the comparison of control visits according to the Dunnett test. To confirm the null hypothesis there has been calculated value of the standard error in each control visit. All the analysis was made using software Microsoft Excel 2017 and XLSTAT 2017 by Addinsoft SARL, Paris, France.

RESULTS

During the period from November 2022 to November 2024 there were 134 patients with varices of the 174 GSV (VV of both lower limbs were in 40 patients) were treated at Poltava 2-nd City Hospital. Among 134 patients treated by RFA on the 12th month of the investigation 124 patients with varices of the 158 GSV (92.5%) were left. Thus, the contact with 10 of them (7.46%) was lost before the deadline of investigation.

The average age of the patients was 49.33 ± 13.03 (range 18-71) years. At the start of the investigation there were 98 (73.13%) females. At each control visit the number of women was not less than 67%. The average body mass index of 134 patients was 27.5 ± 6.2 (range 18.4-42.1) kg/m^2. Concomitant diseases were observed less than in 1/3 of the patients: 34 patients (25.37%) with hypertension history. Treatment of VV in both lower limbs by RFA was performed in 40 of 134 patients (30%). There were 84 right lower extremities and 90 left once of 174 lower limbs involved in the investigation. The average length of GSV from saphenofemoral junction (SFJ) to the venipuncture site in the most distal reflux point was 35.2 ± 10.3 cm. During the RFA procedure tumescent anesthesia with standard Klein's solution was used, its averaged volume was 400 ± 100 ml and it was injected into the soft tissue surrounding the GSV. Taking into consideration the length of the vein the volume of Klein's solution was average 11.4 ± 4.4 ml per cm. 12 patients (9%) were operated on for the veins without affecting GSV before the investigation. These surgeries included 10 sclerotherapies and 2 unspecified surgeries. If it was impossible to finish the surgical procedure only by means of RFA on the VV additional manipulations were used, such as: conventional stripping operation (phlebectomy) of 40 lower limbs (22.9%); foam sclerotherapy of 30 lower limbs (17.2%); the usage of CR30i wire for perforator veins in 20 lower limbs (11.49%).

The average preoperative GSV diameter was 10.87 ± 3.63 (range 2,0-22) mm at the distance of 3 cm from the SFJ. Most veins' diameter was measured in the supine position, in accordance with the guidance provided in the investigation design.

The occlusion of all length the GSV was achieved in the 134 patients (100%) and was confirmed according DUS. The average GSV diameter at the distance of 3 cm from the SFJ immediately after the RFA procedure was 3.16 ± 1.81 (range 1,0-9) mm. The difference between the average preoperative and postoperative diameter of GSV on the same segment was 7.86 mm. The occlusion of GSV on the next day after RFA procedure was observed in 172 lower limbs (98.85%), with an average distance from the SFJ 13,4 ± 2,8 (range 10-18) mm. In one case the occlusion of the GSV was delayed, it was confirmed on the 7th day during the control DUS.

Ultrasound observation was carried out during all control visits to measure the veins diameters treated by RFA. The frequency of DUS control on the first day after the RFA was 100% of all 87 lower limbs of the investigation group; on the 7th day they were 98.51%; in a month – 76.12%, in 3 months – 56.72%; in 6 months – 40.3%; in 9 months – 16.42% and in 12 months they were 7.46%. The average diameter of the fibrous strand of GSV after the RFA, measured at 3 cm from the SFJ, decreased from 3.16±1.81 mm on the first day to 2.88±1.56 mm in 7 days (86 lower limbs) and 2.8 ± 1.39 mm in a month (66 lower limbs); 2.61 ± 1.39 mm in 3 months (48 lower limbs); 2.39 ± 1.13 mm in 6 months (29 lower limbs); 2.08 ± 0.79 mm in 9 month (15 lower limbs); 1.8 ± 1.1 mm in 12 months of investigation (5 lower limbs), it is represented in Fig. 1. Tukey-Kramer's analysis stated

Table 1. Analysis Tukey, honestly significant difference test (HSD)

Contrast	Difference	Standardized difference	Critical value	Significant
0 vs 365	9.0657	9.2482	3.0507	Yes
0 vs 270	8.7823	13.2503	3.0507	Yes
0 vs 180	8.4728	17.8067	3.0507	Yes
0 vs 90	8.2604	19.2370	3.0507	Yes
0 vs 30	8.0618	20.5169	3.0507	Yes
0 vs 7	7.9869	21.7802	3.0507	Yes
0 vs 1	7.7015	21.0813	3.0507	Yes

Source: compiled by the authors of this study

statistically significant reduction in the diameter of the GSV fibrous strand between the examination before surgery and subsequent visits with 95% confidence interval. It is confirmed in (Table 1).

The table presents the data portion according to the ANOVA analysis, which shows a significant and stable distinction of GSV diameter at the distance of 3 cm from the SFJ within the visit before surgery - 0 and analogically for 12 months with 95% confidence intervals. It shows the comparison of difference, a standardized difference, the critical value, the significance of differences. When comparing the data of control visits after surgery there were not revealed significant differences.

The average stump length at the SFJ remained stable in time: 13.4 ± 2.76 mm on the first day; 13.35 ± 2.73 mm in 7 days; 13.33 ± 2.89 mm; 13.34 ± 2.74 mm; 13.11 ± 2.74 mm; 13.00 ± 2.73 mm; 12.2 ± 2.39 mm in 1, 3, 6, 9, 12 months after treatment accordingly (Fig 1).

On the third month of observation during the control DUS there was diagnosed perforator vein reflux in the distal third of the GSV and treated as re-opened in two patients. Reflux was accompanied by appropriate clinical picture, there were observed varicose veins below the knee. Besides, the patients mentioned the presence of heavy leg and diffuse brown pigmentation on it.

The burn of the skin appeared in the other two patients in the lower third of the leg after RFA using CR12i and CR30i wires for performant veins. Its size was 3×1.5 cm, which consequently healed by secondary intention. In a month of observation, the site of the burn was determined by minor skin pigmentation.

Kaplan-Meier analysis showed no recurrence probability VV outcome using the RFA 98.77% (Fig. 2). Confidence interval at 95% was the bottom bound - 0.936, the top bound -1.000. Standard errors for the evaluation were 0.0123. The average survival time (without recurrence of VV) was totally 238.15 days.

During the observation period, all the patients were diagnosed according to CEAP during the control visits at the time of the admission, on the first and on the seventh day and in 1, 3, 6, 9, 12 months after the RFA (Fig. 3).

In the test group before the surgery, the patients with C2 were 56.72%, with C3, C4, respectively 34.33% and 8.96%. By the third month of the observation, the patients with C3 were 21.05%. And by the sixth month, their part decreased to 3.70%. At the same time, the number of the patients with C2 increased from 15.79% in 3 months to 29.63% in 6 months. A part of the patients with C4 did not rise above 9% and tended to decrease clinical class according to the CEAP. As in 3 months the number of patients with C4 was 5.26%, then in 6 months there were no finds.

By the 9th month of the investigation and further in all the observed patients there was determined C0 (45.45%) and C1 (54.55%) clinical classes, according to the CEAP classification.

At the beginning of the investigation, the average meaning of VCSS for 174 lower limbs was 4.69 ± 2.04 (range 1-9) points. It was represented by VV without complications or venous edema not above the ankle in the evening. The showings according to VCSS improved significantly at the following visits. Thus, on the seventh day since the RFA procedure VCSS evaluation made on the average 2.52 ± 1.92 (range 0-7) points, in a month – 1.27 ± 1.22 (range 0-5) points and reached a value of 0.6 ± 0.55 (range 0-1) points for the 10 lower limbs till the 12-th month of the observation (Fig. 4).

Varicose veins were present in 100% of the lower limbs at the beginning of the investigation, 54% of them were coming out of GSV (score on the scale VCSS - 2). According to VCSS assessment, in all patients in 7 days varicose veins were not revealed in the lower limbs. A part of the lower limbs with varicose veins (score on the scale VCSS - 1) increased to 4.6% in 12 months. However, at the same time, VV appeared in 2.3% of the lower limbs in 3 months of investigation (determined by the reflux of blood through the re-opening of perforating vein in the leg) with the corresponding clinical symptoms (score on the scale VCSS - 4).

Swelling of the lower limbs with VV in the preoperative period was observed in 55.17% of the patients. In 1 month after the RFA procedure, it was observed in

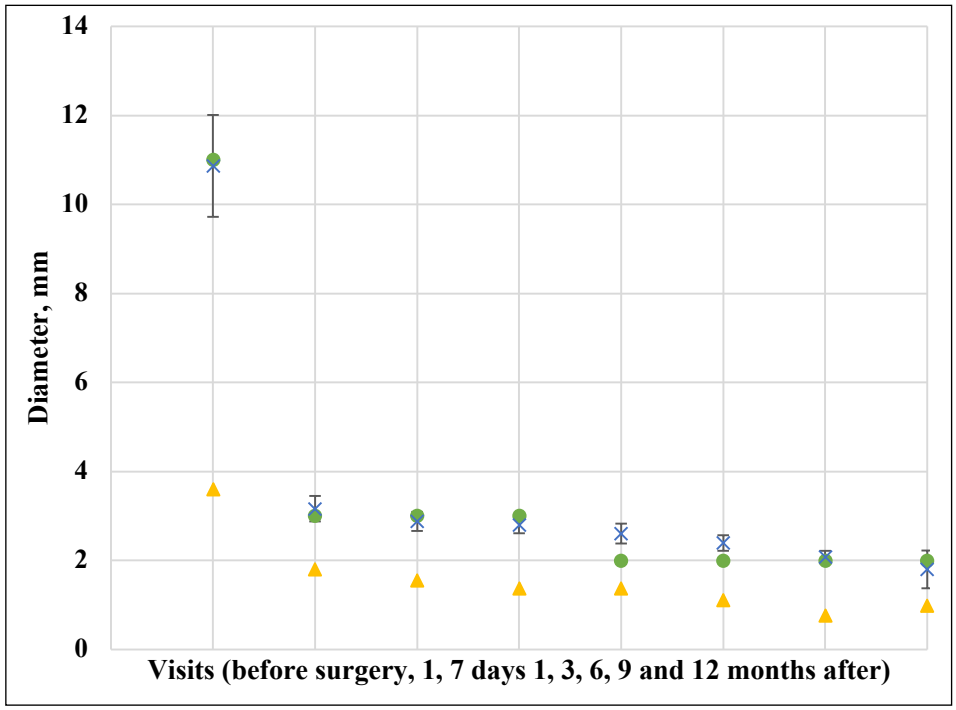


Fig. 1. The GSV diameter at the distance of 3 cm from the SFJ before and after RFA – in 1, 7 day's and 1, 3, 6, 9, 12 months of observation. Mean (blue cross), the median (green dot), standard error of variance at all study visits (black line with horizontal outside) and the standard deviation - n (yellow triangle)
Picture taken by the authors

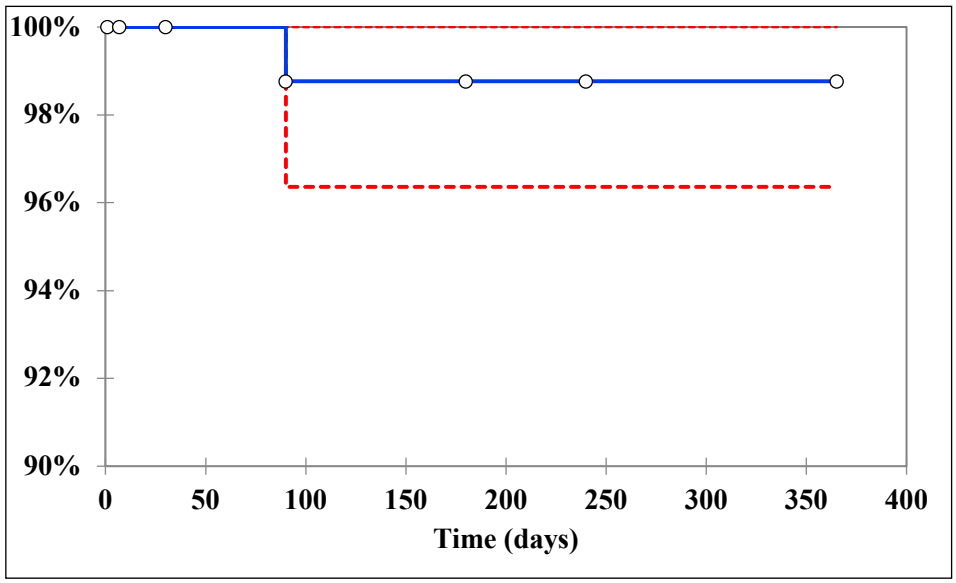


Fig. 2. The distribution function of Kaplan-Meier shows the probability of the outcome without recurrence of VV using RFA (blue line). The black circles indicate the control visits of the patients in the sample. The red dotted line marks the upper and lower limit of the confidence interval. Standard error throughout the investigation was less than 0.05
Picture taken by the authors

12.64% of the lower limbs. It appeared in the evening and did not extend above the leg (VCSS 2 points). In 12 months, swelling was observed during the control visit at 20.68% of the patients, but it did not extend above the ankle and appeared only in the evening (score on the scale VCSS - 1).

According to the survey, 62.06% of the respondents at the time of admission complained of pain according to VCSS. At 40.23% it was an episodic pain without affecting the activity and did not require analgesics. At 19.54% it was a daily pain, which moderately restricted the activity and required to take analgesics episodically. At 2.3 % of cases, it was a daily pain, which essentially restricted the activity and required to take analgesics regularly. In a month after the treatment, the pain reduced to 22.99%, and in 3 months it reduced to 19.54%. There was assessed episodic pain (17.24%) and daily pain, which moderately restricted the activity and required to take analgesics episodically (2.3%). In 9 months after the treatment that index decreased to 9.2% of the lower limbs: episodic (6.9%) or daily (2.3%) respectively. In 12 months, only 1.15% of patients complained of episodic pain.

DISCUSSION

The results of 12 months observation of used firstly the monopolar RFA EVFR system in patients with GSV

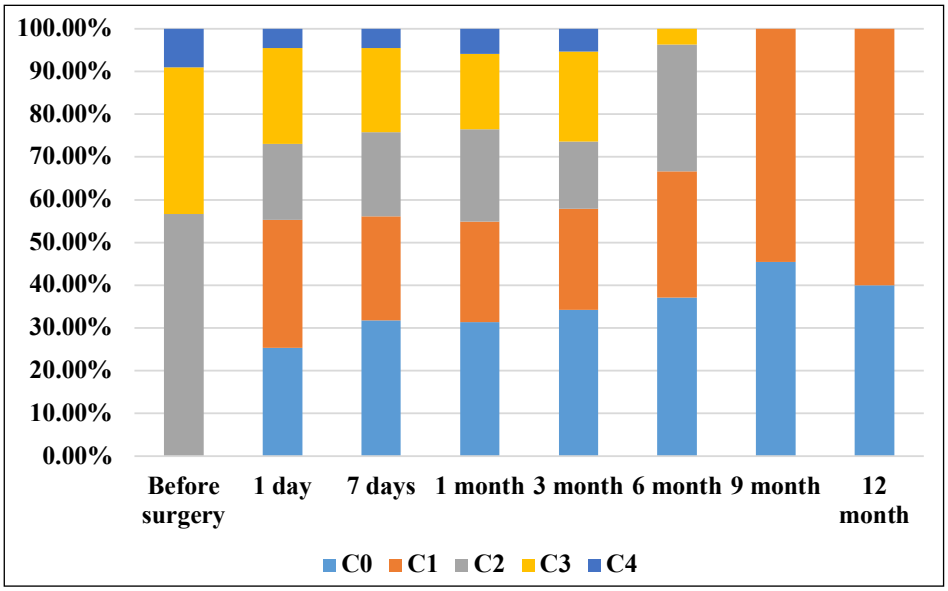


Fig. 3. The proportion of the patients according to the CEAP classification before the procedure, on the first and seventh days and in 1, 3, 6, 9, 12 months of observation. C0 (blue column), C1 (brown column), C2 (gray column), C3 (yellow column), C4 (blue column)
Picture taken by the authors

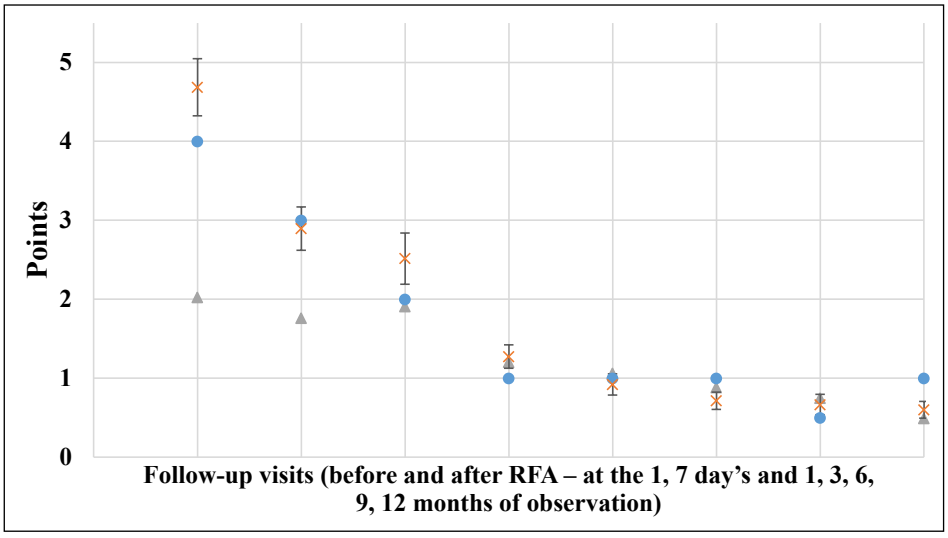


Fig. 4. The average meaning of VCSS before the procedure, on the first and seventh days and in 1, 3, 6, 9, 12 months after RFA (orange cross). The blue circles show the median of VCSS at the time of control visit. Standard error variance at all visits of the investigation was less than 0.8 (the black line with the horizontal limits) and the standard deviation - n (gray triangle)
Picture taken by the authors

incompetence are submitted in the mentioned above investigation. A limited set of the side effects of RFA combined with a high frequency of long-term occlusion of GSV is confirmed with prospectively multicenter comparisons with endovenous laser and classical surgery [5]. Different investigations have acknowledged the auspicious by-effect profile of RFA versus endovenous laser ablation (EVLA) [6-7]. The pain and swelling were observed more rarely for the first fortnight after the RFA procedure versus EVLA procedure. The patients who got the RFA came back to work 4 days sooner than those ones who got EVLA [8]. When we compare the RFA with modern technology of Mechanochemical ablation (MOCA), we can see that MOCA is not so painful and contributes to patients returning to work earlier [9]. Nevertheless, there is evidence that after a year observation at RFA group occlusion of the lumen of the GSV was achieved in 100% cases, while the successful occlusion of the GSV in MOCA group was 82%, and the quality of life of the patients was similar in the groups of comparison [10]. According to the recent investigation, the RFA provides a low frequency to reflux recurrence in 12 months [11]. This method is characterized as one having minimal side-effect profile.

At present, most modern methods of treatments of saphenous vein incompetence are concentrated on minimum by-effects and qualitative well-being after the procedure [12]. By the way, it is worth taking into consideration the original goal of reliable and long-term absence of vein reflux. For example, there is the foam sclerotherapy of the saphenous veins, which looks attractive at first sight due to its easy and quick use and minimal decline in the quality of life [13]. In reference to foam sclerotherapy, we mustn't choose it as the therapy of the first-line at saphenous veins incompetence, at their large diameter in particular, because the risk of unsuccessful grows if the vein diameter is larger than 6 mm [14]. Taking into consideration the mentioned

above data, the anatomic long-term outcomes of MOCA show that the degree of recanalization and detection of reflux in treated veins is higher than after RFA and EVLA [14]. Besides, the revision of protocols for using EVLA with lower doses of energy is necessary, as there are the data showing that the rate of postoperative pain and hematoma was higher than using the wavelength 1470 nm. With the reduction of the energy dose of laser radiation side effect profiles may be improved, but the anatomical success rates may fall below standard benchmarks.

The definition of occlusion was very specific and limited key criteria in this investigation: complete occlusion and the absence of reflux in the treated vein 3 cm below of the SFJ. There was determined blood flow in the perforator vein of GSV in the leg 5 cm length at 1 patient after the RFA in 3 months. In such cases, a real unsuccess of treatment is doubtful since the localization of venous tributaries can affect the final occlusion of GSV.

CONCLUSIONS

In 12 months, the RFA demonstrated its efficiency as a method of endovenous treatment of incompetent great saphenous vein from the point of view of sustainable clinical and anatomic success for the majority of treated patients.

In spite of the fact that in this investigation the quality patients' life was not evaluated, the VCSS and a few of its items distinctly showed the improvement of long-term clinical progress after the RFA. Within 12 months of the observation VCSS assessment improved significantly and tended to decrease the number of points. According to the evaluation VCSS, the presence of VV (sum score 1-3) fell to a minimum of less than 3% just on the first day after the procedure and then remained stable of about 2% in 12 months. It reflects the natural course of vein disease. According to VCSS pain index improved a lot in the medium term. On the baseline only 37.94% of the affected lower limbs didn't feel pain, while its part was more than 98.85% till the 12th month of investigation. The RFA is an efficient way to treat the GSV incompetence in the medium term. Complex investigation of other techniques within 12 months is necessary to select the optimal method of treatment for GSV incompetence.

No adverse events or serious side effects related to the procedure were recorded, and local manifestations were less pronounced compared to laser ablation, allowing patients to return to work faster. Based on our evaluation of clinical data, it can be concluded that RFA is an effective treatment for GSV insufficiency in the medium term. However, to select the optimal treatment method and prevent recurrence of varicose veins and GSV insufficiency after one year, a comprehensive study of other methods over 12 months and long-term follow-up of all patients is necessary.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Justification of the choice of surgical treatment of varicose veins of the lower extremities depending on the physical and morphometric indicators of the thigh and the great saphenous vein

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ABSTRACT

Aim: To improve the results of traditional treatment and combined in patients with varicose veins, evaluate the long-term results of treatment depending on the magnitude of varicose transformation and morphometric parameters of the lower extremities.

Materials and Methods: We analyzed the results of examination and treatment of 228 patients with varicose veins (VV) of the lower extremities who underwent treatment at the Andriy Novak Surgical Clinic from 2012 to 2023. The inclusion criteria in the study were patients with VV (C2-C6 according to CEAP) who gave their voluntary consent to the examination and measurement. In order to compare the results of sclerosurgical and surgical treatment the ultrasound, and the frequency of pathological signs were used.

Results: It was obtained during the measurement of the limbs, when calculating the taper expressed in percentage, it was found that in people with a height of 180 cm and more and a weight of 80+ kg, taper reaches ≥15% in 68 (70%) patients ($p<0.001$). Analysis of long-term results in the two studied groups of patients revealed a statistically significant difference in the frequency of good results from medical interventions with an advantage in favor of surgical treatment (Fisher's criteria $p=0.014$).

Conclusions: To prevent and reduce the rate of unsatisfactory results when choosing a method of surgical treatment of varicose veins, morphometric indicators and thigh taper should be taken into account.

KEY WORDS: sclerotherapy, venous reflux, varicose disease, phlebectomy, crossectomy, conicity

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INTRODUCTION

Varicose vein disease (VVD) is a very common surgical pathology. Despite significant progress in the diagnosis and treatment of VVD, dissatisfaction with their results persists, due to the steady increase in the number of patients, a large percentage of complicated forms of the disease, significant treatment costs, and a high frequency of VVD relapses (VVR), which significantly reduces the quality of life of patients [1, 2].

Despite the active introduction of modern technologies, surgical treatment of VD is considered the most radical and rightfully constitutes the basis of medical care for patients with this pathology [3, 4].

According to the group of authors, currently extraction of the main trunks of the saphenous veins using probes of various modifications (stripping) is the main stage of radical surgical intervention in VD [5, 6]. However, many years of international experience in the application of the Babcock operation in practice has allowed us to identify not only its advantages, but also possible complications that may accompany such an intervention. Unlike surgical treatment, phlebosclerooblitteration is undoubtedly less traumatic, economically affordable, can be performed in outpatient settings, is accompanied by less cosmetic losses and a shorter period of medical and social rehabilitation [7]. In some countries, sclerotherapy is used in more than 80% of VD treatment [8].

According to modern thinking, sclerotherapy itself with subsequent compression is an equivalent stage of phlebosclerosis treatment. Unfortunately, it is practically impossible to predict the adequacy of great safein vein (GSV) compression in the postoperative period, and as a result, the effectiveness of sclerooblitteration in general.

Vertical trunk reflux along the GSV is a well-established pathogenetic mechanism for the development and

Table 1. Clinical distribution of patients according to the international CEAP classification before treatment

Class	Clinical characteristics of the class	Distribution of lower extremities by clinical classes	
		Group I n=189	Group II n=96
C0	No signs of venous pathology were detected during examination and palpation	-	-
C1	Telangiectasias, or reticular veins	-	-
C2	Varicose dilated subcutaneous veins	147(77.8%)	38(39.6%)
C3	Edema	5 (2.6%)	18(18.8%)
C4	Skin changes (hyperpigmentation, venous eczema, lipodermatosclerosis)	4(2%)	21(21.9%)
C5	Healed trophic ulcer	21(11.7%)	13(13.5%)
C6	Active trophic ulcer	12(6.3%)	6(6.2%)

Source: compiled by the authors of this study

progression of venous insufficiency in patients with GSV [9].

Today, the arsenal of funds aimed at its correction is very diverse, including radiofrequency or laser ablation. This is all due to the lack of a single universal treatment method for any clinical situation, on the one hand, the indication to improve the functional outcome of treatment, the cosmetic nature of the intervention, reduce the terms of medical and social rehabilitation, and improve the quality of life of patients with VD.

At the same time, a sufficiently large number of varicose vein recurrences, especially in ovGSVeight patients after traditional venectomy and trunk scleroobliteration, is noteworthy [1, 4, 7].

AIM

To improve the results of traditional treatment and sclerosurgery in patients with varicose veins, evaluate the long-term results of treatment depending on the magnitude of varicose transformation and morphometric parameters of the lower extremities.

MATERIALS AND METHODS

We analyzed the results of examination and treatment of 228 patients with varicose veins (VV) of the lower extremities who undGSVent treatment in the surgical clinic named after A. Novak from 2012 to 2023. The inclusion criteria in the study were patients with VD (C2-C6 according to CEAP) who gave their voluntary consent to the examination and measurement (Table 1). The exclusion criteria from the study were severe concomitant diseases, the presence of thrombosis or thrombophlebitis, and the patient's refusal to participate in the study.

At the same time, 153 patients in group I had two limbs treated in 36 patients, and in 75 patients in group II, two limbs were treated in 21 patients.

Group I consisted of 153 patients (189 limbs operated on), who undGSVent sclerosurgery. For the convenience of analysis, the number of operated limbs in patients with CEAP VD classes from C2 to C6 was counted - a total of 189 limbs. Thus, patients with C2-C4 classes undGSVent crossectomy+trunk catheter scleroobliteration, which amounted to 156 (82.5%) limbs. Crossectomy+trunk catheter scleroobl iteration+echosclerotherapy of perforating veins was performed in patients with C5, which amounted to 21 (11.1%) limbs. Patients with CEAP class C6 undGSVent short stripping of the great saphenous vein (GSV), distal scleroobliteration, and echoscleroobliteration of perforating veins on 12 (6.3%) limbs.

The second group of patients included 75 patients (96 limbs), who undGSVent surgical treatment, mainly phlebectomy according to Babcock-Narat. Similarly to group I, the classes and volume of surgical treatment were correlated with the number of operated limbs. In C2-C4 class, crossectomy + trunk phlebectomy + removal of varicose tributaries was performed - 77 (80.2%) limbs. In C5 class, crossectomy + trunk phlebectomy + ligation of incompetent perforating veins was performed - 13 (13.5%) limbs. In patients with C6 class, crossectomy + trunk phlebectomy + removal of varicose tributaries + ligation of incompetent perforating veins was performed according to CEAP, which amounted to 6 (6.2%) limbs.

Of the 228 patients, 59 were men (25.9%), 169 were women (74.1%). The age of the patients at the time of surgical treatment did not exceed 50 years, on average 47±2.8 years.

As can be seen from Table 1, patients were divided into 2 groups. During the treatment process, patients were provided with complete information about possible treatment options and the subsequent cohort of surgical or sclerosurgical treatment was chosen by the patient based on his own beliefs – that is, it was

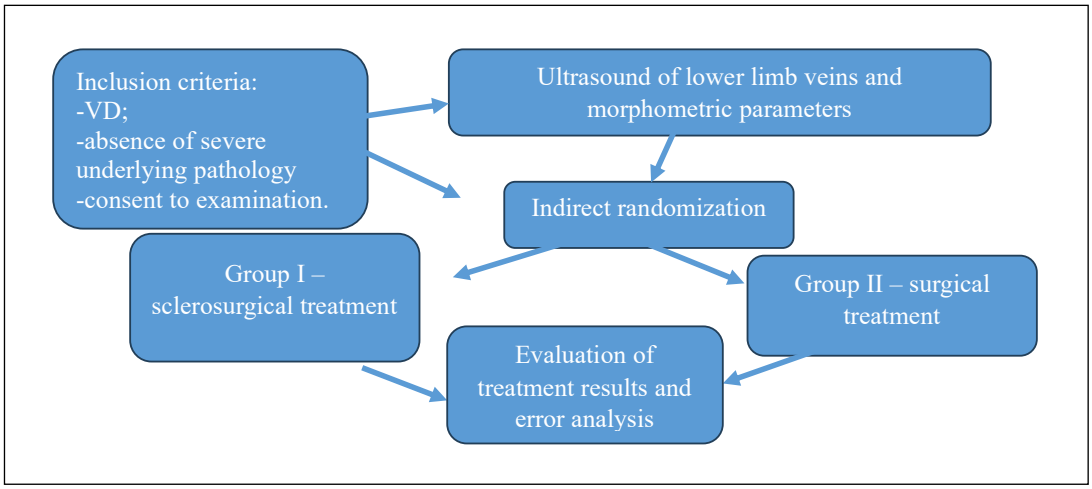


Fig. 1. Study design
Picture taken by the authors

carried out in a randomized manner. The study design is presented in (Fig. 1).

Ultrasound examination was performed on SonoAce R3, Medison (Korea) and Philips En Visor HD (USA) devices, equipped with linear sensors of 7-10 MHz and convex - 3.5-5 MHz. During ultrasound examination, visual inspection of the vessels of the venous bed in real time (B-mode) was combined with the study of the color cartogram and assessment of its spectral characteristics using functional tests of proximal and distal compression, cough test, Valsalva test, Siegel compression test. Angioscanning was necessarily performed in the longitudinal and transverse planes, both lower extremities were examined. Angioscanning was used to measure the diameter of the GSV. In order to compare the results of sclerosurgical and surgical treatment using the ultrasound method, the frequency of pathological signs such as: Varicose transformation of the ostium ducts (length of the GSV stump > 3 cm); GSV obliteration; partial recanalization; complete recanalization; incompetent femoral perforating veins; incompetent tibial perforating veins. The diameter of the GSV in the two groups of patients was not statistically different and did not exceed 10 mm in the upper third of the thigh.

The examination began in the horizontal position, with visualization of the great saphenous vein, peroneal veins, and groups of perforating veins of the leg. The common femoral vein was scanned medial to the artery of the same name, below the inner third of the inguinal fold.

In the upright position, the source of reflux, the distribution paths and the channels of reflux return to the deep venous system were monitored.

Using blind randomization, 98 patients were selected, who were measured for morphometric parameters such as: weight, height, thigh diameter in the upper and lower third, thigh length between diameters, and thigh conicity was determined. From group I, 58 (25.4%) patients (90 limbs operated) were examined, who undGSVent sclerosurgical intervention, from group II, 40 (17.5%) patients (54 limbs) were included, who undGSVent only surgical treatment.

All 98 patients had their femoral conicity measured in the upper and lower thirds according to mathematical modeling. Conicity (C) [10] is the ratio of the diameter of the base of the cone to its height. In the case of a truncated cone, it is the ratio of the difference in diameters of the cross sections D and d of the truncated cone to the distance between them:

$$C = \frac{D - d}{H}$$

where C is the conicity, D is the base diameter, d is the diameter of the smaller base and H is the distance between the bases (Fig. 2).

The severity of VD symptoms (before treatment and in the postoperative period) was assessed using the international CEAP classification. In the long-term postoperative period (5-9 years), three disease variants were recorded: VD regression compared to the initial status, progression, and lack of dynamics.

The criterion for regression of VD was considered to be a decrease in the clinical class and was assessed as good results. An increase in the clinical class according to CEAP was regarded as progression of VD despite the treatment performed and was assessed as an unsatisfactory result. The lack of dynamics in the long-term observation period was assessed on the basis of preservation of the initial clinical class and / or its return to the initial status after 5-9 years as a satisfactory result.

Statistical data were calculated using the Jamovi 2.38.8 software package. When analyzing the distri-

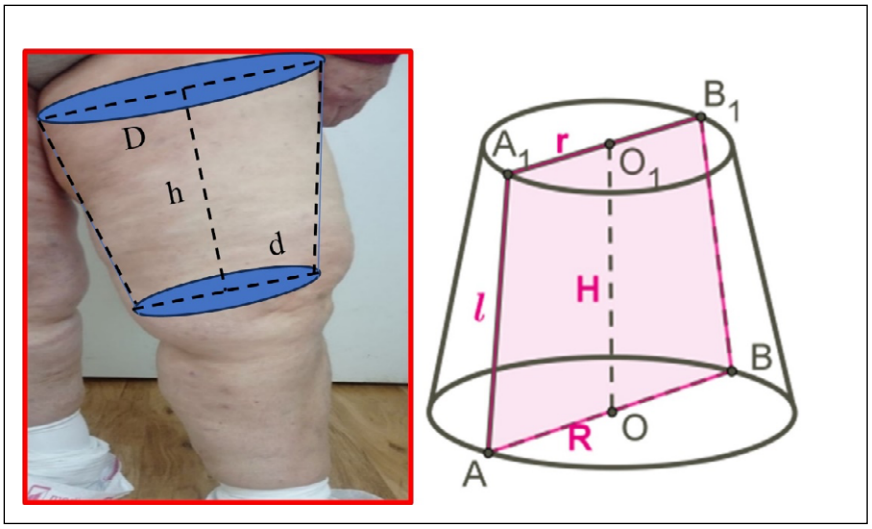


Fig. 2. Schematic representation of the conicity of the hip: R- large radius, r- small radius, H – height
Picture taken by the authors

bution of indicators, it was found that the distribution was correct, so the Student's T test was used. When calculating the average values, the average value and standard deviation (M ± sd) were used. The results are presented in percentage values and absolute values. The Spearman test was used to calculate the correlation dependence.

RESULTS

The postoperative observation period was 6-10 years after the operations. In order to compare the results of sclerosurgical and surgical treatment, the frequency of pathological signs was assessed using the ultrasound method (Table 2).

As can be seen from Table 2, a pathologically long stump of the GSV with varicose transformation of the ostium tributaries was observed in 8 (4.2%) and 6 (6.2%), respectively, and an «additional» erroneously left GSV trunk in 5 (2.6%) and 11 (11.5%) of the examined groups. Partial recanalization of the GSV trunk after sclerotherapy was observed in 54 (28.6%), complete recanalization in 30 (15.5%), which in our opinion was associated with poor compression in the postoperative period after sclerotherapy and an incompetent penetrating femoral vein in 18 (9.5%) cases.

The results of measuring lower limb circumference in patients with chronic venous insufficiency are presented in Table 3.

According to the data obtained during the measurement of the limbs, when calculating the conicity expressed in percentages, it was found that in people with a height of 180 cm and more and a weight of 80+ kg, conicity reaches ≥15% in 68 (70%) patients ($p<0.001$). When assessing the correlation according to Spearman, a high correlation was established between conicity in patients and morphometric indicators such as weight, height and diameter of the upper third of the thigh ($p<0.001$), when analyzing the influence of indicators on conicity of the thigh using linear regression, it was established that weight and height and diameter of the thigh in the upper third strongly affect the change in conicity of the thigh ($R2=0.745$). After analyzing the dependence of conicity on morphometric indicators using Student's T test for independent samples, it was found thatin healthy people with a height of <180 cm and a weight of less than 80 kg, the percentage of conicity was two times less - no more than 10% ($p<0.001$).

Sclerotherapy requires adequate compression for 7-9 days after surgery, which is impossible to achieve with a similar thigh shape.

If the pathological stump of the GSV with varicose transformation of the ostium tributaries, varicose transformation of the anterior accessory saphenous vein of the thigh were observed approximately equally in both groups of patients, and partial recanalization of the GSV (28.6%) and complete recanalization of the GSV (15.9%) were characteristic only with sclerotherapy.

Thus, when analyzing the results of DAS of the veins of the lower extremities performed in patients with VD in the long-term period after sclerosurgical and surgical interventions, no statistically significant difference was found between patients of the two studied groups for 10 out of 15 ultrasound criteria for comparison. Sonographic characteristics of patients with VD after sclerosurgical treatment and phlebectomy, the difference in which is statistically significant (6 out of 15), are not specific to one or the other method of treatment.

It should be noted that out of 10 signs that statistically significantly differ in frequency of observation after different treatment methods, 5 were registered more often after phlebectomy. Postoperative ultrasound monitoring not only objectifies the results of the

Table 2. Comparative assessment of the frequency of pathological ultrasound signs after sclerosurgical and surgical treatment

Pathological ultrasound signs	Sclerotherapy abs. and relative number of limbs n=189 (100%)	Phlebectomy abs. and relative number of limbs n=96 (100%)	Statistical significance (p)
Technical and tactical errors in operational interventions			
Pathological stump of the GSV, varicose transformation of the estuarine tributaries	8(4.2%)	6(6.25%)	p>0.05
"Additional" mistakenly left-over GSV trunk	5(2.65%)	11(11.5%)	p<0.05
Varicose transformation of the anterior accessory saphenous vein of the thigh	42(22.2%)	25(26.04%)	p>0.05
Partial recanalization of the GSV	54(28.6%)	-	-
Complete recanalization of the GSV	30 (15.9%)	-	-
"Lateral varicose veins"	5(2.6%)	18(18.7%)	p<0.05
Possible errors and/or progression of IH			
Varicose transformation of the estuarine tributaries (stump length is normal)	17(9%)	7(7.3%)	p<0.05
Varicose transformation of the intersaphenous vein	13(6.9%)	3(3.1%)	p>0.05
Insufficiency of the perforating veins of the lower leg	85(45%)	41(42.7%)	p<0.05
Insufficiency of the perforating veins of the thigh	18(9.5%)	9(9.4%)	p>0.05
Common femoral and femoral vein valve insufficiency	6(3.17%)	4(4.17%)	p>0.05
Popleteal vein valve failure	21(11.1%)	17(17.7%)	p<0.05
Insufficiency of the valves of the deep veins of the lower leg	31(16.4%)	28(29.2%)	p<0.05
Progression of VD			
"Cavernoma"	4(2.1%)	3(3.13%)	p>0.05
Varicose veins of the small safein vein (SSV) (previously intact)	19(10.1%)	24(30.2%)	p<0.05

Source: compiled by the authors of this study

Table 3. Results of basic topometric indicators in patients with CVI (n=98)

Sex	Weight (abs. and relative amount in %)		Growth (abs. and relative number in %)		Conicity (abs. and relative amount in %)	
Men n=24	≥80kg	12 (12.2%)	≥180cm	10(10.2%)	≥15%	19 (12.2%)
	<80kg	12 (12.2%)	<180 cm	14(14.3%)	<15%	5(12.2%)
Women n=74	≥80kg	50 (51.1%)	≥180cm	39(39.8%)	≥15%	49(12.2%)
	<80kg	24 (24.5%)	<180 cm	35(35.7%)	<15%	25(12.2%)

Source: compiled by the authors of this study

performed interventions, but also helps to determine the strategy of further treatment.

Analysis of long-term outcomes in the two studied groups of patients revealed a statistically significant difference in the frequency of good results from medical interventions with an advantage in favor of surgical treatment (for Fisher's criteria p=0.014) (Table 4).

The difference in the frequency of registered unsatisfactory results depending on the treatment methods in the long-term postoperative period is not statistically significant (p = 0.64). At the same time, according to the clinical class of the CEAP group of patients with the C4-C6 class who underwent sclerosurgical treatment amounted to only 37 (19.6%) of the total number of lower extremities (189), and in the control group (96 cats) patients with the C4-C5-C6 clinical class amounted to 40 (41.7%) cases, which is 29.5% more than in the main group. In addition, in the main group, crossectomy (CE) + short stripping with distal scleroobliteration was performed on 12 (6.3%) extremities. At the same time, in these patients with CEAP-6, an unsatisfactory result was not observed in any case.

Table 4. Summary table of the dynamics of regression of the clinical condition of CEAP after appropriate treatment by groups in the long-term postoperative period

Group II n=96 limbs			Group I n=189 limbs		
Clinical class according to CEAP		Treatment result	Clinical class according to CEAP		Treatment result
Before treatment	After treatment		Before treatment	After treatment	
C2-38	C1 – 16	16 - good	C2 -147	C1 - 39	39 - good
	C2 - 11	11- satisfactory	C2 - 91	91- satisfactory	
	C3 - 12	12 - unsatisfactory	C3 - 17	17 - unsatisfactory	
C3 - 18	C2 - 6	6 - good	C3 - 5	C2 - 0	0 - good
	C3 - 9	9- satisfactory	C 3 - 3	3- satisfactory	
	C4 - 3	3 - unsatisfactory	C4 - 2	2 - unsatisfactory	
C4 - 21	C3 - 5	5 - good	C4 - 4	C3 - 1	1 - good
	C4 - 16	16 - satisfactory	C4 - 3	3- satisfactory	
	C5 - 0	0 - unsatisfactory	C5- 0	0 - unsatisfactory	
C5 – 13*	C4 - 0	0 - good	C5 - 21	C4 - 0	0 - good
	C5 - 13	13- satisfactory	C5 - 21	21- satisfactory	
	C6 - 0	0 - unsatisfactory	C6 - 0	0 - unsatisfactory	

The presence of a significant number of observations with a return to the initial clinical class of CEAP in the long-term observation period was recorded in both groups of patients (in the main group - 114 (60.3%) limbs, in the comparison group - 52 (54.2%) limbs), apparently due to the fact that the use of the presented treatment methods in a number of cases is unable to control the progression of VD.

The prevalence of unsatisfactory results was observed in patients with C2 and C3 clinical scales according to the CEAP, in the main group 20 (10.6%) cases, in the control group 13 (13.5%) cases. Study of resultss-cleroobliteration (189 limbs 100%) in the long-term observation period based on the dynamics of the clinical picture of CVI with CEAP demonstrated a sufficiently high effectiveness of this type of intervention: the total number of positive results (good and satisfactory results 170 (90%), and with standard phlebectomy 83 (93%).

When analyzing the treatment results of patients who were measuredhip conusity it was found that in 98 patients, of which 58 (25.4%) patients (90 limbs operated) were examined from the first group of patients, unsatisfactory results were observed in 19 patients, the conusity of which was 20%. In the second group, 40 (17.5%) patients (54 limbs) were examined, who undGSVent only surgical treatment - unsatisfactory results were observed in 12 patients, but the conusity of the hip of these patients did not exceed 10%, which is probably due to an incorrectly chosen treatment tactic or an error made during the surgical treatment of VD.

DISCUSSION

Modern and high-quality medical compression hosiery should have several important properties, namely, the pressure should decrease in the proximal direction in a certain sequence: 100% - in the supraosteal area, 70% - at the level of the upper third of the lower leg, 40% - at the level of the upper third of the thigh. Such a regressive pressure gradient contributes to a better implementation of the mechanisms of compression effect and good tolerability of the hosiery by the patient.

When using elastic knitwear in patients with a pronounced conicity of 15% or more, the effect of compression knitwear will be proportionally worsened due to the increase in pressure on the proximal part of the limb.

In such cases, in our opinion, it is advisable to use such treatment methods as radiofrequency and/or laser ablation or phlebectomy and/or crossectomy with short stripping in this group of patients.

In our opinion, defining clear indications for this type of treatment is necessary in order to reduce the risk of possible complications, reduce the frequency of IH recurrence, and improve the results of scleroobliteration. In addition, the analysis of the specialized literature demonstrates different views of scientists on this problem. One group of scientists recommends catheter scleroobliteration in the case of uncomplicated forms of IH with an IH diameter of no more than 7-10 mm and the absence of varicose deformation of the tributaries [9, 11, 12].



Fig. 3. A – lower limb with a conicity of more than 20%; B – lower limb with a conicity of less than 20%
Picture taken by the authors

Another group of authors consider the limiting diameter of the trunk to be 15 mm, and even positive results of scleroobliteration at a diameter of 20 mm [13-15]. In addition, the circumference and shape of the thigh are important criteria for patient selection (Fig. 3 AB).

With pronounced fatty tissue, with a conical shape of the thigh, the probability of full compression in the postoperative period is minimal. In this case, surgical methods of correction should be preferred, namely phlebectomy.

Unfortunately, based on practical experience, even precise selection of patients for scleroobliteration with strict control of intraoperative algorithms and techniques for performing the procedure does not guarantee the expected treatment outcome.

CONCLUSIONS

1. The total number of positive (good and satisfactory) treatment outcomes demonstrated comparable efficacy of phlebectomy and sclerosurgery at 93% and 90%, respectively.
2. In people with a height of 180 cm and more and a weight of 80 kg and more, conicity reaches ≥15% in 68 (70%) patients ($p<0.001$). When assessing the correlation according to Spearman, a high correlation was established between conicity in patients and morphometric indicators such as weight, height and diameter of the upper third of the thigh ($p<0.001$)
3. When choosing a method of surgical treatment of varicose veins in order to prevent and reduce the rate of unsatisfactory results, morphometric indicators and thigh conicity should be taken into account.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Hepatoprotective role of vildagliptin in CLP-induced sepsis in mice

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ABSTRACT

Aim: The current study aimed to determine the underlying mechanisms of protective effect of vildagliptin against sepsis induced liver injury.

Materials and methods: Male mice were randomly allocated to sham group, Cecal Ligation and Puncture group, Dimethyl sulfoxide group and vildagliptin group (20 mg/kg). Cecal Ligation and Puncture operation was established for mice. After 24 hours of Cecal Ligation and Puncture, serum levels of Alanine Transaminase, Aspartate Transaminase and histopathological changes of liver tissue were evaluated. Our study assessed IL-6, Macrophage migration inhibitory factor, Intercellular Adhesion Molecule 1, caspase-11, Malondialdehyde, Superoxide dismutase and Microtubule-associated protein 1A/1B-light chain 3 by ELISA method as well as Wnt and B-catenin by RT-qPCR.

Results: The results of current study showed that vildagliptin administration significantly declined serum levels of Alanine Transaminase and Aspartate Transaminase in addition to reduced histopathological changes of liver. Vildagliptin markedly inhibited elevation of IL-6, MIF, Intercellular Adhesion Molecule 1, MDA, caspase-11 and Wnt/B-catenin. Vildagliptin elevated Superoxide dismutase and Microtubule-associated protein 1A/1B-light chain 3 in hepatic tissue.

Conclusions: Vildagliptin has Hepatoprotective effect during endotoxemia induced liver injury through modulation inflammatory and oxidative stress markers in addition to down regulation of Wnt/B-catenin pathway.

KEY WORDS: vildagliptin, Cecal Ligation and Puncture, sepsis, Wnt/B-catenin pathway, oxidative stress, autophagy, apoptosis

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ABBREVIATIONS

CLP - Cecal Ligation and Puncture
DMSO - Dimethyl Sulfoxide
ALT - Alanine Transaminase
AST - Aspartate Transaminase
MIF - Macrophage Migration Inhibitory Factor
ICAM-1 - Intercellular Adhesion Molecule 1
MDA - Malondialdehyde
SOD - Superoxide Dismutase
LC3 - Microtubule-associated protein 1A/1B-light chain 3
GLP-1 - Glucagon-Like Peptide-1

INTRODUCTION

Sepsis can be defined as a serious inflammatory response syndrome caused by infection with high morbidity and mortality [1]. They have been reported the number of deaths caused by multiorgan failure is about 11 million, which represent a great threat to human worldwide [2]. The liver is considered as substantial organ and is involved in various physiological process including detoxification, metabolism and immunity. Sepsis induced liver injury resulting in abnormalities of biochemical investigations and hepatic insufficiency or even liver failure [3]. Early identification and intervention of liver injury result in improvement survival rates [4]. Sepsis can be initiated by a number of pathogens such as parasites, bacteria, viruses and fungi. The main characteristics of sepsis including intense inflammatory response, activation of coagulation and immune suppression [5-6]. The pathogenesis of sepsis includes various mechanisms such as microcirculation disorder, immune response, inflammatory mediators' release and pathogen invasion [7]. The Wnt /B- catenin pathway has concerned as topic of interest among several molecular mechanisms contributed to pathogenesis of sepsis. Wnt/B-catenin elevation has been detected in sepsis and inhibition attenuated inflammatory response and mitigated sepsis induced organ injury. Additionally, aberrant activation of Wnt/ B-catenin signaling pathway

has been implicated in different diseases including degenerative disease and cancer [8]. Vildagliptin is a hypoglycemic agent related to class DPP-4 inhibitor, used for treatment type 2 diabetics through inhibit degradation of glucagon-like peptide-1 (GLP-1) [9]. Previous studies documented that vildagliptin ameliorated hepatic damaged induced by ischemia/reperfusion *via* suppression inflammatory, oxidative stress and apoptotic markers [10]. The present study aimed to investigate the potential Hepatoprotective effect of vildagliptin against sepsis induced liver injury.

MATERIALS AND METHODS

A total of twenty-four Swiss albino male mice, weighting 25-35 gm and aged 8-12 weeks, were randomly distributed into following groups 1- sham group: mice subjected to anesthesia and laparotomy procedure, 2- CLP group: mice subjected to CLP procedure, 3- DMSO group: mice administered an equivalent volume of DMSO intraperitoneal 1h before CLP procedure 4- Vildagliptin group: mice received 20 mg/Kg intraperitoneal 1 hour prior CLP procedure. CLP procedure was established as described previously [11-12]. Anesthetized all experimental mice by intraperitoneal solution of ketamine (100 mg/kg) and xylazine (10 mg/kg). The abdomen was shaved, disinfected and one cm midline laparotomy to exposed cecum. Then cecum was tightly ligated at the base below ileocecal valve, perforated twice with 20-gauge needle size and squeezed in order to extrude feces from perforation regions. Consequently, the cecum was returned to peritoneal cavity and the skin was closed with 6.0 silk. The experimental mice were resuscitated by subcutaneous injection 1 ml 0.9% saline solution and supplied free access of food and water. Blood samples were collected to investigate liver enzymes (aspartate transaminase, AST and alanine transaminase, ALT) and macrophage migration inhibitory factor (MIF). A small part of liver was excised for histopathological examination and the rest of liver tissue was homogenized to measure intercellular adhesion molecule 1 (ICAM-1), interleukin-6 (IL-6), caspase-11, malondialdehyde (MDA), superoxide dismutase (SOD) and microtubule-associated protein 1A/1B-light chain 3 (LC3) in addition to the gene levels of Wnt (F CAAATAGGCAGCCGAGAGAC, R TGCAACCACAGGTAGACAGC) and B-catenin (F GTCAGCTCGTGTCCTGTGAA, R GATCTGCATGCCCTCATCTA) using qPCR. All laboratory and histopathological tests were performed after CLP procedure.

REAGENTS

Vildagliptin was purchased from AK Scientific, USA. ELISA kit of SOD and MDA were obtained from Bioassay Technology Laboratory BT LAB. Additionally, ELISA kit of caspase-11, ICAM-1, MIF, LC3 and IL-6 were purchased from SunLong Biotech CO. Ketamine vial (100 mg/ml) was received from Alfasan and xylazine (20 mg/ml) was from Micropets.

HISTOPATHOLOGICAL EXAMINATION

Liver tissues were kept in buffered formaldehyde solution 10%, then processed and embedded in paraffin. The section was cut to 5 µm thick and stained with hematoxylin and eosin (H&E). Liver histopathological score from 0 to 3. Score 0 when no pathological changes observed, score 1: is considered mild, score 2: moderate, score 3 is sever [11].

STATISTICAL ANALYSIS

Prisms 9.5 software used for analysis. All measurement data were expressed as mean ± standard error of mean. One-way ANOVA followed by Bonferroni test for comparison between groups. Kruskal-Wallis test was used for comparison histology scores. $P<0.05$ was considered statistically significant.

ETHICS APPROVAL

All experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at Kufa University after submitting the required applications (letter number 20549-29/8/2024).

RESULTS

EFFECT OF VILDAGLIPTIN ON SERUM LEVELS OF ALT AND AST

Significantly elevated serum concentration of both ALT and AST were detected in the CLP group in comparison with sham group. Pretreatment with vildagliptin significantly decreased the level of ALT and AST in septic mice. DMSO showed no significant effect on ALT and AST levels when given to septic mice (Fig. 1A-B).

Mice of sham group exposed to laparotomy procedure only. The mice of CLP group were exposed to CLP operation for 24 h. Mice received either vildagliptin (20 mg/kg), DMSO or left untreated (CLP and sham groups) 1 h prior CLP operation. Serum liver enzymes were measured using spectrophotometry. Statistical analysis was performed using one-way ANOVA followed by Bonferroni test.

EFFECT OF VILDAGLIPTIN ON HEPATIC IL-6, HEPATIC ICAM-1 AND SERUM MIF LEVEL

Significantly elevated tissue concentration of IL-6, ICAM-1 and serum MIF were detected in the CLP group

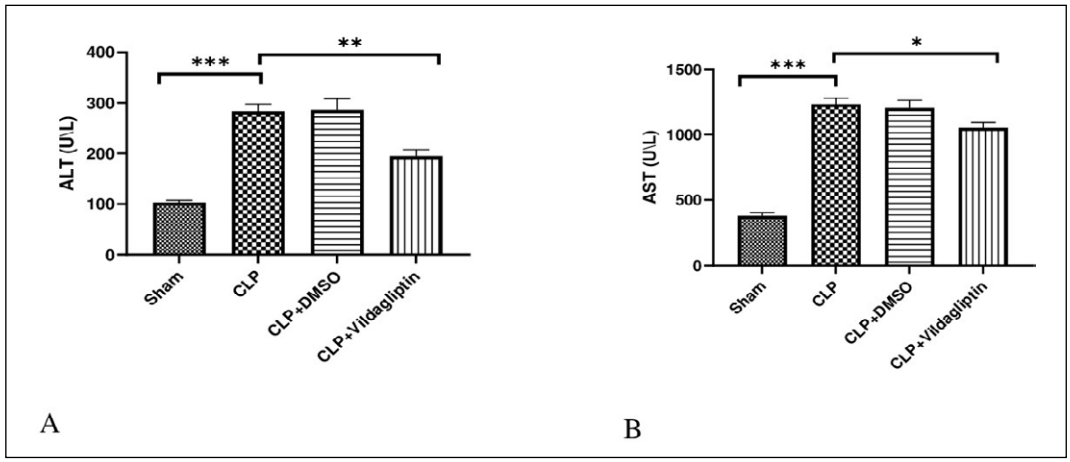


Fig 1. Effect of vildagliptin on serum levels of ALT (A) and AST (B)
Data are presented as mean ±SEM, n=6/group; *p<0.05, **p<0.01, ***p<0.001.
Source: Own materials

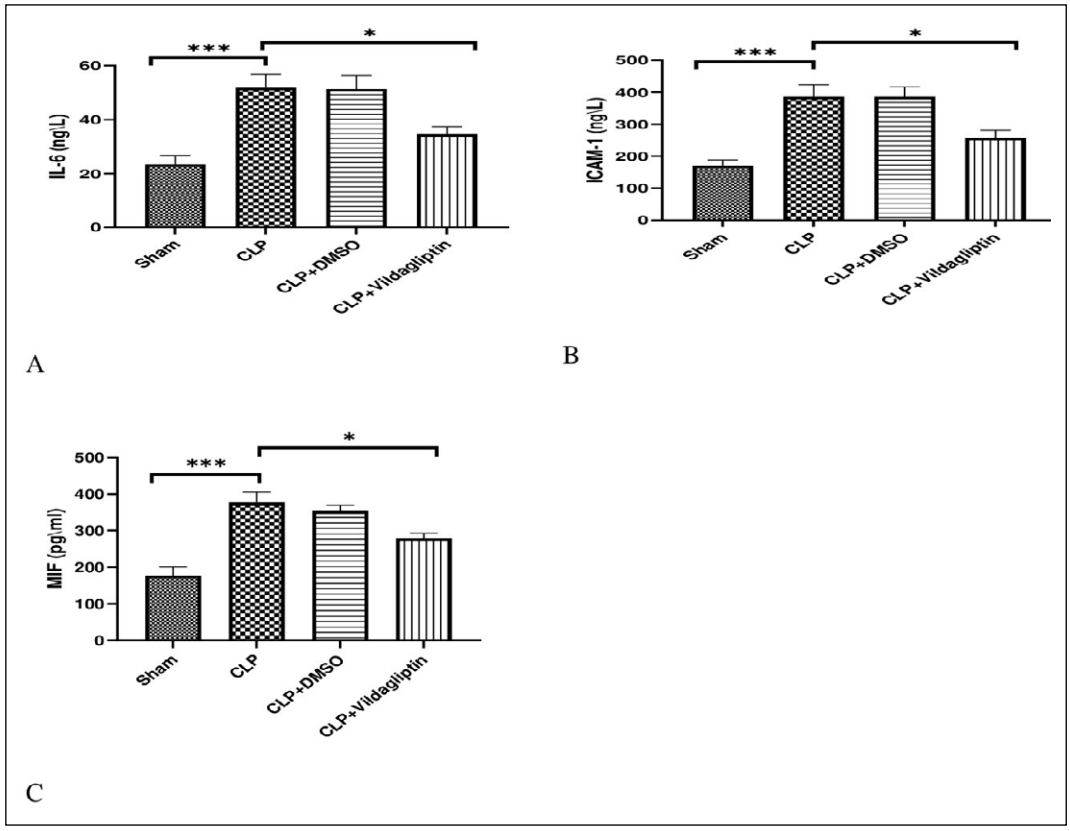


Fig. 2. Effect of vildagliptin on hepatic levels of IL-6 (A), hepatic ICAM-1 (B) and serum MIF (C)
Data are presented as mean ±SEM, n=6/group; *p<0.05, ***p<0.001.
Source: Own materials

in comparison with sham group. Pretreatment with vildagliptin significantly decreased levels of IL-6, ICAM-1 and MIF. DMSO did not affect IL-6, ICAM-1 and serum MIF levels when given to septic mice (Fig. 2A-C).

Mice of CLP group were exposed to CLP operation for 24 h, mice of sham group exposed to laparotomy procedure only. Mice received either vildagliptin (20 mg/kg), DMSO or left untreated (CLP and sham group) 1h before CLP operation. Liver IL-6, liver ICAM-1 and serum MIF were measured using ELISA kit. Statistical analysis was performed using one-way ANOVA followed by Bonferroni test.

EFFECT OF VILDAGLIPTIN ON HEPATIC LC3 LEVEL

Level of LC3 in the liver was significantly declined in the CLP group in comparison with sham group. Pretreatment with vildagliptin significantly elevated level of LC3. DMSO showed no significant impact on LC3 levels when given to CLP mice (Fig. 3).

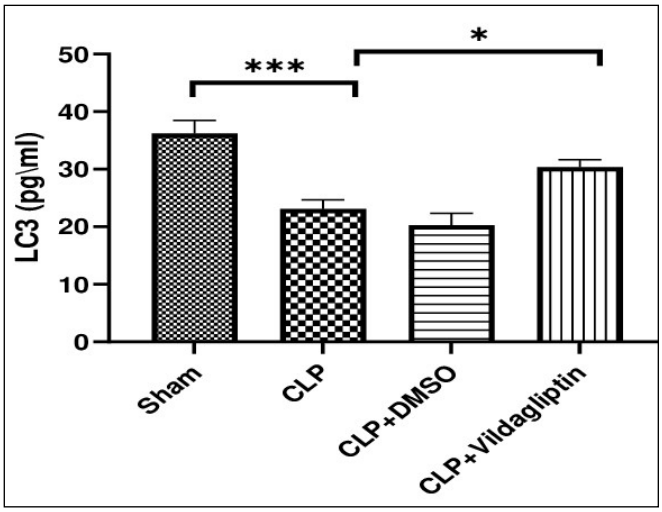


Fig. 3. Effect of vildagliptin on hepatic LC3 level
Data are presented as mean ±SEM, n=6/group; *p<0.05, ***p<0.001
Source: Own materials

Mice of CLP group were exposed to CLP operation for 24 h, while mice of sham group exposed to laparotomy procedure only. Mice were administered either vildagliptin (20 mg/kg), DMSO or left untreated (CLP and sham groups) 1h before CLP operation. Liver LC3 was measured using ELISA kit. Statistical analysis was performed using one-way ANOVA followed by Bonferroni test.

EFFECT OF VILDAGLIPTIN ON HEPATIC MDA AND HEPATIC SOD LEVELS

Significantly lowered tissue concentration of MDA was detected in the CLP group in comparison with sham group. Pretreatment with vildagliptin significantly lowered level of MDA (Fig. 4A). Hepatic level of SOD was significantly declined in the CLP group in comparison with sham group. Pretreatment with vildagliptin significantly elevated level of SOD (Fig. 4B). Pretreatment of septic mice with DMSO showed no significant impact on MDA and SOD levels.

Mice of sham group exposed to laparotomy procedure only. The mice of CLP group were exposed to CLP operation for 24 h. Mice were administered either vildagliptin (20 mg/kg), DMSO or left untreated (CLP and sham group) 1h before CLP operation. Liver MDA and SOD were measured using ELISA kit. Statistical analysis was performed using one-way ANOVA followed by Bonferroni test.

EFFECT OF VILDAGLIPTIN ON HEPATIC CASPASE-11 LEVEL

Significantly elevated tissue concentration of caspase-11was detected in the CLP group in comparison with sham group. Pretreatment with vildagliptin significantly decreased hepatic level of caspase-11. DMSO showed no significant change on caspase-11 levels when given to septic mice (Fig. 5).

Mice of sham group exposed to laparotomy procedure only. Mice of CLP group were exposed to CLP operation for 24 h. Mice received either vildagliptin (20 mg/kg), DMSO or left untreated (CLP and sham groups) 1h before CLP operation. Liver caspase-11 was measured using ELISA kit. Statistical analysis was performed using one-way ANOVA followed by Bonferroni test.

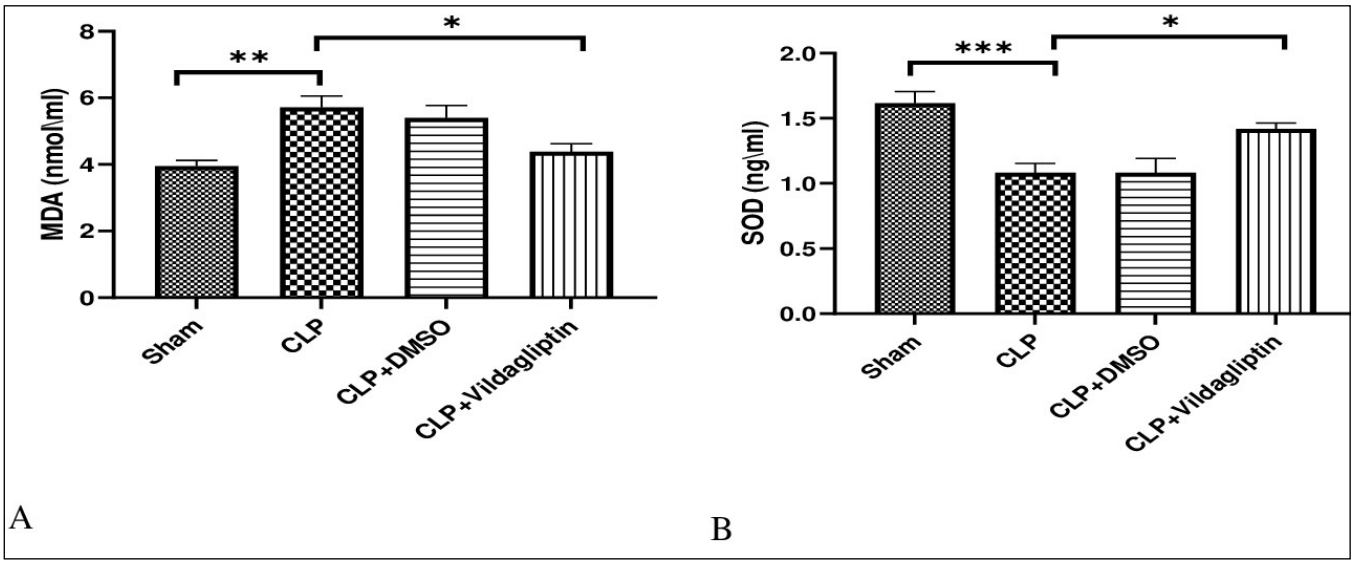


Fig. 4. Effect of vildagliptin on hepatic MDA and SOD levels
Data are presented as mean ±SEM, n=6/group; *p<0.05, **p<0.01, ***p<0.001
Source: Own materials

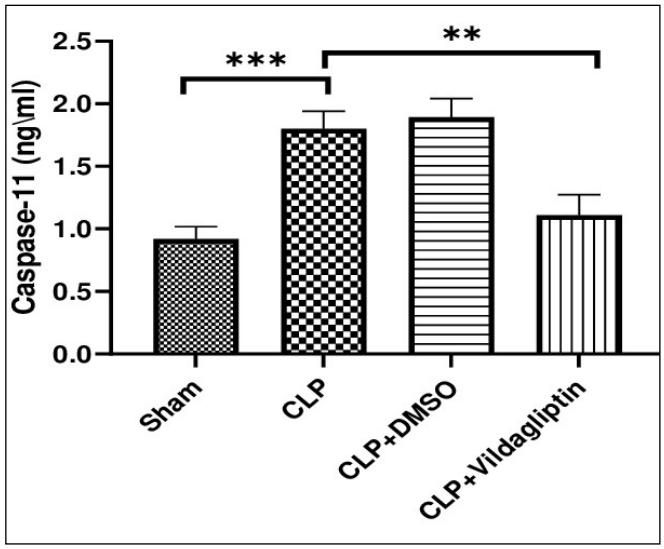


Fig. 5. Effect of vildagliptin on hepatic caspase-11 level
Data are presented as mean ±SEM, n=6/group; **p<0.01, ***p<0.001.
Source: Own materials

EFFECT OF VILDAGLIPTIN ON HEPATIC WNT/B-CATENIN PATHWAY

Significantly lowered tissue ΔCT levels of Wnt and B-catenin were detected in the CLP group in comparison with sham group. Pretreatment with vildagliptin significantly elevated ΔCT levels of Wnt and B-catenin. DMSO showed no significant impact on both tissue ΔCT levels of Wnt and B-catenin when given to septic mice (Fig. 6A-B).

Mice of sham group exposed to laparotomy procedure only. Mice of CLP group were exposed to CLP operation for 24 h. Mice received either vildagliptin (20 mg/kg), DMSO or left untreated (CLP and sham groups) 1h before CLP operation. Liver Wnt and B-catenin were measured using RT-PCR. Statistical analysis was performed using one-way ANOVA followed by Bonferroni test.

VILDAGLIPTIN TREATMENT ALLEVIATES HISTOLOGICAL CHANGES OF THE LIVER IN SEPTIC MICE

In sham group, there is no histological changes as showed in figure 7A. The CLP group exhibited sever histopathological changes characterized by inflammatory cell infiltration, cytoplasmic eosinophilia and necrosis. The histopathological change of CLP was significantly elevated as compared with sham group. Vildagliptin treatment was significantly reduced sepsis induced pathological changes (Fig. 7B-E).

Liver histopathological score in study group. The mice of CLP group were exposed to CLP operation for 24 h, mice of sham group exposed to laparotomy procedure only (sham group score 0). Mice received either (20 mg/kg), DMSO or left untreated (CLP and sham group) 1h before CLP operation. Histology changes were assessed by Kruskal-Wallis test (Fig. 8).

DISCUSSION

Sepsis, a life threating condition characterized by severe organ dysfunction resulted from uncontrolled host response to infection [13]. The pathophysiology mechanisms of sepsis induced organ dysfunction are related to intense inflammatory response, tissue hypoperfusion and intravascular coagulation [14]. The

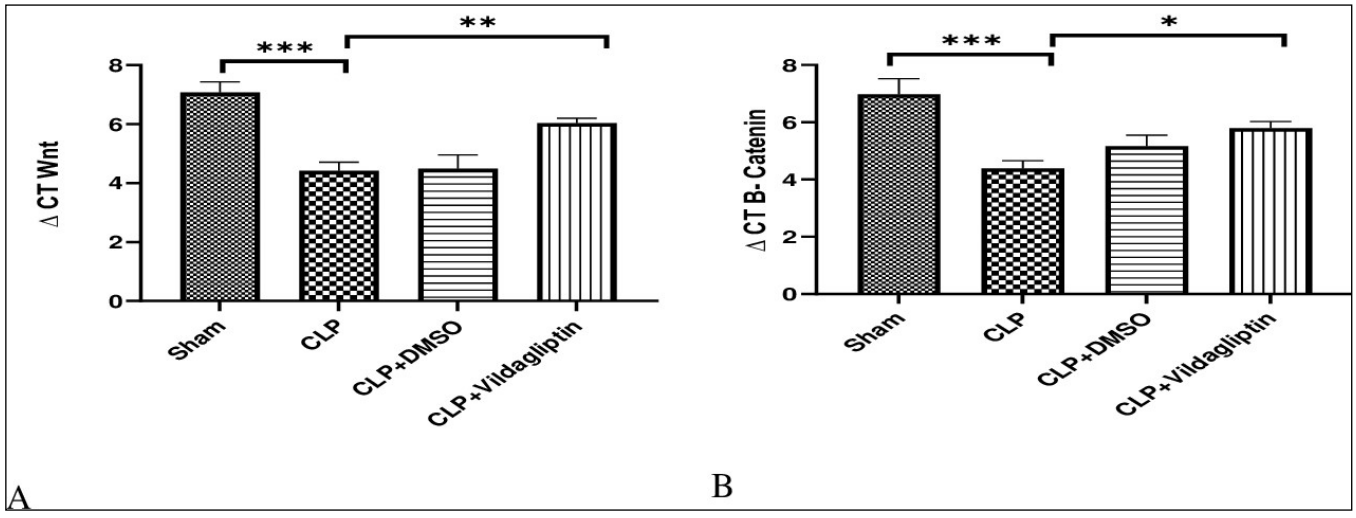


Fig. 6. Effect of vildagliptin on hepatic Wnt **(A)** /B-catenin pathway **(B)**
Data are presented as mean ±SEM, n=6/group *p<0.05, **p<0.01, ***p<0.001
Source: Own materials

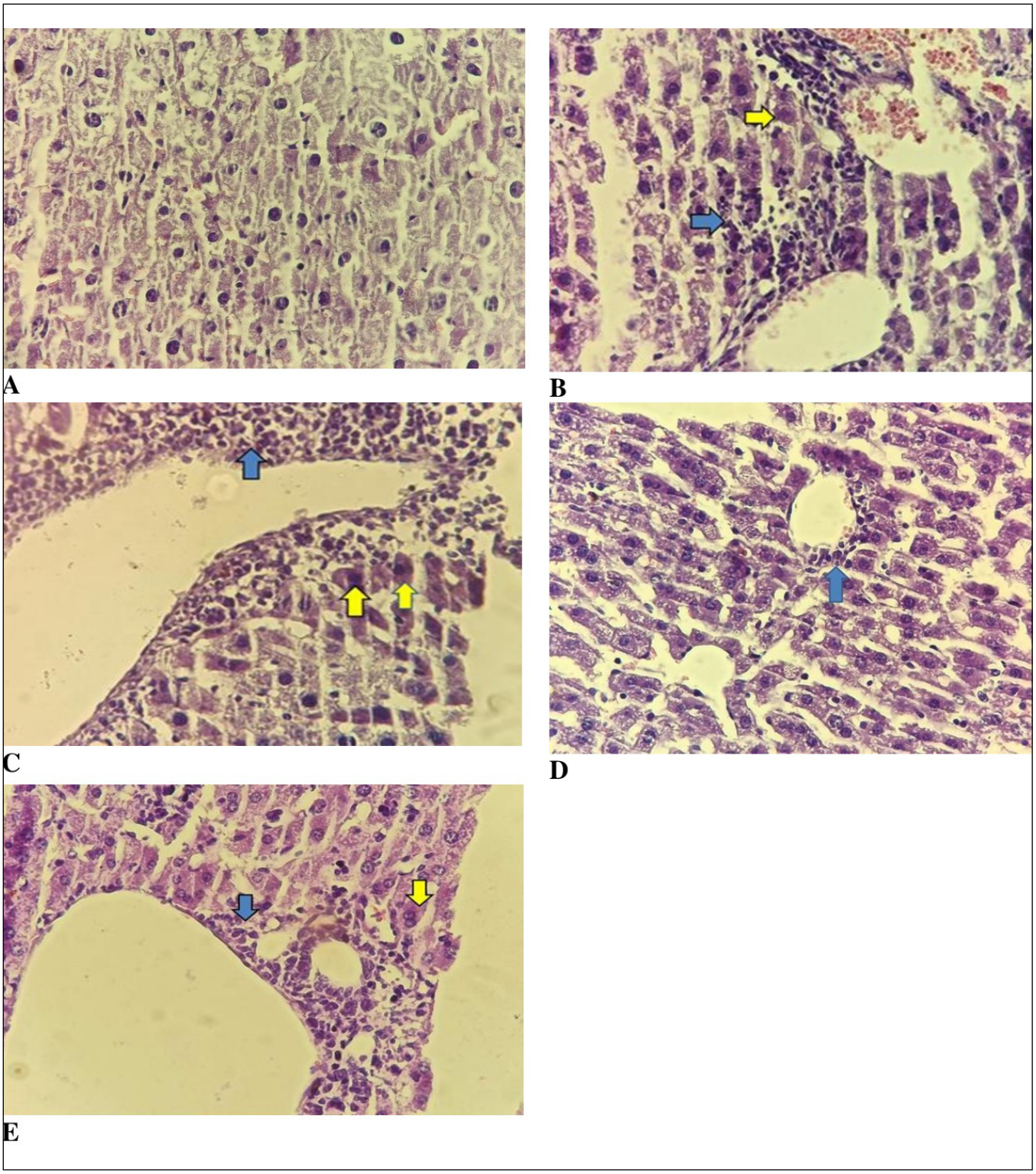


Fig. 7. A - Photomicrograph of hepatic section for sham group showed no evidence of inflammation or necrosis; score 0, H&E, x400; **B** - Photomicrograph of hepatic section for DMSO group showed sever periportal chronic inflammatory cell infiltration (blue arrow), with regions of pyknosis, cytoplasmic eosinophilia and necrosis (yellow arrows); score 3, H&E, x400; **C** - Photomicrograph of hepatic section for CLP group showed sever periportal and centrilobular chronic inflammatory cell infiltration (blue arrow), with focal regions cytoplasmic eosinophilia (apoptotic bodies) pyknosis and necrosis in the interface area (yellow arrows); score 3, H&E, x400; **D** - Photomicrograph of hepatic section for Vildagliptin group showed mild centrilobular chronic inflammatory cell infiltration (blue arrow); score 1, H&E, x400; **E** - Photomicrograph of hepatic section for sham group showed moderate periportal chronic inflammatory cell infiltration (blue arrow), with regions of pyknosis and apoptotic bodies in the interface area (yellow arrows); score 2, H&E, x400

Source: Own materials

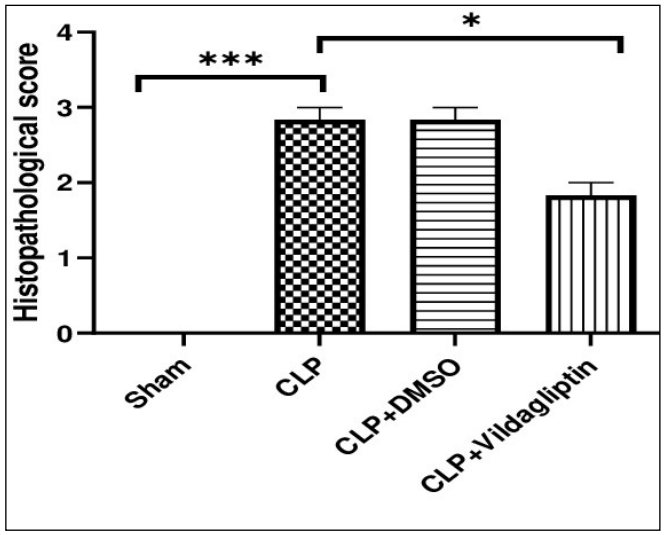


Fig. 8. Impact of vildagliptin on liver injury score. Sham group score 0 Data are presented as mean ±SEM, n=6/group *p<0.05, ***p<0.001
Source: Own materials

current study detected that the serum levels of ALT and AST were elevated in mice subjected to CLP operation in comparison with sham group, coincided with previous report indicating that CLP operation induced liver injury in mice with increased levels of ALT and AST [12, 15]. By contrast, pretreatment with vildagliptin significantly lowered serum concentration of ALT and AST in comparison with CLP group confirming that vildagliptin has protective effect against sepsis induced liver injury. This results are coincided with other study indicating that vildagliptin significantly decreased elevated liver enzyme in rat model with hepatic I /R [16] . Our study represents the first to address vildagliptin protective effect against sepsis induced liver injury. Our results detected marked elevation of IL-6 in liver tissue of CLP group compared to sham group. Several researches are in agreement with our finding [11, 17]. IL-6 is considered a key cytokine up regulated in pathogenesis of sepsis and induced organ injury [18-19]. IL-6 played main role in diagnosis of bacteremia in addition to elevation levels of IL-6 correlated with lowered survival rate in septic patients [20]. Vildagliptin administration showed significant declined of IL-6 in liver tissue. Our result in agreement with previous study documented that vildagliptin treatment significantly lowered IL-6 in rat model with hepatopulmonary syndrome [21]. The present study exhibited marked elevation serum level of MIF in mice exposed to CLP operation compared to sham group. Our finding is in accordance with previous studies [22]. MIF is a main factor played essential role in the development of sepsis and increasing production of IL-6 [23]. To best of our knowledge, no previous research demonstrated the effect of vildagliptin on serum MIF. In addition, current study exhibited significant elevation of hepatic ICAM-1 of CLP group compared to sham group and this result in agreement with previous study [11]. ICAM-1 is regarded as crucial adhesion molecules regulating migration and infiltration of neutrophil during sepsis in addition, Blocked ICAM-1 mitigated sepsis induced organ injury and death [24]. Vildagliptin administration markedly lowered hepatic 1CAM-1compared to CLP group. Furthermore, Wiciński and colleagues confirmed vasculoprotective effects of vildagliptin through reduction expression of ICAM-1 [25]. This study demonstrated a significant elevation of hepatic caspase-11 in CLP group compared to sham group. This results coincided with previous study [15]. LPS induced activation of caspase -11, mediated pyroptosis. However, it considered the main regulator in endotoxemia [26]. Cell pyroptosis played important role in progression of sepsis [27]. In contrast, vildagliptin treatment markedly reduced hepatic caspase-11, no previous researches investigated the effect of vildagliptin administration on caspase-11. To examine the role of autophagy in liver injury induced by CLP operation, LC3 level was determined in hepatic tissue. Current study showed that CLP was associated with significant reduction of hepatic LC3 as compared to sham group. Vildagliptin administration markedly elevated hepatic LC3 compared to CLP group. There is no previous research that documented the effect of vildagliptin on hepatic LC3 during sepsis induced liver injury. Autophagy activation can mitigate sepsis induce organ injury [28], in addition to suppress oxidative stress [29]. In sepsis, the limitation of oxygen supply in addition to damaged antioxidant system resulted in increased ROS and mitochondrial dysfunction [30]. Previous study showed that oxidative stress and inflammation caused tissue damage [31-34]. To investigate the role oxidative damaged MDA, the product of lipid peroxidation was assessed in addition to antioxidant enzymes that played important role in scavenging of free radicles [35-37]. Our results documented significant elevation of MDA alongside with lowered SOD in hepatic tissue of mice which subjected to CLP procedure and this results in agreement with previous study [38]. Vildagliptin administration showed marked declined of hepatic MDA reflecting decreased oxidative stress condition, additionally vildagliptin showed significant elevation of hepatic SOD demonstrating antioxidant effect. Our finding is consistent with study done by Sherif who showed that vildagliptin declined hepatic MDA alongside with elevated antioxidant enzyme activity in rat subjected to hepatic ischemia and reperfusion [10]. The anti-inflammatory and antioxidant of vildagliptin may be related to elevated level of GLP-1 which led to activation GLP-1 receptor [39]. That could have protective effect against mitochondrial

dysfunction [40]. Sharma et al documented that Wnt /B-catenin signaling pathway involved in pathogenesis of sepsis [8]. Our finding revealed that gene expressions of hepatic Wnt and B-catenin markedly elevated in CLP group compared to sham group. Vildagliptin treatment markedly reduced hepatic Wnt/B-catenin. No previous study investigated the effect of vildagliptin on hepatic Wnt/B-catenin during sepsis induced liver injury. Additionally, it was found that sitagliptin inhibit renal Wnt/B-catenin signaling pathway in rat model with diabetic nephropathy [41]. Our study revealed that CLP procedure led to inflammatory cell infiltration, necrosis, pyknosis and cytoplasmic eosinophilia [11]. Vildagliptin administration markedly reduced histopathological changes of hepatic tissues. The Hepatoprotective effect of vildagliptin may be attributed to its anti-inflammatory, antiapoptotic, antifibrotic and antioxidant [42-45].

CONCLUSIONS

Vildagliptin has Hepatoprotective effect during endotoxemia induced liver injury through modulation inflammatory and oxidative stress markers in addition to down regulation of Wnt/B-catenin pathway.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Features of disturbances in the antioxidant-prooxidant balance of the liver in conditions of mechanical trauma of various localizations, complicated by acute blood loss

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ABSTRACT

Aim: To determine the features of disturbances in the antioxidant-prooxidant balance of the liver in conditions of mechanical trauma of various localizations complicated by acute blood loss.

Materials and Methods: Traumatic brain injury(TBI), blunt abdominal trauma (BAT), and skeletal trauma (ST) were modeled in mature male Wistar line rats under conditions of thiopental sodium anesthesia. Additionally, acute blood loss in the amount of 1,5 % of body weight was modeled in the traumatized rats. After 3, 7, 14, 21, and 28 days of the post-traumatic period, catalase activity and malondialdehyde content were determined in the liver homogenate extract, and the antioxidant-prooxidant index (API) was calculated based on their ratio.

Results: Modeling mechanical traumas of different localizations, compared to the control, caused a shift in the antioxidant-prooxidant balance in the liver towards the predominance of prooxidant mechanisms. In the case of traumatic brain injury, the result was statistically significant starting from the 7-th day of the post-traumatic period, and for blunt abdominal trauma and skeletal trauma – starting from the 3-rd day. The greatest decrease in the antioxidant-prooxidant index at all times of the post-traumatic period was observed with blunt abdominal trauma. Complication of the modeled mechanical traumas with acute blood loss was accompanied by a deepening of metabolic disorders in the liver. In the case of traumatic brain injury, the liver API reached its minimum after 14 days of the post-traumatic period; for traumas of other localizations – already after 3 days. The amplitude of impairments at all times of the post-traumatic period was greater in the group of rats with blunt abdominal trauma.

Conclusions: Modeling mechanical traumas of different localizations compared to the control is accompanied by a shift in the liver's antioxidant-prooxidant balance towards the prevalence of prooxidant mechanisms, which is intensified by additional acute blood loss in the amount of 1,5 % of body weight. The largest disorders in the index are noted with blunt abdominal trauma.

KEY WORDS: trauma, blood loss, liver, antioxidant-prooxidant balance

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INTRODUCTION

In modern conditions, trauma occupies one of the key places among the urgent medical and social problems of humanity. In high-income countries, injuries are the third leading cause of death among victims of all age groups, second only to cardiovascular and oncological pathology [1]. It has been established that the share of combined trauma, characterized by high mortality, an increase in long-term complications and disability, has significantly increased in the structure of injuries, ultimately negatively affecting the quality and duration of life [2, 3].

According to [4], in combined trauma during peacetime, traumatic brain injuries and thoracoabdominal traumas are most common. Their causes are road traffic accidents and falls from a height. In the structure of combat trauma, the wounded with injuries to the extremities (56,7 %), chest (10,1 %), and abdomen (5,1 %) dominate [5]. Among them, shrapnel wounds occur in 35,3 % of military personnel, bullet wounds – 48,3 %, and mine-explosive injuries – 16,6 %. Combined trauma prevails in 48,9 % of the wounded.

The lack of a noticeable trend toward reducing mortality due to severe combined trauma encourages an in-depth study of its mechanisms, and the variety of traumatic injuries in terms of location and severity creates the problem of determining the contribution of different types of trauma to the aggregate of systemic homeostatic disorders that determine the consequences of the trauma, the prognosis for life and working capacity [6].

It is currently proven that in half of the cases, mortality from injuries is due to hemodynamic instability associated with acute blood loss [7]. Tissue and organ hypoperfusion, which causes hypoxia, contributes to restructuring of metabolism to an anaerobic mode with the simultaneous generation of reactive oxygen species (ROS). The consequence of their direct effect is the degradation of cell membranes due to lipid peroxidation (LPO) and proteins of cell membranes, leading to loss of their functions and the formation of multiple organ dysfunction and failure syndrome [8]. The balance between prooxidant mechanisms and antioxidant defense mechanisms is decisive in the appearance of a cascade of reactions that leads to secondary multi-organ damage, which in the late period of trauma poses the main threat to the survival of the affected individuals [9, 10].

AIM

The aim of the study is to determine the features of impairments in the antioxidant-prooxidant balance of the liver in conditions of mechanical trauma of various localizations complicated by acute blood loss.

MATERIALS AND METHODS

The experiments used 316 mature white male Wistar line rats weighing 200–220 g. The rats were selected randomly from the vivarium of I. Horbachevsky Ternopil National Medical University, Ministry of Health of Ukraine, and divided into seven groups: a control group and six experimental groups. All experiments involving the infliction of trauma were performed under conditions of thiopental sodium anesthesia (40 mg·kg^{-1}). In experimental group 1, traumatic brain injury (TBI) was modeled; in experimental group 2 – blunt abdominal trauma (BAT); and in experimental group 3 – skeletal trauma (ST), which were standardized according to mortality rates [11].

For modeling TBI, rats were inflicted with a single, dosed blow to the skull with an energy of 0,375 J [12]. For modeling BAT, rats were inflicted with a single, dosed blow to the epigastric region with a device of 2,5 cm diameter and an energy of 0,177 J·cm^{-2} [13]. For modeling ST, rats were inflicted with a single, dosed mechanical blow to each thigh with a wedge-shaped attachment and an energy of 0,637 J, which caused a closed fracture of both femurs [13].

In experimental groups 4, 5, and 6, in rats with TBI, BAT, and ST, respectively, acute blood loss in the amount 1,5 % of body weight was additionally induced by cutting the femoral vein. The volume of blood loss was determined by the gravimetric method. Hemostasis was ensured by applying a ligature.

After 3, 7, 14, 21, and 28 days of the post-traumatic period, under conditions of anesthesia, rats from each experimental group were euthanized by total bloodletting from the heart. A piece of liver was taken for the study, cooled, washed free of blood, and homogenized in a Silent Crasher 75000 homogenizer (Germany). In the extract of a 10 % liver homogenate, using a LabAnalyt SP-V1000 spectrophotometer (Granum, China), the content of malondialdehyde (MDA), which belongs to stable LPO metabolites [14], was determined. In addition, catalase activity, which characterizes the initial stage of protection against reactive oxygen species [14], was determined in the liver homogenate extract. Based on these data, the antioxidant-prooxidant index (API) was calculated as the ratio of catalase activity to the content of malondialdehyde. This index indicates the balance of prooxidant and antioxidant mechanisms [15]. Control group rats were only anesthetized and taken out of the experiment under similar conditions after 14 days.

The experiments were conducted in compliance with the «General Ethical Principles of Experiments on Animals,» approved by the First National Congress on Bioethics (Kyiv, 2001) and consistent with the provisions of the «European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes» (Strasbourg, 1986), as well as the conclusion of the Bioethics Commission of I. Horbachevsky Ternopil National Medical University, Ministry of Health of Ukraine No. 82 of 03.09.2025.

The obtained digital material was processed using the STATISTICA software package ("StatSoft Inc.", USA). The median (Me), lower and upper quartiles (LQ, UQ) were determined. For independent comparison of the degree of deviation of indicators in animals with trauma of different localization and different age groups, the average ratio of individual values of the studied indicators to the average value of the control group was calculated. The significance of differences was assessed by the non-parametric Mann-Whitney criterion.

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Table 1. API value in the liver (conventional units) after modeling traumatic brain injury, blunt abdominal trauma, and skeletal trauma ((Me (LQ;UQ)) – median (lower and upper quartiles)

Group of rats	Examination period				
	3 day	7 day	14 day	21 day	28 day
Control			0.698 (0.617; 0.731)		
Group 1 TBI	0.605 (0.595; 0.690)	0.456* (0.448; 0.514)	0.220* (0.214; 0.226)	0.420* (0.410; 0.438)	0.514* (0.467; 0.576)
Group 2 BAT	0.338* (0.281; 0.369)	0.357* (0.341; 0.382)	0.270* (0.262; 0.299)	0.353* (0.350; 0.391)	0.418* (0.370; 0.431)
Group 3 ST	0.482* (0.461; 0.510)	0.351* (0.341; 0.369)	0.416* (0.402; 0.472)	0.348* (0.334; 0.355)	0.557* (0.507; 0.568)
p_{1-2}	<0.05	<0.05	<0.05	<0.05	<0.05
p_{1-3}	<0.05	<0.05	<0.05	<0.05	>0.05
p_{2-3}	<0.05	>0.05	<0.05	>0.05	<0.05

Notes:
1. * – differences relative to the control group are statistically significant (p<0.05)
2. p_{1-2} – significance of differences between experimental groups 1 and 2
3. p_{1-3} – significance of differences between experimental groups 1 and 3
4. p_{2-3} – significance of differences between experimental groups 2 and 3
Source: compiled by the authors of this study

RESULTS

The study showed that under the influence of TBI, the liver API decreased compared to the control (Table 1, Fig. 1). The result became statistically significant starting from the 7- th day of the post-traumatic period. At this time point, the index was 34,7 % lower than in the control and 24,6 % lower compared to the result of the 3-rd day of the experiment (p<0,05). After 14 days, the index reached its minimum value and was 68,4 % lower than in the control (p<0,05), and 63,6 % and 51,8 % lower compared to the results of the 3-rd and 7-th days of the post-traumatic period (p<0,05). Subsequently, the index increased and after 28 days became 2,34 times higher compared to the result of the 21-st day (p<0,05), exceeded the result of the 21-st day of the experiment by 22,4 % (p<0,05), but did not reach the control level and was 26,4 % lower (p<0.05).

After modeling BAT, the liver API significantly decreased compared to the control already after 3 days of the post-traumatic period—by 51,6 % (p<0,05). The index remained at the same level until the 7-th day of the experiment (p>0,05), and then decreased until the 14-th day, reaching a minimum value and being 61,3 % lower than in the control (p<0,05). Subsequently, the index increased and by the 28-th day of the post-traumatic period became higher compared to the result of the 14-th day (by 54,8 %, p<0,05), but remained 40,1 % lower than in the control (p<0,05).

In turn, with ST, the liver API also decreased compared to the control starting from the 3-rd day of the post-traumatic period (by 30,9 %, p<0,05). Subsequently, the index fluctuated from the 7-th to the 21-st day, but remained significantly lower than the control (by 49,2 %, 40,4 %, and 51,1 %, respectively, p<0,05). By the 28-th day of the post-traumatic period, the index increased, exceeding the results of the 7-th, 14-th, and 21-st days (by 58,7 %, 33,8 %, and 60,0 %, respectively, p<0,05), but remained substantially lower than in the control (by 20,3 %, p<0,05).

Comparison of the experimental groups showed that after 3 and 28 days of the post-traumatic period, the liver API was significantly lower in rats with BAT than in rats with TBI (p_{1-2}<0,05) and ST (p_{2-3}<0,05). After 7 and 21 days of the post-traumatic period, the index was lowest in the groups of rats with BAT and ST (p_{1-2}<0,05, p_{1-3}<0,05). After 14 days of the post-traumatic period, the index was substantially lower in the group of rats with TBI (p_{1-2}<0,05, p_{1-3}<0,05).

Under conditions of acute blood loss, impairments in liver API on the background of modeled traumas of different localizations were more pronounced (Table 2, Fig. 2). In rats with TBI, the index decreased by 51,4 % compared to the control already after 3 days of the post-traumatic period (p<0,05). Subsequently, the index fluctuated with a period of increase after 7 days of the experiment (by 24,5 % compared to the 3-day result, p<0,05), but after 14 days, the index decreased again, reached its minimum value, and became 73,0 % lower than in the control, and 44,5 % and 55,4 % lower, respectively, compared to the results of the previous observation periods (p<0,05). Subsequently, the index increased and after 28 days of the post-traumatic period

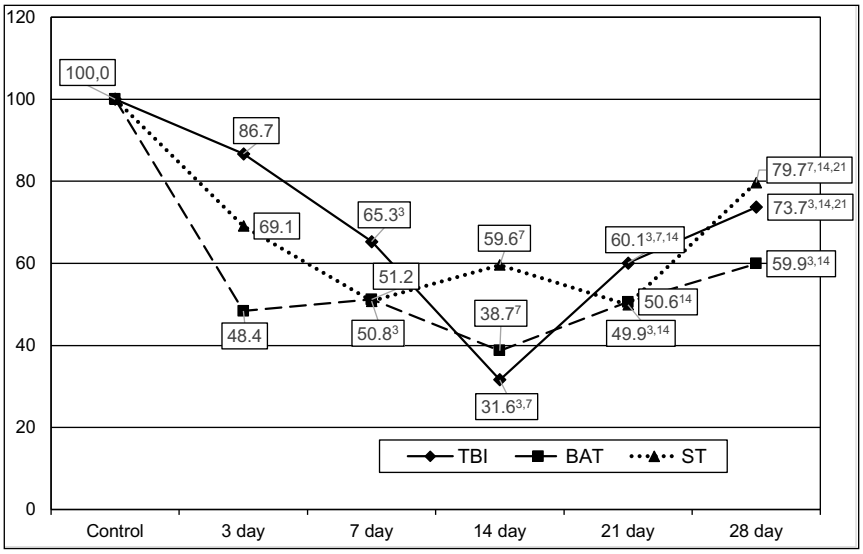


Fig. 1. API value in the liver (as a percentage of the control group level) after modeling traumatic brain injury, blunt abdominal trauma, and skeletal trauma ((Me (LQ;UQ)) – median (lower and upper quartiles)
Note. Here and in Fig. 2: [3, 7, 14, 21] – differences in relation to the results of 3, 7, 14, and 21 days of the post-traumatic period are statistically significant. (p<0,05)
Picture taken by the authors

Table 2. Influence of acute blood loss on catalase activity in the liver (mcat • kg^{-1}) after modeling traumatic brain injury, blunt abdominal trauma, and skeletal trauma ((Me (LQ; UQ)) – median (lower and upper quartiles))

Group of rats	Examination period				
	3 day	7 day	14 day	21 day	28 day
Intact	0.698 (0.617; 0.731)				
Group 4 TBI + blood loss	0.339*# (0.301; 0.389)	0.422*# (0.404; 0.436)	0.188*# (0.173; 0.195)	0.266*# (0.259; 0.291)	0.448* (0.426; 0.506)
Group 5 BAT + blood loss	0.225*# (0.203; 0.267)	0.191*# (0.170; 0.197)	0.199*# (0.194; 0.210)	0.237*# (0.209; 0.249)	0.291*# (0.245; 0.301)
Group 6 ST + blood loss	0.284*# (0.253; 0.303)	0.247* (0.233; 0.279)	0.332*# (0.303; 0.332)	0.380*# (0.364; 0.401)	0.479*# (0.425; 0.484)
p_{4-5}	<0.05	<0.05	>0.05	<0.05	<0.05
p_{4-6}	>0.05	<0.05	<0.05	<0.05	>0.05
p_{5-6}	>0.05	<0.05	<0.05	<0.05	<0.05

Notes:
1. * – differences relative to the control group are statistically significant (p<0,05)
2. # – differences in relation to injured rats without acute blood loss are statistically significant (p<0,05)
3. p_{4-5} – significance of differences between experimental groups 4 i 5
4. p_{4-6} – significance of differences between experimental groups 4 i 6
5. p_{5-6} – significance of differences between experimental groups 5 i 6

exceeded the results of 3, 14, and 21 days of the post-traumatic period (by 32,2 %, 2,38 times, and 68,4 %, respectively, p<0,05), but was 35,8 % lower than in the control (p<0,05).

Complication of BAT with acute blood loss caused a statistically significant decrease in the liver API by 67,7 % compared to the control after 3 days of the post-traumatic period (p<0,05). After 7 days of the post-traumatic period, the index continued to decrease and became 72,6 % lower than in the control (p<0,05). The index remained at the same level until the 14-th day of the experiment (p>0.05). Subsequently, the index increased and after 28 days of the post-traumatic period exceeded the results of 7 and 14 days (by 52,4 % and 46,2 %, respectively, p<0,05), but remained 58,3 % lower than in the control (p<0,05).

In the case of ST, additional acute blood loss also caused a substantial decrease in the liver API compared to the control from the 3-rd day of the post-traumatic period (by 58,8 %, p<0,05). The index continued to remain at the same level until the 7-th day (p>0,05) and subsequently gradually increased until the 28-th day. At this time point, the index statistically significantly exceeded the results of all previous observation periods (by 68,7 %, 93,2 %, 44,3 %, and 26,0 %, respectively, p<0,05) and was 31,4 % lower than in the control (p<0,05).

Comparison of the experimental groups of rats with trauma of various localization complicated by acute

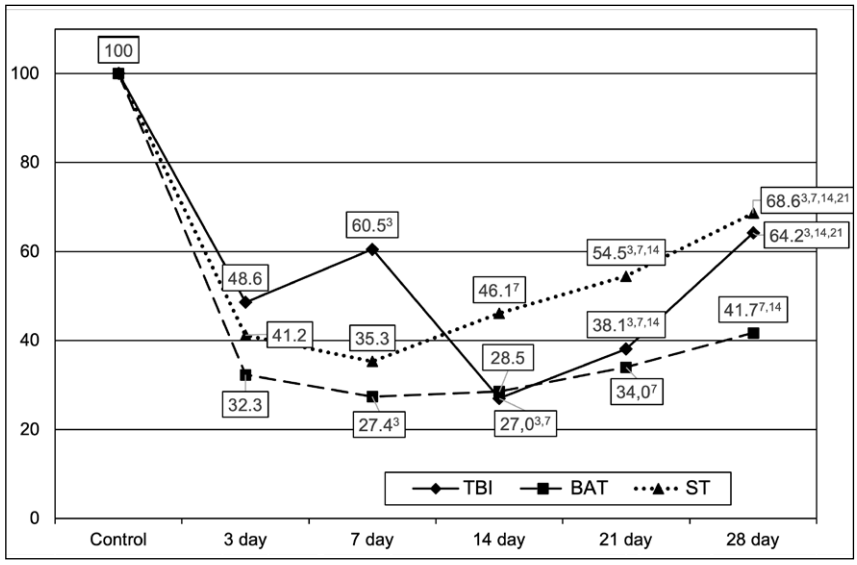


Fig. 2. Dynamics of API values in the liver (as a percentage of the control group) after modeling traumatic brain injury, blunt abdominal trauma, and skeletal trauma complicated by acute blood loss
Picture taken by the authors

blood loss showed that after 3, 7, 21, and 28 days of the post-traumatic period, the liver API was lowest in the group of rats with BAT (p_{4-5}<0,05, p_{5-6}<0,05). It is noteworthy that after 14 days of the post-traumatic period, the liver API was substantially lower in the groups of rats with TBI and BAT compared to the group of rats with ST (by 43,4 %, p_{4-6}<0,05, and 40,1 %, p_{5-6}<0,05, respectively).

An important analysis was the comparison of impairments in liver API between groups of rats with traumas of different localization without acute blood loss and analogous groups in which blood loss was additionally modeled. The studies showed that in all experimental groups at all times of the post-traumatic period, the liver API in traumatized rats with acute blood loss was statistically significantly lower than in rats without blood loss. Thus, in the case of TBI and acute blood loss, the index became lower by 44,0 % after 3 days of the post-traumatic period (p<0,05), by 7,4 % after 7 days (p<0,05), by 14,5 % after 14 days (p<0,05), by 36,7 % after 21 days (p<0,05), and by 12,8 % after 28 days (p<0,05). In rats with BAT and acute blood loss, the index became lower by 33,4 %, 46,5 %, 26,3 %, 32,9 %, and 30,4 %, respectively (p<0,05). In rats with ST and acute blood loss, the index became lower by 41,1 %, 30,4 %, 42,5 %, 9,2 %, and 14,0 %, respectively (p<0,05).

DISCUSSION

The obtained results indicate that modeling TBI, BAT, and ST causes a shift in the antioxidant-prooxidant balance in the liver toward the predominance of prooxidant mechanisms. The result is statistically significant starting from the 7-th day of the experiment with TBI, and starting from the 3rd day with BAT and ST. After modeling TBI and BAT, the API reaches a minimum after 14 days of the post-traumatic period and increases by 28 days, not reaching the control level. After ST, the API changes in a phase manner with periods of decrease after 7 and 21 days and increase after 14 and 28 days of the post-traumatic period. This reaction in the liver generally corresponds to the results of studies by other authors, who noted impairments in the antioxidant-prooxidant balance in internal organs distant from the site of direct trauma under conditions of severe trauma [16]. It should be noted that the decrease in liver API did not depend on the localization of the mechanical trauma and occurred in conditions of TBI, BAT, and ST. This indicates a general biological nature of the impairment in the antioxidant-prooxidant ratio, which is the result of the systemic prooxidant effect of severe trauma [17-19].

It is noteworthy that the greatest decrease in API was observed with BAT at almost all times of the post-traumatic period compared to other experimental groups. Considering the fact that the modeled traumas were standardized by the level of mortality in the acute period of trauma—during the first two days [11]—it can be argued that secondary damage to internal organs comes to the fore in the late period of trauma. In the case of BAT, this involves post-traumatic impairments in the intestines and liver, which were directly affected by the mechanical impact. As shown by the studies of individual authors, among secondary damages in the abdominal organs under conditions of trauma of different localization, an intensification of LPO processes in the small intestine wall, the development of enteral dysfunction with impaired intestinal barrier, microflora translocation, and a powerful endotoxic effect on the body are noted [20, 21]. The liver receives a repeated toxic «blow» [22], which contributes to the intensification of its dysfunction, an increase in

prooxidant impairments, and the activation of the body's systemic inflammatory response, which deepens secondary damage to other organs distant from the site of injury.

The complication of modeled mechanical traumas with acute blood loss in the amount 1,5 % of body weight is accompanied by a deepening of metabolic disorders in the liver. In the case of TBI, the liver API reaches a minimum after 14 days of the post-traumatic period; for BAT and ST – already after 3 days with a subsequent gradual increase in the index, which does not reach the control level by 28 days. The amplitude of impairments at all times of the post-traumatic period is greater in the group of rats with BAT, and after 14 days – simultaneously in rats with TBI.

It is known that on the background of acute blood loss, with a decrease in circulating blood volume, the sympathoadrenal system is compensatorily activated, which, on the one hand, enhances metabolism, and on the other hand, restricts blood flow in organs, primarily the splanchnic area [23]. Reduced perfusion contributes to hypoxia in internal organs, which creates prerequisites for increased generation of reactive oxygen species, peroxidation of lipids and proteins of cell membranes, leading to impaired functions. Thus, there is an overlay of pathogenic mechanisms of both mechanical trauma and acute blood loss (the syndrome of mutual aggravation), which leads to a deepening of the intensity of LPO in the liver and a decrease in the functional capacity of the antioxidant system. That is why acute blood loss most profoundly aggravates impairments in liver API in the case of BAT compared to other experimental groups. In the early period of trauma (3–7 days), the intensity of impairments in the antioxidant-prooxidant balance in the liver was:

TBI ← ST ← BAT.

In the late period (14–28 days), impairments in the case of TBI and acute blood loss became greater:

ST ← TBI ← BAT.

Thus, additional acute blood loss in the amount 1,5 % of body weight in the background of TBI, BAT, and ST significantly aggravates the intensity of impairments in the antioxidant-prooxidant balance in the liver and is substantially greater in the case of BAT. The patterns we identified have significant practical importance for understanding the specificity of secondary oxidative damage to the liver in conditions of mechanical trauma of various localization complicated by acute blood loss and for developing intensive care measures.

CONCLUSIONS

1. Modeling TBI, BAT, and ST compared to the control is accompanied by a shift in the liver's antioxidant-prooxidant ratio towards the predominance of prooxidant mechanisms. The result becomes statistically significant starting from the 7th day of the experiment for TBI, and at all times of the post-traumatic period for BAT and ST. Impairments after 3, 7, and 21 and 28 days of the post-traumatic period are substantially greater in rats with BAT.
2. Additional modeling of acute blood loss in the amount 1,5 % of body weight in rats with mechanical trauma of different origin causes a greater decrease in liver API compared to traumatized rats without acute blood loss, which is statistically significant at all times of the post-traumatic period. The largest impairments in liver API are noted with BAT.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Usage of methods polarization-phase mueller-matrix introscopy for early diagnosis of endometriosis based on study of dehydrated films of peritoneal fluid

Oksana V. Bakun, Oksana I. Yurkiv, Ksenia V. Slobodian, Volodymyr A. Doroshko, Oksana V. Kolesnik, Alla I. Peryzhniak, Yuliya F. Marchuk

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ABSTRACT

Aim: To assess the effectiveness of early diagnosis of endometriosis using polarization-phase Muller-matrix introscopy methods of scattering dehydrated films of peritoneal fluid.

Materials and Methods: Two groups of samples were studied: peritoneal fluid obtained during diagnostic laparoscopy – control group 3.1 - 68 samples; peritoneal fluid obtained during diagnostic laparoscopy in women with endometriosis associated with infertility – experimental group 3.2 - 59 samples.

Results: By statistical analysis of a series of thesiograms of the distributions of the optical anisotropy parameters of biological preparations, the following most effective markers for the diagnosis of endometriosis by the method of polarization-phase Muller-matrix introscopy were experimentally established. Dehydrated films of peritoneal fluid:linear birefringence - unsatisfactory (– 74.6% - 80.1%) diagnostic accuracy - groups "3.1" - "3.2"; circular birefringence - excellent (– 97.6% - 98.4%) and good (– 88.1% - 88.9%) diagnostic accuracy - groups "3.1" - "3.2"; linear dichroism - good (– 90.5%) diagnostic accuracy of endometriosis - groups "3.1" - "3.2"; circular dichroism - very good (– 90.5% - 94.5%) diagnostic accuracy - groups "3.1" - "3.2

Conclusions: The conducted cycle of stusies of the diagnostic effectiveness of the new in biomedical practice method polarization-phase Muller-matrix introscopy of the polycrystalline component of biological preparations (peritoneal fluid) revealed a high level of accuracy of early diagnosis of genital endometriosis.

KEY WORDS: endometriosis, assisted reproductive technologies, infertility, polarization-phase Muller-matrix introscopy

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INTRODUCTION

While efforts are underway to develop biomarker-based tests and improve non-invasive imaging, the current diagnostic pathway remains a complex interplay of clinical assessment and surgical confirmation, underscoring the urgent need for advancements to reduce diagnostic timelines.

Given the high accuracy of the results obtained with polarimetry in transparent media, a search was started for modifications of the method to obtain high-quality results in tissues and biological fluids with different densities. To increase the contrast and improve the ability to visualize tissues, optical visualization was used using a multiply scattering (depolarizing) component of the light beam, which gave a positive result and the ability to evaluate various biological media [1-3]. The results depend on the polarization state of the incident light beam. Thus, a number of modifications have been developed, including linear or circular polarization, Stokes polarimetry or Muller matrix polarimetry, detection geometry (transmission versus reflection), spectroscopic methods for studying light, such as point spectroscopy and spectral imaging (using different wavelengths, diffuse reflection, fluorescence, Raman scattering) [1,4,5], interferometric methods, polarization-guided imaging, optical polarization, laser polarimetry. Each method has its own characteristics. When using different techniques, the anisotropy of biological objects is taken into account, since almost all biological tissues have optical anisotropy [2]. Birefringence of biological tissues is mainly due to the linear anisotropy of fibrous structures that form extracellular environments. The refractive index is more important along the fibers than across them. In this case, we have parallel cylinders forming a uniaxial birefringent medium with an optical axis that is parallel to the axis of the cylinders (shape birefringence), and is characteristic of many biotissues, such as the cornea, cartilage, tendons, sclera, dura mater, muscles, nerves, retina, bone, teeth, and other

biotissues with uniaxial and/or biaxial birefringent structures [5].The traditional microscopic description of the morphological structure of biological tissues of human organs was first developed and supplemented in the practice of biomedical research in systematic studies of polarization, phase and laser (fluorescence) microscopy. As a result, a new direction for biomedical and clinical diagnostics was formed - polarization-phase introscopy of the morphological structure of biological preparations of human organ tissues.

The foundations of this diagnostic direction are based on determining the relationships between polarization, Stokes-polarimetric and Muller-matrix microscopic images of the polycrystalline structure of biological preparations of human organ tissues (thesiograms) and various pathological conditions of human organs [1-5].

AIM

To assess the effectiveness of early diagnosis of endometriosis using polarization-phase Muller-matrix introscopy methods of scattering dehydrated films of peritoneal fluid.

MATERIALS AND METHODS

Two groups of samples were studied: peritoneal fluid obtained during diagnostic laparoscopy – control group 3.1 - 68 samples; peritoneal fluid obtained during diagnostic laparoscopy in women with endometriosis associated with infertility – experimental group 3.2 - 59 samples.

DATA PROCESSING

Fundamentals of statistical analysis

For an objective evaluation of complex, coordinately inhomogeneous distributions of the magnitude of all types of thesiograms (hereinafter we will denote these sets Q), we used statistical analysis, which consists of the following steps:

●Definition of histograms of distributions of the value Q.

●Calculation of the set of central statistical moments of the 1st, 2nd, 3rd and 4th orders, which characterize the average (Z_1), dispersion (Z_2), asymmetry (Z_3) and kurtosis (Z_4) distributions Q by the number of discrete pixels P.

1st order statistical moment Z_1 or the mean of the set Q determines its average value

$$Z_1 = \frac{|\mathbf{Q_1}| + |\mathbf{Q_2}| + \ldots + |\mathbf{Q_{p-1}}| + |\mathbf{Q_p}|}{\mathbf{P}} \quad (1)$$

2nd order statistical moment Z_2 or the variance of a set of values Q determines the degree of its deviation from the mean value Z_1 (ratio (1)).

$$Z_2 = \sqrt{\frac{\mathbf{Q_1^2} + \mathbf{Q_2^2} + \ldots + \mathbf{Q_{p-1}^2} + \mathbf{Q_p^2}}{\mathbf{P}}} \quad (2)$$

3rd order statistical moment Z_3 or asymmetry characterizes the deviation from normality of random values Q

$$Z_3 = \sqrt{\frac{\mathbf{Q_1^3} + \mathbf{Q_2^3} + \ldots + \mathbf{Q_{p-1}^3} + \mathbf{Q_p^3}}{\mathbf{P}}} \Big/ \left(\sqrt{\frac{\mathbf{Q_1^2} + \mathbf{Q_2^2} + \ldots + \mathbf{Q_{p-1}^2} + \mathbf{Q_p^2}}{\mathbf{P}}}\right)^3 \quad (3)$$

The value of this statistical parameter Z_3 characterizes the dynamics of changes in the parameters of thesiograms of optical anisotropy.

4th order statistical moment Z_4 or kurtosis characterizes the dynamics of changes in the distributions of the magnitude of local values of the set of thesiogram points Q

$$Z_4 = \sqrt{\frac{\mathbf{Q_1^4} + \mathbf{Q_2^4} + \ldots + \mathbf{Q_{p-1}^4} + \mathbf{Q_p^4}}{\mathbf{P}}} \Big/ \left(\sqrt{\frac{\mathbf{Q_1^2} + \mathbf{Q_2^2} + \ldots + \mathbf{Q_{p-1}^2} + \mathbf{Q_p^2}}{\mathbf{P}}}\right)^4 \quad (4)$$

ETHICS

Ethical approval for this study was obtained from the Medical Ethics Committee of the Bukovinian State Medical University, Chernivtsi, Ukraine (approval ID: No. 6 from 8.10.2024).

RESULTS

The series of fragments in Fig. 1 presents algorithmically reproduced thesiograms and 3D distributions of the linear birefringence value of samples of dehydrated films of peritoneal fluid of patients from control group 3.1 (fragments (1),(2)) and experimental groups 3.2 (fragments (3),(4))

Installed:

- the presence of linear birefringence of supramolecular networks of tertiary and quaternary structure of proteins of dehydrated films of peritoneal fluid of patients from all groups (Fig. 1, fragments (1), (3));
- increase in the value of linear birefringence of dehydrated films of peritoneal fluid of patients with endometriosis (group 3.2), - (Fig. 1, fragments (2) (4)).

Statistical analysis of the structure of thesiograms of the linear birefringence distributions of supramolecular protein networks of peritoneal fluid of patients from all groups is given in Table 1.

A slight decrease in linear birefringence was found in patients with endometriosis due to the destruction of

Table 1. Statistical moments of the 1st – 4th orders, which characterize the coordinate distributions of the linear birefringence of dehydrated films of peritoneal fluid of patients

Parameters	Group 3.1	Group 3.2
$SM_1 \times 10^{-3}$	0.08±0.006	0.06±0.004
p_2	$p \le 0.05$	
$SM_2 \times 10^{-3}$	0.06±0.003	0.04±0.002
p_2	$p \le 0.05$	
SM_3	1.27±0.069	1.55±0.081
p_2	$p \le 0.05$	
SM_4	1.82±0.097	2.19±0.12
p_2	$p \le 0.05$	

Source: compiled by the authors of this study

Table 2. Specificity, sensitivity, accuracy of the method for statistical analysis of linear birefringence thesiograms of peritoneal fluid films of patients

Groups "1 – 2"			
Parameters	Sensitivity,Se,% N=57	Specificity,Sp,% H=69	Precision,Ac,% N+H=126
SM_1	*A=44*	*B=52*	*A+B=96*
	77.2	75.3	76.2
SM_1	*A=43*	*B=51*	*A+B=94*
	75.4	73.9	74.6
SM_3	*A=46*	*B=54*	*A+B=100*
	80.7	78.3	79.4
SM_4	*A=47*	*B=55*	*A+B=102*
	82.5	79.7	80.1

Note. SM1, SM2, SM3, SM4 – unsatisfactory accuracy
Source: compiled by the authors of this study

tertiary and quaternary proteins of the peritoneal fluid of patients from the experimental group 3.2. Higher-order statistical moments can be used as diagnostic markers of endometriosis, which turned out to be quite sensitive to changes in the tertiary and quaternary structure of proteins in samples of dehydrated films of peritoneal fluid.

Higher-order statistical moments, which have been shown to be sufficiently sensitive to changes in the tertiary and quaternary structure of proteins in samples of dehydrated peritoneal fluid films, can be used as diagnostic markers for endometriosis.

OPERATIONAL CHARACTERISTICS OF THE DIAGNOSTIC POWER OF THE METHOD OF STATISTICAL ANALYSIS OF LINEAR BIREFRINGENCE THESIOGRAMS OF SUPRAMOLECULAR PROTEIN NETWORKS OF DEHYDRATED FILMS OF PERITONEAL FLUID

The diagnostic and clinical effectiveness of using a set of statistical markers of algorithmically reproduced thesiograms of coordinate distributions of the magnitude of linear birefringence in detecting pathological changes - destruction of the tertiary and quaternary structure of supramolecular protein networks of samples of dehydrated polycrystalline films of peritoneal fluid of patients is illustrated by the values of the operating characteristics, the values of which are given in Table 2.

We have established the following ranges of the diagnostic power of the method of polarization-phase Muller matrix introscopy of linear birefringence of supramolecular protein networks of patients' peritoneal fluid:

- unsatisfactory (– 74.6% - 80.1%) accuracy of endometriosis diagnosis - groups "3.1" – "3.2"

Thus, it can be stated that the method of polarization-phase Muller matrix introscopy of changes in linear birefringence of supramolecular networks of dehydrated filmsof patients' peritoneal fluid did not show sufficient efficiency for diagnosing endometriosis, as well as for accurately assessing the effectiveness of its treatment. Therefore, the next step was to study the effectiveness of this polarization-optical technique by determining other markers that characterize the optical activity or chiral properties of molecular complexes of patients' peritoneal fluid.

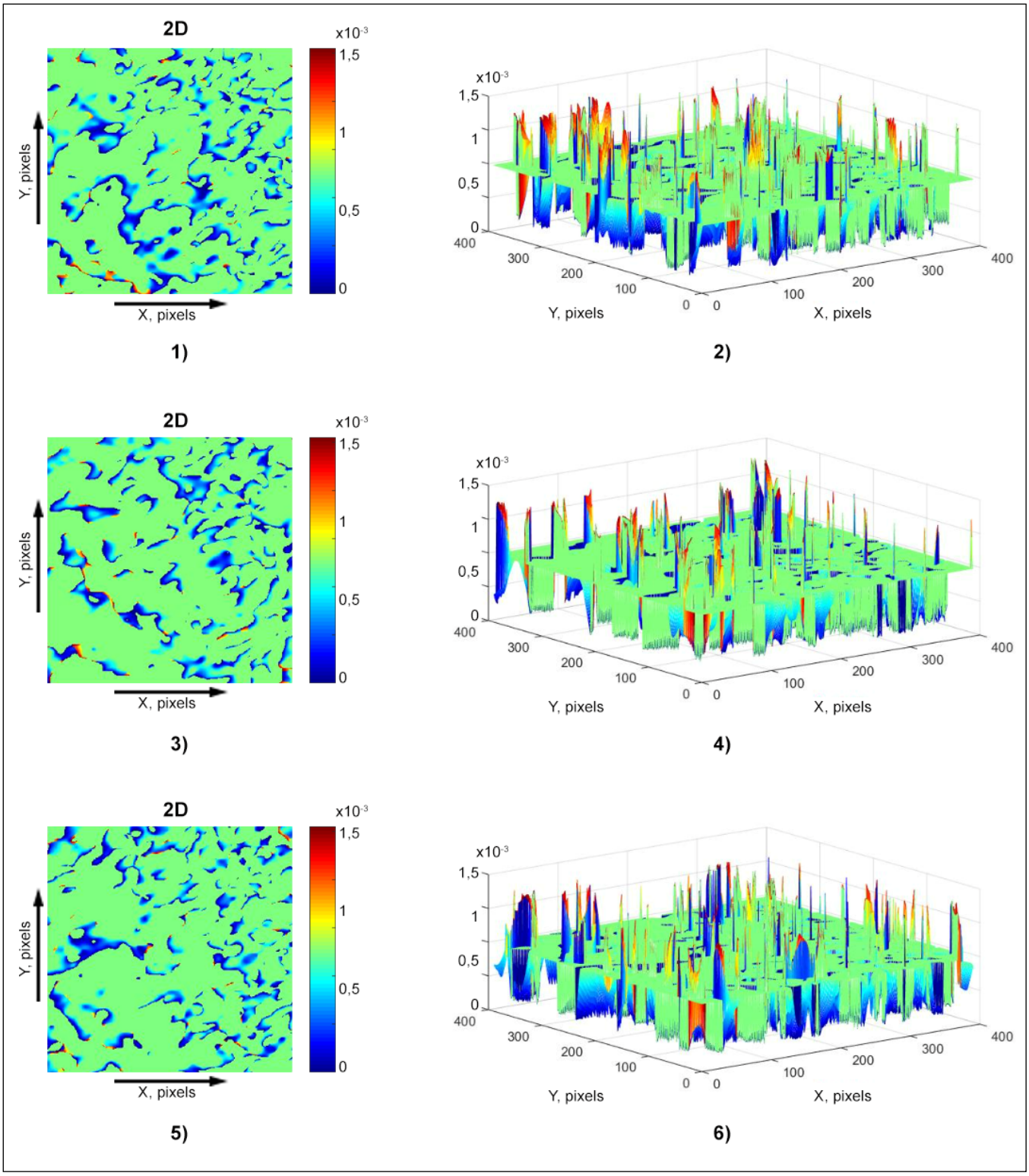


Fig. 1. Thesiograms ((1),(3), and 3D (2),(4),) of the distribution of the linear birefringence value of dehydrated films of peritoneal fluid of patients from group 3.1 ((1),(2)), group 3.2 ((3),(4))
Picture taken by the authors

CIRCULAR BIREFRINGENCE THESIOGRAMS OF DEHYDRATED PERITONEAL FLUID FILMS

In this section, we used the following model approximation. Leukocytes, which are part of the peritoneal fluid of patients, are spherical protein formations and have manifestations of circular birefringence. A reduced or increased number of leukocytes in the peritoneal fluid of patients is an important clinical indicator of the presence of a pathological process. Therefore, an increase in the level of circular birefringence of the dehydrated

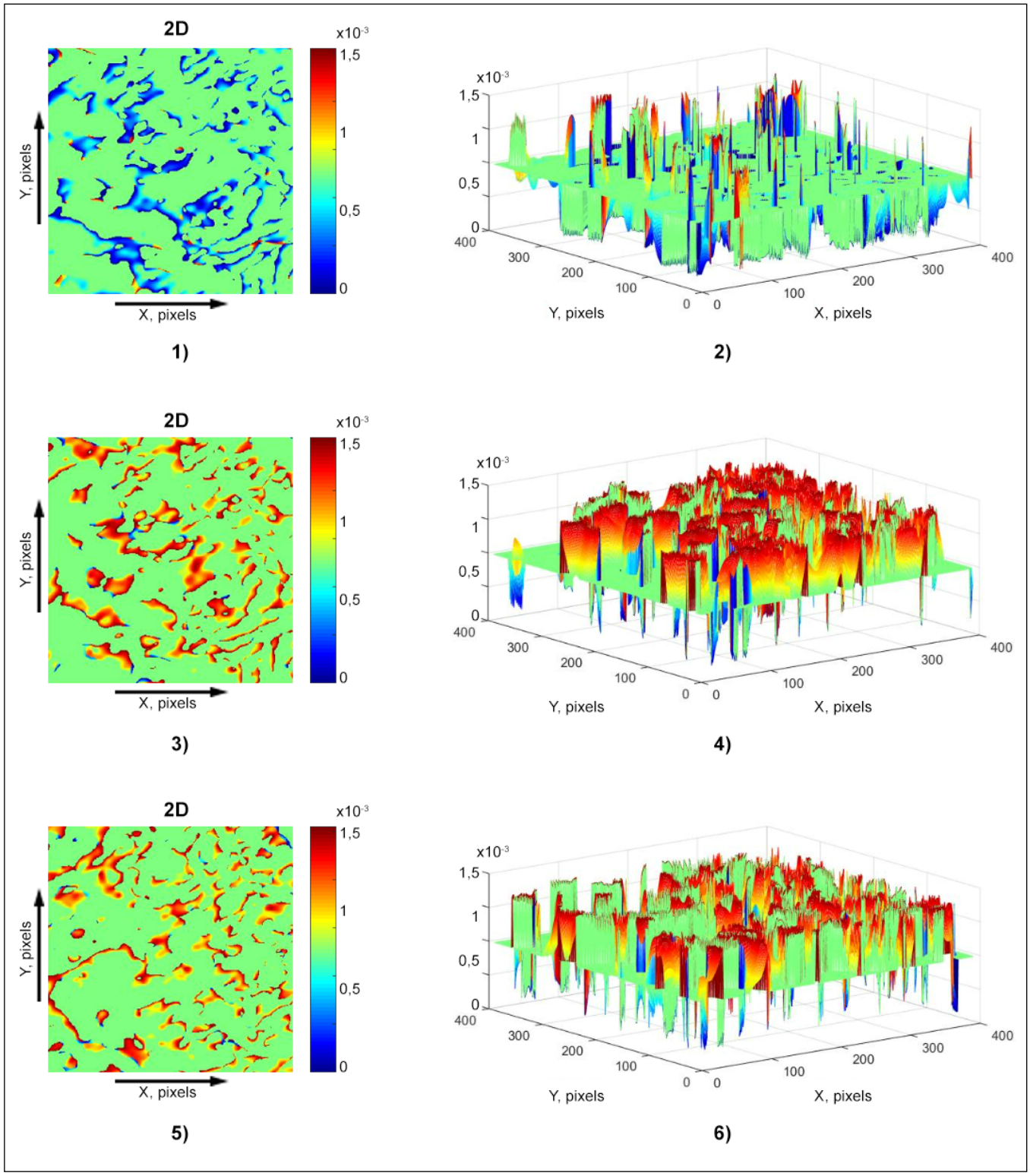


Fig. 2. Thesiograms ((1),(3)) and 3D ((2),(4)) of the circular birefringence value distributions of chiral molecular complexes of dehydrated films of peritoneal fluid of patients from group 3.1 ((1),(2)), group 3.2 ((3),(4))
Picture taken by the authors

film of peritoneal fluid can serve as additional objective criteria for endometriosis.

Algorithmically reproduced thesiograms and 3D distributions of the circular birefringence of chiral protein complexes of samples of dehydrated films of peritoneal fluid of patients from control group 3.1 (fragments (1),(2)) and experimental groups 3.2 (fragments (3),(4)) are shown in a series of fragments in Fig. 2.

Analysis of the obtained experimental data revealed the presence of:

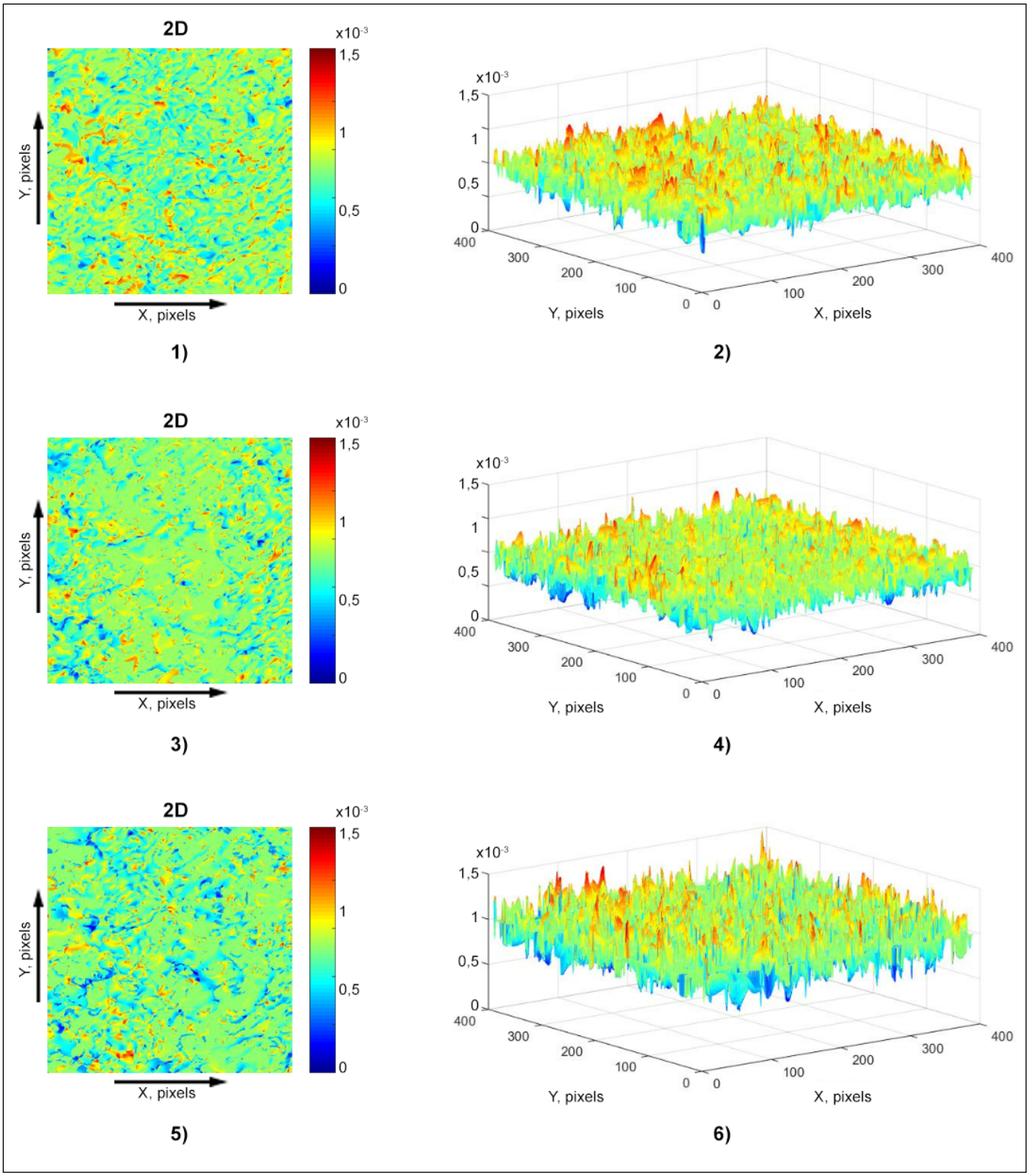


Fig. 3. Thesiograms ((1),(3)) and 3D ((2),(4)) distributions of the linear dichroism value of samples of dehydrated films of peritoneal fluid of patients from control group 3.1 (fragments (1),(2)) and experimental groups 3.2 (fragments (3),(4))
Picture taken by the authors

- coordinate distributions of the circular birefringence value of the supramolecular polycrystalline chiral structure of leukocytes of dehydrated films of peritoneal fluid of patients from all groups (Fig. 2, fragments (1), (3));
- increase in the level of circular birefringence of optically active supramolecular structures of peritoneal fluid samples from patients with endometriosis (Fig. 2, fragments (2), (4)).

Objectively, the differences between the maps of thesiograms of coordinate distributions of the

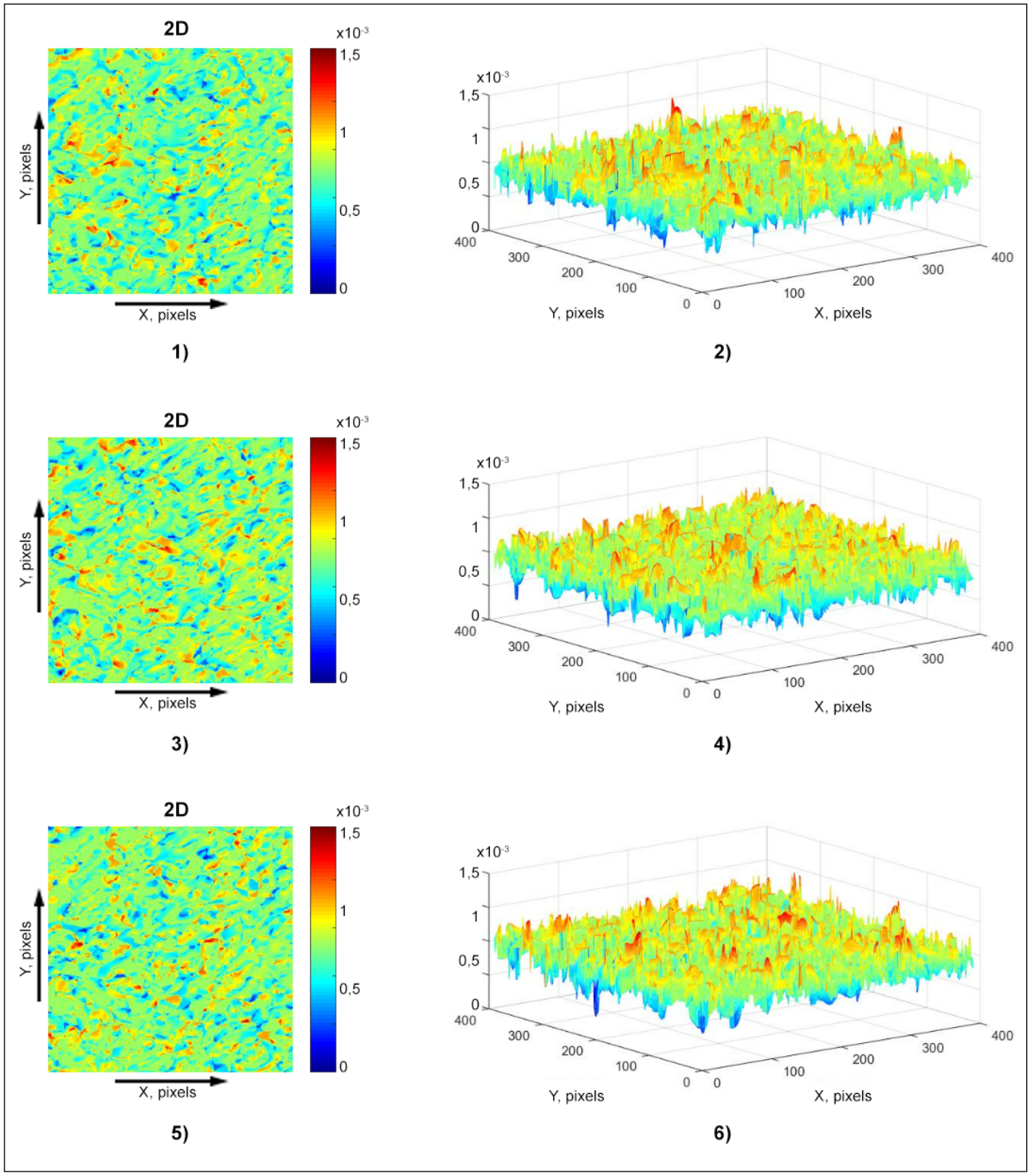


Fig. 4. Thesiograms ((1),(3)) and 3D ((2),(4)) distributions of the circular dichroism value of samples of dehydrated films of peritoneal fluid of patients from control group 3.1 (fragments (1),(2)) and experimental groups 3.2 (fragments (3),(4))
Picture taken by the authors

magnitude of circular birefringence of dehydrated films of peritoneal fluid of patients illustrate the average and spread of the values of the set of central statistical moments of the 1st - 4th orders, which are given in Table 3.

The following scenarios were identified:

- changes in the polycrystalline structure of supramolecular networks of leukocytes in the peritoneal fluid of patients and increased circular birefringence in endometriosis.

Table 3. Statistical moments of the 1st – 4th orders, which characterize the distributions of the magnitude of circular birefringence of dehydrated films of peritoneal fluid of patients

Parameters	Group 3.1	Group 3.2
$SM_1 \times 10^{-3}$	0.07±0.004	0.12±0.007
p_2	$p \le 0.05$	
$SM_2 \times 10^{-3}$	0.05±0.003	0.08±0.005
p_2	$p \le 0.05$	
SM_3	1.44±0.088	1.13±0.062
p_2	$p \le 0.05$	
SM_4	2.33±0.13	1.98±0.104
p_2	$p \le 0.05$	

Source: compiled by the authors of this study

Table 4. Specificity, sensitivity, and accuracy of the method for statistical analysis of circular birefringence maps of peritoneal fluid films

Groups "1 – 2"			
Parameters	Sensitivity, N=57	Specificity, H=69	Precision, N+H=126
SM_1	*A=56*	*B=68*	*A+B=124*
	98.3	98.6	98.4
SM_2	*A=56*	*B=67*	*A=B=123*
	98.3	97.1	97.6
SM_3	*A=51*	*B=61*	*A+B=112*
	89.5	88.4	88.9
SM_4	*A=51*	*B=60*	*A+B=111*
	89.5	87	88.1

Note. SM_1, SM_2 – excellent accuracy; SM_3, SM_4 – good accuracy
Source: compiled by the authors of this study

• Diagnostic, statistically significant markers of this pathological process are the increase in the mean and variance of the circular birefringence thesiograms.

• Similarly, a set of 1st–2nd order statistical moments can be used to monitor the effectiveness of endometriosis treatment.

OPERATIONAL CHARACTERISTICS OF CIRCULAR BIREFRINGENCE THESIOGRAM ANALYSIS OF DEHYDRATED PERITONEAL FLUID FILMS

The diagnostic effectiveness of using statistical markers of the distribution of the circular birefringence value of dehydrated films of peritoneal fluid of patients in detecting pathological changes is illustrated by the values of the operational characteristics (Table 4).

The following parameters of the diagnostic power of statistical analysis of algorithmically reproduced thesiograms were experimentally established:

The following parameters of the diagnostic power of statistical analysis of algorithmically reproduced thesiograms were experimentally established:

• excellent (– 97.6% - 98.4%) and good (– 88.1% - 88.9%) accuracy of endometriosis diagnosis - groups "3.1" – "3.2"

We have already noted that biological films also have optically anisotropic absorption. Therefore, we will consider the effectiveness of the method of polarization-phase Muller matrix introscopy of linear and circular dichroism of dehydrated films of peritoneal fluid.

LINEAR DICHROISM THESIOGRAMS OF SUPRAMOLECULAR ALBUMIN NETWORKS OF DEHYDRATED PERITONEAL FLUID FILMS

The anisotropically absorbing components of peritoneal fluid include aantibodies, immunoglobulins. The tertiary and quaternary structure of an antibody in most cases includes two heavy chains and two light chains. As a result, they acquire the properties of linear dichroism.

The series of fragments in Fig. 3 presents algorithmically reconstructed by the Muller matrix introscopy method thesiograms and 3D distributions of the linear dichroism value of supramolecular networks of dehydrated films of peritoneal fluid of

Table 5. Statistical moments of the 1st – 4th orders, which characterize the linear dichroism distributions of dehydrated peritoneal fluid films

Parameters	Group 3.1	Group 3.2
$SM_1 \times 10^{-3}$	0.07±0.004	0.12±0.007
p_2	$p \le 0.05$	
$SM_2 \times 10^{-3}$	0.05±0.003	0.08±0.005
p_2	$p \le 0.05$	
SM_3	1.44±0.088	1.13±0.062
p_2	$p \le 0.05$	
SM_4	2.33±0.13	1.98±0.104
p_2	$p \le 0.05$	

Source: compiled by the authors of this study

patients from control group 3.1 (fragments (1),(2)) and experimental groups 3.2 (fragments (3),(4)).

The Muller matrix introscopy method revealed the mechanisms of optically anisotropic absorption of dehydrated films of peritoneal fluid, which is illustrated by algorithmically reproduced linear dichroism thesiograms (Fig. 3, fragments (1), (3)).

For dehydrated films of peritoneal fluid from patients with endometriosis (Fig. 3, fragments (2), (4)), a decrease in the linear dichroism of supramolecular polycrystalline networks was found (Table 5).

The results of statistical analysis of linear dichroism thesiograms revealed:

• reduction in the value of linear dichroism due to the destruction of tertiary and quaternary proteins of the peritoneal fluid of patients with endometriosis (study group 3.2).

The following diagnostic statistical markers of endometriosis have been established - asymmetry and excess of the tertiary and quaternary structure of proteins in samples of dehydrated films of peritoneal fluid.

OPERATIONAL CHARACTERISTICS OF THE DIAGNOSTIC POWER OF THE METHOD OF STATISTICAL ANALYSIS OF LINEAR DICHROISM THESIOGRAMS OF PERITONEAL FLUID FILMS

The parameters of the effectiveness of the differential diagnosis of endometriosis, as well as monitoring the effectiveness of its treatment, illustrate the values of the operational characteristics, which are given in Table 6.

The following parameters of the diagnostic power of the method of polarization-phase Muller matrix reconstruction of linear dichroism thesiograms of supramolecular protein networks of dehydrated peritoneal fluid films were experimentally established:

• good (– 90.5%) and very good (– 92.1% - 93.7%) accuracy of endometriosis diagnosis - groups "3.1" – "3.2".

CIRCULAR DICHROISM THESIOGRAMS OF SUPRAMOLECULAR ALBUMIN NETWORKS OF DEHYDRATED PERITONEAL FLUID FILMS

Experimentally determined thesiogram maps and 3D distributions of the circular dichroism value of chiral protein complexes of supramolecular polycrystalline networks of dehydrated films of peritoneal fluid of patients from control group 3.1 (fragments (1),(2)) and experimental group 3.2 (fragments (3),(4)) are shown in a series of fragments in Fig. 4.

The presence of a polycrystalline chiral structure of optically anisotropic absorption of molecular complexes of samples of dehydrated films of peritoneal fluid of patients from all groups was established (Fig. 4, fragments (1), (3)). It was found that for samples of dehydrated films of peritoneal fluid of patients with endometriosis there is a slight increase in the level of circular dichroism of molecular structures. Quantitatively, the processes of change in circular dichroism are illustrated by statistical moments of the 1st - 4th orders, which characterize thesiograms of supramolecular networks of dehydrated films of peritoneal fluid (Table 7).

Analysis of algorithmically reproduced thesiograms of supramolecular networks of dehydrated peritoneal fluid films revealed a correlation of the prognostic scenario of changes in optically anisotropic (chiral) absorption of protein complexes and an increase in the value of circular dichroism in the presence of endometriosis. At the same time, statistically significant (markers of this pathological process are an increase in the value of the central statistical moments of the 1st and 2nd orders.

OPERATIONAL CHARACTERISTICS OF THE DIAGNOSTIC POWER OF THE METHOD OF STATISTICAL ANALYSIS OF CIRCULAR DICHROISM THESIOGRAMS OF SUPRAMOLECULAR GLOBULIN NETWORKS OF DEHYDRATED PERITONEAL FLUID FILMS

Experimental results of information analysis of circular dichroism thesiograms of dehydrated peritoneal fluid films are given in Table 8.

Table 6. Specificity, sensitivity, accuracy of the method of statistical analysis of linear dichroism maps of peritoneal fluid films

Parametersz	Sensitivity, Se, % N=57	Specificity, Sp, % H=69	Precision, Ac, % N+H=126
SM_1	A=54 94.7	B=64 92.8	A+B=118 93.7
SM_2	A=53 92.3	B=63 91.3	A+B=116 92.1
SM_3	A=52 91.2	B=62 89.9	A+B=114 90.5
SM_4	A=53 92.3	B=63 91.3	A+B=116 92.1

Note. SM_1, SM_2, SM_4 – very good accuracy; SM_3 – good accuracy
Source: compiled by the authors of this study

Table 7. Statistical moments of the 1st – 4th orders, which characterize the distributions of the circular dichroism value of dehydrated films of peritoneal fluid

Parameters	Group 3.1	Group 3.2
1	2	3
$SM_1\ 10^{-3}$	0.067±0.004	0.11±0.007
p_2		$p \le 0.05$
$SM_2\ 10^{-3}$	0.035±0.003	0.068±0.005
p_2		$p \le 0.05$
SM_3	1.57±0.089	1.21±0.065
p_2		$p \le 0.05$
SM_4	2.52±0.14	2.19±0.12
p_2		$p \le 0.05$

Source: compiled by the authors of this study

The following parameters of the diagnostic power of the method of statistical analysis of circular dichroism thesiograms of supramolecular networks of dehydrated films of peritoneal fluid were established (Table 8):

• very good (– 90.5% - 94.5%) accuracy of early diagnosis of endometriosis - groups "3.1" – "3.2".

DISCUSSION

In our work, we will use the basic principles of the theory of Muller matrix thesiography of optically anisotropic macro-, micro- and molecular structures of biological tissues in application to the development of objective criteria (markers) for early (preclinical) diagnosis of endometriosis and assessment of the effectiveness of its treatment.Peritoneal fluid is composed of water, electrolytes, antibodies, leukocytes, and biochemicals.

According data other authors [1,3], antibodies, immunoglobulins are large globular proteins secreted by plasma cells of the immune system. They have a Y-shaped shape, at two ends of the molecule there are two identical antigen binding sites, and the third end can be one of several types, depending on which antibodies are classified into one or another class. The tertiary and quaternary structure of one antibody in most cases includes two heavy chains and two light chains. As a result, they acquire the properties of linear birefringence and dichroism.

• The parameters of these anisotropy mechanisms, which form the thesiogram of the hydrated peritoneal fluid film, represent a set of early (pre-clinical) signs of the presence of endometriosis.

• Leukocytes are spherical protein formations and exhibit circular birefringence and circular dichroism.

• A decreased or increased number of leukocytes in the blood is an important clinical indicator of the presence of a pathological process. Therefore, an increase in the level of circular birefringence and circular dichroism of the thesiogram of the hydrated film of peritoneal fluid can serve as additional objective criteria for endometriosis.

Peritoneal fluid is composed of water, electrolytes, antibodies, leukocytes, and biochemicals. Leukocytes are spherical protein formations and have manifestations of circular dichroism. A reduced or increased number of leukocytes in the blood is an important clinical indicator of the presence of a pathological process. Therefore, an increase in the level of circular dichroism of the

Table 8. Specificity, sensitivity, and accuracy of the method for statistical analysis of circular dichroism maps of dehydrated peritoneal fluid films

Groups "1 – 2"			
Parameters	**Sensitivity Se (%) N = 57**	**Specificity Sp (%) H = 69**	**Precision Ac (%) N + H = 126**
SM_1	A = 55 96.5	B = 64 92.8	A + B = 119 94.4
SM_2	A = 54 94.7	B = 64 92.8	A + B = 118 93.7
SM_3	A = 52 91.2	B = 62 89.9	A + B = 114 90.5
SM_4	A = 52 91.2	B = 62 89.9	A + B = 114 90.5

Note. SM_1, SM_2, SM_3, SM_4 – very good accuracy
Source: compiled by the authors of this study

dehydrated film of perineal fluid can serve as additional objective criteria for endometriosis. These data have correlation with our research.

The highest level of linear birefringence for histological sections of endometrial biopsy; the minimum level of optical anisotropy of this type for dehydrated films of peritoneal fluid.

The growth of phase shift dispersion due to extracellular matrix anisotropy is accompanied by reversed changes in the statistical moments of the 3rd and 4th orders.

On this basis, the principles of Jones-matrix classification of the polarization-phase properties of the basic types of histological sections of biological tissues according to the ranges of change of a set of statistical moments characterizing the corresponding matrix elements are established and substantiated.

By statistical analysis of a series of thesiograms of the distributions of the optical anisotropy parameters of biological preparations, the following most effective markers for the diagnosis of endometriosis by the method of polarization-phase Muller-matrix introscopy were experimentally established. Dehydrated films of peritoneal fluid: linear birefringence - unsatisfactory (– 74.6% - 80.1%) diagnostic accuracy - groups "3.1" - "3.2"; circular birefringence - excellent (– 97.6% - 98.4%) and good (– 88.1% - 88.9%) diagnostic accuracy - groups "3.1" - "3.2"; linear dichroism - good (– 90.5%) diagnostic accuracy of endometriosis - groups "3.1" - "3.2"; circular dichroism - very good (– 90.5% - 94.5%) diagnostic accuracy - groups "3.1" - "3.2.

CONCLUSIONS

The conducted cycle of stusies of the diagnostic effectiveness of the new in biomedical practice method polarization-phase Muller-matrix introscopy of the polycrystalline component of biological preparations (peritoneal fluid) revealed a high level of accuracy of early diagnosis of genital endometriosis.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Incidence rate and risk factors of respiratory tract infection for children under five years

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ABSTRACT

Aim: To determine incidence rate and risk factors of respiratory tract infection for children under five years at Hospital, and to find out relationship between incidence rate, risk factors and demographic data of children.

Materials and methods: A Descriptive case-control design is used through the study. The study was carried out from December 5, 2023, to August 8, 2024, in Al-Najaf City, Al-Najaf Al-Ashraf Health Directorate, and Al-Zahra Teaching Hospital. 471 patients with respiratory tract infections below the age of 5 who come to Al-Zahra Teaching Hospital for treatment, follow-up, or both are included in the non-probability (purposive sample). A panel of experts is used to examine clarity in order to determine the face validity of the early version of the questionnaire. Determination of reliability of the questionnaire is based on the internal consistency reliability, Alpha Cronbach's technique.

Results: Results reveal significant risk factors case and control group regarding respiratory tract infection are recent cold or influenza, exposure to smoke, allergy, low birth weight, duration of breastfeeding less than six months, early use of antibiotics before six months, exposure to indoor air pollution, incomplete immunization.

Conclusions: The result shows highly recurrent risk factors in participant are early use of antibiotics less than six months, allergy, and exposure to the smoke and recent cold or influenza. Mass media should utilize to provide health education to increase parents' knowledge and awareness about respiratory tract infection and these risk factors.

KEY WORDS: incidence rate; risk factors; respiratory tract infection

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ABBREVIATIONS

ARTI - Acute Respiratory Tract Infection
LRIs - Lower Respiratory Infections
RSV - Respiratory Syncytial Virus
Hib - Haemophilus influenza type b
WHO - World Health Organization

INTRODUCTION

One of the leading causes of morbidity and mortality among children in underdeveloped nations is acute Respiratory Tract Infections (ARTIs). This infection has the potential to become the most common illness in hospitals if it occurs frequently. One of the main causes of mortality and disability, especially in children under five, is respiratory tract infections, particularly lower respiratory infections (LRIs). The etiology of LRIs can be viral (e.g., respiratory syncytial virus (RSV), parainfluenza virus, influenza virus, adenovirus and coronavirus) or bacterial, e.g., Streptococcus pneumonia and *Haemophilus influenzae* type b (Hib). Moreover, *Streptococcus pneumonia* is the principal aetiological agent of LRIs and is responsible for around half of the deaths. LRIs also have a significant financial impact; on average, RSV-related LRIs cost e3, 452, and e299 for inpatient and outpatient treatment, respectively. These costs may increase if follow-up is necessary or there are underlying medical conditions [1]. Recurrent respiratory tract infections are common in young children because they represent a considerable burden on pediatric outpatient services and hospitalizations, particularly in developing nations. Both the upper and lower respiratory tracts can get infected, and the most common infectious cause of pediatric deaths under the age of five is still recurrent lower respiratory tract infections like pneumonia. The Global Burden of Disease Study from 2016 estimates that lower respiratory tract infections killed 0.65 million children [2]. One of the

most prevalent serious public health issues is respiratory tract infections (RTIs), which are more common among children and the poor in general. In 15% of instances, severe acute RTIs result in hospitalizations and death, especially pneumonia in children under five [3]. The World Health Organization (WHO) estimates that 6% of all diseases worldwide are caused by respiratory infections. Approximately 6.6 million children under the age of five die each year worldwide; 95 percent of these children live in low-income nations, and ARI accounts for one-third of these deaths [4]. The purpose of this study is to determine the prevalence and risk factors of respiratory tract infections in children under five years old at the hospital, as well as the correlation between these variables and the demographic information of the children.

MATERIALS AND METHODS

DESIGN

A Descriptive Case-Control Design is used in the current study to attain the study objectives. The period of the study is from 5th December 2023 to the August 2024.

SETTING

Al-Najaf City, Al-Najaf Al-Ashraf Health Directorate, and Al-Zahra Teaching Hospital are the study's locations.

SAMPLE

The study sample included 471 children under five years of age with respiratory tract infections, selected using non-probability (purposive) sampling. These children visited pediatric wards of Al-Zahra Teaching Hospital for treatment, follow-up, or both. The study group consisted of children diagnosed with respiratory tract infections like (bronchitis, pneumonia, and otitis media) via the pediatrician at Al-Zahra Teaching Hospital during the study period. These children presented with active symptoms and were undergoing treatment or follow-up. While the control group included healthy children under 5 years of age with no current symptoms or recent history of respiratory infections, some had mild enteritis or minor injuries, who had no signs of respiratory infection during admission or hospital stay. The purpose of including such a control group was to assess environmental and demographic risk factors for respiratory infections, not to compare disease outcomes across unrelated illnesses. Both cases and controls had a history and physical exam to check for risk factors. Both groups came from the same patient pool (matched by age and sex) to ensure comparability and were drawn from the same population seen at the hospital during the same time frame.

INCLUSION CRITERIA

1. All participants are medically diagnosed as respiratory tract infection
2. The age of the all participants is children under five years old.
3. All participants are from Iraqis nationality because of the nature of the Iraqi society that differs from other societies.
4. Willing participants who agreed to take part in the study.

EXCLUSION CRITERIA

1- Patients with other disease or acute condition because such cases are critical and the patients' yet not have enough knowledge regarding their case.

Study Instrument: To evaluate patients' incidence rate and risk variables following a respiratory tract infection, the researcher has designed a limited assessment tool. The final study instrument consists of three parts

Part 1: Child Demographic Data: A demographic data sheet consists of 2 items, which included age, gender.

Part II: Parents Demographic Data: A demographic data sheet consists of 6 items, which included level of education, monthly income, residency, Child live with, marital status, occupational status.

Part III: Clinical Data: Second part of the questionnaire is comprised of 5 items, which include diagnosis, duration of the disease, number of previous hospitalizations and risk factors.

Validity of the Study Instrument: The validity of the study instrument refers to its ability to collect the data for which it is intended. A panel of experts is used to assess the questionnaire's face validity by looking into its clarity, applicability, and suitability for measuring the concepts of interest. The consistency and dependability of the study instrument were assessed using internal consistency reliability. Cronbach's Alpha technique was applied to Part III of the questionnaire (Clinical Data), which includes five items: diagnosis, duration of illness, number of previous hospitalizations, risk factors, and incidence. The result yielded a Cronbach's Alpha coefficient of 0.78, indicating acceptable internal consistency.

STATISTICAL ANALYSIS

All data analyses were conducted using IBM SPSS Statistics (version 23). Descriptive statistics were used to

Table 1. Distribution of Socio-demographic characteristics of case and control groups

Socio-demographic	Control 171, Freq. & (%)	Case 300, Freq. & (%)	Total	p-value
Age in Months (Mean ± SD)	10.90(12.57)	11.81(13.04)	11.48(12.87)	0.463
Sex				
Male	93(54.40)	164(54.70)	257(54.60)	1.000
Female	78(45.60)	136(45.30)	214(45.40)	
Father's educational level				
Doesn't Read And Write	24(14.00)	42(14.00)	66(14.00)	0.000
Read and Write	19(11.10)	78(26.00)	97(20.60)	
Primary School Graduated	16(9.40)	29(9.70)	45(9.60)	
Intermediate School Graduated	19(11.10)	21(7.00)	40(8.50)	
Preparatory School Graduated	43(25.10)	31(10.30)	74(15.70)	
Institute Graduated	44(25.70)	32(10.70)	76(16.10)	
College Graduated	6(3.50)	46(15.30)	52(11.00)	
Post Graduated	0(0.00)	21(7.00)	21(4.50)	
Mother's educational level				
Doesn't Read And Write	38(22.20)	43(14.30)	81(17.20)	0.000
Read and Write	21(12.30)	82(27.30)	103(21.90)	
Primary School Graduated	5(2.90)	12(4.00)	17(3.60)	
Intermediate School Graduated	7(4.10)	21(7.00)	28(5.90)	
Preparatory School Graduated	50(29.20)	27(9.00)	77(16.30)	
Institute Graduated	43(25.10)	49(16.30)	92(19.50)	
College Graduated	7(4.10)	44(14.70)	51(10.80)	
Post Graduated	0(0.00)	22(7.30)	22(4.70)	
Economic status				
Not sufficient	44(25.70)	122(40.70)	166(35.20)	0.000
Somewhat sufficient	0(0.00)	6(2.00)	6(1.30)	
Sufficient	127(74.30)	172(57.30)	299(63.50)	
Residence				
Rural	57(33.30)	90(30.00)	147(31.20)	0.470
Urban	114(66.70)	210(70.00)	324(68.80)	
Child's residence				
With both parents	147(86.00)	251(83.70)	398(84.50)	0.597
With one parent	24(14.00)	49(16.30)	73(15.50)	
Marital status				
Married	140(81.90)	247(82.30)	387(82.20)	0.261
Widowed	4(2.30)	15(5.00)	19(4.00)	
Divorced	27(15.80)	38(12.70)	65(13.80)	
Father's occupational status				
Employee	131(76.60)	130(43.30)	261(55.40)	0.000
Housewife	0(0.00)	3(1.00)	3(0.60)	
Freelance	26(15.20)	98(32.70)	124(26.30)	
Unemployed	14(8.20)	60(20.00)	74(15.70)	
Retired	0(0.00)	9(3.00)	9(1.90)	
Mother's occupational status				
Employee	104(60.80)	124(41.30)	228(48.40)	0.000
Housewife	62(36.30)	123(41.00)	185(39.30)	
Freelance	5(2.90)	42(14.00)	47(10.00)	
Unemployed	0(0.00)	6(2.00)	6(1.30)	
Retired	0(0.00)	5(1.70)	5(1.10)	

Freq. = frequency, %= Percentage

Source: Own materials

Table 2. Distribution of clinical characteristics of case and control groups

Diagnosis	Control (171), (%)	Case (300), (%)	Total	p-value
Pneumonia	0(0.00)	171(57.00)	171(36.30)	
Bronchitis	0(0.00)	41(13.70)	41(8.70)	
Allergy	0(0.00)	13(4.30)	13(2.80)	
Otitis media	0(0.00)	27(9.00)	27(5.70)	
Upper airway obstruction	0(0.00)	1(0.30)	1(0.20)	
Influenza (Flu)	0(0.00)	45(15.00)	45(9.60)	
Laryngitis	0(0.00)	1(0.30)	1(0.20)	
Whooping cough	22(12.90)	1(0.30)	23(4.90)	0.000
Urinary tract infection (UTI)	9(5.30)	0(0.00)	9(1.90)	
Enteritis	60(35.10)	0(0.00)	60(12.70)	
Asthma	40(23.40)	0(0.00)	40(8.50)	
Chickenpox	3(1.80)	0(0.00)	3(0.60)	
Eczema	11(6.40)	0(0.00)	11(2.30)	
Allergy	21(12.30)	0(0.00)	21(4.50)	
Constipation	5(2.90)	0(0.00)	5(1.10)	
Duration of illness (Mean±SD)	15.93(17.52)	14.52(20.54)	15.03(19.54)	0.452
Number of hospitalizations				
1	69(40.40)	119(39.70)	188(39.90)	
2	60(35.10)	119(39.70)	179(38.00)	
3	33(19.30)	37(12.30)	70(14.90)	0.221
4	4(2.30)	13(4.30)	17(3.60)	
5 and above	5(2.90)	12(4.00)	17(3.60)	

% = Percentage
Source: Own materials

summarize demographic and clinical characteristics. The p-values presented throughout the results were computed based on these statistical tests, with $p < 0.05$ considered statistically significant.

RESULTS

The study provides a comprehensive overview of patient characteristics in the context of incidence rate and risk factors for respiratory tract infection. In the control group, participants' ages ranged from 10.9 (12.57%) months, with which as the high percentage for age at 11.81(13.04%) months case group. The control and case both showed a predominant male presence, with 54.70% and 54.40%, respectively. Fathers educational attainment varied, with 25.70% of patients in the control being Institute Graduated, while 26% of the case group had just read and write, while mothers educational achievement varied, with 29.20% of patients in the control being Preparatory School graduated, while 27.20% of the case group had just read and write. In terms of monthly income, 74.30% of individuals in the control group and 57.30% in the case group expressed having sufficient income up to a certain limit. Residency patterns revealed that a significant portion of participants were urban residents in both the control 66.70% and case 70% groups. Regarding child's residency most of study participant living with both parents in both the control 86% and case 83.70% groups. Marital status, most of patients in both the control group 81.90% and the case group 82.30% were married. Fathers' occupational findings highlighted that Employee dominated in both the control 76.60% and case 43.30% groups, while the mother's occupational findings highlighted that Employee dominated in the control 60.80% and Housewife is the dominated in the case 41% groups. These insights into patient demographics provide a nuanced understanding of the population under study, facilitating a more informed interpretation of the impact of gastritis and associated lifestyle modifications within different demographic segments (Table 1).

The study presents a thorough examination of the clinical characteristics of patients within the context of incidence rate and risk factors related to children with respiratory tract infection. Within the case group, 57% diagnosed with Pneumonia, while in the control group,

Table 3. Incidence of risk factors among children under five years in both study and control groups

Indicator	Control 171, Freq. (%)	Case 300, Freq. (%)	All 471, Freq. (%)	p-value
Recent cold or influenza	127(74.3)	193(64.3)	320(67.9)	0.03
Exposure to smoke	129(75.4)	118(39.3)	247(52.4)	0.00
Allergy	132(77.2)	81(27)	213(45.2)	0.00
Low birth weight	11(6.4)	43(14.3)	54(11.5)	0.01
Duration of breastfeeding less than six months	18(10.5)	19(6.30)	37(7.9)	0.11
Large family size	18(10.5)	53(17.7)	71(15.10)	0.04
Early use of antibiotics before six months	65(38)	31(10.3)	96(20.4)	0.00
Asthma	29(17)	95(31.7)	124(26.3)	0.00
Exposure to indoor air pollution	3(1.8)	49(16.3)	52(11)	0.00
Incomplete immunization	3(1.8)	77(25.7)	80(17)	0.00

Freq. = frequency, % = Percentage
Source: Own materials

Table 4. Risk factor affecting of respiratory tract infection for children under five years at hospital

Factor	Odd ratio	95% C.I.		p-value
		Lower	Upper	
Recent cold or influenza	2.94	1.53	5.63	0.00
Exposure to smoke	3.78	2.11	6.78	0.00
Allergy	10.49	5.79	19.01	0.00
Low birth weight	0.30	0.13	0.72	0.01
Duration of breastfeeding less than six months	2.81	1.07	7.41	0.04
Large family size	0.95	0.44	2.08	0.90
Early use of antibiotics before six months	12.58	5.77	27.42	0.00
Asthma	0.80	0.41	1.59	0.53
Exposure to indoor air pollution	0.25	0.07	0.95	0.04
Incomplete immunization	0.19	0.05	0.68	0.01

Source: Own materials

35.10% were diagnosed with enteritis. In terms of the duration of the disease, 57% of patients in the case group were diagnosed with Pneumonia within the 14.52 days' range, mirroring the same duration in the control group at 35.1% were diagnosed with enteritis. Notably, a significant proportion of both the control group 40.40% onetime enters to hospital, and the case group 39.9% one time, 39.9% two-time reported admission to hospital (Table 2).

The findings of the study indicate that the during the initial assessment phase, the control group's patients most responses for the highly risk factors allergy, the second risk factor is exposure to smoke and third risk factor recent cold or influenza, while the assessment phase, the case group's patients most responses for the highly risk factors recent cold or influenza, the second risk factors is exposure to smoke and a little less the and third risk factor (Table 3).

The results reveal the significant risk factors case and control group regarding respiratory tract infection are recent cold or influenza, exposure to smoke, allergy, low birth weight, duration of breastfeeding less than six months, early use of antibiotics before six months, exposure to indoor air pollution, incomplete immunization, underscored by a small effect size observed between the case and control groups η^2=0.00. Conversely, there were no significant differences in this risk factors are large family size and asthma at p= 0.000 (Table 4).

DISCUSSION

The result of the current study is illustrated by the frequency and percentage in the tables that provides a comprehensive overview of patient characteristics in the context of incidence rate and risk factors. In the study group, participants' in the control group, participants' ages ranged from 10.9(12.57%) months, with a which as the high percentage for age at 11.81(13.04%) months case group whose result is agree with [5-7] the results indicate that the study

that mean age of the patients less than 23 weeks. Furthermore, is young children had developing immune systems that are not yet fully mature to fight infections and stopped effectively. This makes them more susceptible to respiratory infections. Anatomically airways in the young children are smaller and more prone to obstruction. This can lead to more severe symptoms and complications from respiratory infections. Also may be more exposed to environmental factors such as secondhand smoke, air pollution, and allergens, which can aggravate respiratory problems. The case and control groups both showed a predominant male presence, with 54.70% and 54.40%, respectively. This result is supported by a several descriptive studies conducted in many different countries [8-11], in this studies that the majority of participant are male. The biological differences between female and Male may have differences in immune system function compared to females. Research suggests that females generally had stronger immune responses, which can provide better protection against infections. These factors, combined with the general vulnerabilities of young children, contribute to the higher incidence of respiratory tract infections in male children below 5 years old. Fathers' educational attainment varied, with 25.70% of patients in the control being primary school graduated, while 26% of the case group had just primary school graduated. This result is supported by a several descriptive studies conducted in many different countries [12-14], while mothers educational achievement varied, with 29.20% of children in the control being preparatory school graduated, while 27.20% of the case group had just preparatory school graduated. This result is supported by [15-18] all this study up there reveal the parents of children had middle school, Parents with higher education generally to be more knowledgeable about dietary needs and healthy eating behaviors, leading to diverse dietary practices, and obviously to be aware of the importance of vaccinations, various examinations, and definitive interventions for health problems, risk factors, and are associated with better health after literacy, and inclusion enables understanding of medical preparedness, chronic conditions, and comprehensive health monitoring, which can create more support and stability. Educated parents emphasize more on the influence that has contributed to the development of creativity, such as reading and educational games, which can control the health of their children. Regarding monthly income, 74.30% of individuals in the control group and 57.30% in the case group expressed having sufficient income. This study supported by [19-22] all this study shows the most parents participant had high level income, a good monthly income significantly impacts children's health through improved access to healthcare, better nutrition, safe living conditions, enhanced educational opportunities, and positive psychosocial factors. Financial stability allows families to provide a healthier and more supportive environment, fostering the overall well-being and development of children, Residency patterns revealed that a significant portion of participants were urban residents in both the control 66.70% and case 70% groups. Regarding child's residency most of study participant living with both parents in both the control 86% and case 83.70% groups. This result is supported by [3, 14, 23] all this study shows the parents and their children live in the urban residential area, being in an urban area brings together the opportunities and challenges of children's health. While urban environments often experience better access to health, health and social care, many also have higher levels of air and noise production, which can lead to organ problems and contribute to the onset of heart disease and other health impairments in children, Sedentary life due to limited space for outdoor activities and dependence on screen-based data pattern. Modern social inequalities, life can take over with a higher level of problems due to noise. This includes safety concerns, which can include the general health of children. These challenges require inclusive urban planning and policies to create healthier living requirements for children. In addition, the delayed time to diagnosis in some cases was likely due to resource limitations in our region, including access to diagnostic testing and specialists. This may have affected the timing of treatment and clinical outcomes. Marital status, the majority of patients in both the control group 81.90% and the case group 82.30% were married. Regarding child's residency most of study participant living with both parents in both the control 86% and case 83.70% groups, this study agrees with [24-27]. Married families often feel a sense of security and stability in the family structure, which contributes to improved emotional and psychological health. A harmonious marital relationship can reduce stress within the family, and creating a more positive environment can have a profound impact on children's health, while a stable marriage can provide emotional, economic, and social benefits that promote better health outcomes, marital conflict and divorce can lead to stress and instability that negatively impacts children's well-being. The key is to foster a supportive and healthy family environment, regardless of marital status, to ensure the best possible health outcomes for children. Fathers' occupational findings highlighted that Employee dominated is employee in both the control 76.60% and case 43.30% groups. Behavior et al., (2022) [9] this study shows most participant are employee, the basic good occupation can be significantly unsafe for children's health through social investment, time availability, exposure to working hazards, and psychological factors. Ensuring alignment between work demands and family needs, healthy work environments, and positive medication supplies can contribute to better health outcomes for children, while the mother's occupational findings highlighted that

Housewife dominated in the control 60.80% and Housewife is the dominated in the case 41% groups. These insights into patient demographics provide a nuanced understanding of the population under study, facilitating a more informed interpretation of the impact of associated incidence rate and risk factors within different demographic segments. This study supported by [28-30] this studies reveal most participant are housewife, The role of a mother, either as a wife or we maintain our interest, which greatly impacts the health of the children in many different ways. Here's a quick look at how mothers influence the health and well-being of their children. They often do not include an essential part of their plans and preparations, which directly affects the eating habits of children. We can know by nutrition that a healthy diet is rich in fruits and whole grains, which is important for healthy growth. Mothers are also responsible for scheduling and attending medical appointments for their children, and ensuring that they receive the necessary vaccinations and medical examinations. The study presents a thorough examination of the clinical characteristics of patients within the context of incidence rate and risk factors related to children with respiratory tract infection. Within the case group, 57% diagnosed with Pneumonia. This study supported by [31-35] this studies reveal that most participant diagnosed with pneumonia, it is one of the most common respiratory tract infections in children under five years of age due to several factors. Understanding these factors can help in addressing and mitigating the risk of pneumonia in young children. Pneumonia is the most common respiratory tract infection in children under five years old due to a combination of factors including their immature immune system, high exposure to pathogens, anatomical and physiological vulnerabilities, environmental and nutritional factors, pre-existing health conditions, and lack of access to adequate healthcare. Addressing these factors through preventive measures such as vaccination, improving living conditions, promoting good nutrition, and ensuring access to healthcare can help reduce the burden of pneumonia in this vulnerable age group, while in the control group, 35.10% were diagnosed with gastroenteritis. This study supported by [36-40] all this studies shows the biggest study participant diagnosed with gastroenteritis. Gastroenteritis is the most common gastrointestinal tract infection in children under five years of age due to several factors. Inadequate access to clean drinking water and poor sanitation facilities can increase the risk of children consuming contaminated water or food, Certain viruses that cause gastroenteritis, such as rotavirus, are more prevalent during winter and early spring. This seasonal peak contributes to higher infection rates among young children during these periods. Gastroenteritis is highly prevalent in children under five years of age due to their immature immune systems, high exposure to pathogens, inadequate hygiene practices, and susceptibility to common viral agents, environmental and nutritional factors, and seasonal variations. Addressing these factors through improved hygiene, better sanitation, vaccination, and proper nutrition can help reduce the incidence and impact of gastroenteritis in this vulnerable age group. In terms of the duration of the disease, 57% of patients in the case group were diagnosed with Pneumonia within the 14.52 days' range, mirroring the same duration in the control group at 35.1% were diagnosed with gastroenteritis. The duration of respiratory tract infections can significantly impact children's health through prolonged symptoms, increased risk of complications, effects on growth and development, educational and social disruptions, family and caregiver burden, and strain on the healthcare system. Addressing these infections promptly and effectively is crucial to minimize their impact and support the overall well-being and development of children. In addition, the delayed time to diagnosis in some cases was likely due to resource limitations in our region, including limited access to diagnostic testing, laboratory infrastructure, and pediatric specialists. These constraints may have affected the timing of treatment and, consequently, the clinical outcomes. Notably, a significant proportion of both the control group 40.40% onetime enters to hospital, and the case group 39.9% one time, 39.9% two-time reported admission to hospital, Readmission for respiratory tract infections can significantly affect children's health by increasing the risk of complications, impacting growth and development, causing emotional and psychological stress, disrupting family dynamics, hindering educational and social progress, and burdening the healthcare system. Effective management of RTIs, including preventive measures, timely treatment, and proper follow-up care, is crucial to minimize these impacts and support the overall health and well-being of children. Discussion of their Tables (3-4) incidence of risk factors among children under five years in both study and control groups. The findings of the study indicate that the during the initial assessment phase, the control group's patients most responses for the highly risk factors allergy, the second risk factor is exposure to smoke and third risk factor recent cold or influenza. while the assessment phase, the case group's patients most responses for the highly risk factors recent cold or influenza, the second risk factors is exposure to smoke and a little less the and third risk factor is. It is important to highlight that there were no statistically considerable differences in risk factors for respiratory tract infection scores between the control and case groups during the initial assessment. This emphasizes the similarity in the baseline understanding between the two groups at the outset of the study. This study supported by [41-44] all this study reveal the most frequent risk factors for respiratory tract infection among children under five years old are exposure to smoke, allergy, low birth weight,

duration of breastfeeding less than six months, early use of antibiotics before six months, exposure to indoor air pollution, incomplete immunization, influenza, Air pollution exposure to smoke from cooking stoves, heating devices, or tobacco smoke can irritate the respiratory tract and increase the risk of infections [45]. Also nitrogen dioxide and sulfur dioxide from vehicle emissions, industrial activities, and other sources can impair lung development and function, leading to respiratory illnesses. Poor nutrition can weaken the immune system, making children more vulnerable to infections and hindering their ability to recover from illnesses. Premature infants often have underdeveloped lungs and Low birth weight and premature babies tend to have a weaker immune system increasing their susceptibility to respiratory illnesses. Children who are around secondhand smoke are more likely to get respiratory infections. Lack of vaccination against common respiratory pathogens, such as influenza, pertussis, and *Haemophilus influenzae* type b (Hib), can leave children vulnerable to severe respiratory infections. Malnourished children may experience stunted growth and development, which can include compromised respiratory health. Finally, multiple risk factors, including environmental, socioeconomic, biological, behavioral, and nutritional factors, can significantly affect the respiratory health of children under five years. Young children's respiratory health must be protected and improved by addressing these risk factors through better access to healthcare, better nutrition, better living conditions, full vaccination, and less exposure to secondhand smoke and pollutants.

CONCLUSIONS

This study underscores the vulnerability of infants under 12 months to respiratory tract infections, particularly pneumonia. Key modifiable risk factors include early antibiotic exposure, environmental smoke, and insufficient immunization. These findings highlight the need for targeted community-level interventions and caregiver education programs to reduce preventable respiratory infections in children.

RECOMMENDATIONS

1. Mass media should utilize to provide health education to increase parents' knowledge and awareness about respiratory tract infection and these risk factors.
2. Booklets containing an explanation and instructions about respiratory tract infection and these risk factors should distribute to parents'.
3. Since nurses continue to care for patients 24 hours daily, health management should be implemented, and nurses' roles in the health education process should be expanded to better inform and educate parents.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

The impact of iodine deficiency, combined with the using of thyroid gland function influenced products, on the organs of the cardio-vascular, endocrine, and reproductive systems

Iiliia V.Sobol, Oleksandra T. Harhaun, Ivan S. Kuibida, Oksana H. Popadynets
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ABSTRACT

Aim: To investigate morphofunctional changes in the endocardium, arteries' wall of different types, prostate and adrenal glands, taking into account age-related characteristics on the 60th and 90th days of iodine deficiency combined with goitrogen consumption.

Materials and Methods: The experiment was conducted on 50 white outbred male rats (25 sexually immature, 3–5 months old, and 25 sexually mature, 6–8 months old). Eleven animals from both age groups formed the control group, and 14 animals from the second and third experimental groups were subjected to simulated iodine deficiency combined with goitrogen consumption. Material collection was carried out on the 60th and 90th days of the experiment. Morphological (light and electron microscopy), morphometric, biochemical studies, and statistical data analysis were performed.

Results: On the 60th day of the experiment, the cholesterol content was: in sexually immature rats, 1.66 ± 0.19 mmol/L ($p < 0.01$); in sexually mature rats was 1.44 ± 0.15 mmol/L ($p < 0.01$). Ioduria in sexually immature animals of Group 2 was 1.42 ± 0.16 µg/L, and in sexually mature animals it was 1.92 ± 0.21 µg/L ($p < 0.01$). On the 90th day of the experiment, edema changes in the endocardium progressed. In light microscopy studies, the endocardium appeared as a veil-like strip covering the myocardium. In the light pink cytoplasm, flattened and weakly basophilic endothelial nuclei were visible. The content of endothelin-1 in the blood of sexually immature animals increased by 5.7% ($p < 0.05$), while in sexually mature animals, it rose by 2.4% ($p < 0.05$).

Conclusions: The level and degree of iodine deficiency is confirmed by ioduria indicators and thyroid profile. In all studied organs, edema changes develop in all constituent structures already on the 60th day of simulated iodine deficiency, progressing to the 90th day of the experiment.

KEY WORDS: endocardium, arteries, prostate gland, adrenal gland, iodine deficiency, structural and metabolic changes, postnatal ontogenesis.

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INTRODUCTION

The level of environmental and degree and chemical influences on the functions of the endocrine glands remains an enigma due to an infinite number of potential mechanisms [1, 2, 3, 4]. Processes inhibiting the natrium/iodide symporter (NIS) and the thyroid peroxidase enzyme by certain ions contaminating water bodies have been described. These substances are known as goitrogens [5, 6].

The prostate gland can accumulate both iodide and molecular iodine. The mechanisms of iodine uptake are independent of the transporters used by the thyroid gland (i.e., not via NIS) [7]. Chronic exposure to thiourea, which blocks the conversion of thyroxine (T4) to triiodothyronine (T3) in peripheral tissues, leads to morphological and functional changes in the adrenal glands, particularly in zona fasciculata [1]. Interrelationships between the thyroid and adrenal glands under conditions of alimentary hypothyroidism are being studied [8].

Studies on rats have shown that prolonged consumption of peanut skin reduces T4 and T3 serum levels and inhibits thyroid peroxidase and 5'-deiodinase activity [9]. Adequate synthesis of thyroid hormones is critically important for maintaining cardiovascular health, as protein and enzyme synthesis required for endocardial function depend on sufficient iodine intake. This emphasizes the need for dietary adjustments in cases of iodine deficiency [10, 11, 12]. Given the prevalence of iodine deficiency and the consumption of goitrogens, along with fragmented data on their impact on the endocardium, arteries of different types, adrenal, and prostate glands, comprehensive research became necessary.

AIM

To investigate morphofunctional changes in the endocardium, arteries' wall of different types, prostate and

adrenal glands, taking into account age-related characteristics on the 60th and 90th days of iodine deficiency combined with goitrogen consumption.

MATERIALS AND METHODS

Samples of the endocardium, fragments of the aorta, external carotid, and renal arteries, prostate gland (ventral, dorsal lobes, and coagulating gland) and adrenal gland, blood, and urine were studied. Iodine deficiency was simulated according to the methodology described in [13]. Iodine deficiency combined with goitrogenic product consumption (soybeans, peanuts) was modeled based on our patented methodology [14].

The experiment was conducted on 50 white outbred male rats (25 sexually immature, 3–5 months old, and 25 sexually mature, 6–8 months old). Eleven animals from both age groups formed the control group, and 14 animals from the second and third experimental groups were subjected to simulated iodine deficiency combined with goitrogen consumption. Material collection was carried out on the 60th and 90th days of the experiment. Morphological (light and electron microscopy), morphometric, biochemical studies, and statistical data analysis were performed [15].

RESULTS AND DISCUSSION

The thyroid status of sexually immature animals in Group 1 was: TSH 0.10 ± 0.01 μIU/mL ($p < 0.01$), T3 3.63 ± 0.12 nmol/L ($p < 0.01$), T4 75.44 ± 4.01 nmol/L ($p < 0.01$); in sexually mature animals: 0.08 ± 0.00 μIU/mL ($p < 0.01$), 2.79 ± 0.15 nmol/L ($p < 0.01$), 55.18 ± 2.72 nmol/L ($p < 0.01$), respectively.

Cholesterol content under normal age conditions was: in sexually immature rats, 1.60 ± 0.05 mmol/L ($p < 0.01$); in sexually mature rats: 1.40 ± 0.09 mmol/L ($p < 0.01$). Ioduria in Group 1 sexually immature animals was 97.13 ± 5.40 μg/L, and in sexually mature animals was 101.06 ± 3.44 μg/L ($p < 0.01$).

The thyroid status of sexually immature animals in Group 2 was as follows: TSH 0.18 ± 0.02 μIU/mL ($p < 0.01$), T3 3.44 ± 0.36 nmol/L ($p < 0.01$), T4 74.40 ± 7.10 nmol/L ($p < 0.01$); in sexually mature animals: 0.14 ± 0.01 μIU/mL ($p < 0.01$), 2.39 ± 0.24 nmol/L ($p < 0.01$), 63.60 ± 7.46 nmol/L ($p < 0.01$), respectively.

On the 60th day of the experiment, the cholesterol content was: in sexually immature rats, 1.66 ± 0.19 mmol/L ($p < 0.01$); in sexually mature rats was 1.44 ± 0.15 mmol/L ($p < 0.01$). Ioduria in sexually immature animals of Group 2 was 1.42 ± 0.16 μg/L, and in sexually mature animals it was 1.92 ± 0.21 μg/L ($p < 0.01$).

In the light microscopic examination, the endocardium appeared as an uneven strip lining the myocardium. The nuclei of endothelial cells were not visible in all fields of view and exhibited varying basophilic intensity. The cytoplasm was weakly eosinophilic with indistinct boundaries. In ultrastructural studies, round membrane organelles were observed in the cytoplasm of endothelial cells. The luminal surface formed a relief contour. The subendothelial layer had medium electron density. In the muscle-elastic layer, loci of optical clearance were noticeable (Fig. 1). The content of endothelin-1 in the blood of sexually immature animals increased by 4.3% ($p < 0.05$), while in sexually mature animals, it rose by 2.4% ($p < 0.05$).

In the wall of the different types of arteries, the tunics were distinguishable (Figure 1). The internal elastic membrane exhibited invaginations along the circumference of the lumen. Nuclei of endothelial cells were visible at the apexes of these invaginations. The nuclei of smooth muscle cells in the tunica media were basophilic. The adventitia exhibited localized separations. Morphometric analysis revealed that in sexually immature animals, the thickness of the aorta wall, external carotid artery, and renal artery increased by 0.9% ($p < 0.05$), 1.1% ($p < 0.05$), and 0.8% ($p < 0.05$), respectively. In sexually mature animals, this parameter increased by 0.3% ($p < 0.05$), 0.9% ($p < 0.05$), and 1.4% ($p < 0.05$), respectively.

Submicroscopic studies showed endothelial cells resting on a basal membrane, under which a subendothelial layer of different electron density was observed (Figure 1). In the clarified sarcoplasm of smooth muscle cells, mitochondria and myofilaments were visualized. Membrane organelles in fibroblasts were dilated, and connective tissue fibers were surrounded by amorphous sunstance.

The lumen of the terminal secretory sections of the prostate gland included homogeneous content. Epithelial cells had basally located nuclei, forming a basophilic strip. The cytoplasm was eosinophilic, with pronounced apical clearance. Blood vessels were surrounded by swollen connective tissue fibers and amorphous stromal substance (Figure 2). The height of the epithelium in the terminal secretory sections of the ventral, dorsal lobes, and coagulating gland increased by 0.5% ($p < 0.05$), 0.9% ($p < 0.05$), and 1.1% ($p < 0.05$), respectively, in sexually immature animals. In sexually mature animals, these parameters increased by 0.3% ($p < 0.05$), 0.5% ($p < 0.05$), and 1.0% ($p < 0.05$), respectively.

In ultrastructural studies, euchromatin and heterochromatin concentrated under the nucleolemma were observed in the nuclei of epithelial cells. Canals and cisternae of the granular endoplasmic reticulum, sacs, and vesicles of the endoplasmic reticulum were dilated. Myelin-like bodies were noted. The sarcoplasm of smooth muscle cells exhibited a fine-grained structure, with round mitochondria containing light matrix.

The adrenal glands demonstrated stromal and vascular wall edema in light microscopy studies. Similar edematous changes were observed in zona glomerulosa, zona fasciculata, and zona reticularis of the cortex, as well as in the medulla. Endocrine cells had light pink cytoplasm and basophilic nuclei with indistinct contours (Figure 2C). The thickness of the adrenal cortex in sexually immature animals increased by 0.4% ($p < 0.05$). In sexually mature animals, it rose by 1.6% ($p < 0.05$).

Ultrastructural studies showed light cytoplasm in endocrine cells, with round mitochondria featuring disrupted cristae and

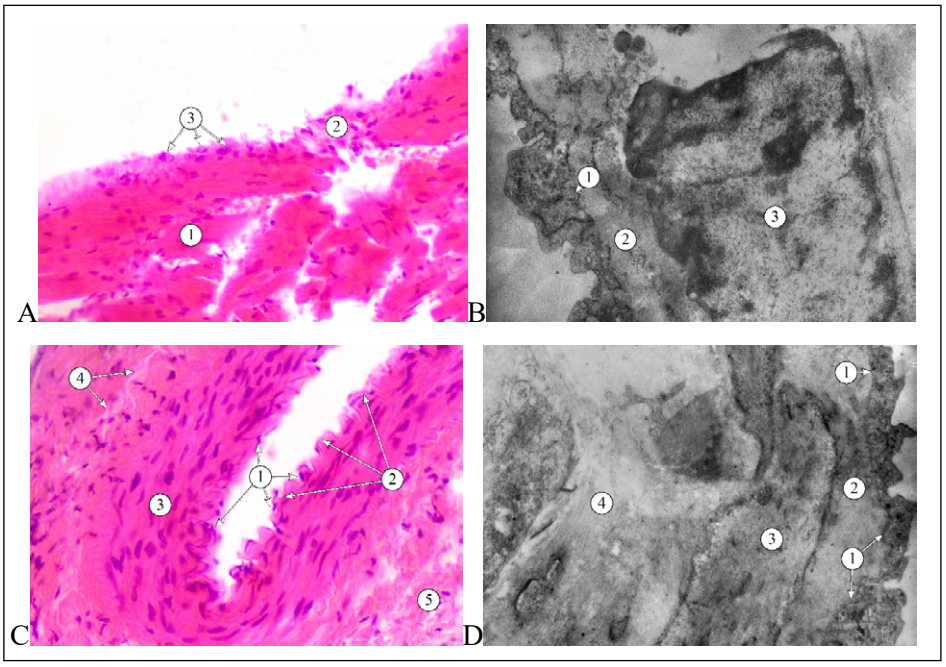


Fig. 1. Structure of the endocardium and wallof the artery on the 60th day of iodine deficiency with using of thyroid gland function influenced products. A. Endocardium of an immature animal. 1 – myocardium, 2 – endocardium, 3 – nuclei of endothelial cells. B. Endocardium of an adult animal. 1 – endothelial cell, 2 – subendothelial layer, 3 – muscular-elastic layer. C. Wall of the renal artery of an immature animal. 1 – endothelial cell nuclei, 2 – internal elastic membrane, 3 – smooth muscle cells of the media, 4 – external elastic membrane, 5 – adventitia. D – wall of the external carotid artery of a sexually mature animal. 1 – endothelial cell, 2 – subendothelial layer, 3 – smooth muscle cells, 4 – connective tissue fibers. A, C – hematoxylin and eosin, magnification: A, C x400. B, D – electron micrograph, magnification: B x9600, D x8000.
Picture taken by the authors

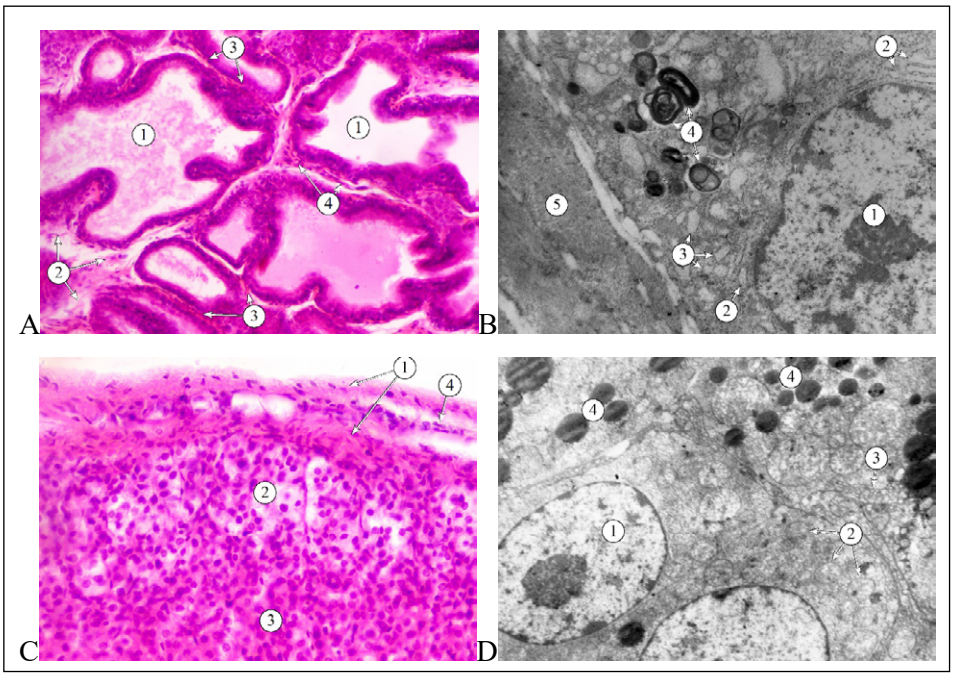


Fig. 2. Structure of the prostate and adrenal glands on the 60th day of iodine deficiency with the consumption of goiter-producing products. A. ventral lobe of the prostate gland of a sexually mature animal. 1 – terminal secretory parts, 2 – fibrous component of the stroma, 3 – smooth muscle cells of the stroma, 4 – blood vessels. B. Coagulation gland of the prostate gland of an immature animal. 1 – nucleus, 2 – granular endoplasmic reticulum, 3 – Golgi apparatus, 4 – myelin-like bodies, 5 – smooth muscle cells. C. Adrenal gland of an adult animal. 1 – capsule, 2 – zona glomerulosa, 3 – zona fasciculata, 4 – blood vessels. D – endocrine cells of the zona fasciculata of the adrenal gland of a sexually mature animal. 1 – nucleus, 2 – mitochondria, 3 – endoplasmic reticulum, 4 – lipid inclusions. A, C – hematoxylin and eosin staining, magnification: A x200, C x400. B, D – electron micrograph, magnification: B x6400, D x4800.
Picture taken by the authors

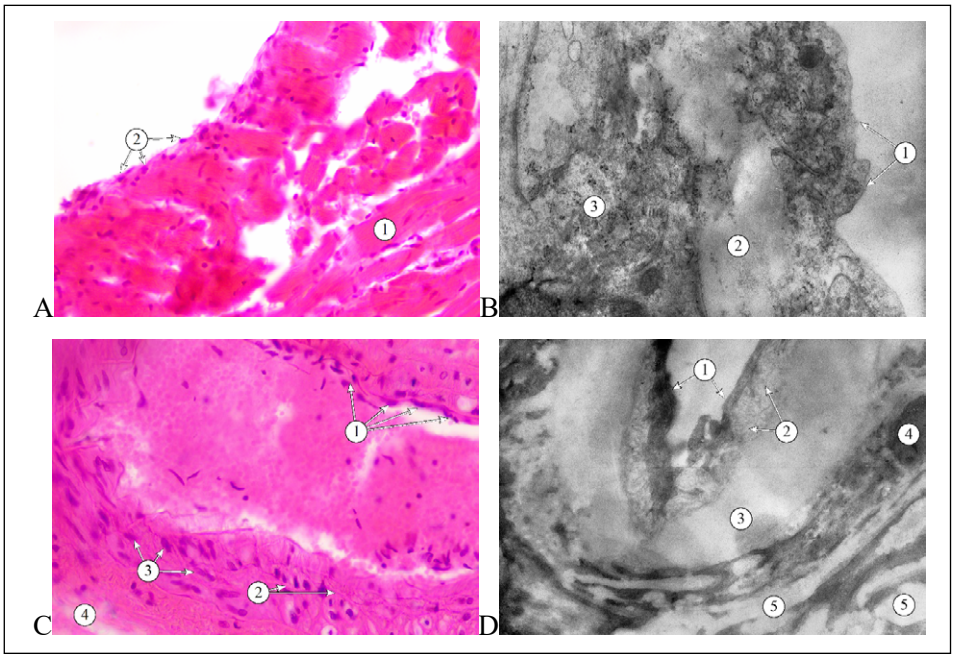


Fig. 3. Structure of the endocardium and wall of the artery on the 90th day of iodine deficiency with the consumption of goiter-producing products. A. endocardium of a sexually mature animal. 1 – miocardium, 2 – nuclei of the endothelial cells of endocardium. B. Endocardium of an immature animal. 1 – endothelial cells, 2 – subendothelial layer, 3 – muscular-elastic layer. C. Wall of the carotis externa artery. 1 – endothelial cell nuclei, 2 – connective tissue fibers of the media, 3 – smooth muscle cells of media, 5 – adventitia. D – wall of the renal artery of a sexually inmature animal. 1 – endothelial cell, 2 – subendothelial layer, 3 – internal elastic membrane, 4 – smooth muscle cell, 5 – elastic fibers. A, C – hematoxylin and eosin, magnification: A, C x400. B, D – electron micrograph, magnification: B x20000, D x6400.
Picture taken by the authors

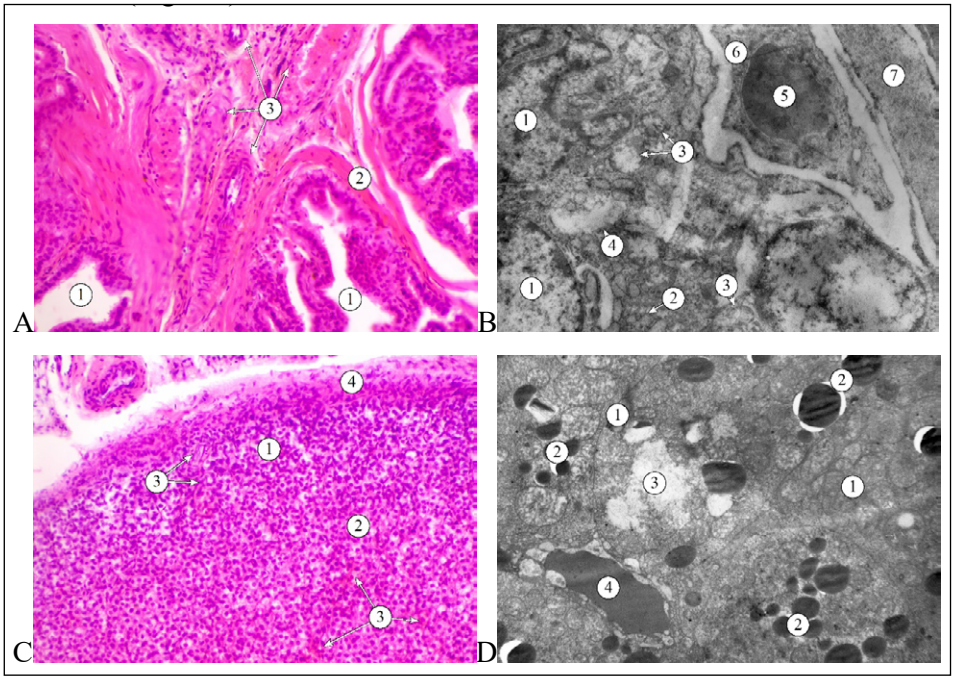


Fig. 4. Structure of the prostate and adrenal glands on the 90th day of iodine deficiency with the consumption of goiter-producing products. A. Coagulation gland of the prostate gland of an immature animal. 1 – terminal secretory sections, 2 – smooth muscle cells of the stroma, 3 – blood vessels. B. Ventral lobe of the prostate gland of a sexually mature animal. 1 – nucleus, 2 – Golgi apparatus, 3 – mitochondria, 4 – vacuoles, 5 – erythrocyte, 6 – endothelial cell, 7 – smooth muscle cell. C. adrenal gland of an immature animal. 1 – zona glomeruloza, 2 – zona fasciculata, 3 – blood vessels, 4 – capsule. D – endocrine cells of the zona fasciculata of the adrenal gland of a sexually mature animal. 1 – mitochondria, 2 – lipid inclusions, 3 – disorganized endoplasmic reticulum, 4 – erythrocyte in the lumen of the blood capillary. A, C – hematoxylin and eosin, magnification: A, C x200, B, D – electron micrograph, magnification: B x6400, D x4000.
Picture taken by the authors

light matrix. The endoplasmic reticulum and Golgi apparatus were vacuolated, and secretory granules of varying optical density were visible (Fig. 2D).

Thyroid status of immature animals of group 3: TSH 0.14 ± 0.02 μIU/ml ($p < 0.01$), T3 3.36 ± 0.33 nmol/l ($p < 0.01$), T4 50.14 ± 5.73 nmol/l ($p < 0.01$); in mature animals – 0.26 ± 0.02 μIU/ml ($p < 0.01$), 2.06 ± 0.18 nmol/l ($p < 0.01$), 50.88 ± 5.69 nmol/l ($p < 0.01$), respectively. The cholesterol content on the 90th day of the experiment is: in immature rats 1.68 ± 0.15 mmol/l ($p < 0.01$, in mature rats – 1.49 ± 0.14 mmol/l ($p < 0.01$). Ioduria in immature animals of group 3 is 0.92±0.08 μg/l, in mature animals – 1.54±0.14 μg/l ($p<0.01$).

On the 90th day of the experiment, edema changes in the endocardium progressed. In light microscopy studies, the endocardium appeared as a veil-like strip covering the myocardium. In the light pink cytoplasm, flattened and weakly basophilic endothelial nuclei were visible (Fig. 3). The content of endothelin-1 in the blood of sexually immature animals increased by 5.7% ($p < 0.05$), while in sexually mature animals, it rose by 2.4% ($p < 0.05$).

Submicroscopically, the cytoplasm of endothelial cells exhibited medium electron density, with dilated membrane organelles and vacuoles present. The subendothelial layer was swollen and homogenized. Sarcoplasmic vacuolization of smooth muscle cells and edema of muscle-elastic fibers were noticeable.

The wall of the arteries also revealed edematous changes. Endothelial cells appeared flattened, and their nuclei were not identifiable throughout. The collagen-elastic framework of the media was deformed, and the coils of elastic fibers were uneven. Smooth muscle cell nuclei appeared misaligned and weakly basophilic. In certain fields of view, sarcoplasm appeared very light.

The adventitia exhibited edematous changes (Fig. 3C). Morphometric analysis showed an increase in wall thickness in the aorta, external carotid artery, and renal artery by 1.1% ($p < 0.05$), 1.3% ($p < 0.05$), and 1.0% ($p < 0.05$), respectively, in sexually immature animals. For sexually mature animals, the parameter increased by 0.6% ($p < 0.05$), 1.4% ($p < 0.05$), and 1.8% ($p < 0.05$), respectively.

In ultrastructural studies, flattened endothelial cells were observed on an indistinctly differentiated basal membrane, with a swollen subendothelial layer beneath. Similar edema changes were found in the fibers and smooth muscle cells of the artery wall (Fig. 3D).

In the prostate lobes on the 90th day of the experiment, edema changes were evident. Connective tissue fibers and smooth muscle cells in the stroma were swollen, accompanied by lymphocytic infiltration. The wall of the blood vessels was thickened, with stasis phenomena in the lumens. The epithelial cells of the terminal secretory sections showed signs of edema. The basal poles of their cytoplasm appeared clarified, with nuclei displaced. The apical light stained cytoplasm formed a veil-like contour in the lumens of the terminal secretory sections (Figure 4).

The height of the epithelium in the terminal secretory sections of the ventral, dorsal lobes, and coagulating gland increased by 3.7% ($p < 0.05$), 4.3% ($p < 0.05$), and 3.9% ($p < 0.05$), respectively, in sexually immature animals. For sexually mature animals, the epithelial height increased by 1.2% ($p < 0.05$), 1.1% ($p < 0.05$), and 1.6% ($p < 0.05$), respectively.

During electron microscopy studies, in the epithelial cells deformed nuclei were visible due to the invagination of their nucleolemma. Membrane organelles were dilated and vacuolated. Mitochondria had clarified matrix and deformed cristae. Stasis phenomena were observed in the lumens of blood vessels, with vacuolized endothelial cell cytoplasm. Perivascular edema was prominent visualized. The sarcoplasm of smooth muscle cells exhibited a homogenized granular appearance.

In the adrenal glands, light-microscopic examination revealed edema changes in the capsule, cortex and medulla. Perivascular edema surrounding the vascular network was evident (Fig. 4C). The thickness of the adrenal cortex in sexually immature animals increased by 1.5% ($p < 0.05$). For sexually mature animals, it rose by 2.5% ($p < 0.05$).

Submicroscopically, stasis of formed elements of blood was observed. Cytoplasm of the endocrine cells contained mitochondria of different sizes with deformed cristae and light matrix. Membrane organelles of the synthetic apparatus were dilated and vacuolated, while secretory inclusions exhibited varying optical densities (Fig. 4D).

Edema changes were observed in all examined organs, progressing by the 90th day of the experiment. In both sexually immature and mature animals, an increase in endothelin-1, a vasoconstrictor exacerbating trophic disorders, was evident [16, 17, 18]. Edematous processes in the walls of elastic, mixed, and muscular-type arteries led to their thickening, confirmed by morphometric analysis [19, 20, 21]. Additionally, lipid profile changes served as a specific marker of hypothyroid conditions [22, 23, 24, 25, 26]. Edematous changes in the connective tissue components of the prostate and adrenal glands also compressed vascular structures, amplifying ischemic manifestations [27, 28]. Consequently, we observed edema-dystrophic changes in epithelial tissues.

The adrenal glands' involvement in lipid peroxidation due to their high content of unsaturated fatty acids in the plasmalemma of endocrine cells was noted [2]. Impaired thyroid hormone synthesis resulted in oxidative stress[10, 22, 29, 30], triggering a cascade of inflammatory and hemodynamic disturbances [31, 19, 32]. The prostate gland, containing key components of thyroid signaling, indicated potential direct action of thyroid hormones on this target organ [33]. Molecular iodine exhibited multi-factorial protective effects on the prostate, functioning as both a thyroid microelement and an antioxidant, anti-inflammatory agent, apoptosis inducer in pathological cells, and normalizer of prostate function in benign hyperplasia [7].

CONCLUSSION

The level and degree of iodine deficiency is confirmed by ioduria indicators and thyroid profile. In all studied organs, edema changes develop in all constituent structures already on the 60th day of simulated iodine deficiency, progressing to the 90th day of the experiment.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Diagnostic measures in patients with thyroid diseases complicated by tracheal compression syndrome

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ABSTRACT

Aim: Aim of the study is analysis of results of diagnostic studies in patients with diseases of thyroid complicated by tracheal compression syndrome which were operated on urgent and immediate indications.

Materials and Methods: The study comprised 167 patients, who were admitted to the clinic of State University «Institute of General and Emergency Surgery named after V. T. Zaitsev National Academy of Sciences of Ukraine» with thyroid diseases complicated by tracheal compression syndrome. All the patients were operated on by urgent and immediate indications. Condition of thyroid and residual tissue were assessed with the help of ultrasound examination, elastography, gland function according to the level of hormones, the results of histological examination and immunohistochemical studies were also taken into account.

Results: The conducted immunohistochemical studies proved presence of three stages of proliferative activity of the thyroid gland; correlation was established between the characteristics of elastography and markers of proliferative activity. Further tactics of patient's management depends on determination of type of elastogram and degree of proliferative activity of thyroid tissue: referral for dynamic observation or surgical treatment with the selection of the scope of surgical intervention including minimally invasive one.

Conclusions: Diagnostic measures in patients with thyroid diseases complicated by tracheal compression syndrome are aimed first of all at receiving comprehensive data on patient's actual condition and disease to determine optimal treatment tactics and decrease the level of postoperative complications and lethality in surgical interventions performed by urgent and immediate indications.

KEY WORDS: diagnostics, cytological examination, elastography, thyroid disease, immunohistochemical study, compression syndrome of trachea

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INTRODUCTION

For our country thyroid diseases including so-called iodine deficiency state, are of particular importance, as far as for weakened attention to iodine prevention some territories turned out to be endemic for goiter. Results of ecological catastrophe in Chornobyl emphasize again the relevance of program research related to pathology of thyroid gland [1-3].

Frequency of nodular forms of goiter among thyroid diseases according to different authors varies from 42 to 98.9%, among which the multiple defeat makes about 25-62% [3, 4].

Clinical concept of "thyroid disease" combines focal and diffuse lesions of thyroid gland with different pathomorphological changes: thyroiditis, cysts, and true nodes, benign and malignant tumors of the thyroid gland. Their nature is completely different: benign proliferation, primary and secondary dysplasia, autoimmune lymphoid infiltration. Great number of existing thyroid disease classifications is represented with isolated from one another histological, cytological, clinical and prognostic schemes [5, 6]. Most of them are devoted to classification of thyroid cancer; this actually determines the necessity of preliminary diagnostics [7].

Thus, the problem of adequate diagnostics of thyroid diseases complicated by tracheal compression syndrome in patients operated by urgent and immediate indications has not been solved and highlighted enough.

AIM

The aim of the study is analysis of results of diagnostic studies in patients with thyroid diseases complicated with tracheal compression syndrome, which were operated by urgent and immediate indications.

MATERIALS AND METHODS

The research comprises 167 patients with tracheal compression syndrome induced by thyroid diseases who were admitted to the clinic of State University «Institute of General and Emergency Surgery named after V. T. Zaitsev National Academy of Sciences of Ukraine» urgently. 88 of them were patients with polynodous euthyroid cervicosternal goiter and 79 patients with differentiated forms of thyroid cancer.

The research met international requirements CONSORT and had corresponding design – retrospective and prospective randomized clinical trial in which the results of diagnostics of polynodous euthyroid cervicosternal goiter complicated with tracheal compression syndrome and thyroid cancer were studied.

Ethical principles of the research were observed. The ethical commission of Educational granted permission for the research and Scientific Medical Institute of National Technical University «Kharkiv Polytechnic Institute» (Protocol of the meeting №3 from 22.01.2024). Written informed consents for the use of the results of treatment for the research were gained from all the patients.

All the patients were operated on and classified into two groups depending on the terms of performing emergency surgical interventions: 1st group of patients comprises those who underwent urgent surgery intrusion during the first day. There were 52 (31.1%) of them; second group of patients were 115 (68.9%) patients who underwent emergency surgery intrusion during the second and third day since the moment of admission.

In the vast majority of observations, (132/79.0%) operations were performed by urgent and immediate indications in patients aged over 60 years. The age of operated patients by urgent indications varied from 54 to 86 years (on average 72.0 ± 1.28 years). In 35 (67.3% of the number of urgently operated) patients the age was more than 70 years. Women prevailed among the patients. The ratio of men and women was 1:9.4.

The age of those operated by urgent indications varied between 54 and 85 years. Average age of such patients (on average 62.6±1.49 years) was considerably lower than in the group of urgently operated ($p < 0.05$). Age of majority (66/57.4%) of patients of this group turned out to be lower than 70 years. There were 108 (93.9%) women operated on urgently, and 7 (6.1%) men. The ratio of men and women was 1:15.4.

When assessing comorbidity of these patients it has been found: 52 (31.1%) of patients have 3 and more concomitant pathologies, all of them had pathology of the cardiovascular system – coronary heart disease, tension angina, arterial hypertension, part of patients had arrhythmic variant of coronary heart disease, including atrial fibrillation. Comorbidity index of these patients made 6.2±0.61, which is characterized as high.

All the patients with tracheal compression syndrome underwent laboratory and instrumental studies on admission to the hospital, with estimation of objective and local status before the operation, condition of thyroid gland and residual tissue during ultrasound examination, as well as function of the gland according to the level of hormones, we took into account the results of histological examination and immunohistochemical examination.

Against the background of the underlying disease, many patients with tracheal compression syndrome had concomitant pathology: ischemic heart disease, hypertension, diabetes, chronic heart failure, chronic obstructive bronchitis and others. Moreover, most patients had more than one concomitant disease.

STATISTICAL PROCESSING OF THE OBTAINED DATA

To compare average absolute values the Student's criteria (t) was used. Because of the comparative analysis of relative indicators, the ratio criterion was used χ^2. Confidence intervals were calculated with the help of Fisher's method. The criterion of statistical reliability of the obtained results was considered to be the value $p<0.05$. Statistical processing of digital data was conducted on a personal computer IBMPC with the help of program system Statistica V 10.0. Patient survival was calculated using Kaplan-Meier survival tables.

RESULTS

ELECTROGRAPHY IN COMPLEX ULTRASOUND EXAMINATION OF THE THYROID GLAND

All the patients in the preoperative period underwent ultrasound examination of the thyroid gland. Following changes in thyroid gland tissue were observed: single nodules of thyroid tissue, diffuse-nodular formations, cystic-nodular formations, adenomas of the thyroid gland. The highest percentage made patients with multiple thyroid nodules - 68 (62%).

For patients of the main group standard ultrasound examination was supplemented with electrography

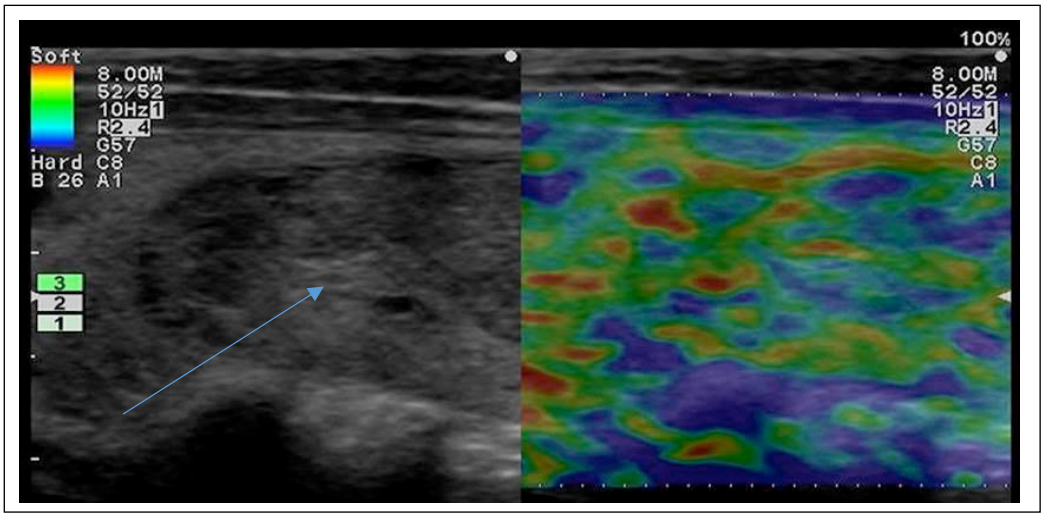


Fig. 1. Nodular goiter. Ultrasound of the neck, gray scale mode: longitudinal scanning and sonoelastography. On the sonoelastography there is intensive yellow staining, stiffness coefficient 1.0 kPa (blue arrow)
Picture taken by the authors

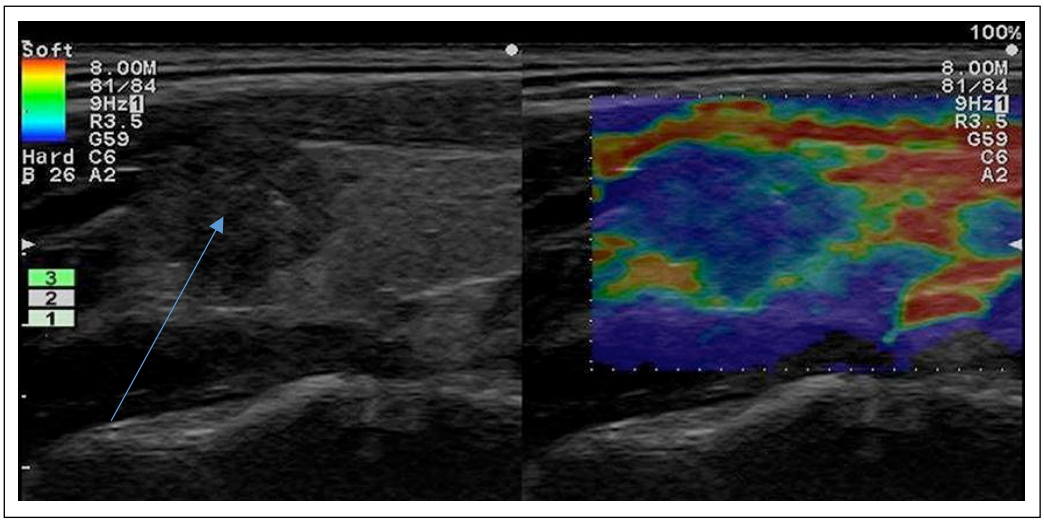


Fig. 2. Nodular goiter. Ultrasound of the neck, gray scale mode: longitudinal scanning and sonoelastography. Hypoechoic nodule with indistinct edges and microcalcifications. On the sonoelastography there is intensive blue staining, stiffness coefficient 5 kPa (blue arrow)
Picture taken by the authors

performance with determination of stiffness coefficient and shear wave speed. Determination of benignity of thyroid gland changes was established based on staining of nodules in relation to the surrounding gland tissue, by measuring stiffness coefficient and speed of shear wave.

The identified changes allowed classifying 3 types of elastograms. Under first type of elastogram the formation is colored red and yellow, as well as the parenchyma of the thyroid gland. When measured, the density of the studied formation did not exceed 1.0 kPa, speed of shear wave did not exceed 2.0±0.4 m/s. This data proves benign changes in thyroid gland. Under second type of elastogram the formation had a mosaic structure with a predominance of green or blue areas with stiffness coefficient 1-4 kPa, speed of shear wave made from 2.5 to 4.5 m/s. This indicates dubious result and requires further examination. Under third type of elastogram the formation has mosaic structure with intense blue areas, with stiffness coefficient over 4 kPa, speed of shear wave exceeded 4.5 m/s. It indicates high probability of malignant changes in thyroid gland tissue.

Types of elastogram are shown on fig. 1, fig. 2, fig. 3, fig. 4.

During the study, it has been established: in 26 patients (43.3%) – 1 type of elastograms, in 28 patients (46.7%) – 2 type of elastograms; in 6 patients – 3 type of elastograms (10%). Among patients with 1 type of elastograms, characteristic of benign changes in the tissue of the thyroid gland, 10 – were patients of old and senile age (over 65 years).

When assessing thyroid gland condition it has been found: 2 degree of increase, absence of compression syndrome, multiple nodes up to 2.0 cm, during elastography red and yellow staining of nodes, stiffness coefficient 0.5 – 1.2 kPa, speed of shear wave 1 5 -2.1 m/s, corresponds to benign nodes. These patients were refused operative treatment, were directed to dynamic supervision under the conditions of the polyclinic. At the follow-up examination after 6 and 12 months growth of nodes according to ultrasound data was not observed, characteristics of elastograms did not change.

16 patients with 1 type of elastograms, all the patients with 2 and 3 types of elastograms were directed to

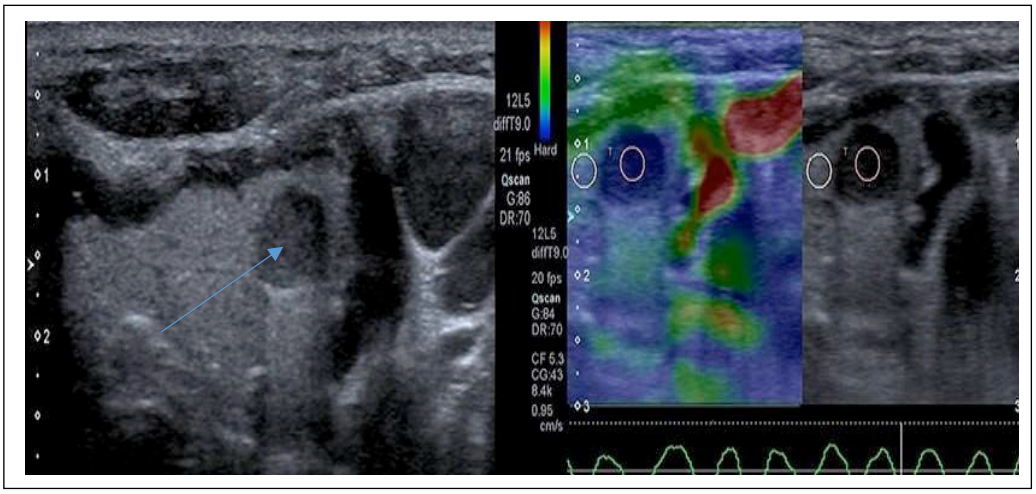


Fig. 3. Ultrasound of the neck, gray scale mode: transverse scanning and sonoelastography. Isoechogenic node (blue arrow). On the sonoelastography speed of shear wave is 1.9 m/s
Picture taken by the authors

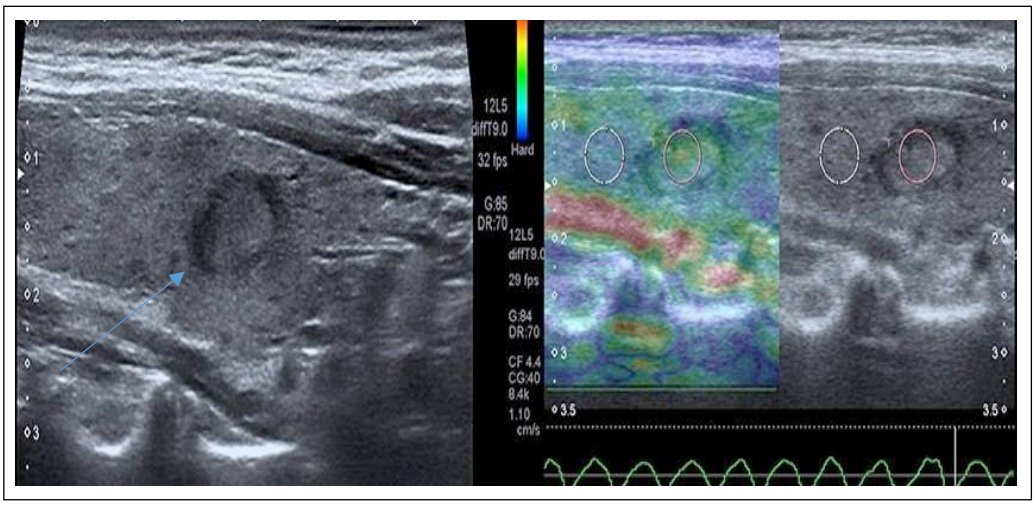


Fig. 4. Ultrasound of the neck, gray scale mode: transverse scanning and sonoelastography. Hypoechoic node (blue arrow). On the sonoelastography there is intensive blue staining, speed of shear wave is 4.8 m/s
Picture taken by the authors

surgical treatment. The indications for surgery in these patients were increased thyroid gland of more than 50 cm^3, presence of nodes of more than 5 cm in diameter, presence of compression syndrome (squeezing of neck vessels, hoarseness of voice).

CYTOLOGICAL CHARACTERISTICS OF THYROID GLAND DISEASES AND EVALUATION OF ELASTOGRAPHY RESULTS COMPARED TO FNA OF THYROID NODULES

Cytological study was conducted taking into account material received with fine needle aspiration biopsy (FNA). Changes in the form of a colloid-proliferating goiter were detected in 45 patients (41%), colloid-cystic – in 32 (29%), colloidal goiter without signs of proliferation – in 21 (19%), B-cell transformation was less common – in 12 (11%). The result is represented in table 1.

We have conducted a comparative analysis of results of elastography and FNA of thyroid nodules. The result is represented in table 2.

The obtained results testify the predominance of benign changes in the tissue of the thyroid gland in patients with 1 type of elastograms, they comprise colloidal goiter without signs of proliferation (11 patients) and colloid-cystic goiter (11 patients). Proliferating forms of goiter predominate in patients with type II of elastograms (19 patients), under III type of elastograms the majority are patients with B-cell adenoma of the thyroid gland.

Thus conducting elastography in complex ultrasound examination of thyroid gland allows not only identifying nodules but also to evaluate their qualitative characteristics based on the color of staining nodular formations, calculating stiffness coefficient and speed of shear wave. Establishing of the I type of elastogram allows predicting benign changes in the thyroid gland tissue, in the absence of reliable indicators for surgical treatment conducting dynamic monitoring of the patient. Establishing of the II type of elastograms provides additional methods of examinations in the form of repeated FNA. Establishing of the III type of elastograms involves risk of malignancy of nodular formations of the thyroid gland and is subject to surgical treatment.

Moreover identification of such characteristics of nodular formations of thyroid gland detected during

Table 1. FNA results in the studied groups

	Main group (60)		Comparison group (50)	
	abs	%	abs	%
Colloid-proliferating goiter	24	40	21	42
Colloid-cystic goiter	18	30	14	28
Colloidal goiter without signs of proliferation	13	22	8	16
B – cellular adenoma	5	8	7	14

Source: compiled by the authors of this study

Table 2. Elastography and FNA results

FNA	Type of elastogram		
	1	2	3
Colloid-proliferating goiter	4	19	1
Colloid-cystic goiter	11	6	1
Colloidal goiter without signs of proliferation	11	2	-
B – cellular adenoma	-	1	4

Source: compiled by the authors of this study

Table 3. Nature of morphological changes

Morphological changes	Main group		Comparison group		Total	
	abs	%	abs	%	abs	%
Nodular colloidal goiter without signs of proliferation	15	30	24	48	39	39
Nodular colloidal goiter with proliferation	12	24	6	12	18	18
Cystic nodular goiter	11	22	11	22	22	22
Follicular adenoma	3	6	5	10	8	8
B – cellular adenoma	4	8	3	6	7	7
Embryonic - fetal adenoma	1	2	1	2	2	2

Source: compiled by the authors of this study

Table 4. Distribution by markers of proliferation activity

N - 30	Ki 67			p 53			Thyroglobulin		
	Up to 49%	50-70%	More than 71%	Up to 49%	50-70%	More than 71%	Up to 49%	50 – 70%	More than 70%
abs.	18	8	4	17	11	2	20	7	3
%	60	27	13	57	37	6	67	23	10

Source: compiled by the authors of this study

Table 5. Correlation of elastography indicators depending on proliferation markers

Indicator	stiffness coefficient		speed of shear wave	
	Correlation	Value	Correlation	Value
Ki 67	0.456**	0.03	0.399*	0.11
P 53	0.547**	0.00	0.553**	0.00
thyroglobulin	0.641**	0.00	0.620**	0.00

** correlation is significant only at 0.01.

* correlation is significant only at 0.05

Source: compiled by the authors of this study

elastography as intense blue staining, stiffness coefficient more than 4.0 kPa, speed of shear wave more than 4.5 m/s, allows identifying the most dubious nodes for FNA conducting, provides high accuracy when conducting cytological examination, decrease number of unfounded puncture biopsies.

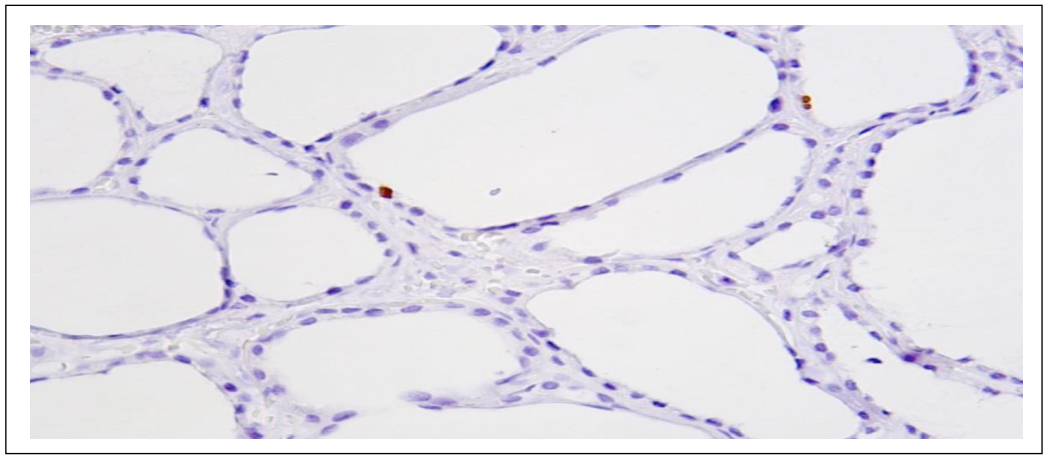


Fig. 5. Expression of Ki67 in macrofollicular goiter. Low proliferation index of follicular epithelium – up to 1% positive cells, Hematoxylin staining, magnification 50
Picture taken by the authors

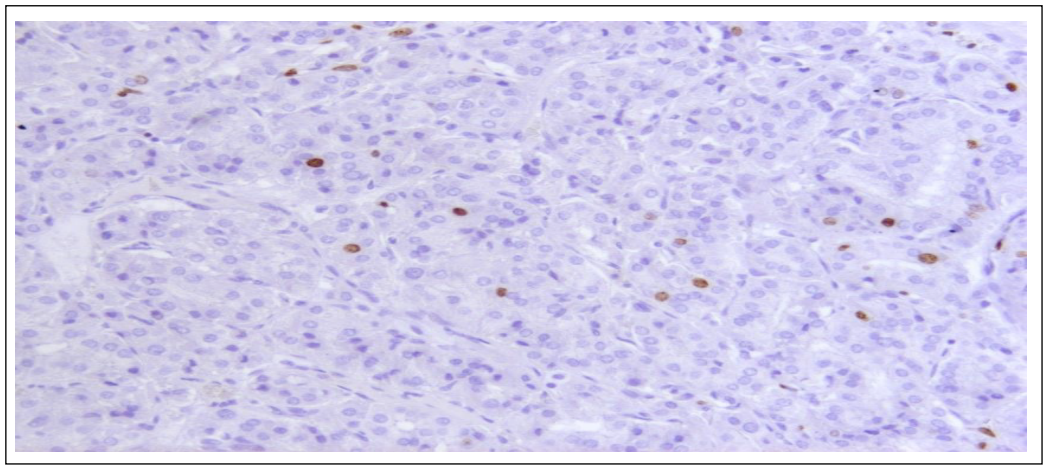


Fig. 6. Expression of Ki67 in microfollicular nodular goiter. Highest proliferation index of follicular epithelium – up to 5% positive cells, Hematoxylin staining, magnification 50
Picture taken by the authors

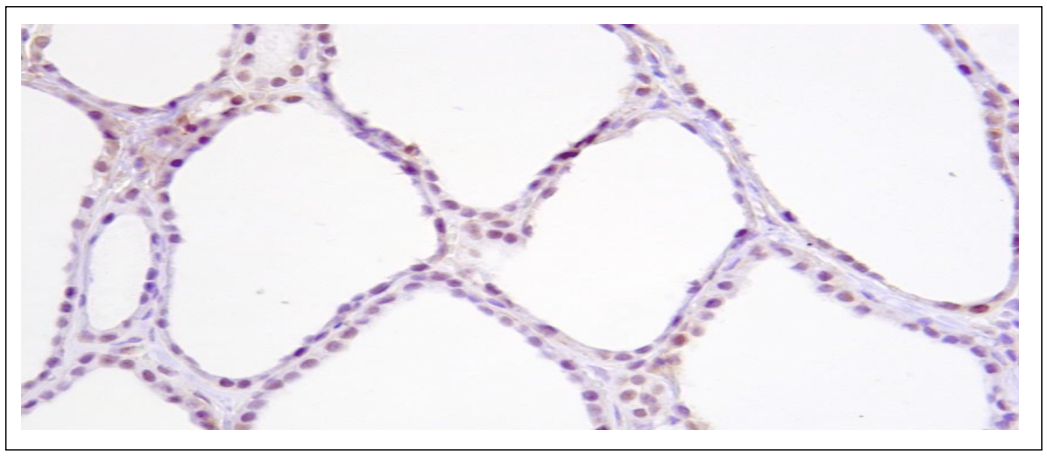


Fig. 7. Expression of p53 protein in normal thyroid gland tissue. Weak yellow-brown staining of nuclei on blue-violet background, p53 gene expression, Hematoxylin staining, magnification 100
Picture taken by the authors

IMMUNOHISTOCHEMICAL ANALYSIS IN NODULAR DISEASES OF THE THYROID GLAND

Histological studies were carried out taking into account the operating material of thyroid gland tissue. The most common is nodular colloidal goiter without signs of proliferation – in 39 patients; colloid-proliferating goiter - in 18; cystic nodular goiter - in 22; follicular adenoma – in 8; B – cellular adenoma – in 7; embryonic - fetal adenoma - in 2; follicular carcinoma – in 1; papillary carcinoma - in 3.

Distribution of histological changes in groups is represented in table 3.

In the main group nodular colloidal goiter without signs of proliferation was identified in 30%, in the comparison group in 48% of cases; colloidal proliferative goiter in the main group was identified in 24%, and in the comparison group in 12% of cases, cystic nodular goiter occurs in the main group - in 22% of cases, in the comparison group - in 22%. The most frequent is nodular colloidal goiter without signs of proliferation in 39% of cases, in equal quantities cystic nodular goiter can be seen – in 22 cases.

We have conducted research on the basis of immunohistochemical analysis of removed thyroid gland

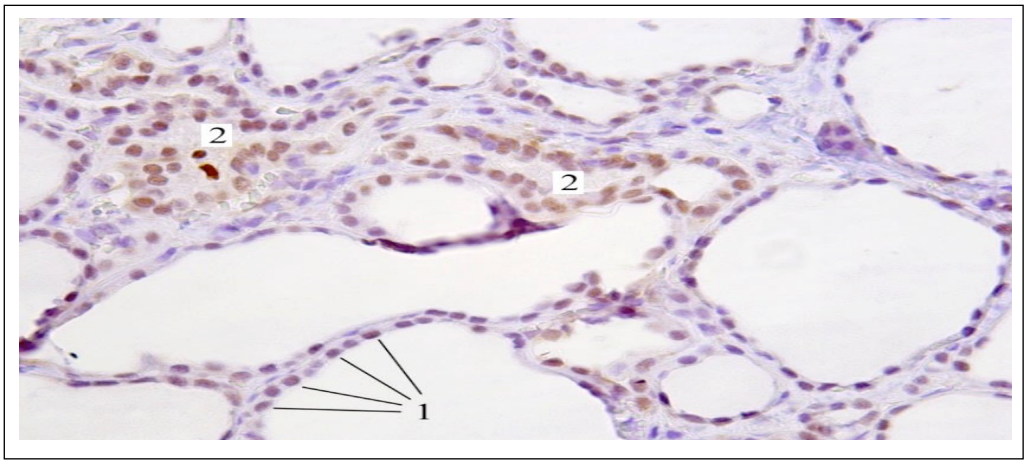


Fig. 8. p53 protein expression in micro-macrofollicular goiter. Weak yellow-brown staining of nuclei. 1, 2 arrows - p53 gene expression in large follicles. Magnification 100
Picture taken by the authors

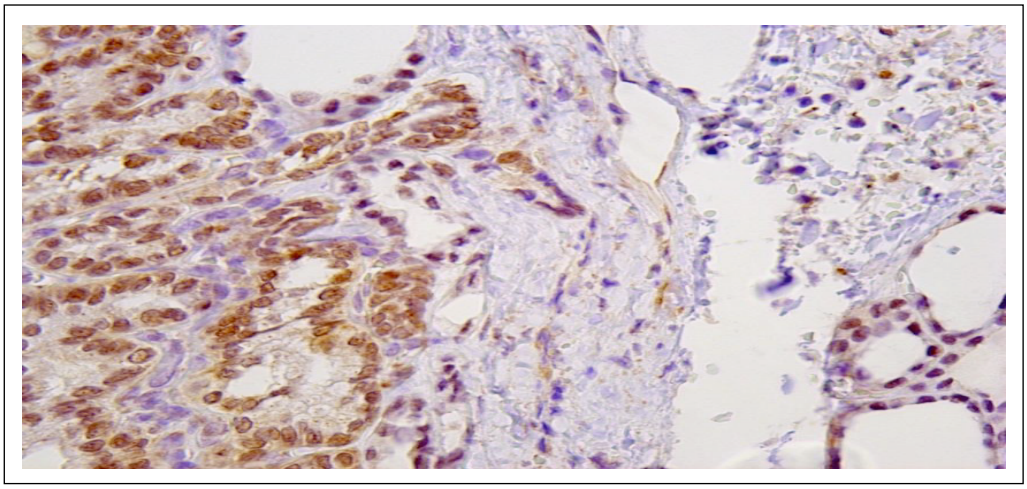


Fig. 9. Hyperexpression of the p53 mutant gene in the area of microfollicular epithelial proliferation with signs of severe dysplasia. Hematoxylin staining, magnification 100
Picture taken by the authors

tissue. We have studied proliferative activity of thyroid gland tissue based on determination of expression of Ki67 proteins (clone MIB-1), p53 (clone DO-7), thyroid transcription factor TTF-1 (clone 8G7G3/1), thyroglobulin (clone DAK-Tg6).

The analysis comprised 30 immunohistochemical studies of removed thyroid gland tissue preparations of patients. Marker of proliferative activity Ki67 is determined by brown staining of nuclei, and then the percentage of positive cells in the population was calculated. The results of immunohistochemical studies are presented in Fig. 5, Fig. 6, Fig. 7, Fig. 8, Fig. 9, Fig. 10, Fig. 11.

Thyroid transcription factor TTF-1 is determined by brown staining of cell nuclei. In all cases of goiter intensive expression of TTF-1 in follicular epithelium is observed.

On the basis of the obtained results 3 degrees of proliferation of nodular formations were revealed: I degree of thyroid gland tissue proliferation: Ki-67 proliferation index makes up to 49%; P53 protein expression made up to 49%; proliferation of follicular and parafollicular epithelium takes up to 49% of follicles.

II degree of proliferation is characterized by: Ki-67 proliferation index makes 50-70%; P53 protein expression makes 50-70%; proliferation of follicular and parafollicular epithelium takes 50 % of follicles;

III degree of thyroid gland tissue proliferation is characterized by: proliferation of follicular and parafollicular epithelium takes more than 70 % of follicles; Ki-67 proliferation index makes more than 70%; P53 protein expression – more than 705-80 %;

The obtained results are represented in table 4.

After conducted immunohistochemical analysis 3 groups of patients were identified according to the degree of proliferative activity of thyroid tissue. Presence of the 1 degree of proliferation was detected in 18 patients (60% cases), this degree is not a risk of disease recurrence and possible malignancy, accordingly, the performance of organ-preserving operations is justified. Second degree of proliferative activity was detected in 9 patients (30% cases), this state is border one, in this case there is high risk of disease recurrence. Under such proliferation it is advisable to perform radical operations. Presence of 3 degree of proliferation was detected in 3 patients (10% cases). Patients with third degree of proliferation have the highest risk of malignancy, therefore, it is more appropriate to perform surgical interventions according to oncological principles.

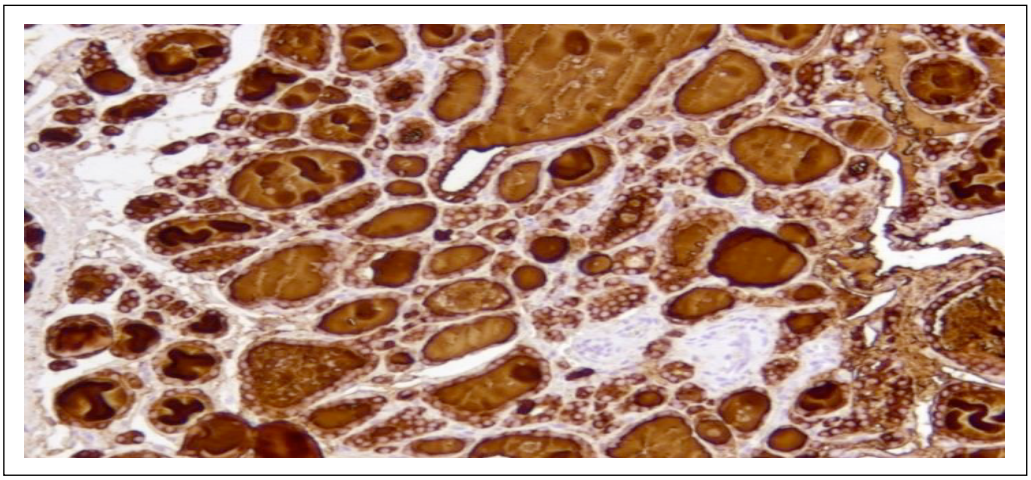


Fig. 10. Thyroglobulin in micromacrofollicular goiter. Intense reaction in the colloid of the follicles and in the cytoplasm of the follicular epithelium. Hematoxylin staining, magnification 100
Picture taken by the authors

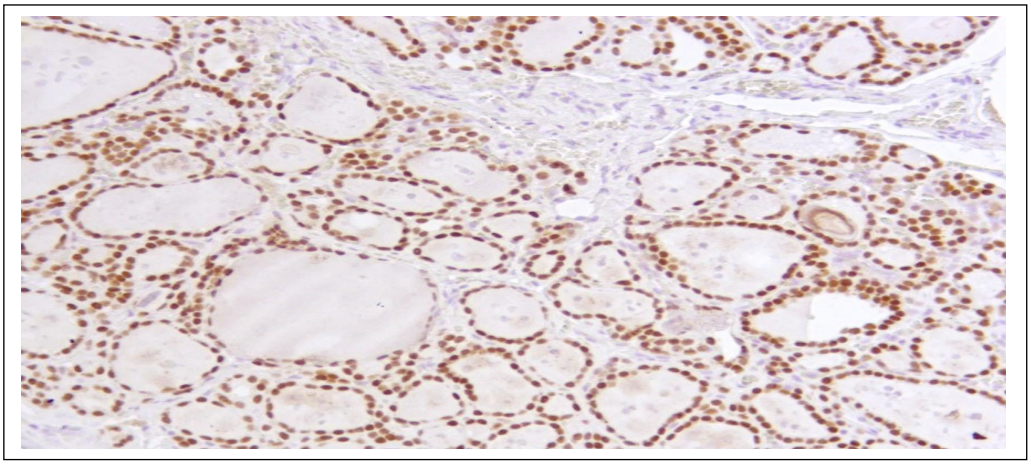


Fig. 11. Micromacrofollicular goiter. Intensive expression of thyroid transcription factor in in the nuclei of all cells of the follicular epithelium. Hematoxylin staining, magnification 100
Picture taken by the authors

We have calculated correlation coefficient of such characteristics of elastography indicators depending on proliferation markers as tissue stiffness coefficient and speed of shear wave depending on the degree of proliferation markers Ki 67, P 53, thyroglobulin. The obtained data is represented in table 5.

According to correlation coefficient relationship between stiffness coefficient, speed of shear wave and proliferation markers in all the cases is moderate and significant, apart from dependence of Ki 67 marker and speed of shear wave.

DISCUSSION

The vast majority of works in emergency thyroid surgery are devoted to the description of compression syndrome in patients with malignant tumors. Undifferentiated (anaplastic) thyroid cancer is the most aggressive human carcinoma, doubling in size 2 times in 117 hours. In the structure of malignant tumors of thyroid origin, it is on average 7-12% [8-10].

In a short period of time (1-3 months), the thyroid capsule sprouts and infiltrates the surrounding tissues and organs, causing compression and deviation of the larynx, trachea, esophagus, vascular-nerve bundle of the neck, and recurrent laryngeal nerves [11].

In most cases, the clinical picture and complaints of patients with undifferentiated thyroid cancer are caused by manifestations of the local spread of carcinoma, its invasion into surrounding structures and mechanical pressure on organs and tissues, which leads to the occurrence of a compression syndrome, the severity of which depends on the size of the tumor and the degree of cervicothoracic spread. The authors emphasize that 45% of their patients with undifferentiated thyroid cancer came to the clinic as an emergency due to deterioration of the condition caused by obstruction by carcinoma of the upper respiratory tract (trachea). Moreover, 5 out of 27 patients needed emergency tracheal intubation to facilitate breathing [12, 13].

According to the data of a number of authors, the progression of compression of the neck and mediastinum in the case of undifferentiated thyroid cancer led to the fact that 36 (36.4%) of 99 patients were operated on urgent indications in conditions of severe respiratory failure. In 21 (21.2%) patients, it was possible to carry out conservative measures, which included the correction of disturbed functions of breathing, blood circulation, digestion, nor-

malization of the acid-base state, and the operation was performed urgently 2-3 days after admission after relative stabilization of vital important functions [14, 15].

Locally spread differentiated carcinomas of the thyroid gland, sprouting into surrounding tissues and organs, such as the trachea, esophagus, recurrent laryngeal nerve, can lead to emergency conditions [16].

The reasons for the appearance of thyroid tumors at the border of the organ can be different. It is possible to formulate the following reasons for the local prevalence of thyroid tumors: late referral of patients; observation, diagnosis and treatment errors; peculiarities of the "biological behavior" of the tumor [16].

In some cases, the course of the tumor becomes aggressive after inadequate primary surgical interventions, which may be related to the phenomenon of non-differentiation of tumors [17].

Respiratory failure caused by compression of the trachea by benign thyroid diseases is usually caused by a giant goiter of the cervical or cervicosternal localization. Compression of the trachea caused by a giant benign goiter is rare. Nevertheless, the cervical-thoracic location of the thyroid gland can lead to progressive respiratory failure up to respiratory arrest [18].

Benign thyroid diseases of cervicosternal localization, chronic obstructive diseases of the respiratory tract, and a long history of thyroid disease are considered risk factors for acute respiratory failure, and therefore, life-threatening conditions [18].

The proliferative activity of a neoplasm is one of the most important characteristics of its phenotype, which determines the growth rate of the neoplasm, its ability to metastasize, and the effectiveness of therapeutic measures. The proliferative activity index as a quantitative criterion is quite actively used in assessing the aggression of the malignant process. Many studies demonstrate a high level of expression in malignant pathology of the thyroid gland, in particular in papillary thyroid cancer, as well as as a prognostically unfavorable factor in the differential diagnosis of follicular adenomas and follicular thyroid cancer [19].

The pronounced expression of Ki-67 in follicular thyroid cancer, according to the results of our studies, is consistent with the generally accepted hypothesis. The closeness of the proliferation marker Ki-67, follicular adenomas and follicular thyroid cancer of fetal-embryonic structure in terms of indicators is noteworthy, which probably indicates the highest potential of the latter for malignancy and subsequent metastasis. The low level of Ki-67 expression indicates the relative efficiency of cell cycle regulation, which is characteristic of unchanged thyroid tissue, which, according to our data, normofolliary adenomas demonstrate. According to numerous studies, increased expression of p53 has been found in many cancer cell lines. In normal tissues, the level of p53 remains low, but in response to DNA damage, p53 is activated with a large accumulation of its level. However, there is evidence that does not support differences in p53 expression in patients with follicular adenoma and follicular carcinoma, or conversely, shows a decrease in expression in differentiated forms of thyroid cancer [19, 20]. Mutations of this gene in patients with benign and autoimmune thyroid diseases are also poorly studied [20].

CONCLUSIONS

The conducted immunohistochemical studies have proved presence of three degrees of proliferative activity of thyroid gland; correlation dependence has been identified between characteristics of elastography and proliferative activity markers. Upon determination of elastogram type and degree of proliferative activity of thyroid gland depends further tactics of patient's management: referral for dynamic observation or surgical treatment with the selection of the scope of surgical intervention, including minimally invasive one.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

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CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Pathogenetic aspects of the features of androgen deficiency state under the condition of experimental varicocele

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ABSTRACT

Aim: The aim of this article is to study of the main pathogenetic aspects of the features of androgen deficiency state under the condition of experimental varicocele caused by serotonin hydrochloride.

Materials and Methods: Simulation of serotonin testicular damage (experimental varicocele) is used to reproduce gonadal pathology. In the mechanism of the development of this gonadopathy was the vasoconstrictor effect of serotonin hydrochloride plays a leading role. Serotonin hydrochloride (Alfa Aesar®, USA) was administered for 14 days at a dose of 5 mg/kg of body weight ("pathology" group).

Results: Experimental varicocele led to disturbance of structural elements of connective tissue (and the content of connective tissue components of glycosaminoglycans in the testes and chondroitin sulfates in the seminal plasma) as a leading link in the pathogenetic chain which promotes the formation of hormone dependent reproductive diseasess.

Conclusions: Experimental varicocele on the background of the androgen deficiency state led to the disturbance of the morphofunctional state of the mitochondria of testicular cells, damage to the nuclear membrane, a decrease in the content of testosterone in the blood and fructose in the seminal vesicles; a decrease in the concentration and motility of spermatozoa.

KEY WORDS: morphology, connective tissue, varicocele, spermatogenesis, reproductive system

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INTRODUCTION

Ensuring adequate demographic condition is the main task of creating resource potential and the health of the nation as a whole. It is known that demographic policy is focused on the effective regulation processes of the population reproduction . The majority of factors determining depopulation depend on the decline in the birth rate. This is due not only to social and economic conditions, but also to the deterioration of the reproductive health of the population of Ukraine and high rates of reproductive losses, especially during the war years [1]. One of the leading conditions characterizing the deterioration of reproductive health is infertility or hypofertility.

Thus, male infertility is a global medical, socio-economic, demographic, medical-biological and individual marital problem throughout the world and in Ukraine, as well as a problem that requires further study of the etiology and pathogenesis to increase awareness of hypofertility and search for new approaches, methods and mechanisms for the treatment of this common pathological condition of the male body [1, 2].

Among the numerous factors of male fertility disoders the leading place is occupied by the spread of chronic somatic diseases (primarily those accompanied by ischemia of the reproductive system, in particular, varicocele) [1, 2]; an increase in the number of cases of chronic inflammatory diseases of the genital system in man of reproductive age [3]. As already noted, with varicocele blood stasis occurs with the development of hypoxia of the germinal epithelium and a violation of the androgen-estrogen balance, which is the cause of a decrease in the fertilizing ability of sperm [4]. It is well known connective tissue takes an active part in the processes of inflammation, apoptosis, destruction of organs and systems [5, 6]. As for its participation in

the mechanisms of development of pathology of the male reproductive system has not been sufficiently studied to date. It is believed that changes and disturbances in the structure of connective tissue can lead to testicular dysfunctions, including idiopathic male infertility. These configurations are characterized by metabolic disturbances, in particular of collagen and proteoglycans. [7]. Transformation of the connective tissue structures of the venous wall and the uncompensated state of the valve apparatus of the testes cause varicocele [4, 8]. It is well known that changes in blood flow, nutrients and oxygen supply, as well as an increase in local temperature, can adversely affect the functioning of Leydig cells and spermatogenesis, which is a background for male hypofertility or infertility [9]. It is also well known that the detailed etiology and pathogenesis of varicocele-mediated infertility are not fully understood. But heat stress, toxin impact and blood stasis, hormonal imbalance, oxidative stress, and testicular hypoperfusion have been discussed as potential mechanisms. However, none of these mechanisms alone can accurately explain the impairment effects of varicocele on testicular function [8].

AIM

The aim of this article is to study of the main pathogenetic aspects of the features of androgen deficiency state under the condition of experimental varicocele caused by serotonin hydrochloride.

MATERIALS AND METHODS

The studies were carried out in compliance with the rules of the European Convention on the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Strasbourg, 1986) [10, 11]. During the experiment, the animals were in the vivarium at t0 18–240C, humidity 50-60 %, natural day-night light mode, on a balanced diet according to the current norms. The experimental investigations were carried on white mature male Wistar rats with basal weight of 340–380 g.

According to the aim and objectives we simulated the androgen deficiency state in male rats taking into account the verified etiopathogenetic role of effects of damaging factors of serotonin hydrochloride in particular.

Simulation of serotonin testicular damage (experimental varicocele) is used to reproduce gonadal pathology. In the mechanism of the development of this gonadopathy was the vasoconstrictor effect of serotonin hydrochloride plays a leading role. As result of the vasoconstriction it is trophic disorders and pathological changes occur, which are usually observed in patients with hypofertility and can occur in case of varicocele, injuries, conditions caused by hypodynamia, etc. [10].

Serotonin hydrochloride (Alfa Aesar®, USA) was administered for 14 days at a dose of 5 mg/kg of body weight *("pathology" group)*. The drug at this dose destroys the metabolism of supporting epitheliocytes (Sertoli cells) and interstitial endocrinocytes of rat testes (Leydig cells), which produce testosterone (Butenko I.V., Laryanovskaya Yu.B., 2010) [10]. To achieve the goal of studying the main pathogenetic features and mechanisms of experimental varicocele caused by serotonin hydrochloride, a series of studies was conducted. Carry out of the mass of androgen-dependent organs as markers of reproductive system function. The levels of intratesticular testosterone in testis homogenates (A. G. Reznikov, 1986) and testosterone in the blood serum was determined by the enzyme immunoassay method using standard kits. The content of fructose in the seminal vesicles (by Bokunyaeva N.I., 1975) , the activity of acid phosphatase (prostatic) in blood serum was determined by the kinetic method (with naphthyl phosphate as a substrate) using a standard kit. After were evaluated features of the morphofunctional state according to the main indicators: the number of spermatozoa (sperm concentration), the percentage of pathological forms, motility and the duration of preservation of their motility according to the generally accepted method (Stefanov O.V., 2001) [10].

A biochemical study of the content of chondroitin sulfate (component of connective tissue) in the spermatoplasm of rats was also carried out according to the Nemeth-Csoka method modified by L.I. Slutsky (Morozenko D.V., Levchenko V.I., Timoshenko O.P., 2012). Morphological examination of spieciments was carried out due to standart method by Merkulov H.P. [10]

Electron microscopic examinations were carried out according to standard methods (Boiko V.V., Prasol V.A., Nevzorova O.F., Nevzorov V.P. et al., 2013). Structural parameters of mitochondria: the number of morphologically normal and altered mitochondria (destruction of the outer membranes, matrix homogenization and edema, lysis of cristae) were determined and counted using an EMV-100BR electron microscope (expressed as a percentage of 100%) (Rozovaya E.V. et al., 2015) [10].

Detection of glycosaminoglycans (GAGs) on sections was shown as a metachromasia reaction by staining with toluidine blue at pH 2.5 (Kern M., Modish L., Dedukh N. V. et al., 1985). The degree of color saturation was assessed according to the principle of counting cells in histological samples, using a semiquantitative method of visual assessment of the intensity of coloration of

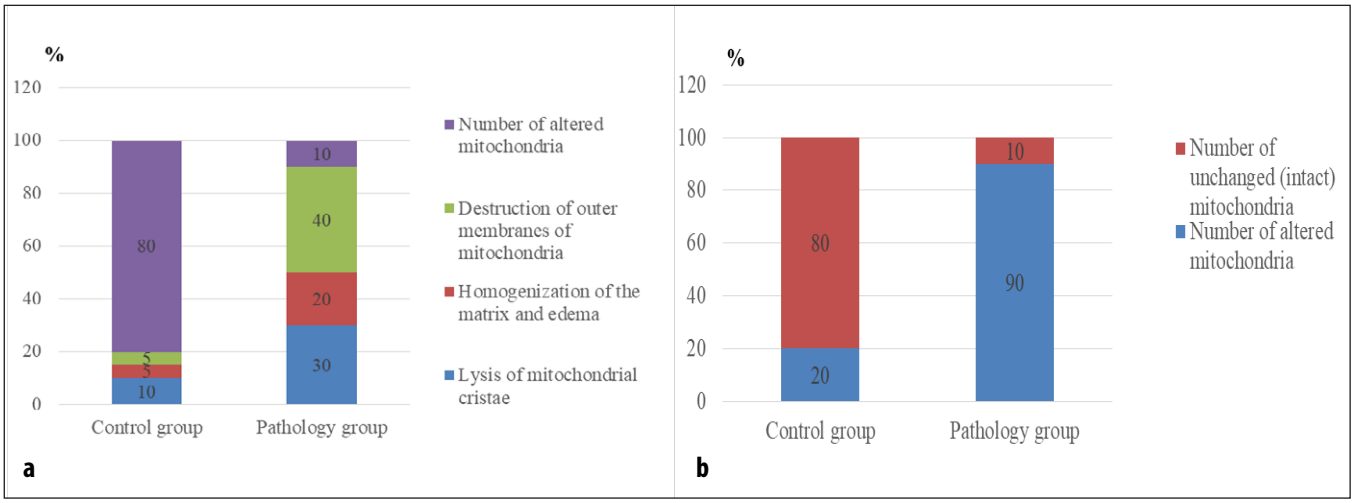


Fig. 1. Frequency of mitochondria structural changes of Leydig cells under the conditions of experimental varicocele
a – structural changes of mitochondria (in %% of the total number of morphologically changed mitochondria, taken as 100%). b – violation of the morphofunctional state of mitochondria.
Picture taken by the authors

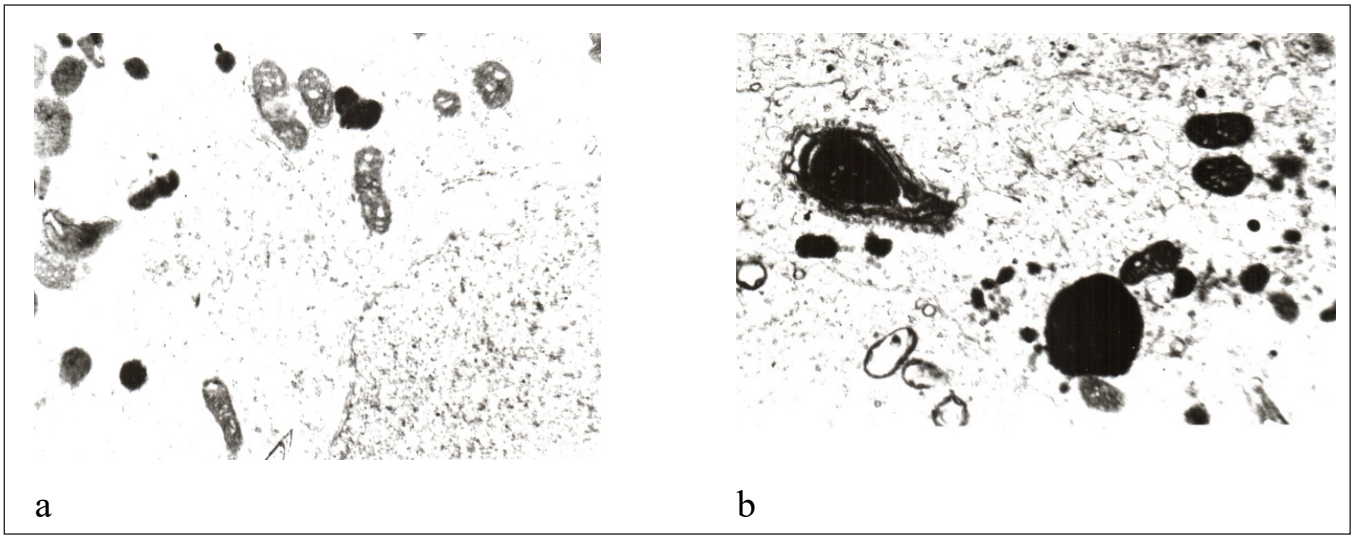


Fig. 2. Ultrastructure of testicles cells of the "pathology" group:
a – Sertoli cells. Lysis of the nuclear membrane. Decondensed chromatin. x32000
b – Leydig cells. Homogenization of the mitochondrial matrix. x35000. Contrasted by lead citrate

microstructures by histochemical reactions (Sokolovsky V.V., 1971). Quantitative assessment of the intensity of staining was expressed in point (+4) [10].

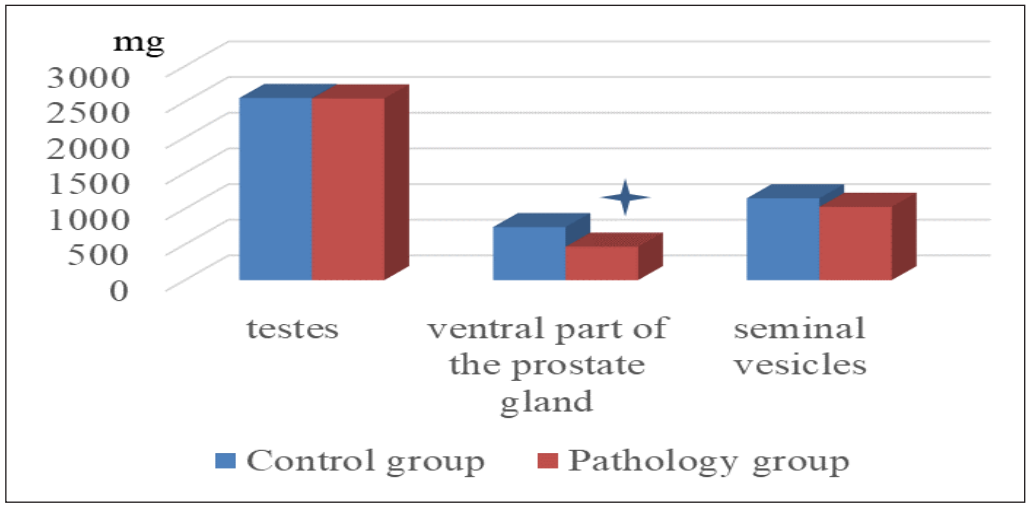
Statistical processing of the obtained results was performed using parametric and non-parametric methods. As a result of the normal distribution, the arithmetic mean and its statistical error were calculated $(\bar{x} \pm s_{\bar{x}})$; if the data were not consistent with the nor mal distribution, the median (Me), minimum and maximum data (min ÷ max) were calculated. Newman-Keuls method, nonparametric Wilcoxon-Mann-Whitney U-test and Kruskal Wallis H-test were used to analyze differences. Critical level of significance was assumed to be equal to or less than 0,05 ($p \leq 0,05$).

FRAMEWORK

The research was carried out as part of investigation works at the SI «V. Danilevsky Institute for Endocrine Pathology Problems of NAMS of Ukraine»: 1) «Mechanisms of development, diagnosis and therapy of infertility in individuals with gonadal dysfunction and comorbid pathology» (state registration number 0116U007259);

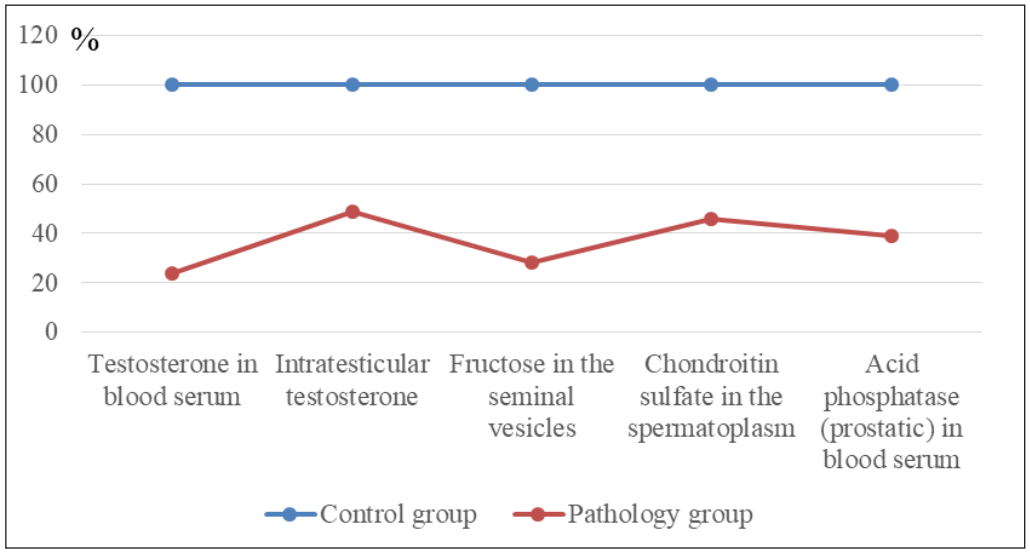
2) "Determination of the role of vitamin D deficiency and insufficiency in the development of gonadal dysfunction, substantiation of approaches to their therapy" (state registration number 0119U102387);

3) Determination of the role and deficiency of vitamin D in the development of the sexual glands dysfunctions,



Picture taken by the authors

Fig. 3. Mass of androgen-dependent organs as a markers of reproductive system dysfunction
– statistically significant differences compared to the data for the "Control" group, $p \leq 0.05$



Picture taken by the authors

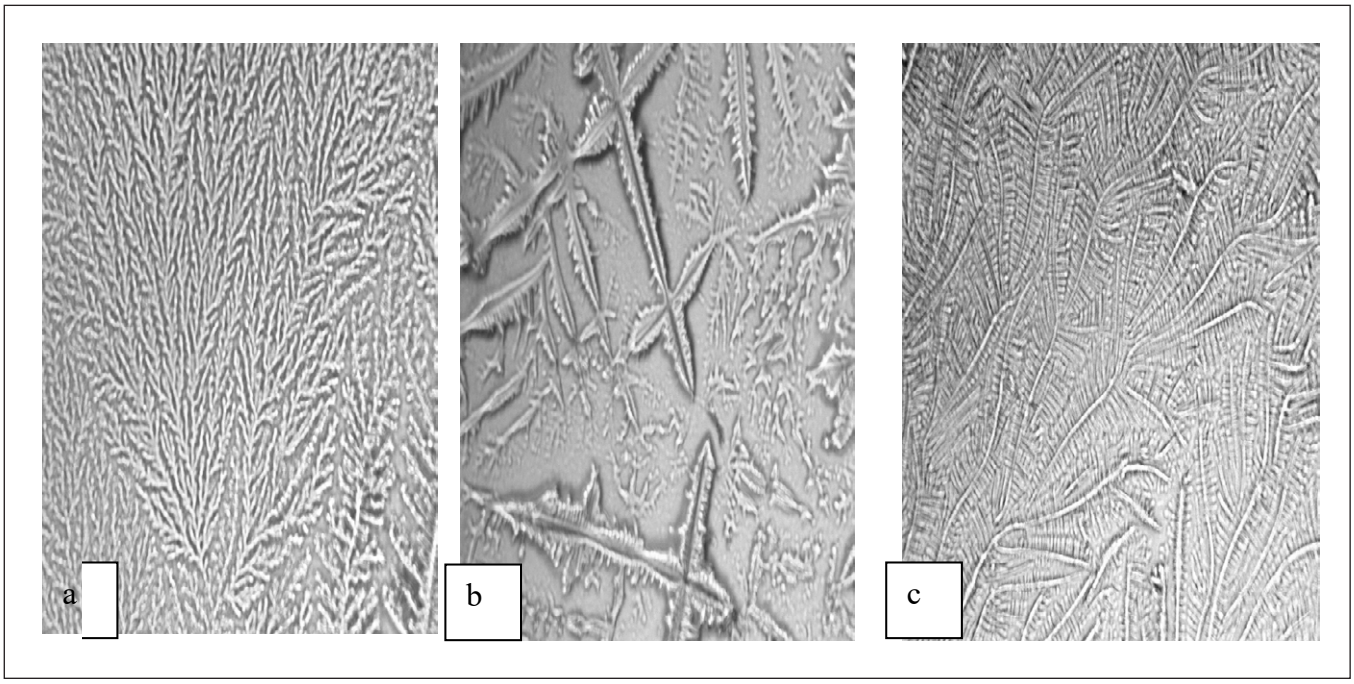
Fig. 4. Indicators of androgen saturation and the content of chondroitin sulfates in the spermatoplasm of male rats under the conditions of experimental varicocele

definition to ways to their therapies (state registration number 0119U 102387).

RESULTS

The main feature of pathogy of gonad (experimental varicocele) was destructive changes in the organelles of testicular cells. It has been seen the percentage of morphofunctional changes of mitochondria under the simulation of serotonin testicular damage. Thus, a significant number of Leydig and Sertoli cells were found to be foci of cytoplasmic necrosis. The mitochondria of the testis cells had destroyed outer membranes, homogenization of the matrix, edema, and foci of lysis of the cristae, increased a number of altered mitochondria and the cisternae of the endoplasmic reticulum were greatly expanded. A significant increase in the percentage of morphologically altered mitochondria (90 %) was observed. Only 10 % had an unchanged structure (Fig. 1). The cytoplasmic membrane of endotheliocytes had small foci of destruction. The cytoplasm contains a small amount of ribosomes and polysomes. Such changes indicate a slowdown in the recovery of structures against the background of gonadopathy.

nder the simulation of serotonin testicular damage (experimental varicocele) destruction of the nuclear membrane (lysis of the nuclear membrane) of Leydig cells was noted, which can lead to DNA destruction and, as a result, inhibition of transcription and translation processes (Fig. 2). The accumulation of mitochondria and the location of small and large vacuoles, as well as myelin-like structures near the membranes, indicate a violation of synthetic processes in the cell. These factors also caused a violation of the process of spermatogenesis, which is confirmed by anomalies in the ultrastructure of the flagellum, which indicates a violation of the motor apparatus of spermatozoa. Ultramicroscopic examination of testis specimens revealed foci of connective tissue degeneration also.



Picture taken by the authors

Fig. 5. Types of crystallization of the prostate gland secretion of rats under the conditions of experimental varicocele:
a - type of crystallization of the secretion of the prostate gland of an intact rat (typical phenomenon of «fern leaves», androgen saturation of the body is normal);
b – single atypical crystals on the background of an amorphous mass;
c – divergence of transverse branching of branches from the main stem at a much greater angle, reduction in size, reduction in the number of side branches.
Androgenic saturation of the body is reduced. Photomicrograph: imprint of the secret. x200

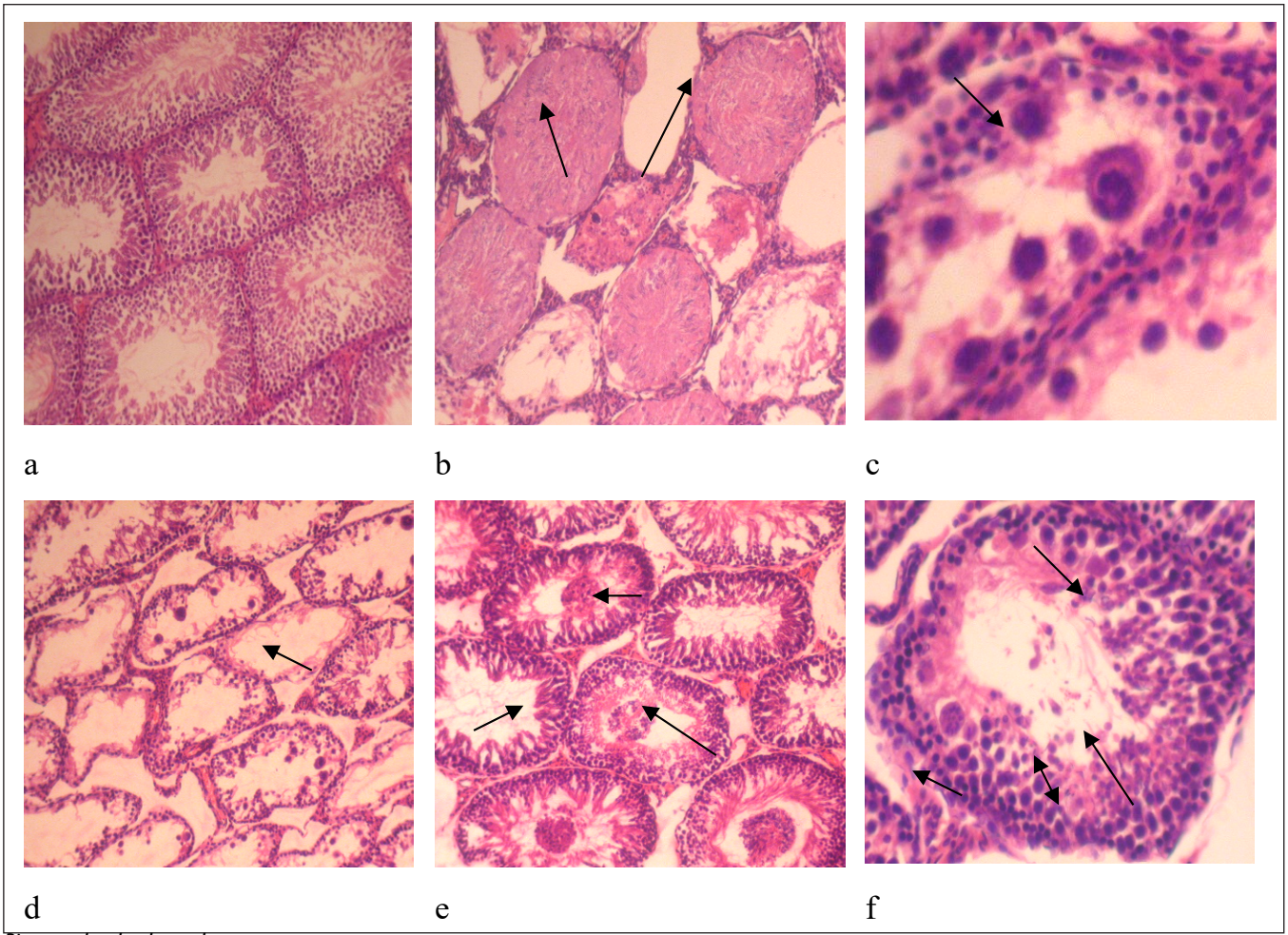
Table 1. Indicators of the spermogram, morphometry of spermatogenesis and the type of crystallizing prostate secretion under the under conditions of experimental varicocele (n=8), $(\bar{x} \pm S_x)$; Me (Q25; Q75)

Indices	"Control" group, n=8	"Simulation of serotonin testicular damage" group ("Pathology" group) n=8
The number of spermatozoa, million/ml	52.42±3.93	25.85±2.42*
Pathological forms, %	21.86±1.97	35.14±4.54*
Sperm motility, %	81.86±3.48	39.57±8.17*
Sperm motility preservation time, min	187.86±12.10	115.71±6.81*
The number of spermatogonia in the seminiferous tubules, pcs	57.12±1.05	13.21±2.03*
The number of seminiferous tubules with the 12th stage of meiosis, %	5.0 (4; 6)	1.0 (0; 7)*
Index of spermatogenesis, points	3.36 (3.28; 3.44)	0.99* (0.36; 2.76)
The type of crystallization of prostate secretion, points,	3.58 (3.2; 3.83)	2.04 (1.33; 2.36)*

notes: * - statistically significant differences compared to the Control group, $p \leq 0.05$
Source: compiled by the authors of this study

Simulation of serotonin testicular damage as well was accompanied by changes in the mass of androgen-dependent organs as markers of reproductive system dysfunction, a decrease in the mass of the ventral part of the prostate gland by 36% was observed compared to the "Control" group, which may be caused by a violation of the morphostructure of the gland as a result of the action of serotonin hydrochloride (Fig. 3).

Experimental varicocele demonstrated the development of androgen deficiency: its formation indicates a



Picture taken by the authors

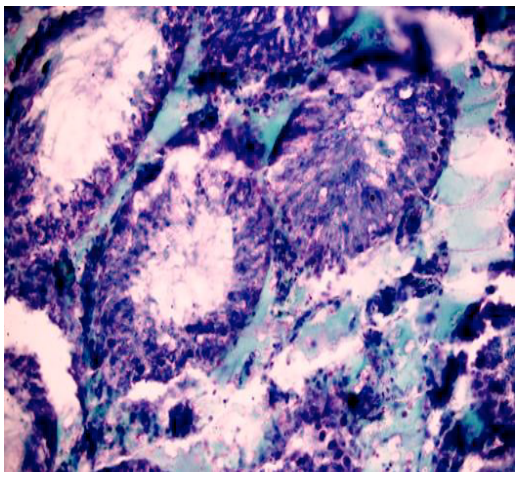
Fig. 6. Testicles of experimental animals (a – "Control" rats; b-e – «Pathology» group (rats with experimental varicocele):
a – normal size of seminiferous tubules, germ cells are presented in full content. x100;
b – necrotic detritus in seminiferous tubules. x100;
c –multinucleated giant cells in the seminiferous tubules. x250;
d – emptying of the seminiferous tubules, reduction in their size, expansion of the intertubular space. x100;
e – desquamation of germ cells in seminiferous tubules (arrow). x100;
f – section of seminiferous tubule wall exposed (arrow), sphere cells (double arrow). x250. Staining with hematoxylin-eosin

decrease in intratesticular testosterone in testicles by 2 times (14.20 (11.60; 17.10) and 28.80 (24.50; 31.30); ($p<0.05$) and general testosterone in blood serum by 4 times (3.84±0.98 and 16.04±0.82; $p<0.05$) in male rats, which leads to decrease in the mass of androgen-dependent organ (ventral part of the prostate gland), disrupting its functioning, as evidenced by a decrease activity of acid phosphatase (prostatic) in blood serum by 2.5 times (8,84±0,70 and 22,46±0,97; $p<0.05$) and content fructose seminal vesicles by 3.6 times (0,98±0,05 and 3,48±0,08; $p<0.05$) and then spermogram parameters (Fig. 4).

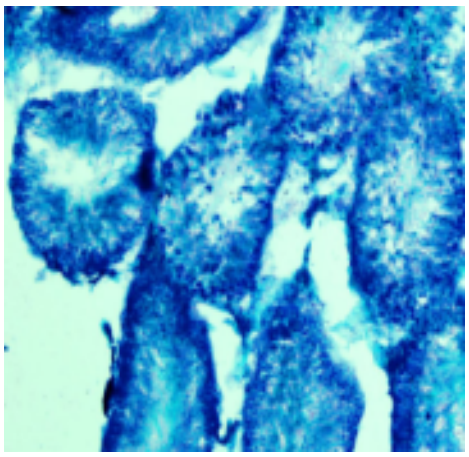
Androgen saturation of the organism which was determined by the type of crystallization of the secretion of the prostate gland of animals, significantly decreased and amounted to 2,04 points ($p<0.05$) (Fig. 5, Table 1).

Against the background of a testosterone decrease impact of serotonin hydrochloride led to damage of spermatogenesis, there was a significant decrease in the number of spermatozoa almost by 2 times (25,85±2,42 and 52,42±3,93; $p<0.05$), their motility and sperm motility preservation time. It has been increased pathologically altered forms of spermatozoa (21,86±1,97 and 35,14±4,54; $p<0.05$) in comparison with "Control" group (Table 1).

According to the obtained data the presence of probable changes in all of the above parameters of the semen analysis which affected the morphometric characteristics of spermatogenesis in the seminiferous tubules, namely, the number of spermatogonia in the seminiferous tubules decreased sharply) and respectively it has been decreased the number of spermato-



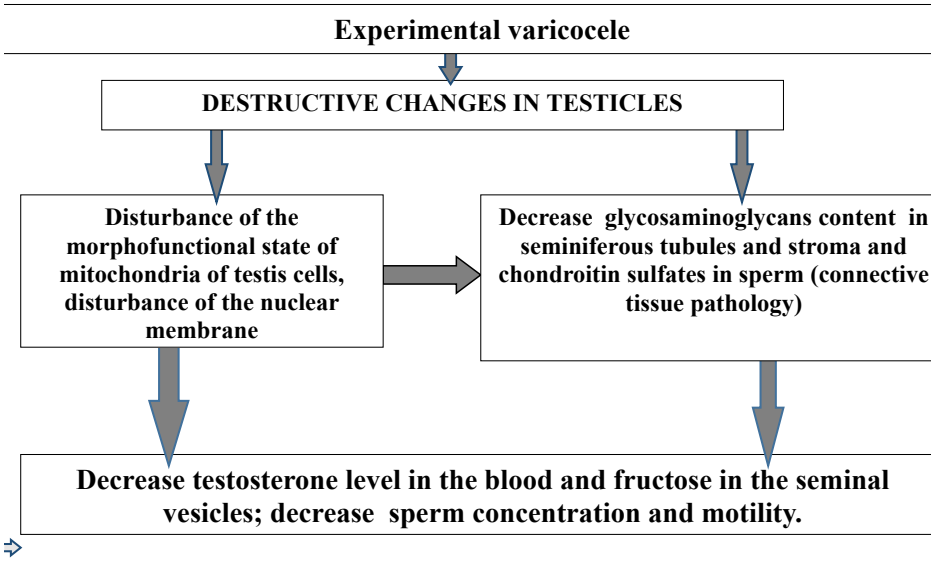
A."Control" group (×250)



B."Pathology" group (×250)

Picture taken by the authors

Fig. 7. Histological preparations of rat testicles. Staining with toluidine blue



Picture taken by the authors

Fig. 8. Probable diagram of the pathogenetic mechanism of experimental varicocele
Picture taken by the authors

cytes of the second order which entered the metaphase of the second division of maturation (stage 12 of meiosis) - 5 times (5,0 (4; 6) and 1,0 (0; 7) in comparison with "Control" group ($p<0.05$).

All these processes led to a 3-fold drop in index of spermatogenesis (3,36 (3,28; 3,44) and 0,99 (0,36; 2,76) (Table 1). Simulation of serotonin testicular damage confirms the violation of the excretory function of the testes and indicates the development of experimental gonadopathy [9].

It should be noted in animals from the "pathology group" a decrease in total gonadotropic activity by 32% was observed, but this was not reflected in the mass of the testis and pituitary glands.

Therefore, serotonin testicular damage (experimental varicocele) leads to central dysregulation of sexual function.

The histological picture of the testes of experimental groups of rats has been shown at Fig. 6.

Morphological changes of a typical nature were found in the testicles of male rats under the influence of serotonin hydrochloride («Pathology» group). In animals, the vast majority of seminiferous tubules are reduced in size, completely emptied or contain several damaged cells, some of the tubules are filled with necrotic detritus.

Supporting cells are often "bloated", the nucleolus is sharply ectopic or absent. Disorganization of the spermatogenic epithelium was observed in a limited part of the tubules. Spermatids are dystrophically changed, small vacuoles are visible in the nucleus, the shape of the cells is changed. Often, against the background of devastation, spherical cells are visible - giant multinucleated spermatids (containing 3-10 nuclei), formed when their division is disrupted.

The shell of the seminiferous tubules is sclerosed. Interchannel spaces are increased. Interstitial cells (Leydig cells) were characterized by expressive monomorphism in the size

of the nuclei (mostly small), few, in some of them the nuclei were pyknotic. It is possible that this state of interstitial cells is a morphological reflection of a certain decrease of the steroid-producing function of these structures (Fig. 6, b-f).

Under the conditions of simulation of serotonin testicular damage, the content of connective tissue components - chondroitin sulfates in the seminal plasma of rats was reduced by 2,2 times 13,10 (12,40; 13,90) and 28,40 (26,80; 29,30) ($p<0,05$) compared to the "Control" group (Fig. 4).

The results of the study of the content of glycosaminoglycans (GAGs) in testicular samples and seminal plasma under the conditions of serotonin testicular damage are presented in Fig. 7. In the gonads of rats of the "Control" group, when stained with toluidine blue in the seminiferous tubules, purple coloration was detected in the band of the spermatogenic epithelium (see Fig. 7).

Small zones of metachromasia (the same purple color) were also visualized in the intertubular stroma, which indicates the presence of GAG. The degree of color saturation was 4.00±0.53 (4.50; 4.00) points.

During reconstruction of experimental varicocele in the seminiferous tubules and intertubular stroma, an orthochromatic reaction (almost complete absence of purple coloration) was observed when stained with toluidine blue, which indicates a deficiency of GAG compared to the "Control" group (see Fig. 6). The degree of color saturation was 0.00±0.20 (0.00; 0.08) points ($p<0,05$). The obtained results give reason to believe that androgen deficiency caused by serotonin hydrochloride leads to a decrease in the level of GAG in the sperm of rats, which can be a marker of the development of infertility in males.

Such a histochemical pattern in group of "simulation of serotonin testicular damage" can explain the fact that the testosterone level decreases in animals of this group, because the decrease in the amount of loose connective tissue that nourished the Leydig cells led, most likely, to a decrease in the level of their functional activity. Violation of the functional state of the testes under the conditions of "simulation of serotonin testicular damage" was also described by other authors [12].

DISCUSSION

Summing up our long-term research we can conclude that spermatogenesis changes of the "pathology" group (experimental varicocele) associated with change in the morphological structure of the testes, as a result of the effect of vasoconstriction by impact of serotonin hydrochloride, which leads to hypoxia, trophic disorders and pathological conditions that occur in male under the conditions of varicocele, injuries, dysfunctions caused by hypodynamia and lead to hypofertility [12], as well as a decrease in testosterone secretion.

There are data, although sometimes contradictory, about the contribution of connective tissue to the development of many pathological processes in the male reproductive system, disorders of testosterone secretion and spermatogenesis [13]. Thus, sex hormones affect the processes of connective tissue remodeling: estrogens increase the intracellular water content, testosterone causes proliferation of fibroblasts and the synthesis of connective tissue components [10]. Considering that in all types of male infertility there is androgen deficiency of varying degrees of severity [8], a decrease in testosterone level can negatively affect the state of connective tissue. However, in andrological and obstetric-gynecological practice attention is paid to disorders of metabolism connective tissue and its effect on reproductive potential [14].

It has been proven that a decrease in the part of chondroitin sulfate and an increase in the part of keratan sulfates in the composition of glycosaminoglycans of the reproductive system can be used for ecological assessment of areas with probable contamination by toxic pollutants, where an increase in the number of infertile men is observed. The study of the spectrum of proteoglycans in blood serum and seminal plasma can be used as a criterion for predicting fertility (Vetoshkin R.V., Nikolaev A.A., 2015) [7].

Summarizing the data on experimental modeling (simulation) animals pathology of gonad, we can confirm that a general distinguishing feature for experimental varicocele is a disturbance of the morpho-functional state of mitochondria, destruction of the nuclear membrane of Sertoli and Leydig cells, a disturbance of the endocrine and spermatogenic functions of the testes against the background of a decrease in the content of GAGs in the testes and chondroitin sulfates in the spermatozoa. Consequently, we observe the formation of the so-called the "perverse circle": violation of testosterone secretion and pathology of reproductive system → changes in the structure of connective tissue → violation of testosterone secretion and pathology of reproductive system → deepening of violations in the structure of connective tissue. Therefore, the pathogenetic mechanism of experimental varicocele development can be presented schematically (Fig. 8).

CONCLUSIONS

1. Experimental varicocele led to disturbance of structural elements of connective tissue (and the content of connective tissue components of glycosaminoglycans in the testicles and chondroitin sulfates in the seminal plasma) as a leading link in the pathogenetic

chain which promotes the formation of hormone dependent reproductive diseasess.
2. Experimental varicocele on the background of the androgen deficiency state, there is a disturbance of the morphofunctional state of the mitochondria of testicular cells, damage to the nuclear membrane, a decrease in the content of testosterone in the blood and fructose in the seminal vesicles; a decrease in the concentration and motility of spermatozoa.
3. The conducted studies show that under the conditions of experimental varicocele in sexually mature male rats there is a significant impairment of the functional state of the testicles against the background of a decrease in the gonadotropic activity of the pituitary gland.
4. Experimental varicocele associated with violation of the morphofunctional state of the mitochondria of Sertoli and Leydig cells and damage to the nuclear membrane.
5. Pathological changes in the testicular tissue on the background of experimental varicocele are accompanied by suppression of the spermatogenesis process: decrease the number of stem cells (spermatogonia), a delay of the differentiation of germ cells (spermatids → spermatozoa), as a result of these processes spermatogenesis index decreases.
6. Under the condition of experimental varicocele the level of androgen saturation of the body also decreases, there is obviously related to changes in the synthesis of steroidogenesis in glandulocytes.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

The potential effects of cinnamon, cumin, and Rosuvastatin on lipid profile among Iraqi patients with dyslipidemia

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ABSTRACT

Aim: To investigate effects of three spices on lipid profile and compare between them in patients with dyslipidaemia.

Materials and Methods: This is randomized prospective clinical trials study; it is randomized prospective clinical trials study. Study includes patients that admitted to hospital and diagnosed with dyslipidaemia and have high levels of lipid profile, while patients with normal level of lipid profile and patients who discharged from hospital without complete their tests are excluded. The sample includes 30 patients who admitted to hospital with dyslipidaemia disease.

Results: Highest readings are of Rosuvastatin that effected on triglyceride with 53.1, cholesterol with 75.4, high density lipoprotein with 11.8 and low density lipoprotein with 65.5 were noticed, second highest readings are of cumin that effected on triglyceride with 31.6, cholesterol with 47.5, high density lipoprotein with 9.3 and the lowest reading is low density lipoprotein with 32 were noticed, lowest readings are of cinnamon that effected on triglyceride with 25.7, cholesterol with 44.8, high density lipoprotein with 8.4 and the second highest reading is low density lipoprotein with 43.9 were noticed on dyslipidaemia patients living in Al-Najaf City.

Conclusions: The study provides evidence that indicates the cumin, cinnamon both have antihyperlipidemic effect but less than Rosuvastatin in dyslipidaemia patients. It can be used as an adjuvant treatment with other drugs to decrease triglyceride, cholesterol, low density lipoprotein and increase high density lipoprotein levels.

KEY WORDS: cumin, cinnamon, rosuvastatin, dyslipidaemia, triglyceride

Wiad Lek. 2025;78(10):2008-2018. doi: 10.36740/WLek/210069 DOI

ABBREVIATIONS

HDL - High Density Lipoprotein
TG - Triglyceride
LDL - Low Density Lipoprotein
RCTs - Randomized Controlled Trials
SEM - Standard Error Mean
TC - Total Cholesterol
LDL-C - Low Density Lipoprotein Cholesterol
HDL-C - High Density Lipoprotein Cholesterol
CHOL - Cholesterol

INTRODUCTION

Dyslipidaemia is one of the important of cardiovascular diseases risk factors. It is rising in developed and developing countries [1-3]. Nowadays, many pharmaceutical treatments such as statins are being used to control blood lipids [4]. Consumption of statins results in different complications like digestive problems, flushing, high blood sugar and rhabdomyolysis [5]. Cumin, as one of these medicinal plants, contains more than 100 different chemicals, including essential fatty acids and volatile oils [6-7]. Moreover, some studies have shown that cumin has a decreasing effect on blood lipids and body weight [8]. Furthermore cinnamon with more than 50 different combinations [9] came up as a nutraceutical to improve health such as glycaemic control in humans without documented potential toxic effects and a high therapeutic effect [10-11]. It is found that consumption of 1 to 6 grams cinnamon has a reducing effect on fasting blood glucose, triglycerides and low density lipoprotein cholesterol (LDL-c) [7]. Consumption of 1 gram cinnamon for 3 months has reduced lipid profile in diabetic patients [12]. Data from clinical trial suggests, from a group of comparator statins, Rosuvastatin, give improvement in lipid profiles [13]. *Cuminum Cyminum L.* is an annual plant of the family apiaceae; seeds of cumin contain various phytochemicals which may have carminative, antioxidant and anti-flatulent characteristics. The cumin active principles may enhance the gastro-intestinal tract motility in addition to increase the force of digestion by enhancing secretions gastro-intestinal enzyme. Cumin is considered

an excellent major source of minerals like copper, iron , selenium, potassium, calcium, manganese, magnesium and zinc .The seeds are also represent rich source of numerous flavonoid phenolic anti-oxidants like lutein, carotenes and zeaxanthin [14]. Cinnamon contain many ingredients and most important one is cinnamaldehyde or trans-cinnamaldehyde which present as essential oil, for this reason contributing to the diverse biological activities and fragrance that occur with cinnamon [15]. One of the major constituents of essential oil that obtained from *C. zeylanicum* named (E)-cinnamaldehyde possessing an antityrosinase activity while cinnamaldehyde is the major compound responsible for this activity [16-17]. Cinnamon prevent bleeding because has coagulant property [18]. Cinnamon also enhances the circulation of blood in the uterus and regeneration of advances tissue [19]. This plant plays an important role as a spice, but other constituents and essential oils also have another important activities, including antifungal, antimicrobial, antidiabetic and antioxidant properties. Cinnamon has been used as antiemetic, anti-inflammatory, nematicidal, larvicidal, and antimitotic, mosquito insecticidal, and anticancer agent. Cinnamon has also been classically used as tooth powder for treatment of toothaches, oral microbiota, dental problems, and bad breath (Table 1) [20-21].

Table 1. Classification of total LDL, HDL, cholesterol and triglycerides

Total Cholesterol	
<200 mg/dL	Desirable
200-239 mg/dL	Borderline high
240 mg/dL	High
LDL Cholesterol	
<100 mg/dL	Optimal
100-129 mg/dL	Near or above optimal
130-159 mg/dL	Borderline high
160-189 mg/dL	High
190 mg/dL	Very High
HDL Cholesterol	
<40 mg/dL	Low
60 mg/dL	High
Triglycerides	
<150 mg/dL	Low
150-199 mg/dL	Borderline high
200-299 mg/dL	High
500 mg/dL	Very high

MATERIALS AND METHODS

This is randomized prospective clinical trials study; it was conducted in November-December 2023 in Al-Sader Medical City laboratory and in Al-Hakeem General Hospital Laboratory. Al-Sader Medical City it is one of the biggest medical school in al Najaf in Iraq and Al-Hakeem General Hospital Laboratory.

Inclusion criteria include middle-aged women from 30-59 years that admitted to hospital and diagnosed with dyslipidaemia and have high levels of lipid profile based on the following classification (defined hypercholesterolemia: total cholesterol >250 mg/dl and triglycerides <200 mg/dl , hypertriglyceridemia: total cholesterol <200 mg/dl and triglycerides >200 mg/dl; or mixed hyperlipidemia: total cholesterol >200 mg/dl and triglycerides >200 mg/dl.

Exclusion criteria include patients with normal level of lipid profile and patients who discharged from hospital without complete their tests are excluded. The presence of conditions that may cause secondary dyslipidemia and need medications; previous cardiovascular events or other chronic diseases such as diabetes or chronic obstructive pulmonary disease, cancer, chronic liver or renal failure, alcohol or other substance abusers; patient who were unable to comply with the study procedures or to be subject to loss to follow-up; having allergic reaction to any one of the agents used in the study, pregnant or nursing women; and patients prescribed hypolipidemic drugs during the study.

SAMPLE SIZE

Sample of study include 30 patients who admitted to hospital with dyslipidaemia disease. Arrangement were done to take approvals from two medical schools in government to seek agreement for data collection. The method of collecting information depends on data from laboratories of these two Hospitals Al-Sader Medical City Hospital and Al-Hakeem General Hospital. The data collection occurs in most days of week.

STUDY GROUPS

The sample of study include 30 patients divided into three groups each group contain 10 patients:

Group I: received Rosuvastatin 20mg tablet once daily for 8 weeks;

Group II: received cinnamon 3g/d (6 capsule for 8 weeks);

Group III: received cumin 3g/d (6 capsule for 8 weeks).

PREPARATION OF AGENTS USED IN THE STUDY

Cinnamon and cumin were obtained from local market and verified by a pharmacognosists. Rosuvastatin is obtained

from pharmacy participants were invited to receive advices about the study protocol and were allocated to their groups. Anthropometric measurement, dietary intake and physical activity were assessed. The weights of participants were recorded in light clothing with electronic scales, with accuracy of 0.1 kg (Omron, Japan). Heights were recorded at the first visit with a wall mounted stadiometer with accuracy of 0.5 cm. The waist circumference was measured at the high point of the iliac crest at minimal respiration, using a nonstretched tape meter, without any pressure to body surface to the nearest 0.1 cm. The body mass index (BMI) was calculated as weight in kilograms (kg) divided by height in meters (m) squared. Physical activity was calculated using the formula Metabolic Equivalent of Task – minutes / week. Participants were instructed not to change their usual dietary habits and physical activities for the duration of the study and to take the experimental supplements with meals. To estimate the frequency of supplements consumption, a checklist was used and the consumption of supplements was controlled by telephone at the end of every week. Participants were instructed to return unused capsules and their patient's compliance was measured by counting the remained capsules at the end of the study. In this study, two 24-hour recalls were completed for each participant (before and after the study). The principal researcher, the participants, and laboratory staff were not aware of patients' group assignments. The capsules were produced in laboratory and packed in packages labeled A, B or C by a third person. A code could belong to one of cumin, cinnamon or control groups. This sample size was 30 with power 80%, assuming a two-sided alpha level of 0.05. Supplements were taken three times a day for 8 weeks. Also, the consumption of supplements was controlled by telephone at the end of every week.

MATERIALS AND METHODS

The patients referred to a laboratory for biochemical assessment. After 12-14 hours of fasting, 5 ml venous blood was taken from all the individuals to measure blood triglycerides, total cholesterol, LDL-C, and HDL-C concentrations. Triglyceride and total cholesterol were measured using commercial assay kits (with sensitivity 3 mg/dl (0.08 mmol/l), with glycerol oxidize and cholesterol oxidize enzymatic methods by the auto-analyzer (Echo plus Company, Italy). HDL cholesterol was measured after the precipitation of beta-lipoproteins by dextran sulfate and chloride magnesium using oxidizes cholesterol method and the auto-analyzer (with sensitivity 1 mg/dl). LDL cholesterol was calculated by Friedewald's formula. Dosage of cumin and cinnamon was selected based on previous studies. Duration of the study was 8 weeks. This period was a bit short but it was enough to show the decrease in lipid profile. We could not extend this period due to possible high attrition rate.

STATISTICAL ANALYSIS

Statistical analysis were performed using SPSS 16.0. Data of quantitative variables were represented in Mean + SEM. Differences between each pair were compared using paired-sample Student's t-test in addition to use Microsoft Office Excel 2007. In all tests, P-value less than 0.05 was interpreted to be statistically significant.

RESULTS

EFFECT OF CUMIN ON SERUM TRIGLYCERIDE LEVELS

There was a significant difference between the mean serum concentration of triglyceride before and after 2 months treatment with cumin among study population as shown in figure 1.

EFFECT OF CUMIN ON SERUM CHOLESTEROL LEVELS

There was a significant difference between the mean serum concentration of cholesterol before and after 2 months treatment with cumin among study population as shown in figure 2.

EFFECT OF CUMIN ON SERUM HIGH DENSITY LIPOPROTEIN LEVELS

There was a significant difference between the mean serum concentration of HDL before and after 2 months treatment with cumin among study population as shown in figure 3.

EFFECT OF CUMIN ON SERUM LOW DENSITY LIPOPROTEIN LEVELS

There was a significant difference between the mean serum concentration of LDL before and after 2 months treatment with cumin among study population as shown in figure 4.

EFFECT OF CINNAMON ON SERUM TRIGLYCERIDE LEVELS

There was a significant difference between the mean serum concentration of triglyceride before and after 2 months treatment with cinnamon among study population as shown in figure 5.

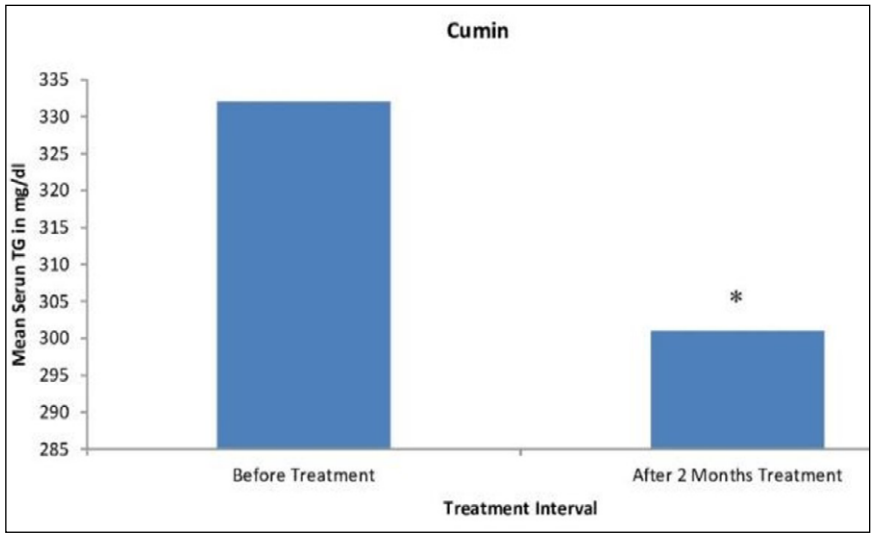


Fig. 1. Comparison between the effect of cumin on serum triglyceride before and after 2 month treatment among study population
* Mean statistically significant
Source: Own materials

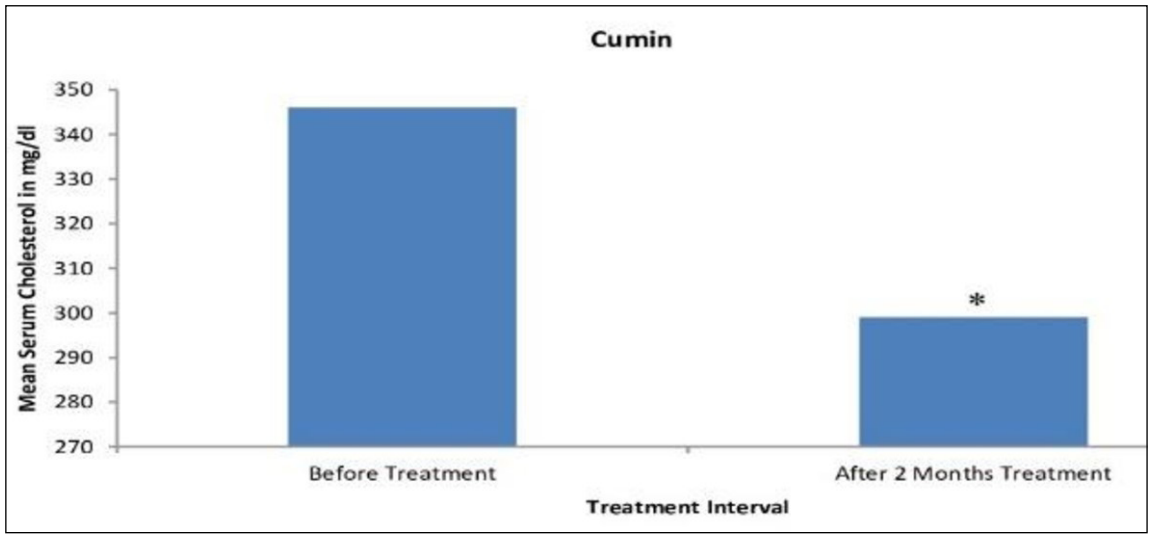


Fig. 2. Comparison between the effects of cumin on serum cholesterol before and after 2 month treatment among study population
* Mean statistically significant
Source: Own materials

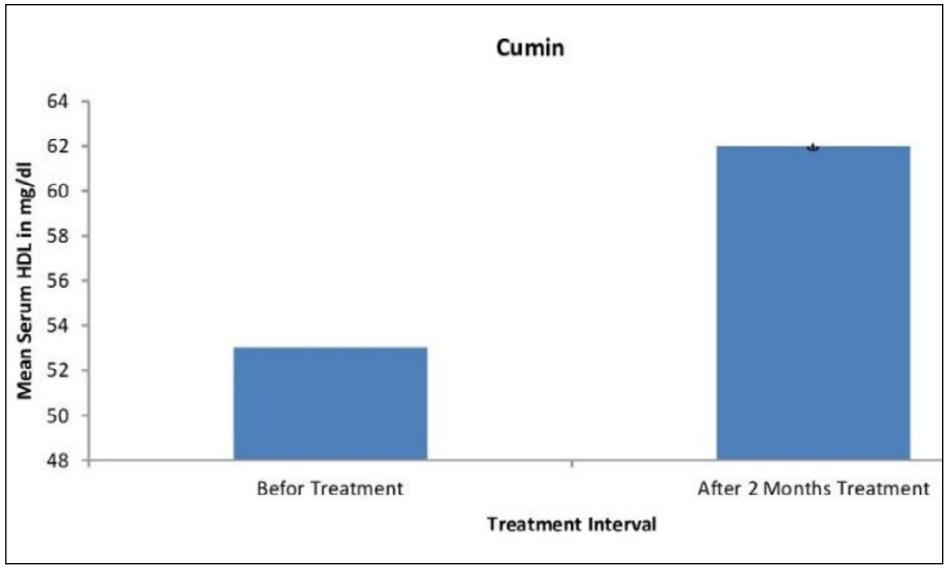


Fig. 3. Comparison between the effect of cumin on serum HDL before and after 2 month treatment among study population
* Mean statistically significant
Source: Own materials

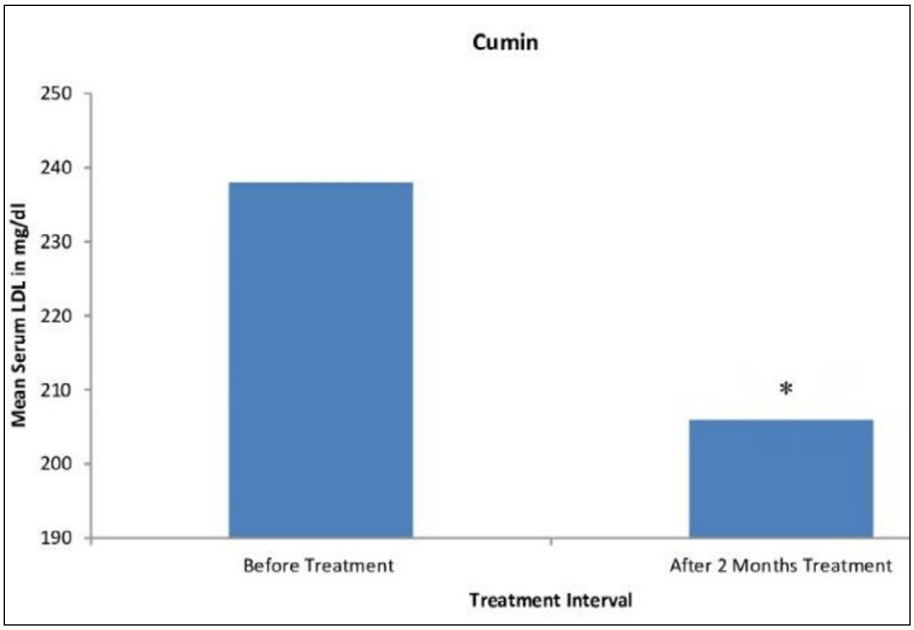


Fig. 4. Comparison between the effect of cumin on serum LDL before and after 2 month treatment among study population
* Mean statistically significant
Source: Own materials

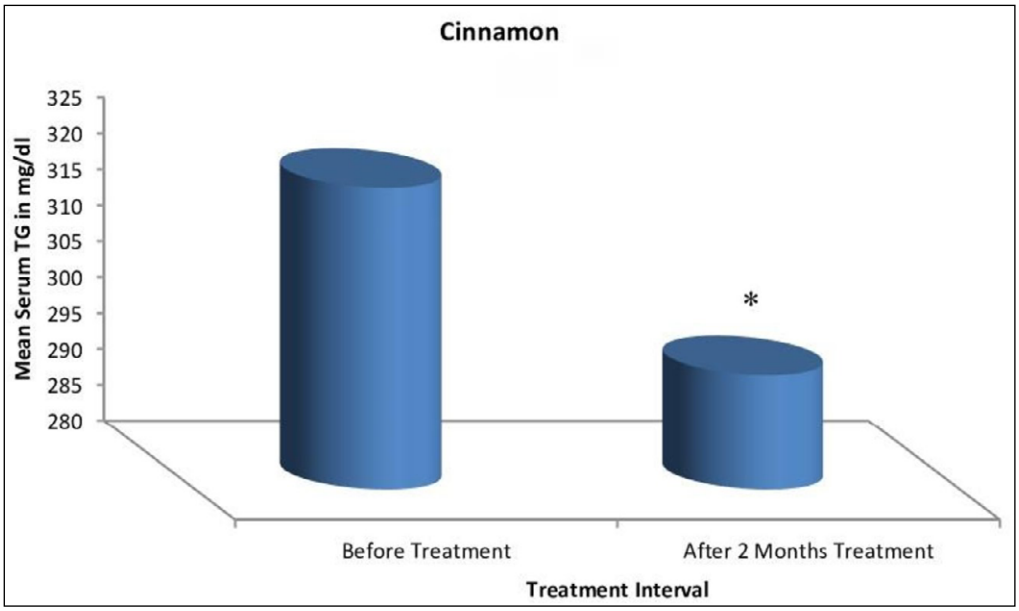


Fig. 5. Comparison between the effect of cinnamon on serum triglyceride before and after 2 month treatment among study population
* Mean statistically significant
Source: Own materials

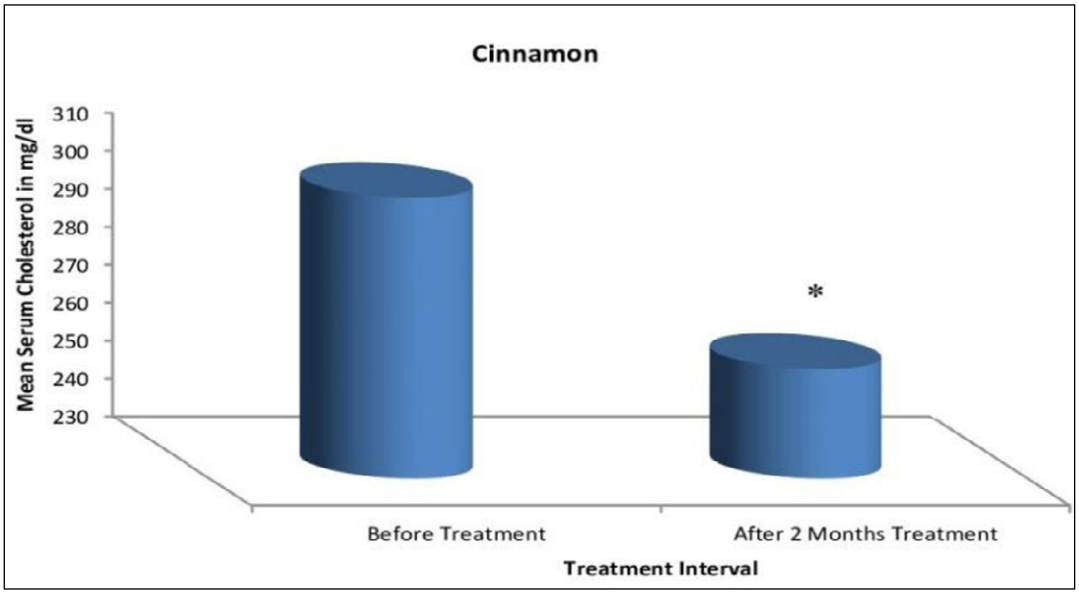


Fig. 6. Comparison between the effect of cinnamon on serum cholesterol before and after 2 month treatment among study population
* Mean statistically significant
Source: Own materials

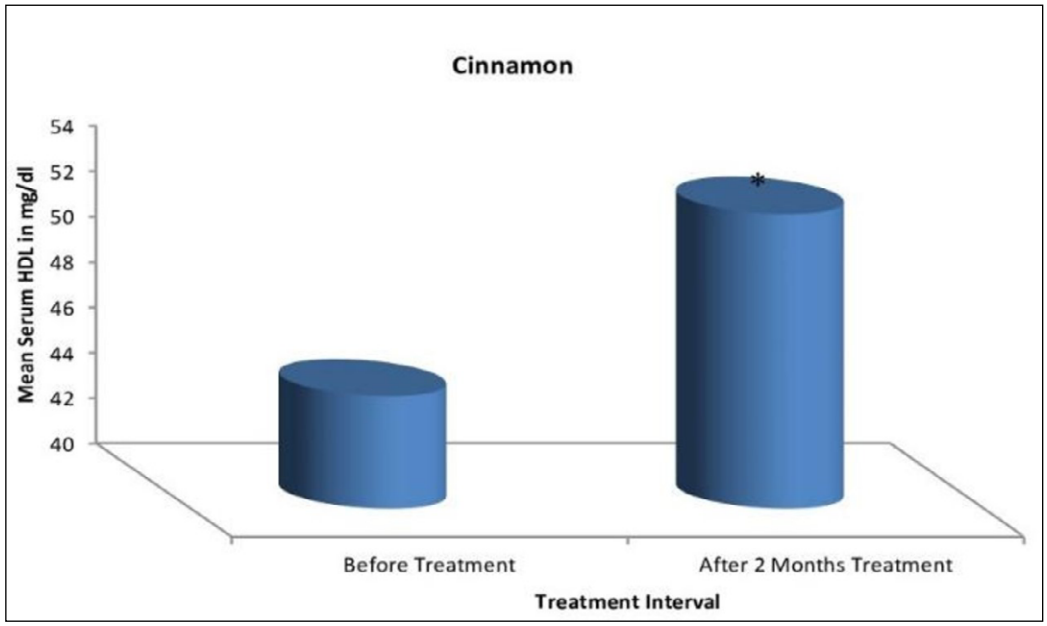


Fig. 7. Comparison between the effect of cinnamon on serum HDL before and after 2 month treatment among study population
* Mean statistically significant
Source: Own materials

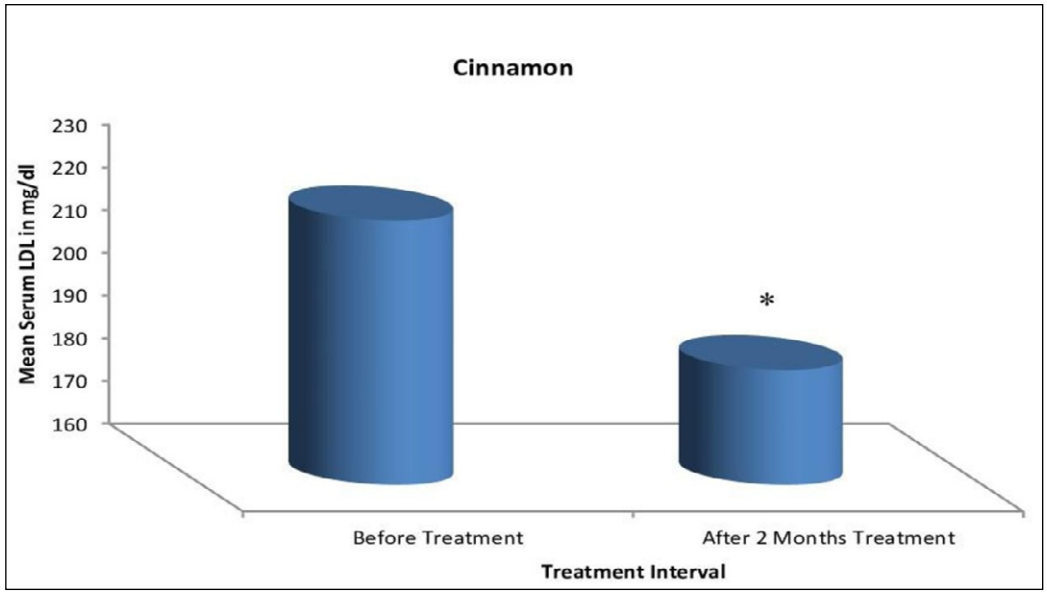


Fig. 8. Comparison between the effect of cinnamon on serum LDL before and after 2 month treatment among study population
* Mean statistically significant
Source: Own materials

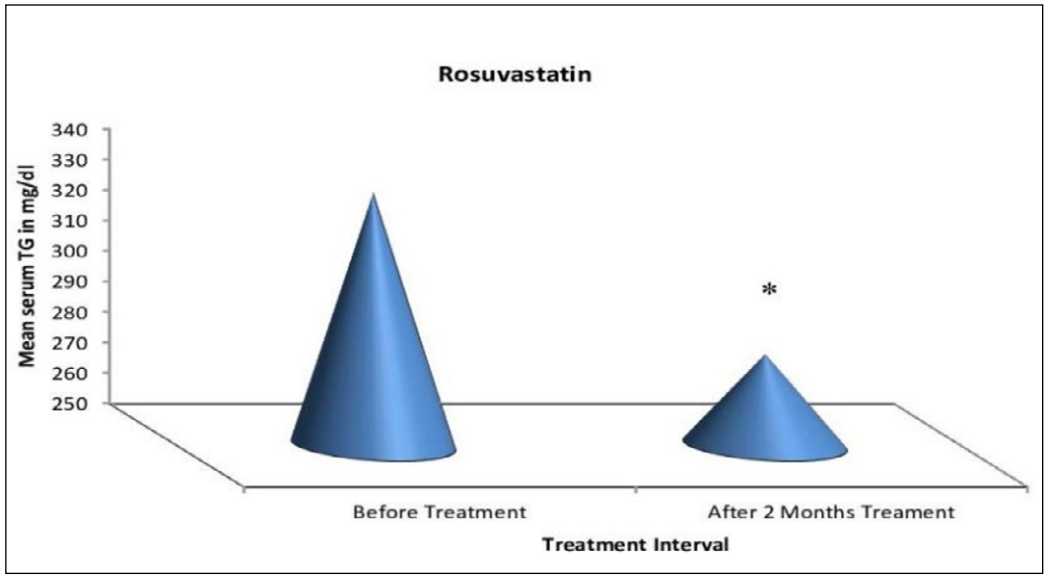


Fig. 9. Comparison between the effect of Rosuvastatin on serum triglyceride before and after 2 month treatment among study population
* Mean statistically significant
Source: Own materials

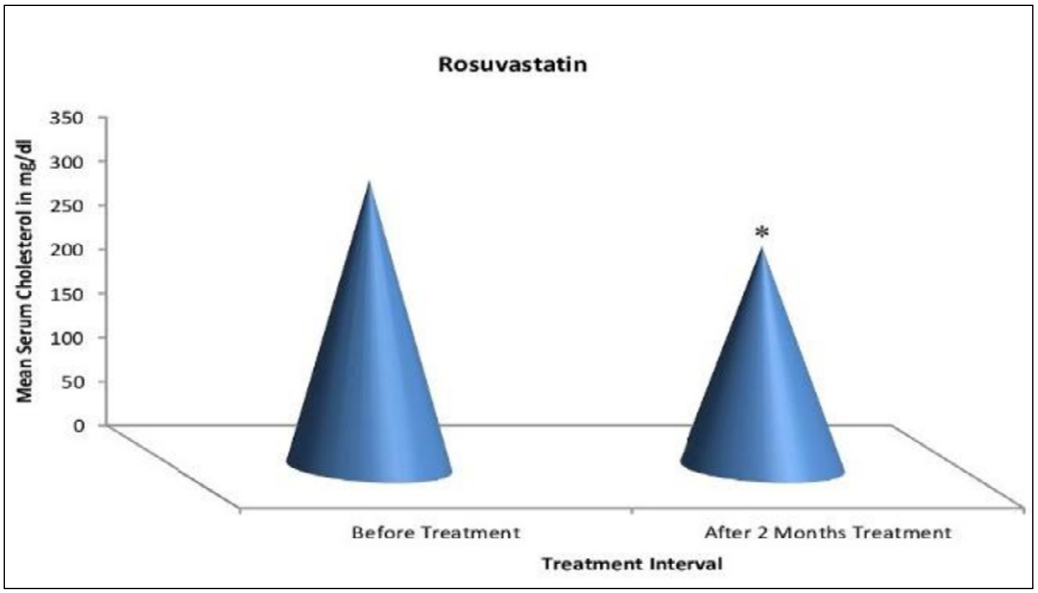


Fig. 10. Comparison between the effect of Rosuvastatin on serum cholesterol before and after 2 month treatment among study population
* Mean statistically significant
Source: Own materials

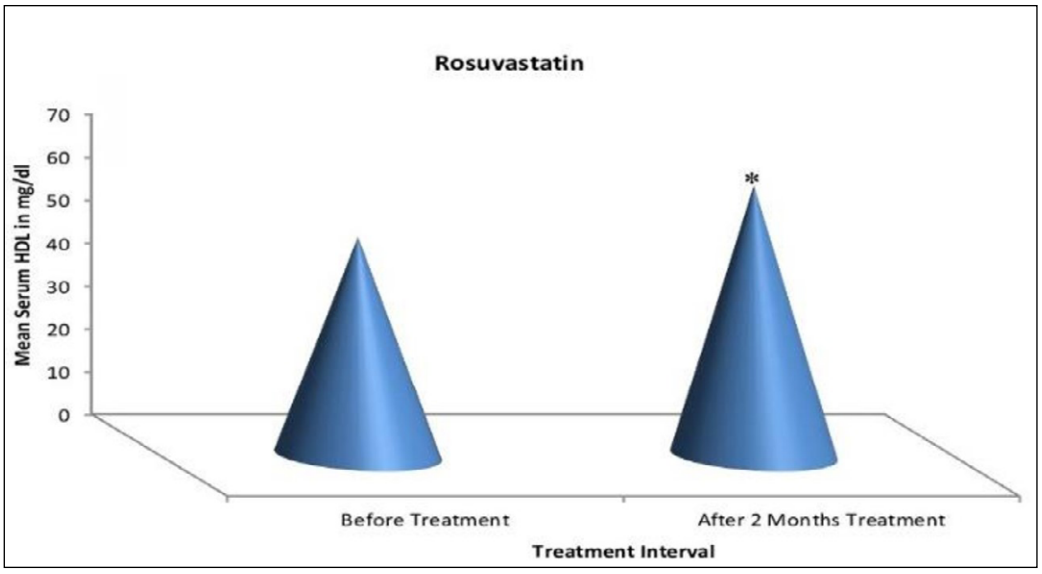


Fig. 11. Comparison between the effect of Rosuvastatin on serum HDL before and after 2 month treatment among study population
* Mean statistically significant
Source: Own materials

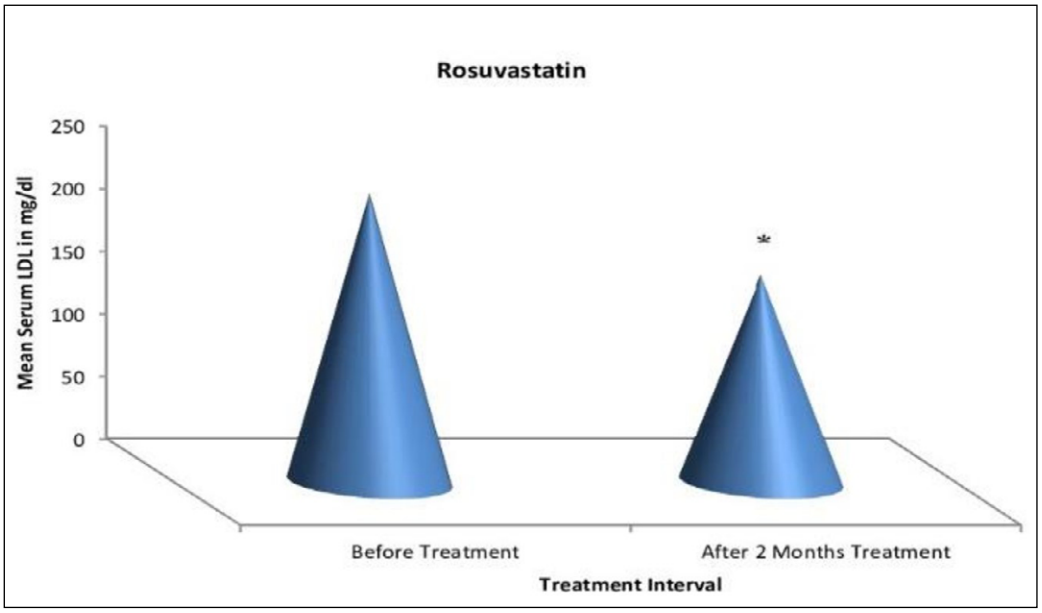


Fig. 12. Comparison between the effect of Rosuvastatin on serum LDL before and after 2 month treatment among study population
* Mean statistically significant
Source: Own materials

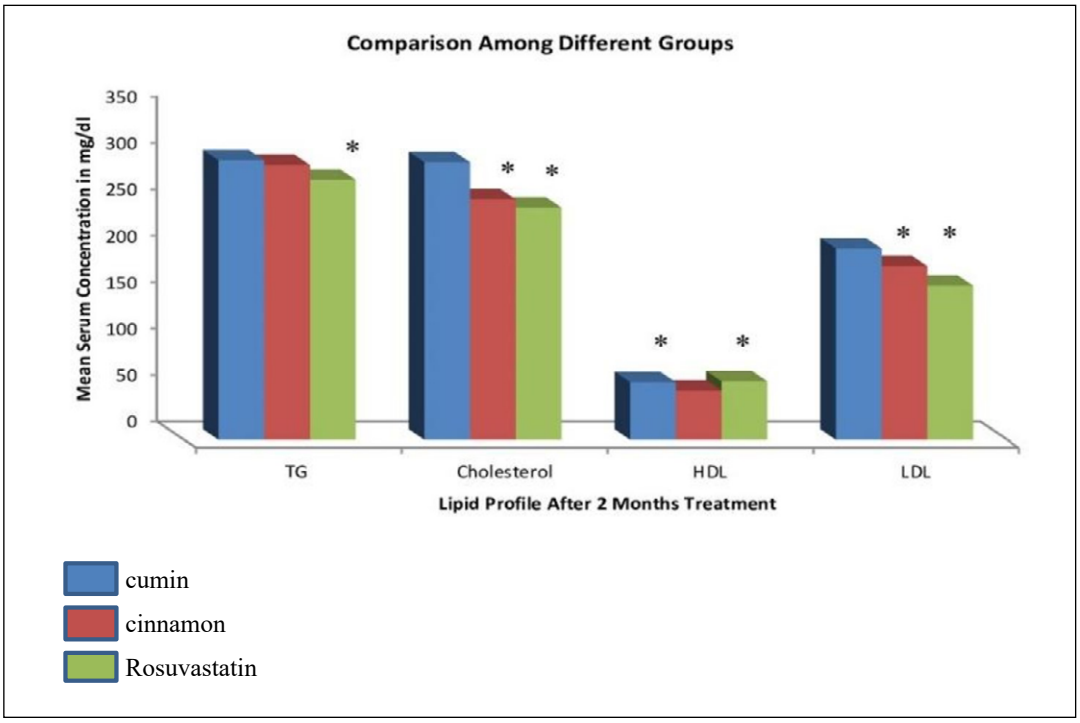


Fig. 13. Comparative effects of different treatment on different parameters after 2 months among study population
* Mean statistically significant
Source: Own materials

EFFECT OF CINNAMON ON SERUM CHOLESTEROL LEVELS

There was a significant difference between the mean serum concentration of cholesterol before and after 2 months treatment with cinnamon among study population as shown in figure 6.

EFFECT OF CINNAMON ON SERUM HIGH DENSITY LIPOPROTEIN LEVELS

There was a significant difference between the mean serum concentration of HDL before and after 2 months treatment with cinnamon among study population as shown in figure 7.

EFFECT OF CINNAMON ON SERUM LOW DENSITY LIPOPROTEIN LEVELS

There was a significant difference between the mean serum concentration of LDL before and after 2 months treatment with cinnamon among study population as shown in figure 8.

EFFECT OF ROSUVASTATIN ON SERUM TRIGLYCERIDE LEVELS

There was a significant difference between the mean serum concentration of triglyceride before and after 2 months treatment with Rosuvastatin among study population as shown in figure 9.

EFFECT OF ROSUVASTATIN ON SERUM CHOLESTEROL LEVELS

There was a significant difference between the mean serum concentration of cholesterol before and after 2 months treatment with Rosuvastatin among study population as shown in figure 10.

EFFECT OF ROSUVASTATIN ON SERUM HIGH DENSITY LIPOPROTEIN LEVELS

There was a significant difference between the mean serum concentration of HDL before and after 2 months treatment with Rosuvastatin among study population as shown in figure 11.

EFFECT OF ROSUVASTATIN ON SERUM LOW DENSITY LIPOPROTEIN LEVELS

There was a significant difference between the mean serum concentration of LDL before and after 2 months treatment with Rosuvastatin among study population as shown in figure 12.

COMPARATIVE EFFECTS OF CUMIN, CINNAMON AND ROSUVASTATIN ON DIFFERENT PARAMETERS AMONG STUDY POPULATION

There was a significant difference in mean serum concentration of triglyceride regarding to the group treated with Rosuvastatin as compare with groups treated by

Table 2. Average reads of Cumin, Cinnamon, and Rosuvastatin on TG, CHOL, HDL and LDL

	Cumin		Cinnamon		Rosuvastatin	
Lab test	Before	After	Before	After	Before	After
TG	332.8	301.2	322.3	296.6	333.3	280.2
CHOL	346.7	299.2	304.2	259.4	325.5	250.1
HDL	52.9	62.2	44.9	53.3	50.9	62.7
LDL	237.9	205.9	221.5	186.6	231.2	165.7

Table 3. Difference between before and after reads

	Cumin	Cinnamon	Rosuvastatin
TG	*31.6	25.7	*53.1
CHOL	*47.5	44.8	*75.4
HDL	*9.3	8.4	*11.8
LDL	32	* 34.9	*65.5

cinnamon and cumin but nonsignificant differences in mean serum concentration of triglyceride between cinnamon and cumin treated groups. Also there was a significant difference in mean serum concentration of cholesterol regarding to the groups treated with cinnamon and Rosuvastatin as compare with cumin treated group but nonsignificant differences in mean serum concentration of cholesterol between cinnamon and Rosuvastatin treated groups. There was a significant difference in mean serum concentration of HDL regarding to the groups treated with cumin and Rosuvastatin as compare with cinnamon treated group but nonsignificant differences in mean serum concentration of HDL between cumin and Rosuvastatin treated groups. There was a significant difference in mean serum concentration of LDL regarding to the group treated with Rosuvastatin as compare with groups treated by cinnamon and cumin in addition to significant differences in mean serum concentration of LDL between cinnamon and cumin treated groups (Tables 2-3 and Fig. 3).

DISCUSSION

According to table 2 show the highest readings are of Rosuvastatin that effected on triglyceride with 53.1, cholesterol with 75.4, high density lipoprotein with 11.8 and low density lipoprotein with 65.5 were noticed on dyslipidaemia patients living in Al-Najaf city. This finding is in agreed with previous study conducted by [22]. Statins are particularly useful in patients with severe forms of hypercholesterolemia. Rosuvastatin when compare with most potent statins is capable of performing the major goals for the treatment of dyslipidemic patients as reduction of LDL cholesterol. In addition to that, Rosuvastatin possess the property of enhancing HDL cholesterol and decreasing small-dense LDL levels [22]. Table 3 shows ssecond highest readings are of cumin that effected on triglyceride with 31.6, cholesterol with 47.5, high density lipoprotein with 9.3 and the lowest reading is low density lipoprotein with 32 were noticed on dyslipidaemia patients living in Al-Najaf city. This finding is in agree with previous study conducted by [23] with the reading of triglyceride and cholesterol and not agree with the reading of high density lipoprotein and low density lipoprotein. Study [23] reported a significant decrease in plasma concentration LDL cholesterol and total cholesterol after taking cumin as compared with control treatment, and HDL cholesterol plasma concentration were found to be enhanced. Despite of finding of present study recorded that supplementation with cumin don't alter the concentration of triglyceride. Table 4 shows lowest readings are of cinnamon that affected on triglyceride with 25.7, cholesterol with 44.8, high density lipoprotein with 8.4 and the second highest reading is low density lipoprotein with 43.9 were noticed on dyslipidaemia patients living in Al-Najaf city. This finding is not agreed with previous study conducted by [24] with the reading of triglyceride and cholesterol and high density lipoprotein but agrees with the reading of low density lipoprotein. Cinnamon reduce total cholesterol (TC), LDL, triglycerides (TG), LDL and increase HDL levels. There is one study suggests that extract from *Cinnamomum aromaticum* in a dose of 120 mg capsule show significant reduction in triglyceride levels after three months of treatment as compared with baseline [24].

LIMITATIONS

One of the limitations of this study is considering only women with dyslipidemia. The reason is the availability of them. So, the results cannot be generalized to men

or healthy women. Considering the patients of both sexes can help reach better results. Although, physical activity and dietary intake were considered as confounding factors among three groups. The short period of study was another limitation of this study. Further investigations with a longer period are warranted. As far as we know, this is the first study that compared the effect of cumin and cinnamon consumption. The strengths of the present study were the double blind placebo-controlled design and low drop-out. Probably, this study can be effective on treatment of high cholesterol levels. However, due to no significant impact on reducing LDL and TG and no increase of HDL, more research is needed.

CONCLUSIONS

Overall, this study provides evidence that indicates the cumin, cinnamon has a hypocholesterolemic effect with Rosuvastatin with dyslipidaemia patients. It can be used as an adjuvant treatment with other drugs used to decrease TG, cholesterol, LDL and increase HDL levels.

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CONFLICT OF INTEREST

The Author declare no conflict of interest

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A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

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CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Assessment of carbacetam effect on the mitochondria of hippocampal neurons in rats of different sexes with experimental metabolic syndrome

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ABSTRACT

Aim: To study carbacetam effect on the mitochondria of hippocampal neurons in rats of different sexes simulating metabolic syndrome.

Materials and Methods: The experiments were conducted on non-linear laboratory albino male and female rats with the body weight of 0,220-0,250 kg. To create the pattern, the rats were kept (60 days) on a high-fat diet (fat enrichment was provided by the addition of solid pork lard) with free access to fructose solution (100 g/L). Carbacetam was injected into the peritoneum in the dose of 5 mg/kg once a day during 14 days.

Results: Simulated metabolic syndrome was found to manifest by a decreased light scattering and an increased relative rate of mitochondrial swelling in the hippocampal mitochondrial fraction; increased free radical lipid and protein oxidation with more marked changes in males. When rats with metabolic syndrome receive carbacetam during 14 days, in their mitochondrial fraction light scattering and relative rate of mitochondrial swelling decrease, both in males and females. The content of products reacting with 2-thiobarbituric acid and protein oxidative modification decrease, and catalase activity in males and females increases, superoxide dismutase activity increases in males only.

Conclusions: Thus, a decreased intensity of mitochondrial swelling and improved condition of the antioxidant system of the hippocampal mitochondria of rats with metabolic syndrome irrespective of their sex is indicative of the effective correction of GABA receptors by means of carbacetam under conditions of the experiment.

KEY WORDS: metabolic syndrome, carbacetam, lipid and protein peroxide oxidation, mitochondrial functional state

Wiad Lek. 2025;78(10):2019-2025. doi: 10.36740/WLek/210015 DOI

INTRODUCTION

Urbanization, associated sedentary lifestyle, and overeating are the main cause of current health epidemic – metabolic syndrome [1, 2]. Excessive caloric intake and reduced energy loss lead to lipid accumulation and obesity. Accumulated toxic lipids inhibit insulin signaling in the liver and skeletal muscles provoking insulin resistance [3, 4]. Metabolic syndrome is a complex and multifactorial disorder affecting a large part of the world's population and is characterized by a group of interrelated conditions that increase the risk of cardiovascular diseases, stroke and type 2 diabetes mellitus. Although definite etiology of the syndrome requires further investigation, the recent researches have determined a crucial role performed by mitochondria in its pathogenesis.

According to science mitochondria that today are considered as keys to solve numerous mysteries play an important role in energy metabolism and oxidative stress, which is one of the molecular mechanisms of damaging different organs and tissues with metabolic syndrome [5, 6, 7]. Since the neurons are restricted in glycolytic abilities, their functional activity depends on mitochondrial energy production more than other body cells. Accumulation of mitochondria in the synapses is a well-known fact. They provide the mechanisms of transmission of nerve impulses [8]. Therefore, mitochondrial dysfunction is one of the pathogenic links of metabolic disorders. At the same time, mitochondria are known to contain gamma-aminobutyric acid (GABA). Its functional cycle is closely associated with glucose metabolism [9]. Considering cerebroprotective effects of GABA agents with functional and organic disorders of the CNS, learning carbacetam effect as a modulator of GABA-ergic system with metabolic disorders taking into account sexual differences is of certain interest.

AIM

To study carbacetam effect on the mitochondria of hippocampal neurons in rats of different sexes simulating metabolic syndrome.

MATERIALS AND METHODS

The experiments were conducted on non-linear laboratory albino male and female rats with the body weight of 0,220-0,250 kg, kept under standard vivarium conditions with a natural change of day and night at a temperature of 18-22 °C and relative humidity of 40-60 %. The study was conducted keeping to the main principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasburg, 1986); the EU Directives № 609 of 24.11.1986 and the Order of the Ministry of Health of Ukraine № 690 of 23.09.2009.

All the rats were divided into two groups by random sampling method: 1 – control group; 2 – group with simulated metabolic syndrome. To create the pattern, the rats were kept (60 days) on a high-fat diet (fat enrichment was provided by the addition of solid pork lard) with free access to fructose solution (100 g/L) [10]. The reproduction of the syndrome was confirmed by determining the fasting blood glucose concentration in the blood plasma and positive glucose tolerance test. Rats with hyperglycemia lower than 7,0 mmol/L were isolated from the experiment. After that, the group of rats with syndrome (7 rats) was injected with carbacetam into the peritoneum in the dose of 5 mg/kg during 14 days. The groups of comparison including the control one and rats with simulated pathology (7 ratsin each group) 0,9 % NaCl solution was injected in the same way.

Euthanasia of rats was conducted under chloroform. The brain was removed in the cold and carefully washed with cool 0,9 % NaCl solution. The hippocampus was isolated according to a stereotaxic atlas [11]. Then it was washed with cool (2-4°C) 0,9 % KCl solution, grinded, homogenized in a 10-fold volume of pH 7,4 buffer (saccharose 250 mM, EDTA 1 mM, tris-HCl 10 mM) [12].

The mitochondrial fraction was isolated by means of differential centrifugation of the examined structure homogenates: 700 g during 10 min (4°C), supernatant –11 000 g during 20 min (4°C). The sediment was re-suspended in 5 ml of pH 7,4 buffer (without EDTA), then it was centrifuged again under similar conditions. The mitochondrial fraction (the sediment obtained) was re-suspended in the same buffer and immediately taken for the examination.

Mitochondrial swelling was registered according their ability to extension-contraction and optic density changes. Mitochondrial suspension was placed into the incubation pH 7,4 buffer (mmol/L): saccharose – 150, KCl – 50, KH_2PO_4 – 2, succinate – 1, tris-HCl – 5 (final volume of 3 ml). The optic density level of the mitochondrial suspension with λ 520 nm was registered during 60 min of swelling with Ca^{2+} inductor (50 mcmol/L) available. The change in the level of mitochondrial swelling was determined as the difference between the rate of organelle swelling at 5, 10, 15, 20, 30, 40, 60 min relative to the initial value. The mitochondrial suspension in the incubation medium without the inductor was used as the control followed by further registration of the optic density during 60 min. Protein concentration in the incubation medium was 0,4 mg/ml. The change of E520 parameters in the incubation medium was used to calculate a relative rate of mitochondrial swelling [12].

Lipid peroxide oxidation (LPO) in the mitochondria was assessed according to the levels of thiobarbituric acid active products (TBAAP) [13]; carbonylation of the mitochondrial proteins – by the amount of 2,4-dinitrophenylhydrazone derivatives with the formation of carboxylphenylhydrazone (CPH). It was presented in nmol of carbonyl derivatives per 1mg of protein [14]. The state of the antioxidant defense system in the mitochondria was estimated according to the enzymatic activity of superoxide dismutase (SOD) and catalase [12]. Protein content in the mitochondria was determined by Lowreh method [15].

The results of the study was statistically processed by means of Student criterion. To confirm reliability of the conclusions the non-parametric Mann-Whitney comparison test was used that demonstrated similar results of the calculations obtained by the Student criterion concerning p value. Differences were statistically reliable with $p \leq 0,05$.

RESULTS

Participation of the mitochondria in various critical processes within the cell is a well-known fact. These are oxidative phosphorylation, tricarboxylic acid cycle, fatty acid oxidation, calcium ion homeostasis, and apoptosis regulation [16]. Although, their main function is ATP production in order to obtain energy. Disorders of the main mitochondrial functions is usually characterized as mitochondrial dysfunction. Recently the scientists have associated it with numerous diseases. Therefore, many studies are being carried out in order to find how mitochondrial dysfunction is connected with pathology of different diseases, for example, with metabolic syndrome and GABA-receptors.

Results of our studies demonstrated in Fig.1 and Fig.2 show the dynamics of intensity changes of the hip-

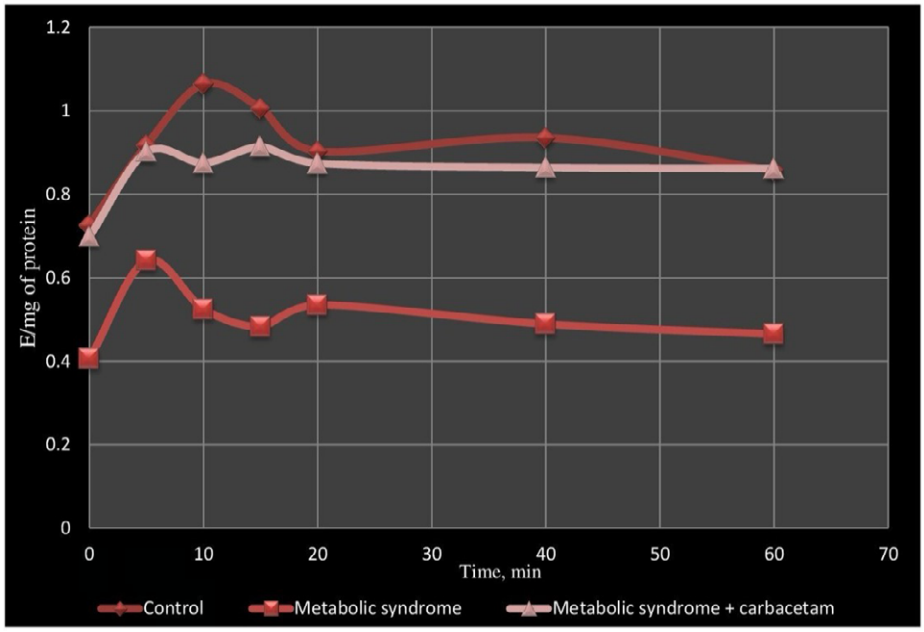


Fig. 1. Intensity of mitochondrial swelling in the hippocampus of male rats with metabolic syndrome after carbacetam administration during 14 days in the dose of 5 mg/kg
Picture taken by the authors

pocampal mitochondrial swelling in male and female rats. Thus, light scattering level of the mitochondrial suspension in the control group of male and female rats from the 1st to the 15th minute increased, and after the 20th to 60th – decreased. Thereby, our results obtained coordinate with literature data concerning physiological role of the mitochondria in maintenance of their own homeostasis due to their ability to maintain Ca2+ balance in the matrix.

Rats with metabolic syndrome in comparison with those from the control group presented decreased light scattering of the mitochondrial suspension after60 minutes of incubation by 45,71 % in males and 39,2 % – in females (Fig. 1, 2). After a 14-day administration of carbacetam rats with metabolic syndrome presented gradual increase of light scattering both in males and females – by 85,3 and 60,8 % respectively compared to the simulated pattern of the pathology. Thus, simulation of metabolic syndrome provoke more pronounced mitochondrial lesions in male rats, but they respond to carbacetam administration better.

Results of our studies showed (Fig. 3) that a relative rate of mitochondrial swelling in rats with metabolic syndrome decreased in comparison with the control group both in males and females – by 50,0 % and 25,0 % respectively. It should be noted that after carbacetam administration, compared to the results obtained from the group of simulated pathology, a relative rate of mitochondrial swelling increased in both research groups: by 59,5 % – in males and 16,0 % – in females.

That is, inn rats of both sexes with metabolic syndrome, the sensitivity of the mitochondrial pore to the action of calcium ions increases, which is probably related to the overload of these ions under pathological conditions. A probable cause of these processes is a disturbance of the membrane permeability. However, administration of carbacetam to rats caused a decrease in the level of mitochondrial swelling. It is possible that the mechanism of carbacetam action is associated with prevailing intensification of NAD-dependent oxidation, which is one of the ways to increase the resistance of the mitochondrial respiratory link [17].

In this respect, the next stage of our research was to study the processes of lipid and protein oxidation in the mitochondria. The results of our experiment are presented in the Table 1. As we can see, the content of products of lipid and protein peroxidation increases in rats with metabolic syndrome. Thus, in rats with simulated pathology the content of TBAAP increased in male and female rats by 81,0 % and 97,6 % respectively. At the same time, CPH content in these groups increased as well. It was 56,3 % in males, and 41,9 % in females compared to the control group of rats.

The results obtained are indicative of the activation of lipid and protein peroxidation processes in both sexes of rats with metabolic syndrome. At the same time, in the research groups the activity of the major enzymes of the antioxidant defense decreases. Thus SOD activity 32,6 % decreased in male rats with simulated pathology, and the same tendency to decrease is found among females. Catalase activity 21,5 % decreased in male rats and 18,1 % in females.

Administration of carbacetam to rats promoted a decrease in the content of TBAAP and CPH in both groups, in comparison with the rats with simulated pathology. Thus, inn male rats the content of TBAAP and CPH decreased by 31,9 %, 21,3 %, and in female rats by 27,3 %, 17,6 %, respectively. At the same time,

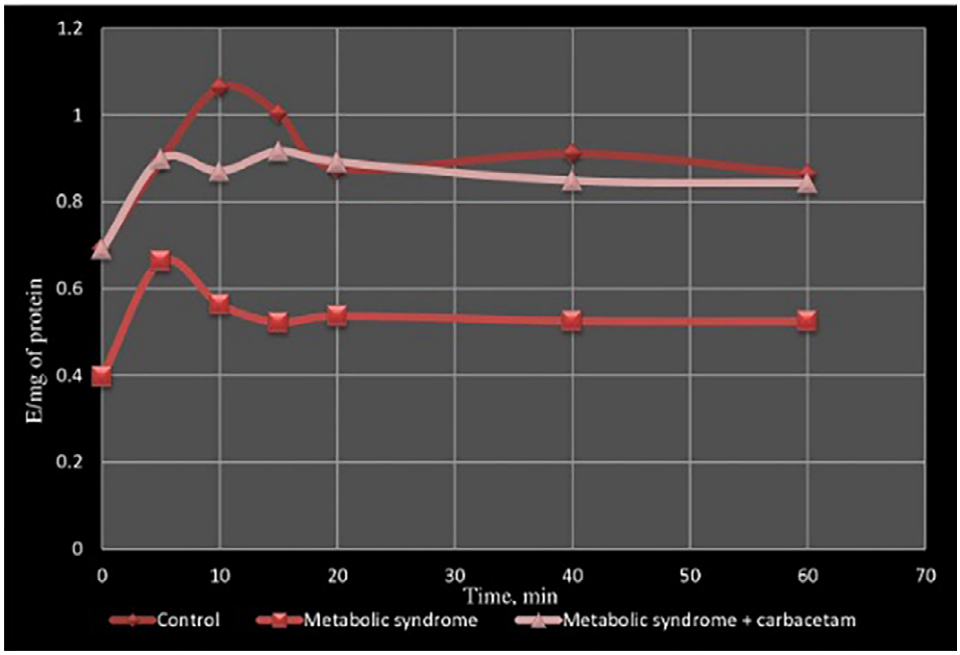


Fig. 2. Intensity of mitochondrial swelling in the hippocampus of female rats with metabolic syndrome after carbacetam administration during 14 days in the dose of 5 mg/kg
Picture taken by the authors

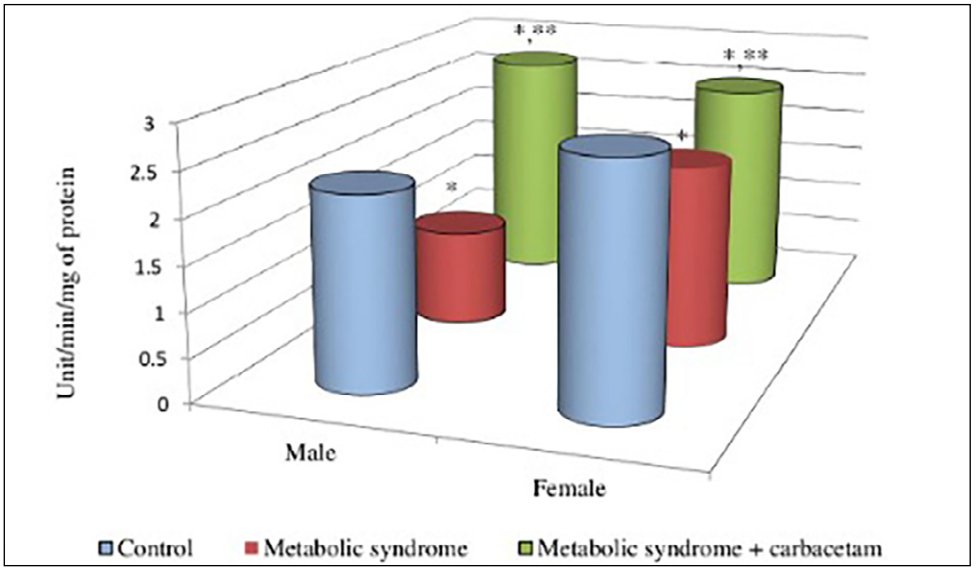


Fig. 3. Relative rate of mitochondrial swelling in the hippocampus of rats with metabolic syndrome after carbacetam administration during 14 days in the dose of 5 mg/kg (M±м, n=7)
Notes:* – reliability of differences compared with the control group of rats,
** – reliability of differences compared with the group of rats with metabolic syndrome
Picture taken by the authors

the activity of the antioxidant defense enzymes increased. SOD activity increased by 24,1 % in males, but among females only a tendency to increase the enzymatic activity was found. Meanwhile, catalase activity increased both in males and females – by 14,8 % and 11,2 % respectively.

Therefore, disorders of the mitochondrial functional state and prooxidant-antioxidant imbalance is found in both sexes with metabolic syndrome, but more pronounced in males. Nevertheless, modulation of GABA-receptors with carbacetam promotes improved mitochondrial functional state and decreased processes of lipid and protein peroxidation with activation of the antioxidant defense enzymes. Male rats are exposed to the modulator effect better than females.

DISCUSSION

Our experimental data are consistent with the publications of other scientists. In particular, it is believed that males are more sensitive to oxidative stress than females [18]. First, this is due to increased leakage of superoxide anion in the mitochondrial chain. In addition, they have lower expression and activity of important antioxidant enzymes, such as SOD and glutathione peroxidase. These facts contribute to a greater accumulation of oxidative damage over time in DNA, proteins and lipids, disrupting the proper function of cells and tissues. Therefore, taking into account sex differences in metabolic syndrome will improve the understanding of pathogenesis and contribute to the development of personalized medical approaches to more effectively address the problems of metabolic disorders.

Table 1. Carbacetam effect on free radical lipid and protein oxidation in the mitochondria of the hippocampus of rats with metabolic syndrome (M±m, n=7)

Indices	Research groups	Control	Metabolic syndrome	Metabolic syndrome + carbacetam
The content of TBAAP, nmol/mg of protein	Males	11.6±0.66	21.0±1.14*	14.3±0.69* **
	Females	12.4±0.91	24.5±0.99*	17.8±0.59* **
The content CPH, nmol/mg of protein	Males	18.3±0.89	28.6±1.31*	22.5±0.79* **
	Females	22.4±0.66	31.8±0.73*	26.2±0.88* **
The activity of SOD, units / mg of protein	Males	0.43±0.039	0.29±0.008*	0.36±0.015**
	Females	0.37±0.050	0.28±0.023	0.32±0.024
The activity of catalase, mcmol H2O2/ min of mg of protein	Males	191.8±7.97	150.5±4.31*	172.7±3.17**
	Females	180.4±5.62	147.9±2.89*	164.4±2.21* **

Notes:* – reliability of differences compared with the control group of rats,
** – reliability of differences compared with the group of rats with metabolic syndrome.
Source: compiled by the authors of this study

In addition, the more pronounced sensitivity of GABA receptors to the action of carbaracetam in males due to the increase in the activity of antioxidant defense enzymes is probably due to a certain ratio of sex hormones. For example, it is known that significant differences in GABA concentrations are observed during the estrous cycle in females. This indicates the relationship between the neural system and sex hormones and their significant role in the expression of feedback [19]. This assumption is based on the data of other scientists, since in our experiment we did not determine the levels of hormones, which is a prospect for further research. After all, hormonal differences are the main cause of behavioral and physiological changes observed between individuals. At the same time, there are few studies that show sex-related differences in the functioning of the GABA-ergic system. Sex bias is observed in many pathological mechanisms [19]. Taking into account all the above, it confirms the importance of this study to determine the relationship between gender and GABAergic receptors observed in metabolic syndrome. Understanding these processes will provide an opportunity to influence the prevalence and progression of the disease, and will serve as a basis for the development of new preventive and therapeutic strategies.

Modulation of type A GABA-receptors, which regulate permeability of chlorine channels, is a probable mechanism of correction of the obtained changes [20]. According to the science, it is this type of receptors located in the walls of the cerebral vessels. Their stimulation promotes vasodilation and improvement of blood supply to the brain [21]. Therefore, these processes improve blood flow volume and brain oxygenation. In its turn, it slows the intensity of lipid and protein peroxidation processes. It results in a decreased generation of oxygen active forms and insulin resistance [22, 23]. Accordingly, the balance between energy production and its use is restored leading to better cellular metabolism. Since disturbed metabolism is the main cause of metabolic syndrome, it is one of the possible mechanisms of its prevention and treatment.

In addition, in our studies, we observe an increase in the activities of antioxidant defense enzymes - SOD and catalase when using carbaracetam. This indicates the ability of this drug, through the modulation of GABA receptors, to restore antioxidant defense when it is reduced, in particular - metabolic syndrome. Its binding to these type A receptors causes conformational changes in ion channels of cell membranes, due to which the permeability of the central part of the channel for chloride ions increases [24]. It provokes conformation changes in the ion channels of the cellular membranes due to which permeability of a central part of the channel for chlorine ions increases. Increased entrance of chlorine ions causes hyperpolarization and metabolism correspondence to the functional requirements of the cells. At the same time, glutamate-calcium excitotoxicity cascade is modulated and calcium-dependent pathologic reactions decrease. The changes indicated result in decreased formation of oxygen active forms, LPO, and increased activity of the antioxidant protection enzymes.

One of the probable protective mechanisms of carbacetam action is increasing affinity of the nerve cells to GABA-benzodiazipine receptor complex, which decreases hyperexcitability of glutamate receptors and glutamate excitotoxicity respectively [25]. It results in decreased activity of NO-synthase, reduced production of NO, increased content of reduced glutathione and its enzymes. As a result, functional stability of neurons increases [25].

Scientific literary sources report that oxidative stress and mitochondrial dysfunction participate in the pathology of metabolic diseases, which is evidenced in our studies [26]. Based on the obtained results, we observe improvement of the functional state of mitochondria in the hippocampus of rats after carbacetam administration, which is indicative of an important value of GABA-receptors in metabolic disorders. Meanwhile, further studies are required to learn the role of GABA-receptors in metabolic disorders and the ways responsible for the mitochondrial function and sensitivity to insulin in the human body.

CONCLUSIONS

1. When simulating metabolic syndrome in the mitochondrial fraction of the hippocampus of rats, light scattering decreases and a relative rate of mitochondrial swelling increases; free radical oxidation of lipids and proteins increases with more pronounced changes in males.
2. 14-day administration of carbacetam to rats with metabolic syndrome promotes a decrease in light scattering and a relative rate of mitochondrial swelling in both males and females observed in the mitochondrial fraction. The content of products reacting with 2-thiobarbituric acid and protein oxidative modification decrease. Catalase activity in both males and females increases, and superoxide dismutase increases in males only.
3. A decreased intensity of mitochondrial swelling and improved condition of the antioxidant system of the hippocampal mitochondria of rats with metabolic syndrome irrespective of their sex is indicative of the effective correction of GABA receptors by means of carbacetam under conditions of the experiment.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Resilience, hardiness and social support in combatants with non-psychotic mental disorders that have suffered from Covid-19

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ABSTRACT

Aim: To establish the features of resilience, hardiness and social support in combatants with non-psychotic mental disorders who have suffered from COVID-19 in a comparative aspect.

Materials and Methods: 252 male combatants with non-psychotic mental disorders who suffered from COVID-19 were clinically examined using Connor–Davidson resilience scale-10, Brief Hardiness Scale and MSPSS. The examination included a clinical interview organized by the method of a semi-structured clinical interview, during which the presence and detail of complaints from the affective sphere was established, as well as an assessment of the severity of resilience, hardiness and social support. Statistical analysis of differences in categorized traits was performed using Fisher's exact test (one-sided), quantitative traits - using the nonparametric Mann-Whitney test.

Results: In combatants with non-psychotic mental disorders that have suffered from COVID-19 were found lower levels resilience: 18.08±10.06 points vs 23.41±8.80 points ($p<0.0001$); hardiness: 12.28±4.20 points vs 15.67±6.88 points ($p<0.001$) and his components: commitment: 3.50±1.81 points vs 4.89±2.76 points ($p<0.0001$), control: 4.44±1.70 points vs 5.38±2.40 points ($p<0.01$) and challenge: 4.33±1.79 points vs 5.40±2.42 points ($p<0.001$); social support: 11.63±4.29 points vs 14.68±4.62 points ($p<0.0001$) and his components: family support: 3.85±1.54 points vs 4.95±1.58 points ($p<0.0001$), friends support: 3.95±1.48 points vs 5.00±1.77 points ($p<0.0001$) and significant other support: 3.83±1.51 points vs 4.73±1.53 points ($p<0.0001$). Among combatants with non-psychotic mental disorders who did not suffer from COVID-19, people with average and high levels of resilience, moderate resilience and a high level of social support predominated, and among combatants who recovered from COVID-19, people with low resilience, hardiness and social support.

Conclusions: Combatants with non-psychotic mental disorders are characterized by a low level of resilience and hardiness, as well as a moderate level of social support, significantly worse in combatants that have suffered from COVID-19.

KEY WORDS: combatants, non-psychotic mental disorders, COVID-19, resilience, hardiness, social support

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INTRODUCTION

Restoring the health and social functioning of combatants is one of the most important tasks of the state during the war and in the post-war period. Participation in an armed conflict entails a number of psychosocial, medical and economic consequences characterized by a complex combination of biological, psychological, social, cultural and political factors [1]. Combatants have an increased risk of depression, anxiety disorders, post-traumatic stress disorder, various forms of addictions, which often become chronic and long-lasting, significantly reducing the adaptive capacity and quality of life of combatants [2].

An important role in the psychosocial rehabilitation and readaptation of combatants is played by the features of stress response, which is a component of personal potential; these features can be used as predictors of psychosocial maladaptation and should be taken into account when planning rehabilitation measures [3].

In recent years, the attention of researchers has been drawn to personality characteristics such as resilience and hardiness, which are important for combating the severe psycho-emotional stress of war [4, 5].

Resilience is considered as a dynamic process that determines the individual's ability to restore adaptive and effective psychosocial functioning and ensure personal growth after a period of maladaptive functioning, which is a consequence of the disorganizing action of psychotraumatic factors [6]. Resilience is one of the key factors that determines a serviceman's ability to counteract the stress of combat operations and restore normal mental functioning [7]. It is important that resilience

in modern clinical psychology and psychotherapy is not considered as a static innate trait, but as a dynamic personality characteristic that can change in the course of life and is subject to correction [8, 9].

Along with resilience, hardiness is important for resistance to psycho-emotional stress, which is considered a psychological trait that allows an individual to maintain a normal psychological state under the influence of extraordinary stressful events and remain healthy despite severe stress [10, 11]. In the structure of hardiness, three components are distinguished: commitment (an individual's conviction that participation in events gives him a chance to find something important and interesting), control (confidence in the fact that the struggle with circumstances gives an opportunity to influence the result, even if this influence is not absolute , and success is not guaranteed), and challenge (the belief that all events contribute to personal development through the acquisition of knowledge and experience - both positive and negative) [12]. Hardiness is an important component of the personal characteristics of a serviceman, which determine his professional suitability and effectiveness of functioning in combat conditions, as well as affect the emotional state and behavioral strategies [13, 14].

Social support of combatants is an important factor in their resocialization and psychological adaptation, helping to reduce the severity of post-traumatic symptoms, depression and anxiety [15, 16]. Importantly, social support has been shown to moderate genetic and environmental vulnerability and increase resilience, as confirmed by neurobiological studies; this opens up new perspectives for the development of intervention methods to prevent the development of mental disorders [17].

The COVID-19 pandemic caused a significant negative impact on the mental activity of people around the world, including military personnel [18]. During the pandemic, military personnel experienced greater psychological stress compared to civilians, which, in combination with the impact of professional stress, led to a high risk of developing mental illnesses [19, 20]. A special danger for mental health is the transferred coronavirus infection, which was found to be associated with the presence of both acute and long-term mental disorders, in particular, affective disorders, cognitive dysfunction, somatovegetative manifestations and various forms of dyssomnias [21-23]. At the same time, the impact of negative psychological factors associated with the COVID-19 pandemic. can be effectively reduced by increasing resilience and using constructive behavioral strategies [18]. Hardiness was also found to moderate the impact of stress due to COVID-19 on depressive and anxiety symptoms [24].

The research hypothesis was that combatants with non-psychotic mental disorders who recovered from COVID-19 have a worse resilience, hardiness and social support compared to combatants who did not have COVID-19.

AIM

The aim of the study was to establish the features of resilience, hardiness and social support in combatants with non-psychotic mental disorders who have suffered from COVID-19 in a comparative aspect.

MATERIALS AND METHODS

In compliance with the principles of biomedical ethics on the basis of informed consent, we clinically examined 252 male patients who were directly involved in combat operations, and who applied for psychiatric help in the Ternopil Regional Neuropsychiatric Hospital and for consulting and medical assistance in the Department of Psychiatry, Narcology and Medical Psychology of the Ternopil National Medical University named after I. Y. Gorbachevskiy during 2020-2022. The selection of patients for the study was carried out using a sampling method based on the following criteria: participation in hostilities and the establishment of a diagnosis of non-psychotic mental disorder to the criteria of ICD-10. the criterion for exclusion from the study was the presence of mental disorders of the psychotic register, narcological pathology, as well as concomitant severe somatic diseases. Among these patients, we identified two groups: patients who did not have COVID-19 numbering 132 people (group 1 – G1), and patients who had COVID-19. which was confirmed by enzyme immunoassay or polymerase chain reaction and recorded in the relevant medical documentation, numbering 120 people (group 2 – G2). Comparison of the results of clinical and psychopathological, psychodiagnostic and psychometric studies was carried out by comparing the indicators of patients of the first and second groups.

STUDY DESIGN

The study was carried out in three stages. At the first stage, patients were selected for the study and their medical records were studied. At the second stage, a clinical examination of patients and a psychodiagnostics study using resilience, hardiness and social support scales were carried out. At the third stage, statistical processing, data analysis and generalization of the research results were made.

The study used clinical, anamnestic, psychodiagnostic and statistical methods. Clinical and anamnestic meth-

ods were used to assess the subjective manifestations of affective pathology and their dynamics, the psychodiagnostic method – for a standardized assessment of the level of resilience using the Connor–Davidson resilience scale-10 [25], hardiness using the Brief Hardiness Scale [26] and social support using the Multidimensional Scale of Perceived Social Support (MSPSS) [27], the statistical method – to analyze the differences in indicators.

The examination included a clinical interview organized by the method of a semi-structured clinical interview, during which the presence and detail of complaints from the affective sphere was established, as well as an assessment of the severity of resilience, hardiness and social support.

Statistical analysis of differences in categorized traits was performed using Fisher's exact test (one-sided), quantitative traits - using the nonparametric Mann-Whitney test.

RESULTS

In general, combatants with non-psychotic mental disorders had a moderate level of resilience. At the same time, in combatants who not suffered from COVID-19 the resilience index corresponded to a moderate level (within 21-25 points), and in combatants who were have suffered from COVID-19, it was lower than the average level (Table 1). Differences in resilience scores between groups statistically significant.

When studying the peculiarities of the distribution of the examined combatants into groups depending on the resilience indicator, differences were also found depending on the transferred COVID-19 (Table 2).

Most combatants with non-psychotic mental disorders who have suffered from COVID-19 belonged to the group with a low level of resilience (37.5%), moderate (25.0%) and higher than moderate (24.2%) levels of resilience. The specific weight of combatants with lower than average (7.5%) and high (5.8%) levels of resilience turned out to be the smallest in this group.

In a group of combatants with non-psychotic mental disorders who did not have suffered from COVID-19 the majority of those examined had a higher than moderate level of resilience (36.4%). The specific weight of persons with moderate (22.7%), low (18.9%) and high (16.7%) levels of resilience was also significant, and the smallest – with a lower than moderate level (5.3 %). Statistically significant differences between groups 1 and 2 were found for low, above average and high levels.

Combatants with non-psychotic mental disorders also had a low level of hardiness and its components (table 1). Indicator of commitment in combatants with non-psychotic mental disorders who did not have suffered from COVID-19 turned out to be moderate and the lowest among all components of hardiness. Commitment is associated with the ability to get satisfaction from one's own activity, and its low level creates in the individual a feeling of rejection, detachment from life, a feeling that he is "on the sidelines" of life, and the surrounding world is unfair and indifferent to him. In combatants with non-psychotic mental disorders who have suffered from COVID-19 the commitment rate was low, and significantly lower compared to combatants who did not have suffered from COVID-19.

The control indicator among combatants who did not have suffered from COVID-19 turned out to be significantly higher, than in combatants who have suffered from COVID-19. Control implies the individual's confidence that he chooses his own path, and low control indicators give rise to a feeling of helplessness, powerlessness in the face of circumstances.

Challenge in combatants who did not have suffered from COVID-19 also turned out to be significantly higher, although the quantitative values of the indicator are also not very high. Challenge is associated with the tendency to take risks and the willingness to act despite the lack of reliable guarantees of success through the active assimilation of knowledge and experience and their further use to achieve life goals. A low level of challenge is associated with passivity, reluctance to take risks even in situations when the goal is attractive, a tendency to "go with the flow", and a desire for peace and security.

The general level of hardiness in combatants who have and not have suffered from COVID-19 corresponded to a moderate level, while in persons who did not have suffered from COVID-19 it was significantly higher.

The study of hardiness levels also confirmed these patterns (table 2). Although in groups G1 and G2, most of the examined belonged to moderate hardiness (54.3% and 64.7%, respectively), among those who did not have suffered from COVID-19 there were more people with high hardiness - 30.2% against 11.8%, and among those who were have suffered from COVID-19 - more people with low hardiness - 23.5% against 15.5%.

Interesting data were obtained when studying the features of social support. In general, a high level of social support among combatants with non-psychotic mental disorders was found for the social support of friends (Table 1). Level of family social support in combatants with non-psychotic mental disorders who did not have suffered from COVID-19 also approached a high level, and the level of social support significant other was moderate. Combatants with non-psychotic mental disorders who have suffered from COVID-19 also rated the level of social support from friends the high-

Table 1. Indicators of the resilience, hardiness and social support in combatants with non-psychotic mental disorders

Indicators	Value, points M±SD (conf. limits 95%) / Me [Q_{25}-Q_{75}]		p
	G1, n=132	G2, n=120	
Connor Davidson Brief Resilience Scale			
Resilience	23.41±8.80 (21.89–24.92) / 26.00 [21.00–30.00]	18.08±10.06 (16.26–19.89) / 22.00 [7.00–27.00]	<0.0001
Brief Hardiness Scale			
Commitment	4.89±2.76 (4.42–5.37) / 4.00 [3.00–6.00]	3.50±1.81 (3.17–3.83) / 3.00 [3.00–4.00]	<0.0001
Control	5.38±2.40 (4.96–5.79) / 5.00 [4.00–6.00]	4.44±1.70 (4.13–4.75) / 4.00 [4.00–5.00]	0.0049
Challenge	5.40±2.42 (4.98–5.82) / 5.00 [4.00–6.00]	4.33±1.79 (4.01–4.66) / 4.00 [3.00–5.00]	0.0004
Hardiness	15.67±6.88 (14.49–16.86) / 13.00 [11.00–20.00]	12.28±4.20 (11.52–13.03) / 12.00 [11.00–14.00]	0.0001
Multidimensional Scale of Perceived Social Support			
Family Subscale	4.95±1.58 (4.68–5.22) / 5.00 [3.75–6.50]	3.85±1.54 (3.57–4.13) / 4.00 [2.50–5.25]	<0.0001
Friends Subscale	5.00±1.77 (4.70–5.31) / 5.25 [3.50–6.63]	3.95±1.48 (3.68–4.22) / 4.00 [3.00–5.00]	<0.0001
Significant Other Subscale	4.73±1.53 (4.47–4.99) / 4.75 [3.75–6.00]	3.83±1.51 (3.56–4.10) / 3.75 [2.88–5.00]	<0.0001
Total score	14.68±4.62 (13.89–15.48) / 15.25 [11.00–18.75]	11.63±4.29 (10.85–12.40) / 11.63 [8.63–15.00]	<0.0001

Source: compiled by the authors of this study

Table 2. Structure of levels of the resilience, hardiness and social support in combatants with non-psychotic mental disorders

Level	G1, n=132		G2, n=120		p
	abs.	%	abs.	%	
Connor Davidson Brief Resilience Scale					
Low resilience	25	18.9	45	37.5	0.0008
Below moderate resilience	7	5.3	9	7.5	0.3239
Moderate resilience	30	22.7	30	25.0	0.3913
Above moderate resilience	48	36.4	29	24.2	0.0245
High resilience	22	16.7	7	5.8	0.0056
Brief Hardiness Scale					
Low hardiness	20	15.5	28	23.5	0.0679
Moderate hardiness	70	54.3	77	64.7	0.0480
High hardiness	39	30.2	14	11.8	0.0004
Multidimensional Scale of Perceived Social Support					
Family Subscale					
Low support	15	11.4	32	26.7	0.0015
Moderate support	52	39.4	55	45.8	0.1827
High support	65	49.2	33	27.5	0.0003
Friends Subscale					
Low support	20	15.2	26	21.7	0.1212
Moderate support	42	31.8	70	58.3	<0.0001
High support	70	53.0	24	20.0	<0.0001
Significant Other Subscale					
Low support	20	15.2	30	25.0	0.0359
Moderate support	54	40.9	61	50.8	0.0731
High support	58	43.9	29	24.2	0.0007

Source: compiled by the authors of this study

est, but the rate was significantly lower compared to combatants who did not have suffered from COVID-19. In a group of combatants with non-psychotic mental disorders who have suffered from COVID-19 indicators of family support and significant other support were moderate and significantly worse.

Most combatants who did not have suffered from COVID-19 belonged to the group with a high level of family support, friends support, and significant other support: 49.2%, 53.0%, and 43.9%, respectively, the percentage of people with a moderate level of support was smaller in this group: 39.4%, 31.8%, and 40.9%, respectively. The smallest among combatants who did not have suffered from COVID-19 there was a specific weight of people with a low rating of family support, friends support, and significant other support: 11.4%, 15.2%, and 15.2%, respectively (Table 2). Instead, in the group of combatants who have suffered from COVID-19 persons with a moderate assessment of family support, friends support and significant other support prevailed: 45.8%, 58.3% and 50.8%, respectively. In this group, there were slightly more people with a high level of family support (27.5%) than people with a low level (26.7%), and more people with a low level of friends support (21.7%) and significant other support (25.0%) than with high level (20.0% and 24.2%, respectively).

DISCUSSION

The differences we identified in the resilience indicators of combatants with non-psychotic mental disorders depending on the experienced COVID-19 are consistent with modern concepts of resilience, which consider it as a dynamic process that is closely related to the state of the affective sphere, cognitive functioning and can change under the influence of external factors (Assonov D., 2021; Niederhauser M. et al., 2023; Zueger R. et al., 2023). In previous studies, we found them in combatants who have suffered from COVID-19 higher levels of depression and anxiety, as well as a worse state of cognitive functioning compared to combatants who have suffered from COVID-19 [28, 29]. The neuropsychiatric consequences of COVID-19 include persistent affective and cognitive disorders that significantly affect the general state of functioning of patients (Ismael F. et al., 2021; Renaud-Charest O. et al., 2021) [30, 31], which can also cause a decrease in the ability to resist stress, and therefore, a decrease in resilience indicators. Resilience is formed by a set of interrelated competencies that influence each other (Bekesiene S. et al., 2024) [7], and the psychological mechanisms linking such a psychological construct as resilience with behavior are still very complex unclear (Sefidan S. et al., 2021), although the connection of greater resilience with a lower level of stress and better functioning in military personnel is not in doubt [4].

Similarly, hardiness is related to the state of the affective sphere, and less hardiness is associated with high levels of depression and the use of non-constructive behavioral strategies (Bartone P.T., Homish G.G., 2020) [14]. Our study confirmed these patterns, and our data are consistent with the data of other researchers who found an inverse relationship between hardiness and the severity of the consequences of stress, as well as the influence of psychosocial factors on hardiness (Bartone P.T. et al., 2022 [24]; Bekesiene S. et al., 2024 [7], et al.). Hardiness, like resilience, is a dynamic characteristic and can change over time (Kokun O. et al., 2023) [13], which determines the need to analyze not only the impact of hardiness on the state of the affective sphere and the effectiveness of an individual's functioning, but also the dynamics of hardiness in the process of life, especially in critical periods of life.

In general, we tend to believe that lower levels of resilience and hardiness are found in combatants who have suffered from COVID-19 mediated by neuropsychiatric consequences of coronavirus infection, primarily persistent depressive, anxiety, dyssomnic and somatovegetative manifestations, as well as deterioration of cognitive functioning. These persistent changes in some patients, who probably have a certain personal predisposition, acquire the character of patho-characteristic transformations, leading to a change in the general psychological model of response and behavioral strategies, while others do not undergo significant patho-characteristic changes. This is confirmed by the heterogeneity of the studied groups: among combatants who did not have suffered from COVID-19 there were individuals with low resilience and hardiness, and among combatants who were sick with COVID-19 – individuals with high resilience and hardiness.

Lower ratings of social support in combatants who have suffered from COVID-19 in our opinion, they are also associated with a greater expressiveness of depressive and anxiety manifestations in them, which confirms the connection of worse social support with the expressiveness of psychopathological symptoms and resistance to stress found in other studies (Ozbay F. et al., 2007 [17]; Blais R.K. et al., 2021; Pollmann J.B. et al., 2022) [16]. At the same time, a greater expressiveness of psychopathological symptoms, a lower level of resilience and hardiness contributes to psychosocial maladjustment, worsening microsocial relations and leading to a decrease in social support.

CONCLUSIONS

1. Combatants with non-psychotic mental disorders are characterized by a low level of resilience, significantly lower in combatants who have suffered from COVID-19: 18.08±10.06 points vs 23.41±8.80 points in combatants who were not sick with COVID-19 (<0.0001).
2. To combatants with non-psychotic mental disorders who have suffered from COVID-19 inherent reduced levels of hardiness (12.28±4.20 points vs 15.67±6.88 points, p<0.001) and its components: commitment (3.50±1.81 points vs 4.89±2.76points, p<0.01), control (4.44±1.70 points vs 5.38±2.40 points (p<0.01) and challenge (4.33±1.79 points against 5.40±2.42 points, p<0.001).
3. Combatants with non-psychotic mental disorders who did not have suffered from COVID-19 rate the level of social support of family, friends, and significant other as moderate (4.95±1.58 points, 5.00±1.77 points, and 4.73±1.53 points, respectively), and combatants who have suffered from COVID-19 as lower than moderate (3.85±1.54 points, 3.95±1.48 points and 3.83±1.51 points, respectively). General level of social support in combatants who have suffered from COVID-19 significantly lower compared to combatants who did not have suffered from COVID-19 (11.63±4.29 points vs. 14.68±4.62 points, p<0.0001).
4. Reduction of resilience, vitality and level of social support in combatants who have suffered from COVID-19 probably mediated by long-term neuropsychiatric consequences of coronavirus infection, in particular, persistent anxiety, depressive, dyssomnic, somatovegetative and cognitive disorders.

LIMITATION

This study used short scales for assessing resilience and hardiness, one for each of these psychological characteristics. The impact on the results of measuring resilience, vitality and social support of depressive and other psychopathological disorders present in the examined persons was not evaluated. The study contingent is represented only by male combatants with non-psychotic mental disorders.

PROSPECTS FOR FURTHER RESEARCH

Related to the study of the mutual influence of psychopathological symptoms and resilience, vitality and social support in both combatants and the civilian population, as well as improvement of existing methods of developing resilience and vitality in combatants.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Carbenoxolone attenuates renal ischemia-reperfusion injury in rats by modulating inflammatory cytokines, oxidative stress markers, and apoptotic pathways

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ABSTRACT

Aim: This study investigates the protective effects of Carbenoxolone (Cbx) in attenuating renal injury through the modulation of the FOXO3 gene.

Materials and methods: Twenty-eight adult male Wistar Albino rats were randomly divided into four groups (N=7): Sham (laparotomy without ischemia), Control (bilateral renal ischemia for 30 min followed by 2 h reperfusion), Vehicle (DMSO injection prior to IRI), and Carbenoxolone-treated (Cbx injection three days before IRI).

Results: Tissue levels of TNF-α, IL-1β, MDA, Caspase-3, and KIM-1 were significantly elevated in the control and vehicle groups, while GSH levels were reduced. Cbx treatment significantly decreased TNF-α, IL-1β, MDA, Caspase-3, and KIM-1 while increasing GSH levels, indicating enhanced antioxidant defense. FOXO3 expression was notably lower in the Cbx group compared to the control and vehicle groups. Histopathological analysis further confirmed reduced renal damage in Cbx-treated rats. These findings suggest that Cbx exerts renoprotective effects by mitigating oxidative stress, inflammation, and apoptosis.

Conclusions: Carbenoxolone significantly reduces RIRI-induced kidney damage, demonstrating its potential as a therapeutic agent for renal ischemic injuries.

KEY WORDS: Carbenoxolone, RIRI, IL-1β, TNFα, KIM-1, FOXO3

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ABBREVIATIONS

Cbx - Carbenoxolone,
I/R - ischemia reperfusion
KIM-1 - kidney injury molecule 1
TNFα - tumor necrosis factor alpha,
IL-1β - interleukin -1 beta,
FOXO3 - Forkhead Box O3,
DMSO - dimethylsulfoxide

INTRODUCTION

Renal ischemia-reperfusion injury (RIRI) is a major cause of acute kidney injury (AKI), with the inflammatory response playing a critical role in its pathogenesis [1-2]. Following re-oxygenation, ischemia-reperfusion injury occurs primarily due to the excessive production of reactive oxygen species (ROS), leading to various cellular outcomes, including programmed cell death, necrosis, and the release of injury-associated factors that serve as initiators of the inflammatory response [3-4]. Apoptosis has two diverse mechanisms: the first pathway (extrinsic) is activated by the death receptors, a subgroup of the tumor necrosis factor (TNF) receptor family and the intrinsic pathway which focuses on mitochondria [5]. The renal injury resulting from ischemia-reperfusion is a dynamic process involving complex interactions among inflammatory mediators, oxidative stress, and lipid peroxidation, all of which exacerbate tissue damage [6]. The PI3K/Akt signaling pathway plays a significant role in this process by phosphorylating FOXO3, a transcription factor involved in regulating apoptosis, oxidative stress response, and cell cycle progression [7]. Oxidative stress, characterized by the excessive generation of ROS, contributes to renal cell damage by triggering lipid peroxidation and depleting crucial antioxidants such as glutathione (GSH). The accumulation of malondialdehyde (MDA), a lipid peroxidation by-product, serves as a marker of oxidative stress and correlates with the severity of renal injury. These reactive oxygen species may lead to damage of mitochondrial functional, energy imbalances and cause apoptosis [8]. Additionally, inflammatory cytokines, including tumor

necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), are key mediators in the pathogenesis of AKI, further amplifying the inflammatory response. Renal function is commonly assessed through biomarkers such as kidney injury molecule (KIM-1), which provide insight into the extent of ischemia-reperfusion injury[4] Multifunctional compounds with antioxidant, anti-inflammatory, and anti-inflammatory capabilities serve as the best defenses in cases of kidney damage. The protective properties of antioxidants can be explained by their ability to restore intracellular processes related to oxidative damage kinetics [9-10]. In addition, antioxidant therapy can protect against the oxidative damage caused by infrared radiation. Antioxidant compounds have been found to restore intracellular processes related to oxidative damage, potentially contributing to their protective properties [11]. Inflammation caused by ischemia-reperfusion injury (IRI) can lead to further renal damage, but protecting against this occurrence is possible [12]. Pro-inflammatory cytokines, such as tumor necrosis factor (TNF-α), interleukin-1-B (IL-1B), and interleukin-6 (IL-6), play a primary role in renal disease [13-14] This study aimed to investigate the nephron-protective effects of Carbenoxolone on renal ischemia-reperfusion injury in rats by measuring the following parameters: Kidney injury molecule 1 (KIM-1), interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), GSH, MDA, Caspase-3, Foxo3 gene expression and histological examination.

MATERIALS AND METHODS

PREPARATION OF ANIMALS

In this study, 28 adults male Wistar Albino rats aged more than 20 weeks and an average weight of 300±50 g were used. All animals had free access to food and water and were subjected to a 12:12 light-dark cycle. The temperature and humidity were controlled at 25 °C and 60-65%, respectively. The rat handling, experiments, and tests complied with the Ethical Conduct for Use of Animals guidelines and regulations. The animals were housed at the animal house of the College of Sciences, University of Kufa. The materials were procured from Medchem express, Sunlong Technology Lab, China and Macrogen/ Korea.

ETHICAL STATEMENT AND APPROVAL

The study was done in the department of pharmacology and toxicology, Faculty of Pharmacy, University of Kufa and in Middle Euphrates Unit for Cancer Researches, Faculty of Medicine, University of Kufa. The study was accepted by Committee center of Bioethics in the University of Kufa and its representative in Faculty of Pharmacy. Whole procedures were done according to the recommendations of the Committee.

All procedures involving the handling and experimentation on rats, as well as the conducted tests, were carried out in compliance with the applicable guidelines and regulations for the ethical use of animals, Kufa University (20545 in 29/8/2024). The animals were housed in the animal facility at the College of Sciences, University of Kufa.

CARBENOXOLONE

The pure powder of Carbenoxolone disodium was gutted from medchemexpress, USA Company. Formal name: (3β,20β)-3-(3-carboxy-1-oxopropoxy)-11-oxo-olean-12-en-29-oic acid, disodium salt Chemical name: Carbenoxolone disodium, Chemical Formula: $C_{34}H_{48}O_7 \cdot 2Na$, CAS Number: 7421-40-1, Molecular Weight: 614.7, Physical Description: A crystalline solid, Color: White to off-white, Solubility: In DMSO: 12.5 mg/mL. DMSO was considered the standard vehicle for preparation this drug before use. The dose of drug that used was 200mg\kg of rat weight intraperitoneal [15].

EXPERIMENTAL DESIGN

In this study, Wistar Albino rats were randomly selected and divided into four groups, each consisting of seven rats, subjected to different handling procedures. The sham group served as the negative control and underwent no IRI procedure. The control group underwent bilateral renal ischemia by clamping the renal pedicles for 30 minutes, serving as the positive control. The vehicle-treated group received an intraperitoneal injection of DMSO given every 24 hr. for 2 consecutive days (before the day of decapitation) and 1 h before ischemia induction on third day and undergone bilateral renal ischemia for 30 min and reperfusion for 2 hours. Rats in the last group received Cbx. intraperitoneal injection 200 mg/kg given every 24 hr. for 2 consecutive days (before the day of decapitation) and 1 h before ischemia induction on third day.

RENAL ISCHEMIA REPERFUSION INJURY RAT MODEL

Rats were anesthetized, maintained at 37°C, and underwent midline incisions to expose renal pedicles. Ischemia was induced by clamping both pedicles for 30 minutes, followed by reperfusion. Hydration was ensured with sterile saline. After suturing, bilateral nephrectomy was performed, and kidneys were washed

Table 1. Primers used in this study

Host	Gene		5′-3′	Product (bp)	Accession number	Reference
Rattus	FOXO-3	F	AAAGGGGAAATGGGCAAAGC	83	XM_032888496.1	Nayakanti, 2022
		R	GGCTGAGAGCAGATTTGGCA			
Rattus	GAPDH	F	ATGACTCTACCCACGGCAAG	89	NM_017008	Kunst et al., 2012
		R	CTGGAAGATGGTGATGGGTT			

with precooled PBS to remove blood. Finally sacrificed the rat by heart puncture [16]. The left kidney was cut sagittal into 2 halves. The first half was kept in deep freeze for bimolecular assessment. While the second half was inserted in 10% formalin then embedded in paraffin for histopathological.

PREPARATION OF TISSUE FOR MEASUREMENT OF IL1-B, TNF-A, GSH, MDA, CASPASE-3 AND KIM-1 BY ELISA

At the end of the surgical procedure, the left kidney was taken from the dead rat and rinsed by ice-cold normal saline to remove any blood clot and then dissected to two parts. One section was taken and homogenized with a high intensity ultrasonic liquid processor in 1:10 (w/v) phosphate buffered saline that contained 1% Triton X-100 and a protease inhibitor cocktail. The homogenate was centrifuged at 26,310 x g for 20 min at 4°C. The supernatant was collected for determination of IL1-β, TNF-α, GSH, MDA, KIM-1 and Caspase-3 by ELISA technique (Bio-Elisa Reader-BioTek Instruments, USA).

TISSUE SAMPLING FOR HISTOPATHOLOGY ANALYSIS AND DAMAGE SCORES

The left kidney tissue sample was fixed in 10% formalin, processed through alcohol dehydration, xylene clearing, and paraffin embedding. Thin sections (5 µm) were stained with Hematoxylin and Eosin (H&E) for histopathological analysis. A blinded investigator assessed tissue damage using a quantitative scoring system under light microscopy at 40× magnification. Renal tubular injury was evaluated based on epithelial swelling, vacuolar degeneration, brush border loss, necrosis, and cast formation. The histological damage was categorized as follows: Score 0 (normal), Score 1 (<25% damage), Score 2 (25%–50%), Score 3 (50%–75%), and Score 4 (>75%).

ASSESSMENT OF TISSUE FOXO3 GENE EXPRESSION BY RT-QPCR

1. Total RNA Extraction Using Easy-spin™ (DNA free) Total RNA Extraction Kit
2. cDNA Synthesis (Using Add Script cDNA Synthesis Kit)
3. Preparation of Primers
4. Primers Used in this Study (Table 1)[17-18]
5. Protocol of GoTaq® RT-qPCR System for Real-Time qPCR (Gene expression assay).

STATISTICAL ANALYSIS

Statistical analyses were performed using GraphPad Prism and SPSS 28.0 for window. Inc. Data were expressed as mean ± SD. Analysis of variance (ANOVA) was used for the multiple comparisons among all groups followed by post–hoc tests using Bonferroni method. For the histopathological renal changes, the Kruskal-Walli's test was used to assess the statistical significance of difference across multiple groups in total severity score (mean score) for histopathological renal changes. In all tests, P≤0.001 was considered statistically significant.

RESULTS

EFFECTS OF CARBENOXOLONE ON KIDNEY INJURY MOLECULE-1 (KIM-1)

The renal tissue level of (KIM-1) in control and vehicle groups were significantly ($p<0.001$) higher than that in the sham group, alternatively, the level of KIM-1 in the Cbx treated group is significantly ($p<0.001$) lower than that in the control group (Fig 1A).

EFFECT OF CARBENOXOLONE ON THE OXIDATIVE STRESS MARKER GLUTATHIONE (GSH)

The mean GSH levels were significantly lower in the Control and control vehicle group compared to the Cbx treated groups ($P<0.001$) while slightly decreased when compare with the sham group (Fig 1B)

EFFECT OF CARBENOXOLONE ON THE OXIDATIVE STRESS MARKER GLUTATHIONE (GSH)

The renal tissue level of MDA in control and vehicle groups were significantly $p<0.001$ higher than that in

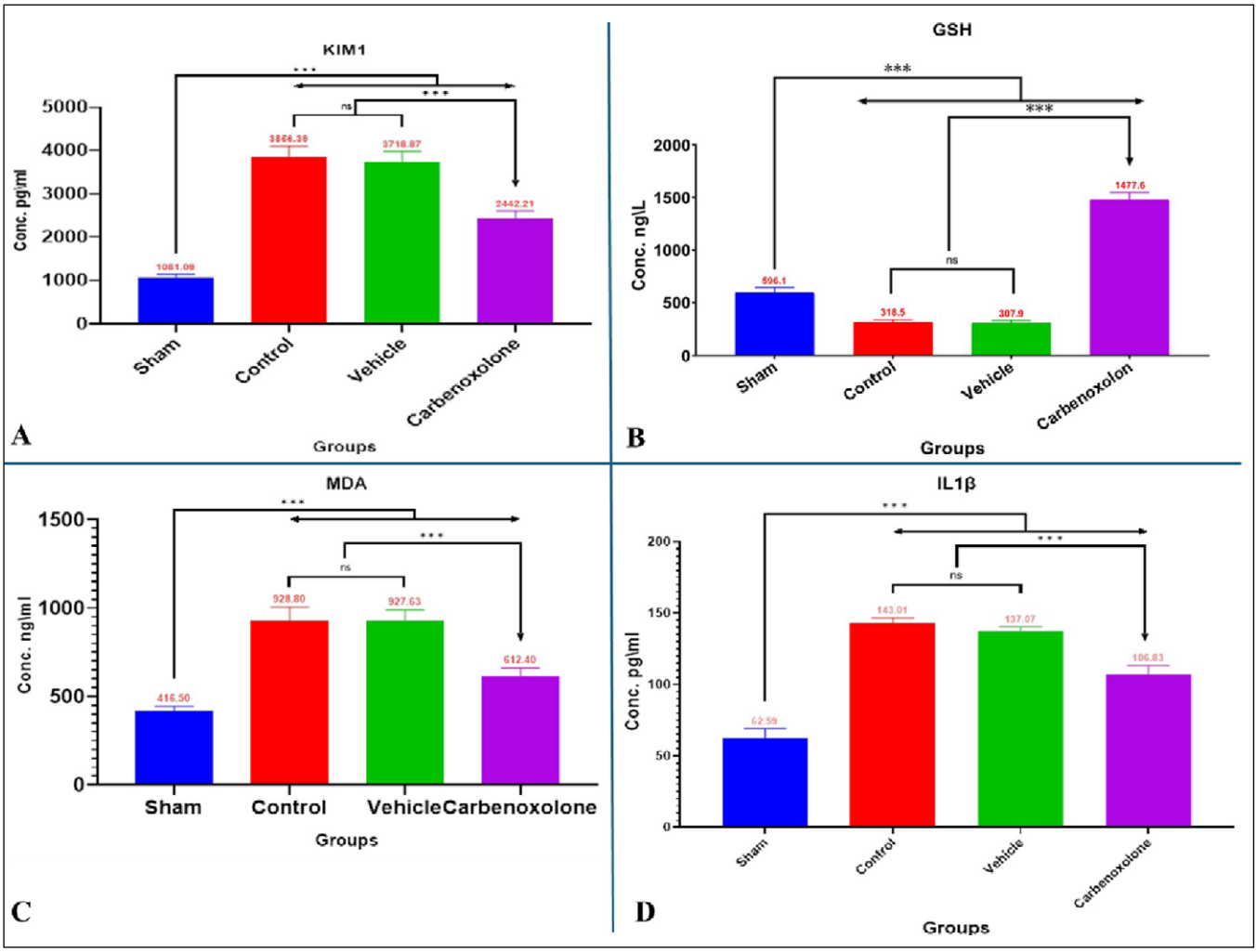


Fig 1. A. The statistical analysis of KIM1 concentrations mean (pg/ml) in renal tissues in the four experimental study groups at the end of the study (No of rats = 7 in each study group) Sham group *vs.* vehicle & control groups, ***P. value < 0.001. Cbx. *vs*. vehicle & control groups, ***P. value < 0.001, **B.** The statistical analysis of GSH concentrations mean (ng/L) in renal tissues in the four experimental study groups at the end of the study (No of rats = 7 in each study group). Sham group *vs.* vehicle & control groups, ***P. value < 0.001. Cbx. *vs.* vehicle & control groups, ***P. value < 0.001**,* **C.** The statistical analysis of MDA concentrations mean (ng/ml) in renal tissues in the four experimental study groups at the end of the study (No of rats = 7 in each study group). Sham group *vs*. vehicle & control groups, ***P. value < 0.001. Cbx *vs*. vehicle & control groups, ***P. value < 0.001, **D.** The statistical analysis of IL-1β concentrations mean (pg/ml) in renal tissues in the four animal study groups at the finishing of the research (No of rats = 7 in each study group).
Sham group *vs.* vehicle & control groups, ***P. value < 0.001. Cbx. *vs*. vehicle & control groups, ***P. value < 0.001
Source: Own materials.

the sham group, in contrast, their levels in the Cbx were significantly p<0.001 lower than that in the control group (Fig. 1C).

EFFECT OF CARBENOXOLONE ON THE INTERLEUKIN -1 BETA

There was a significant P<0.001 difference in IL-1β levels among the studied groups. The sham group had the lowest levels than all other groups (P<0.001 for all comparisons). Conversely, IL-1β levels were significantly (P<0.001) lower in the Cbx treated group compared with control groups (Fig. 1D).

EFFECT OF RIRI, CARBENOXOLONE ON THE TUMOR NECROSIS FACTOR-ALPHA

The renal tissue level of (TNF-α) in control and vehicle groups were significantly p<0.001 higher than that in the sham group, On the other hand, their levels in the Cbx were significantly p<0.001 lower than that in the control group (Fig. 2).

EFFECTS OF CARBENOXOLONE ON THE MARKER OF APOPTOSIS, CASPASE3

The renal tissue level of Caspase3 in control and vehicle groups were significantly (p<0.001) higher than that in the sham

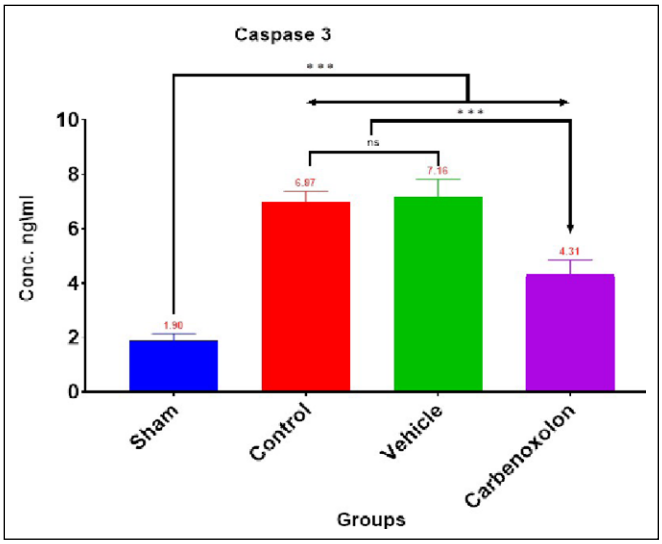


Fig. 2. The statistical analysis of TNFα concentrations mean (ng/L) in renal tissues in the four animal study groups at the end of the study (No of rats = 7 in each study group)
Sham group *vs*. vehicle & control groups, ***P. value < 0.001, Cbx *vs*. vehicle & control groups, ***P. value < 0.001
Source: Own materials.

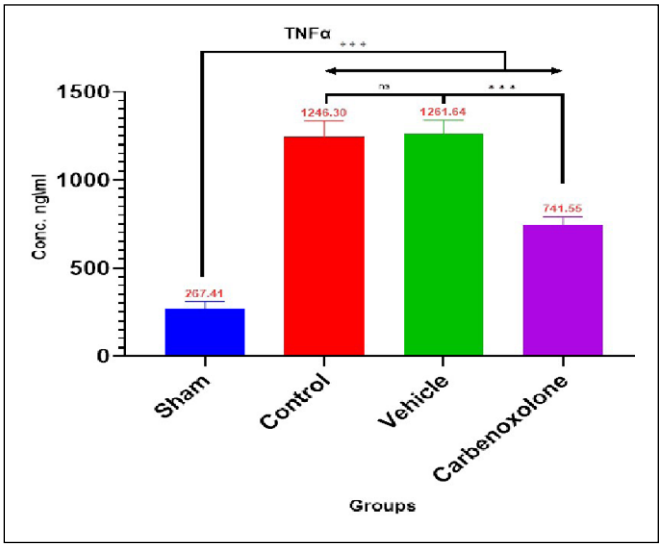


Fig. 3. The statistical analysis of caspase-3 concentrations mean (ng/L) in renal tissues in the four animal study groups at the end of the study (No of rats = 7 in each study group)
Sham group *vs*. vehicle & control groups, ***P. value < 0.001, Cbx vs. vehicle & control groups, ***P. value < 0.001
Source: Own materials.

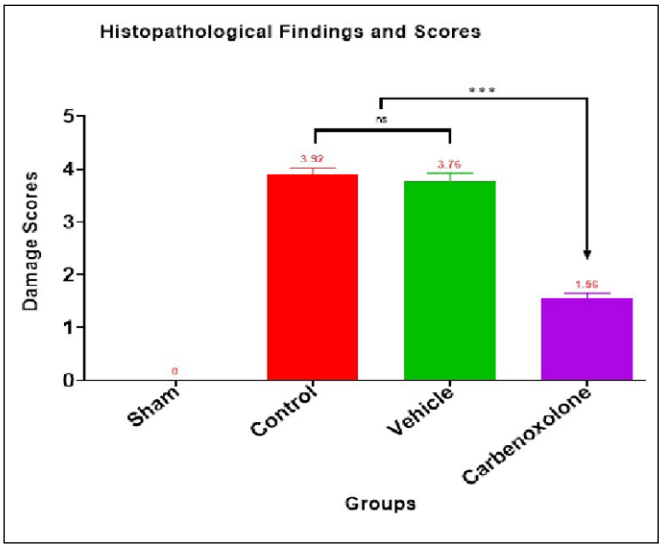


Fig. 4. Mean of fold change of FOXO3 gene expression in renal tissue of the four experimental groups at the end of the study (No of animals = 7 in each group)
Sham group *vs*. vehicle & control groups, ***P. value < 0.001, Cbx *vs*. vehicle & control groups, ***P. value < 0.001
Source: Own materials.

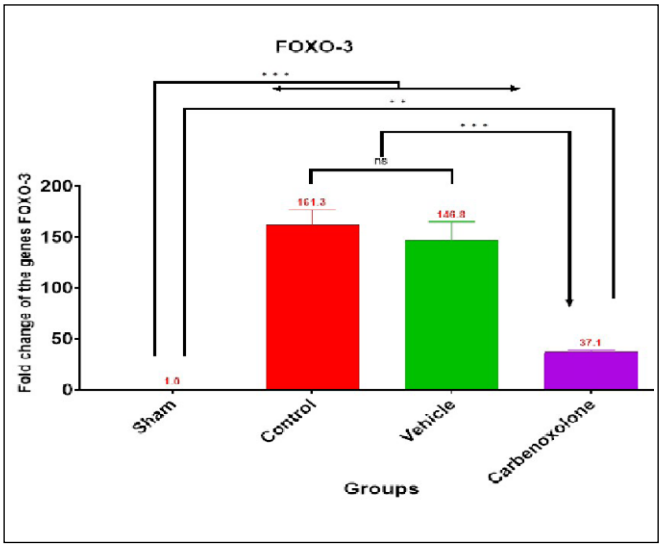


Fig. 5: Score severity mean of renal tissue histopathology of the four experimental groups at the end of the study (No of animals = 7 in each group)
Sham group vs. vehicle & control groups, ***P. value < 0.001, Cbx vs. vehicle & control groups, ***P. value < 0.001
Source: Own materials.

group, On the other hand, their levels in the Cbx were significantly (p<0.001) lower than that in the control group (Fig. 3).

THE EFFECT OF CARBENOXOLONE ON THE INTRACELLULAR SIGNALING (FOXO3) AFTER RIRI BY PCR TECHNIQUE

The renal tissue levels of FOXO3 expression in control and vehicle groups were significantly P<0.001 higher than that in the sham group, Meanwhile, the expression level of FOXO3 in the Cbx treated group is significantly P<0.001 lower than that in the control group (Fig. 4).

HISTOPATHOLOGY FINDINGS

Results of the histopathological examination of renal tissuesc from the four study groups are shown in figures 5, 6A-D, 7.

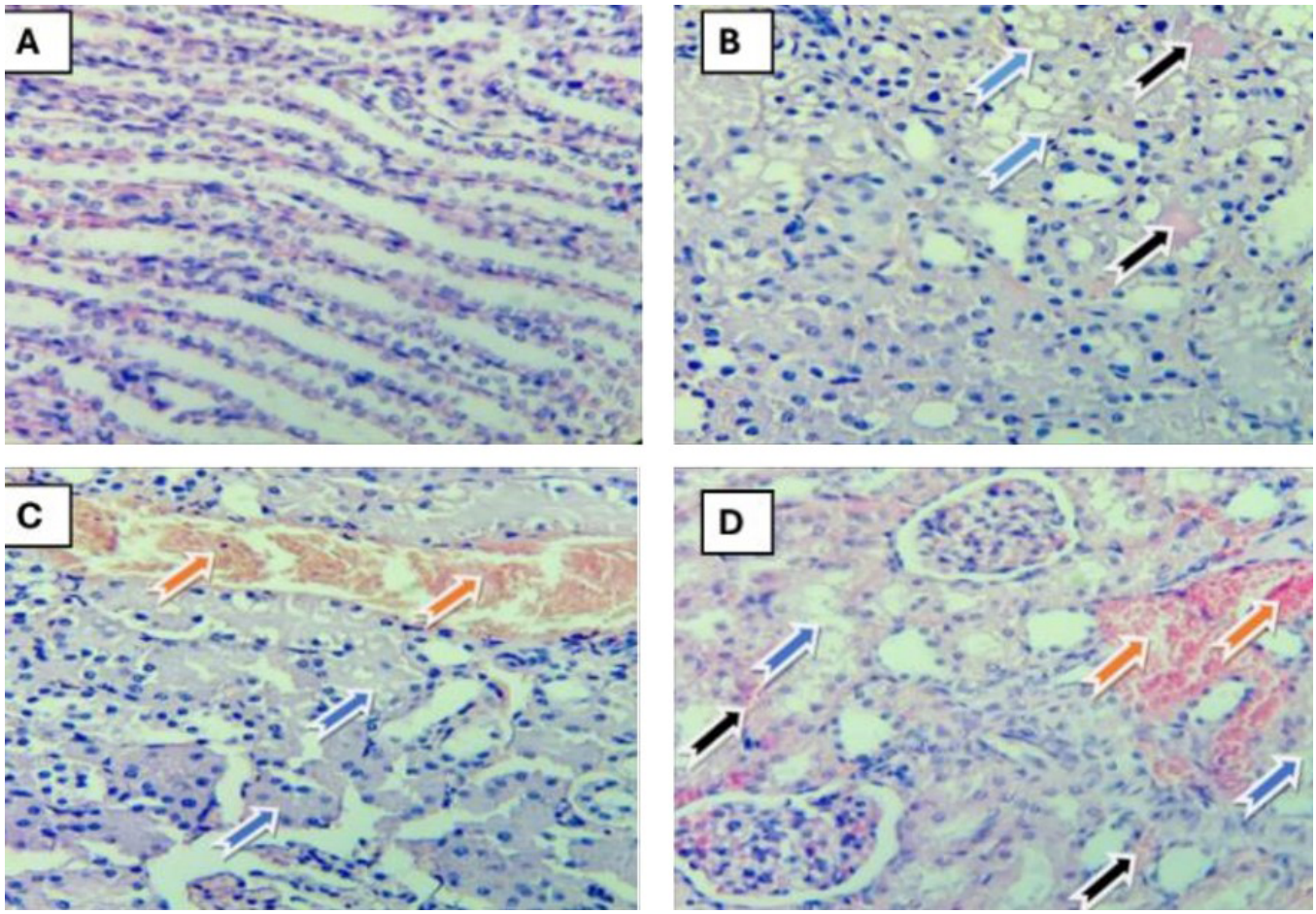


Fig 6. Results of the histopathological examination of renal tissues from the four study groups: **A**-Sham group, a microscopic cross section of left kidney represented normal tissues histology, normal renal tubules, and normal cell size and there is no cast formation, cells edema or loss of brush boarder. Sham group. H & E stain × 400.The mean of the severity score was zero (score severity mean = 0 and represent 0% of damage renal tubules), **B** Control group. Rat kidney, renal tubules with score 4 damage involving 100% of the examined tubules. Cytoplasmic vacuoles (blue arrows) and eosinophilic cast (black arrows). H&E. X400, **C** Control group. Rat kidney, renal tubules with score 4 damage involving 100% of the examined tubules. Vascular congestion (orange arrows) and cytoplasmic eosinophilia (blue arrows). H&E. X400, **D** Vehicle group. Rat kidney, renal tubules with score 4 damage involving 95% of the examined tubules. Cytoplamic swelling and increased cytoplasmic eosinophilia (black arrows), cytoplasmic vacuoles (blue arrows), hemorrhage (orange arrows), H&E. X400
Source: Own materials.

DISCUSSION

Acute kidney injury caused by renal IRI is often associated with high morbidity and mortality and ROS play a critical role in this pathological process [19] ROS can trigger inflammation, increase vasoconstrictors production, and induce tubular cell apoptosis and necrosis [20]. This study investigated the nephroprotective effects of Cbx in RIRI. Cbx treatment reduced Kidney Injury Molecule-1 (KIM-1), indicating renal protection, and attenuated inflammation by lowering TNF-α and IL-1β levels. It also decreased oxidative stress (MDA) while enhancing antioxidant defenses (GSH). Additionally, Cbx downregulated Caspase-3, suggesting anti-apoptotic effects, and modulated FOXO3, implicating its role in cellular survival. These findings highlight Cbx's potential as a therapeutic candidate for AKI management through antioxidant, anti-inflammatory, and anti-apoptotic mechanisms.

EFFECTS OF RIRI, CARBENOXOLONE ON KIDNEY INJURY MOLECULE-1 (KIM-1)

In this experimental work, notably, the groups treated with Cbx exhibited significantly P≤0.001 lower levels of KIM-1 compared to the control and vehicle groups. In contrast, the control and vehicle groups exhibited a marked increase in KIM-1 levels of renal tissue relative to the sham group. These findings are consistent with the results of [21] who demonstrated that KIM-1 is primarily expressed in kidney and drastically upregulated in injured renal tubules. Therefore, KIM-1 is regarded as a sensitive and specific biomarker for kidney inju-

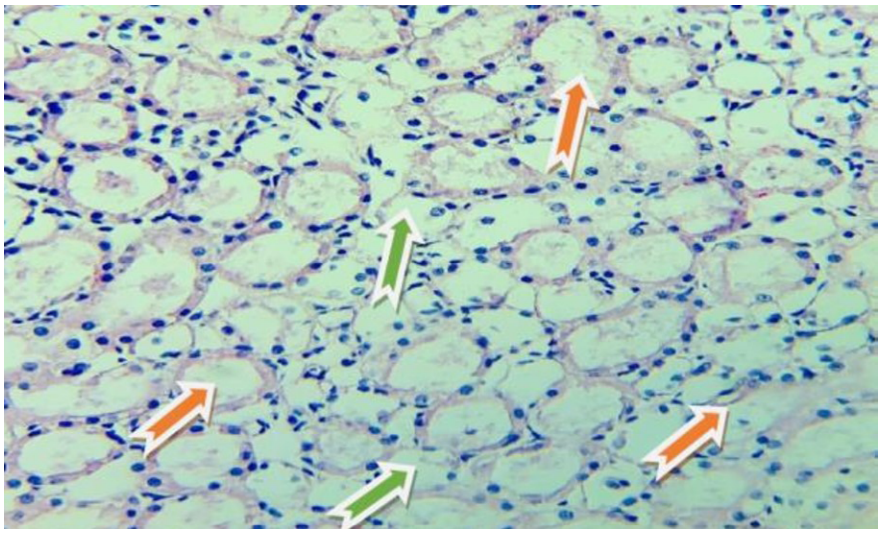


Fig. 7. Cbx treatment group
Rat kidney with score 2 damage involving 35% of the examined tubules. Damaged tubules (orange arrows) and normal tubules (green arrows). H&E. X400
Source: Own materials.

ry which has been approved by the FDA to evaluate nephrotoxicity. Previous studies have established that KIM-1 expression on the apical surface of renal proximal tubule epithelial cells is induced by ischemia and toxic damage [22-23]. The lower KIM-1 levels observed in the treatment groups in this study suggest a potential nephroprotective effect of Cbx in RIRI. To the best of our knowledge, no previous studies have investigated the impact of Cbx on KIM-1 levels in the subject of RIRI. The reduction in KIM-1 levels observed in the present study is likely attributable to the anti-inflammatory and antioxidant properties of this agent. These findings align with the results of [24] who demonstrated that, the antioxidant effect of pioglitazone by reducing the inflammation, oxidative stress, and renal tubular damage through targeting various cellular pathways, including TNF-α, NF-κB signaling, KIM-1, and NGAL pathways.

EFFECTS OF RIRI, CARBENOXOLONE ON TUMOR NECROSIS FACTOR-ALPHA (TNF-A)

The present study revealed that pretreatment with Cbx prior to ischemia induction significantly $P \leq 0.001$ reduces the level of TNF-α in renal ischemic tissues, as compared to the levels of these inflammatory cytokines in the IR control and vehicle groups these findings are consistent with previous studies shown that the levels of TNFα was increased after 30 min of ischemia followed by 2hrs of reperfusion in rats, and there was significant changes in endothelial function and the elevation in this parameter contributes to the endothelial dysfunction [25], also another study in a rat model showed that the level of the TNFα gene was highly elevated in injured kidney tissues in rats that are undergone nephrectomy of the right kidney and ischemia in the left kidney for 45 min then reperfusion [26]. Additionally, [27] demonstrated that the treatment with Cbx could significantly decrease levels of inflammatory factors such as IL-6 (Interleukin 6) and TNF- a (Tumor necrosis factor-a).

EFFECTS OF RIRI, CARBENOXOLONE ON INTERLEUKIN 1 BETA (IL-1B)

In the current study, IL-1β levels were significantly ($P \leq 0.001$) higher in the control group receiving RIRI without treatment, whereas the vehicle group did not differ significantly from the control, suggesting that there were no anti-inflammatory effects. These findings are consistent with earlier research [28], which found that rats that experienced 30 minutes of ischemia and two hours of reperfusion had higher levels of serum creatinine, urea, IL-1β, and caspase-3. In contrast, Cbx-treated groups showed significantly ($P \leq 0.001$) lower levels of IL-1β, indicating that it may have anti-inflammatory effects by inhibiting IL-1β. According to [29], Cbx has anti-inflammatory, antiviral, antitumor, antibacterial, antifibrotic, and neuroprotective qualities. This finding is in line with their findings. By raising IL-10 and decreasing TNF-α, IL-1β, IL-6, and monocyte infiltration, Cbx treatment decreased lung inflammation in pulmonary hypertension models. Cbx reduces IL-1β. By lowering IL-1β, Cbx may mitigate RIRI-induced inflammation and renal damage.

EFFECTS OF RIRI, CARBENOXOLONE ON MALONDIALDEHYDE (MDA)

In this experimental rat model study, the results indicated that the control and vehicle group, which experienced RIRI without any therapeutic intervention, exhibited significantly $P \leq 0.001$ higher levels of MDA as compare with sham group. The increase reflects the oxidative stress induced by ischemic injury. This result are

agreement with those reported by [30] which revealed that RIRI is characterized by a burst of ROS formation, reduction of antioxidants, significantly reduced SOD activity and increased MDA content, resulting in increased oxidative stress and tissue damage. In contrast, the group treated with Carbenoxolone demonstrated significantly P≤0.001 lower level of MDA compared to both the control and vehicle groups. This reduction in MDA levels indicates that Cbx may exert antioxidant effects, potentially through mechanisms that involve the scavenging of free radicals or the enhancement of endogenous antioxidant defenses. These findings align with the work of [31] who demonstrated that the administration of Cbx leads to a significant reduction in MDA levels, a well-established biomarker for lipid peroxidation and oxidative damage. Also, another study reported by [32], demonstrated that treatment with rotenone, a mitochondrial complex I inhibitor, significantly increased ROS levels, this rise in ROS was associated with increased lipid peroxidation. Importantly, co-administration of Cbx effectively mitigated these effects. Cbx treatment not only reduced ROS levels but also reversed the increase in lipid peroxidation in rotenone-treated animals.

EFFECTS OF RIRI, CARBENOXOLONE ON ANTIOXIDANT MARKER GLUTATHIONE (GSH)

GSH is a critical endogenous antioxidant that plays a vital role in protecting cells from oxidative stress by neutralizing free radicals and reactive oxygen species. The [33] found that the level of kidney glutathione in untreated RIRI rats was significantly lower than that of control rats. Other studies confirmed that the level of GSH significantly decreased, which was accompanied with a significant increase in MDA level of kidney tissue following I/R [34]. These findings are consistent with the results in the current study that exhibited significantly lower levels of GSH in the control and vehicle group which underwent RIRI without any therapeutic intervention. Conversely, pretreatment with Cbx displayed significantly P≤0.001 higher levels of GSH compared to both the control and vehicle groups. This increase in GSH levels indicates that Cbx may enhance the antioxidant capacity of renal tissues, potentially through mechanisms that involve the upregulation of GSH synthesis or the reduction of oxidative stress. By increasing GSH levels, Carbenoxolone may help to restore the antioxidant defense system and protect renal tissue from damage associated with RIRI. This is consistent with the conclusions reached by Bhardwaj et al., 2014, [35] that a significant increase P<0.05 in the levels of GSH in case of Cbx alone treated group was observed when compared to the MG-132 treated group in an animal model of Parkinson's disease induced by proteasome inhibitor.

EFFECT OF RIRI, CARBENOXOLONE ON APOPTOTIC MARKER (CASPASE3)

This experimental study confirmed a significant increase in Caspase3 levels in renal tissue of both I/R control and vehicle groups P≤0.001 relative to the sham group following ischemia-reperfusion injury (IRI). While there is no significantly different between control and vehicle groups. These findings supported earlier research by [36] who found that the level of Caspase3 was increased after 30 minutes of ischemia followed by 2 hours of reperfusion in rats. Another studies in a rat model appeared that the level of Caspase3 was highly elevated in injured kidney tissues in rats that undergone ischemia for 45 minutes then reperfusion for 24 hr. and ischemia for 60 minutes then reperfusion for 2 hours, respectively [37-38]. In contrast, this study revealed that the pretreatment with Cbx before ischemia induction can significantly P<0.001 downregulated the expression of caspase-3 in injured renal tissues in comparison with those in both control and vehicle groups. The outcomes in the current study are in agreement with those reported by [39] who demonstrated that pretreatment with Cbx (5-20 µg/ml) significantly reduced reactive oxygen species (ROS) levels. Furthermore, Cbx effectively decreased the expression of cleaved caspase-3 protein in PC12 cells subjected to SGD-induced stress, indicating its potential role in mitigating apoptotic pathways under oxidative conditions. This reduction in Caspase-3 levels indicates that Cbx may exert anti-apoptotic effects, potentially through mechanisms that involve the inhibition of pro-apoptotic signaling pathways or the enhancement of cell survival pathways. By decreasing the levels of Caspase-3, Cbx may help to mitigate apoptosis and protect renal tissue from damage associated with RIRI.

EFFECT OF RIRI, CARBENOXOLONE ON THE MOLECULAR PROTEINS FOXO3

In the present study we found that the renal tissues from the control and vehicle groups, which underwent IRI without any treatment, demonstrated upregulation of FOXO3 gene while Cbx treated group showed downregulation of Foxo3 gene. FOXO3 is expressed throughout the body. It is phosphorylated and inactivated by the PI3K/AKT pathway. The phosphorylated and inactive form of FOXO3 is localized in the cytoplasm, but it is translocate into the nucleus after dephosphorylation and activation, where it exerts its transcription function. Our results corroborate those of [40] who also observed that treatment with Cbx reduced cerebral injury in rats exposed to transient focal

ischemia and reperfusion (I/R), and this was mediated by the activation of the PI3K/Akt pathways as well as by blocking the caspase 3 apoptosis pathway. This study examined the potential renoprotective effects of Carbenoxolone in cases of acute kidney injury (AKI) caused by renal ischemia-reperfusion injury (RIRI). Significantly, this therapeutic agent had not been previously investigated for their protective effects against acute kidney injury in this model. Our findings demonstrated substantial protective effects of Cbx, underscoring their potential as innovative therapeutic alternatives for alleviating ischemia-reperfusion-induced renal injury. This study is, to our knowledge, the first to assess and demonstrate the efficacy of Carbenoxolone in this model. These findings facilitate subsequent research into their mechanisms of action and their relevance in clinical.

EFFECT OF RIRI ON KIDNEY PARENCHYMA

Histological analysis revealed an increase in tissue damage after IRI, as shown by the presence of Cytoplasmic swelling and increased cytoplasmic eosinophilia, vacuolization, dilated renal tubules, glomerular alterations and hemorrhage, as well as a dilatation of the Bowman's capsule and a lack of brush boundaries. These changes had been in agreement with some other studies [41-43], that showed the same histopathological changes.

EFFECT OF CARBENOXOLONE ON KIDNEY PARENCHYMA

The findings demonstrated that Cbx pretreatment significantly ($P < 0.001$) reduced kidney injury severity compared to control and vehicle groups. The Cbx-treated group exhibited moderate structural changes, while the other groups showed severe damage. Notably, this is the first study to explore Cbx as a protective agent against renal ischemia-reperfusion injury (RIRI). Its antioxidant and anti-inflammatory properties likely contribute to its protective effects by reducing oxidative stress and inflammation—key drivers of RIRI-induced damage. These findings suggest Cbx as a potential therapeutic intervention for preserving renal tissue integrity and mitigating kidney injury. One of them [44] showed that the curcumin-treated group exhibited significant attenuation of these lesions, with only mild congestion and edema observed in the renal medulla. The renal tissue structure of the curcumin group was markedly improved compared to the RIRI group and resembled near-normal histology. The Paller scores, reflecting the severity of renal injury, were significantly higher in the RIRI group than in the sham group $P<0.01$. However, curcumin treatment significantly reduced these scores compared to the RIRI group $P<0.01$, highlighting its protective effects in mitigating renal tissue damage caused by ischemia-reperfusion injury. On the other hand [45] reported that in a transient cerebral ischemia model (30 min middle cerebral artery occlusion, 24 hr. reperfusion), CBX (25 mg/kg, intracerebroventricular) significantly reduced infarction size in rats by decreasing ROS production, thereby alleviating oxidative stress. CBX also inhibited astrocyte and microglia activation, reducing neuroinflammation and improving histopathological outcomes, including decreased apoptosis, preserved tissue architecture, and enhanced cell viability in the ischemic penumbra. Additionally, demonstrated that CBX supplementation provided Neuroprotection against Aβ 1-42 oligomer-induced hippocampal damage by improving neuronal viability.

CONCLUSIONS

Taken together, these results reinforce the potential of Carbenoxolone as a therapeutic agent in AKI management. Its ability to reduce kidney injury markers, suppress inflammation, restore antioxidant defenses, and modulate apoptotic pathways highlights its multifaceted protective effects. The translational significance of these findings underscores the need for further investigations to establish Cbx. as a viable medical intervention, ultimately improving patient outcomes in nephrology and critical care settings.

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CONFLICT OF INTEREST

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ORIGINAL ARTICLE

CONTENTS

Orthobiotics as an education technology for socionomic sphere specialists

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ABSTRACT

Aim: The experimental check up of orthobiotics potential as a health-conservative workability technology for socionomic sphere workers.

Materials and Methods: Theoretical methods: study, analysis and synthesis of psychological, pedagogical scientific literature, educational and methodological publications on the issue, synthesis; comparison and generalisation of data; empirical - diagnostic methods (question-naire, conversation and interview), pedagogical experiment, statistical method.

Results: According to the results of experimental check up of orthobiotics potential as a social workers workability health-conservative technology, it has been discovered that at the formation stage of the experiment, the starting level of knowledge (low level) in higher education students was 14 %, whereas at the establishing stage it equalled 43 %. The quantity of the higher education students for whom the middle level was characteristic at the formation stage, was 42 % (30 % at the establishing stage). The sufficient level has been presented by 44 % of to be social workers (27 % at the establishing stage).

Conclusions: The number of the higher education students who have the middle and the sufficient level of the knowledge of the subject, has grown quantitatively. It gives us an opportunity to affirm that the experimental work has positive tendency towards formation of skills and abilities for auto-conservation of health of social workers in the process of their professional training.

KEY WORDS: social work, healthy lifestyle, health, university

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INTRODUCTION

The socionomic sphere workers activity specific character includes emotional surcharge and professional burnout. It is those specialists that are susceptible to different psychosomatic disorders and taking stress situations by going through introspection, reflection, optimum regulation of their activity when coming across life and professional difficulties.

The influence of working stress on the social-and-pedagogical sphere workers puts a significant pressure upon their physical and emotional conditions, as well as upon the efficiency of their whole activity. So, the study of health-conservative technologies in the professional activity of socionomic sphere (i.e. orthobiotics) specialists, becomes more topical. This problem has been interpreted in scientists' works dedicated to such matters: human health [1], development of theories in the field of orthobiotics [2, 3], ensuring high quality level of specialists professional training process at a university [4-6], problematics of orthobiotics as a science of healthy life-style [7, 8], peculiarities of professional burnout of socionomic sphere workers [9-11].

In order to realize health-conservative technologies in his or her professional activity, a socionomic sphere worker shall have formed certain personal qualities and high level of professional education. That is why we consider that in the process of education of a qualified socionomic sphere specialist, it is expedient to ensure personal competence in orthobiotics. Below in the study, the question will be described using a social worker education as an example.

A social worker health-conservative activity technology presupposes his or her high professional competence (deep knowledge, skills, culture, humane attitude to him- or herself and to other persons, application of system activity bases to the process of education). Health protection pedagogics cannot be expressed in a concrete educational technology. But the conception of a "health protection technology" unites all directions of education organization towards formation, conser-

vation and improvement of health of a higher education student as a social worker. The health protection technology represents the aggregate of forms, modes and methods oriented to the achievement of optimum results of support of physical, psychical, moral and social success of a person [12].

The goal of introduction of health-conservative technologies into social workers professional training consists, first of all, in the improvement of their knowledge of health conservation, improvement of physical education organizational and sanitary methods, and the healthy life-style, for use in the further intensive work.

AIM

The experimental check up of orthobiotics potential as a health-conservative workability technology for socionomic sphere workers.

MATERIALS AND METHODS

The methodological and theoretical basis of the study is the activity approach, systematic approach, psychological theory of personality, psychology of educational activity, the concept of pedagogical support in the educational process, ideas of using specialists in the socio-economic sphere. Theoretical methods: study, analysis and synthesis of psychological, pedagogical scientific literature, educational and methodological publications on the issue, synthesis; comparison and generalization of data; empirical - diagnostic methods (non-standardized questionnaire) to determine the level of awareness of orthobiotics in the process of professional training of future social workers; pedagogical experiment to test the level of understanding by students of the importance of maintaining and improving their own health; statistical - to process the data obtained. The study was conducted during 2022–2023 at the Petro Mohyla Black Sea National University with students of higher education of 4–6 years of the specialty "Social Work". The total number of higher education students was 48 people, who, in turn, were divided into experimental and control groups. By organizing this sample, we tried to reflect the real level of awareness of future social workers with orthobiosis technologies and the shortcomings and problems in implementing these technologies in professional and practical training, and also intended to obtain the necessary scientific information that would help us develop the content of the pedagogical conditions for the training of students.

RESULTS

The conclusions made upon theoretical analysis of the health-conservative educational technologies application to social workers professional training problem, have called for verification. For this purpose, an experimental study was conducted in two stages - ascertaining and formative.

In order to obtain reliable data on the level of awareness of future social workers about health-saving technologies, we developed and used a set of primary and secondary surveys, which included conducting questionnaires with open and closed questions, conversations and interviews with students; statistical data were collected and analyzed.

During the experimental study, we tried to solve the following tasks:

- characterize the general state of the formation of knowledge and skills of orthobiotics in the process of professional training of future social workers;
- outline the concepts of health and health-preserving technologies from the point of view of students of the specialty "Social Work".

A quantitative research method was chosen for the study - a survey, namely a questionnaire (the questionnaire is not standardized and is an independent development of the authors of the study, its form is exclusively of a recommendatory nature and can be changed depending on the needs and circumstances or can serve as a guideline during the survey). Each of the proposed questionnaires included questions related to indicators of the formation of ideas about the health of a social worker in professional activities and in general. The questionnaires, compiled in accordance with all relevant requirements, had control questions to verify the reliability of the answers provided. The survey was conducted in written form and anonymously. Before the survey began, we provided instructions on the features of filling out the questionnaires, as well as provided brief information on the relevance and importance of this study. The data obtained were processed using the SPSS Statistics program.

During the ascertaining stage, we highlighted the following: students have insufficient knowledge of orthobiotics (81%), while their current health status has negative dynamics. This indicates insufficient professional and practical training of future social workers, as well as unpreparedness to use health-saving technologies in their further professional activities. That is why it has become urgent for us to solve the problems associated with enriching the content of professional and practical training of future social workers with health-saving technologies.

The purpose of the formative stage of the experiment was to determine quantitative and qualitative changes in the formation of professionally important qualities, knowledge, skills and abilities of orthobiotics. Groups of

Table 1. Understanding Orthobiotics Level Formation Summary (according to the formation experiment results)

Questions Posed	Stage	Formation Levels					
		Low		Middle		High	
		abs.	%	abs.	%	abs.	%
Are your knowledge of orthobiotics sufficient?	Establishing	43	81	10	19	0	0
	Formation	7	15	15	31	26	54
Is the learning of orthobiotics expedient in the educational process?	Establishing	5	10	24	45	24	45
	Formation	2	4	16	33	30	63
Average index	Establishing	48	91	34	64	24	45
	Formation	9	10	31	32	56	58

Source: compiled by the authors of this study

Table 2. Recognizing the role of orthobiotics in professional education by social workers (according to the formation experiment results)

Questions Posed	Stage	Formation Levels					
		Low		Middle		High	
		abs.	%	abs.	%	abs.	%
Is knowledge of orthobiotics necessary for social workers in their professional activity?	Establishing	13	24	19	36	21	40
	Formation	2	4	9	19	37	77
Is orthobiotics a subject of your professional interest?	Establishing	41	77	12	23	0	0
	Formation	9	19	15	31	24	50
Do you think orthobiotics is a priority for your professional activity?	Establishing	39	74	4	7	10	19
	Formation	0	0	7	15	41	85
Do you think the introduction of new "Orthobiotics" discipline would be expedient for social workers?	Establishing	5	10	24	45	24	45
	Formation	2	4	7	15	39	81
Average index	Establishing	98	46	59	28	55	26
	Formation	13	7	38	20	141	73

Source: compiled by the authors of this study

respondents were formed in the experimental program, and the subsequent final diagnostics gave results that allowed us to compare how this program contributes to psychological changes in students of higher educational institutions.

During the formative stage, we tried to solve such problems: to determine the condition of knowledge and skills formation as regards health-conservative technologies; to confirm the conclusions about efficiency of the proposed recommendations.

The formation experiment has been conducted according to principle of importance of the professionally oriented disciplines in the process of the social workers professional training.

Basing upon the answer results of the higher education students, we have determined the levels of efficiency of the health-conservative technologies application to teaching the social workers. We have marked out three levels: low, middle, and high. The high level group included the higher education students who had deep and sound knowledge of health-conservative technologies, methods and forms of health conservation; they realize the necessity of studying orthobiotics in the professional education process; their amount of knowledge is enough for developing necessary qualities of a social worker, their intellect is brightly expressed, they have skills and abilities of modelling the professional and practical activity of a to-be social worker.

The middle level group have enlisted the higher education students who had certain knowledge about the "healthy life-style" idea, but could not determine concrete technologies in the system of social workers professional education, they possessed middle level abilities and skills for their professional activity, but were not consistent enough in the maintenance.

The low level group embraced the higher education students who had fragmentary knowledge of the health-conservative technologies, possessed practically no skills and abilities of their application, could not tell the "health" conception, were not interested in learning orthobiotics in the course of their professional training; they had low level of social intellect and communicative skills, and their psychological staunchness was insufficient.

The levels described above will allow to determine the efficiency of the use of orthobiotics as a health-conservative technology in the process of the social workers professional education.

To elucidate the results, after the work has been done, the higher education students were posed an open question about determining the "health-conservative technologies" term. If earlier the mentioned question had caused difficulties in more than a half of the higher education students, then after the informative lectures and practical trainings had been conducted, the answers of the respondents became clear and structurized, which confirms understanding of the subject. It has been confirmed during talks and interviews, and it was discovered that higher education students understand content and form of the health-conservative technologies, and can give concrete examples of their use in the future professional activity. The results show that 84 % of the higher education students can be included into the orthobiotics knowledge high level group.

The analysis of the respondents' answers to a number of questions shows a positive tendency as regards realizing the importance of orthobiotics in the social workers professional training. According to average indices, the low level has decreased by 79 %. Thus, at the beginning of the experiment, of the higher education students could not confirm they had sufficient knowledge of orthobiotics, and after, this index has grown, which can be represented as a percentage, namely 54 % (see Table 1).

The obtained data analysis and interpretation have shown better quality indices and opinions of the higher education students; a positive dynamics can be observed.

In the research process, we proceeded from the fact that it was also expedient to take into account the organizational character of the teaching process when assessing the level of higher education students' formation towards health-conservative technologies. The conclusions have shown the presence of positive changes. The higher education students are characterized by professional attitude to the determination of orthobiotics in their professional preparation, the positive dynamics was conditioned by correspondent pedagogical conditions, and represented 77 %. The number of respondents who attributed the health-conservative technologies to the subjects of professional interests, has grown from 0 % to 50 %, which in its turn made an influence on the interest to learn the new educational component, this index showed 73 %. The mentioned direction changes results are summarized in the table (see Table 2).

During the main stage of the formation experiment, the higher education students of "Social Work" speciality begin to realize the importance and the role of orthobiotics in the course of their education. If at the time of the first meeting they have noted that the preparation shall be, but as a minor one, then after the explanatory (talks, lectures, brain-rings) and theoretical-and-practical (trainings, round tables) work has been conducted, the higher education students have determined orthobiotics as a must-have for a social sphere specialist's professional progress. In percentage – 56 % instead of 6 %, which allows to include the mentioned amount into the group of high level understanding of orthobiotics necessity for the process of professional education. This statement has been confirmed also during the question: "What prompts you to improve your knowledge of orthobiotics?", the data of which are shown in histogram (see Fig. 1).

Generalization of the formation experiment results, taking into account basic criteria and indices of formation of social workers' knowledge and abilities in relation to health-conservative technologies, gave an opportunity to obtain final results (see Table 3):

The respondents' questionnaire answer results shown in Table 3, at the control stage of the experiment demonstrate that 7 higher education students (15 %) have not formed the knowledge of the orthobiotics importance as a health-conservative technology, the higher education students nearly did not possess skills and abilities of how to use the technology, they cannot tell the importance of health for a social sphere specialist's professional activity, were not interested in the application of the health-conservative technologies to their professional education, that is, they are at the beginning stage of formation. The number of higher education students whose middle level of formation of subject knowledge has risen quantitatively, and the qualitative indices regarding the concrete application of this technology to the system of social workers professional education, as well as to the future professional activity, cannot be determined efficiently. Thus, we diagnosed 20 higher education students with middle level, or 42 %. The sufficient level characterized by deep and sound knowledge of health-conservative technologies and methods, has been discovered in 21 higher education students (44 %), which is by 18 % more than before the formation work has been conducted. It gives us an opportunity to affirm that the experimental research has a positive tendency to formation of skills and abilities of social workers' self-conservation of health in the process of their professional training.

DISCUSSION

Displays of health protection technologies at higher education institutions include, besides the level of

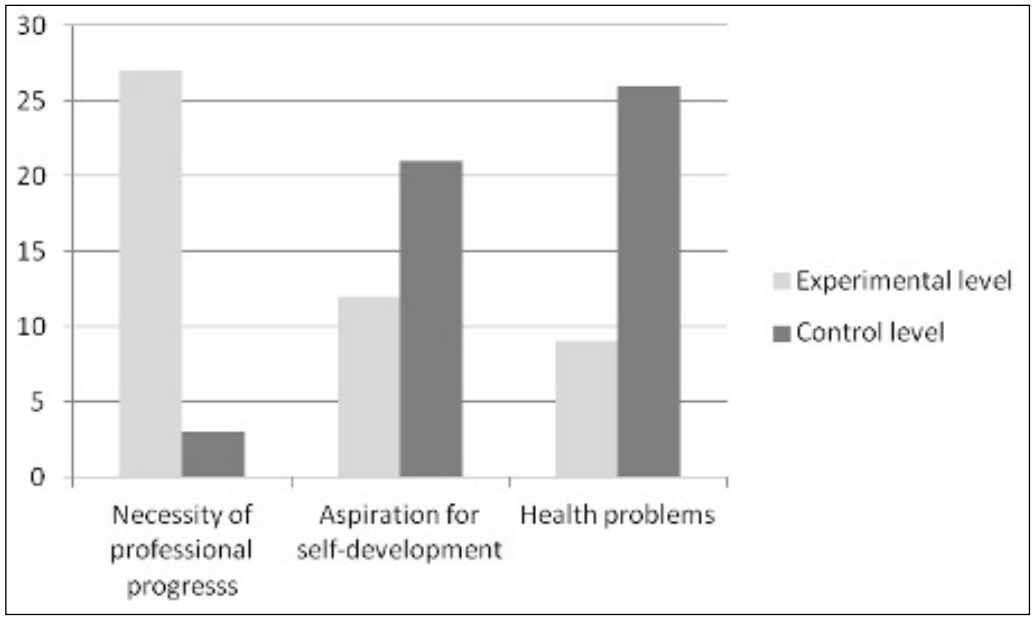


Fig. 1. Factors that influence upon learning the orthobiotics system of knowledge
Picture taken by the authors

mastering the knowledge and skills of this branch, the observance of sanitary and hygienic requirements by teachers and students, and the scientific organization of pedagogical work. From our point of view, the great role is played here by the activity of pedagogical process participants. The further work, in which orthobiotics is used, shall be oriented to application of the health conservation technology in the socionomic sphere specialists professional training process (by the example of to-be social workers preparation). According to the World Health Organization [13], a healthy lifestyle involves a comprehensive system of behavioral practices aimed at supporting physical, mental and social well-being. In particular, in social work, a client's health is viewed as the result of the interaction of social, economic and cultural factors, where lifestyle is one of the key components [14].

The preliminary content analysis of the training schedule, the survey of educational components working programmes, and the thoroughly interpreted contents and nature of the sorts of activity of the higher education students during their studying at the higher education institutions, have afforded an opportunity to determine the units of educational information and to make their further concrete definitions, which can serve as scientific theoretical grounds for social workers preparation.

The further development of the proposed units content has determined the role of each unit in the educational process. In order to enrich the professional training of social workers, we analysed the educational components content according to training schedule, which presupposed a differentiated enrichment of the training components content elements which traditionally expose the nature and the peculiarities of work with different groups of clients, their satiety with new educational material in conformity with modern requirements for the professional social-and-pedagogic activity of this direction, establishing inter-subject links, strengthening the educational components orientation to the ensuring of the social workers professional training efficiency. The amplified content of the educational material about the use of the health-conservative technology, which can be included in the process of general pedagogic and special education of the social workers.

Higher education institutions shall optimize and perfect the process of professional training of social workers in the course of the self-conservation of health. From our point of view, it can assist higher education students in being informed about the importance of health-conservative technologies. Namely: increase of a social worker's responsibility for conservation of his or her own health; forming of professionally important qualities, rational and active way of life; motivation to the improvement of their health; ability of giving before-physician first medical aid in cases of exacerbations and crises; obtaining those skills and abilities of health self-control in their professional activity that can reduce unfavourable influence (control of stress, avoiding emotional, psychological and professional burnout etc.). Some researchers [15] emphasize that social workers often act as intermediaries between health care providers and local communities, explaining the importance of disease prevention, healthy eating, physical activity, and psycho-emotional well-being. At the same time, knowledge about healthy lifestyles helps not only increase the level of awareness of clients, but also prevent professional burnout among social workers themselves.

Table 3. Dynamics of social workers' orthobiotics formation (according to the formation experiment results)

No.	Levels of Orthobiotics Formation	At the Beginning of the Experiment		After the Termination of the Experiment	
		abs.	%	abs.	%
1.	High level	14	27	21	44
2.	Middle level	16	30	20	42
3.	Low level	23	43	7	14
	Total number of higher education students	53	100	48	100

Source: compiled by the authors of this study

Table 4. Experimental programme

Index No.	Kind of Work
1.	Analysis of the establishing experiment data in relation to the research problem.
2.	"Health is Value" subject lesson at a university.
3.	Lessons on professional burnout prophylaxis for tobe social workers – 5th year students.
4.	Organization of dialogue forms of work (discussions, disputes, debates) on "Regional Social Policy" discipline, according to topic: "Health of Young People: in Ukraine; in North Pontic Region; in the Town of Mykolayiv", on "Innovations in Social Work" discipline according to topic: "Introduction of Innovations into Social Work System for Improvement of a Specialist's Health" – 4th to 6th year students.
5.	"Health-Conservative Technologies in Social Work" training with 4th year students.
6.	"Health-Conservative Technologies in Social Work" training with 5th and 6th year students.
7.	Scientific office. Speech of an invited specialist.
8.	Demonstration of video reels on the problematcs.
9.	Meeting with "We Are By Your Side" psychological support group, on the topic: "How to Struggle Against Constant Anxiety, Fear, Tiredness and Other Phenomena Which Cause Discomfort in Your Life".
10.	Lecture (in the form of an informational training) on "Innovations in Social Work" discipline.
11.	Lecture (in the form of a business game) on "Social Work in Different Spheres of Life" discipline.
12.	Lecture on "Applied Methods in Social Work" discipline.
13.	"Sport in My Life" curator educational hours: exchange of experience on health conservation problems between higher education students.
14.	Synthesis of an earlier performed work in the form of talks and discussions.
15.	Filling in the testing questionnaire.
16.	Analysis and comparing of the research results.
17.	Conclusions and recommendations on the research results.

Source: compiled by the authors of this study

The professional training content, in relation to teaching health-conservative technologies to higher education students, shall include practical abilities to analyse causes and factors which influence health, with the further making of plan of individual improvement of health.

In the process of our formation experiment we made an effort of enriching the social workers professional training content with health-conservative technologies, where we used a complex of such methods: filling in the questionnaire, testing, pedagogical observation, investigation talks, analysis of higher education students' creative and practical activity, modelling of pedagogical situations etc. We created an experimental programme which corresponds to the mentioned tasks (see Table 4):

Making the social workers acquainted with necessary knowledge about orthobiotics is the basic pedagogical condition. Orthobiotics is a system that integrates knowledge about the harmonious combination of lifestyle, psycho-emotional state and ecological awareness [16]. In European pedagogical practice, orthobiotics is considered as an interdisciplinary approach that combines the principles of valeology, psychohygiene and environmental education, aimed at developing a responsible attitude towards one's own health and the health of others [17]. This approach is especially valuable for social workers, psychologists, teachers and other socio-economic specialists who often work with vulnerable groups of the population

and require a high level of stress resistance and self-regulation skills. In this relation, there is need of carrying out improved educational components. We chose the educational components: "Regional Social Policy", "Innovations in Social Work", "Applied Methods in Social Work", "Social Work in Different Spheres of Life" and proposed the study of individual topics on application of health-conservative technologies.

For example, the "Social Work in Different Spheres" educational component, "Theories of Healthy Life-Style" lesson subject. The goal of the educational component (study of methods and strategies of a healthy life-style and of the health formation processes in social workers) subject determining acquires new oriented quality (study of approaches and directions of the health-conservative professional work of a social worker), the content of the material undergoes improvement owing to introduction of innovative methods of rapid memorizing of the learned material and because of securing of the skills and abilities by means of introduction of practical health-conservative methods as an active sort of education into the lecture material. The lesson was given for higher education 4th year students of "Social Work" speciality in the form of a business game.

During the study on "Innovations in Social Work" educational component, the higher education students have been proposed the subject "A Modern Social Worker Self-Control Techniques" in the form of a training, the goal of which was working through the learned material on methods of making an individual schedule of one's own life and work for an effective activity; forming of taking decisions abilities and practical mastering of the health-conservative methods. The lesson was given to 5th year students of "Social Work" speciality.

As practice shows, the execution of the improved educational components raises significantly the level of higher education students' knowledge about formation of a social worker health-conservative activity, as well as broadens the higher education students' ideas on levels, forms, methods and technologies of conserving one's own health in social sphere specialists' professional activity.

The achieving of concrete results concerning the orthobiotics formation in the professional training is possible under condition of drawing the higher education students in the processes of conservation and improvement of their own health, which makes necessary the use of different communication techniques of teaching. With this purpose, such forms and methods were introduced: brain-rings, discussions, conferences, round tables etc. The use of these forms of the educational and teaching activity has contributed to the higher education students' attitude to the values of health and healthy life-style, to the development of the health-conservative technology necessity understanding in the process of preparation of to-be social workers.

The training of future workers in the socionomic sphere takes place under rather difficult political, migration, economic, and social conditions, which may lead to a deterioration in psychological and physical health. Such a situation requires students to mobilize both external and internal resources to overcome these challenges. One of the internal resources for overcoming difficulties is considered to be the support of mental health, and therefore, the implementation of health-preserving technologies in the educational process.

We provide an analysis of publications related to the topic of our research.

In the monograph "Research on the Psychology of Human Orthobiosis" [3], theoretical and methodological approaches to assessing human orthobiosis are provided; systematic studies of personality orthobiosis using a competency-based approach are presented; the results of research on an optimistic attitude towards life as an activating personal resource are provided; the issue of the impact of modern technologies on psychological health is examined; for the first time, an approach has been proposed that establishes the correlation between the concepts of 'psychological well-being' and 'personal performance success' in the context of human orthobiosis.

Particular attention is given to the issues of activating personal resources for overcoming difficult life situations within the framework of the orthobiotic approach and harmonization of personal development. Human orthobiosis has been examined as a process of balancing psychological resources, in which a significant role is played by a person's successful activity, their symbolic reality, difficulties in establishing psychological intimacy, the individual's sexual health, as well as issues related to time loss dependency [3].

In the work 'Optimistic Attitude Towards Life as an Activating Personal Resource (orthobiotic approach)', researchers M. Kremenchutska and I. Dobrynina presented the components of an optimistic attitude towards life: optimism as a psychological category; activation of personal resources for overcoming difficult life situations; and the connection between pessimistic and optimistic worldviews with the process of individualization (the current state of the issue).

It was experimentally established that individuals with an active optimistic attitude towards life and the world are characterized by a high degree of life consciousness, internality, and a communication-oriented mindset combined with high communicative tolerance.

Optimists mostly live in the present or are oriented toward the future; for them, the present is pleasant and safe, filled with events and hopes for a better future, easy and interesting, successful and meaningful.

Individuals with a passive pessimistic outlook on life are characterized by inner discomfort, which expressed in a depressed mood, dissatisfaction with their quality of life, and a feeling of unhappiness. In contrast, an optimist is distinguished by inner harmony, vitality, a sense of happiness, and satisfaction with various aspects of life [3].

In T. Cherniavska's study 'Psychological well-being and the success of an individual's performance in the context of human orthobiosis,' the following aspects are presented: the relevance of studying issues of psychological well-being and personal performance success; the definition and nature of the concept of psychological well-being; performance success as a subject of scientific analysis in modern psychology; and the psychological mechanisms of personal success in business [3].

O. Kononenko's work 'The Influence of Orthobiotics and Modern Technologies on Human Psychological Health' is dedicated to highlighting: the issue of the impact of the Internet environment and modern technologies on human psychological health; modern understanding of FOMO syndrome (Fear of Missing Out — an obsessive fear of missing an interesting event or a good opportunity, often triggered by browsing social media) and methods for its correction and prevention; the psychological nature of selfies; cyber-communicative addiction of the individual and modern methods of its psychological correction [3].

The analysis of O. Dotsenko's study 'The Correlation Between Personality Orthobiosis and the Symptom Complex of 'Emotional Coldness' explores the following issues: orthobiosis and various types of contact disruption between partners; orthobiosis and interpersonal dependence and counter-dependence; the impact of the symptom complex of 'emotional coldness' on the formation of personality orthobiosis; and harmonious relationships as an essential part of orthobiosis [3].

A range of issues in the field of personality orthobiosis is examined in A. Yermakov's study 'Psychological Aspects of Human Sexual Health as a Development of the Idea of Orthobiotics', specifically: the relevance of studying and the nature of human sexual health; qualitative parameters of sexual health; a theoretical analysis of psychological issues related to sexual health; and sexual health problems in the modern world [3].

The analysis of the results of O. Vorokhaiev's dissertation research 'Preparation of Future Social Workers for Teaching Health-Preserving Disciplines in Master's Programs' showed that the author highlighted and substantiated the pedagogical conditions for preparing future social workers to teach health-preserving disciplines: The stimulation of the value-motivational sphere of future social workers to teach health-preserving disciplines; improvement of the content of training of future social workers in teaching health-preserving disciplines through the specialized course 'Teaching Health-Preserving Disciplines; involvement of future social workers in health-preserving activities.

Research shows that the introduction of orthobiotics into educational programs allows to increase the level of professional motivation of students, to form a conscious model of a healthy lifestyle, as well as to develop interpersonal interaction skills and the prevention of emotional burnout [17]. Thus, orthobiotics appears not only as an innovative educational technology, but also as a value basis for the formation of a humanistic paradigm for the training of future specialists in the socio-economic sphere.

The effectiveness of the highlighted and experimentally verified pedagogical conditions for preparing future social workers to teach health-preserving disciplines in educational programs is evidenced by the obtained overall efficiency factor of professional training for teaching health-preserving disciplines in higher education institutions for students in specialty "Social Work" of the experimental groups EG 1.1 and EG 1.2, which is 0.4 and 0.34, respectively, and indicates an average level of readiness. The overall training coefficient of students in the experimental groups EG 2.1 and EG 2.2 is 0.72 and 0.69, which reflects a high level of readiness among future social workers for teaching health-preserving disciplines. In contrast, the overall efficiency coefficients of training for teaching health-preserving disciplines among future social workers in the control groups of the formative stage of the experiment indicate an insufficient level of readiness [18].

Thus, we made a conclusion that in the process of enrichment of the professionally oriented disciplines, the social workers' learning-and-cognitive activity and the higher education students' better mastering of special knowledge on the investigated problem, undergo an activation. Orthobiotics ensures practical introduction of health conservation values into education of social workers, contributes to creation of health-conservative consciousness and activates operations in this direction.

CONCLUSIONS

According to the results of the experimental verification of the potential of orthobiotics as a health-preserving technology for the work capacity of specialists in the

socio-economic sphere, during the comparison of the results of the ascertaining and formative experiments, it was found that the analysis conducted at the final stage of the experiment revealed positive dynamics of changes in the level of awareness among students and demonstrated significant advantages of high and medium levels of effectiveness of using orthobiotics as a health-preserving technology for training future social workers. At the formation stage of the experiment the initial level of knowledge (low level) in the higher education students constituted 14 %, whereas at the establishing stage it was 43 %. The number of higher education students characterized by the middle level at the formation stage, was 42 % (30 % at the establishing stage). The sufficient level was shown by 44 % of to-be social workers (27 % at the establishing stage). Thus, the number of higher education students whose level of a subject knowledge formation was middle and sufficient, has grown qualitatively. It gives us the opportunity to affirm that the experimental work has positive tendency towards formation of abilities and skills of self-conservation of health by social workers in the process of their professional training.

The existing situation can be changed by means of the higher education more effective influence upon informational provision of socionomic sphere specialists with the appropriate content, in relation to mastering the health-conservative technologies in the context of orthobiotics. Thus, the professional education of socionomic sphere specialists can be made more effective by using orthobiotics as an educational healh-conservative technology.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Individual psychological features as factors of psychological trauma in police officers–combatants

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ABSTRACT

Aim: The aim is to investigate the peculiarities of the manifestation of psychological trauma symptoms in police officers–combatants depending on their individual psychological features.

Materials and Methods: The research, conducted in 2023-2024, involved 138 police officers–combatants (men) with different types of temperament: choleric (Group C, n = 38), sanguine (Group S, n = 34), phlegmatic (Group P, n = 35) and melancholic (Group M, n = 31). Research methods: bibliosemantic, systematic analysis and generalization, survey, statistical

Results: The main individual psychological features that are factors in the occurrence of psychological trauma in police officers–combatants have been identified: temperament, character, and personality orientation. It has been established that the most common symptoms of psychological trauma in combatants, regardless of the kind of temperament, are gloomy mood, dissatisfaction with oneself and others, increased aggressiveness, fear, anxiety, sleep problems, and cognitive impairment. At the same time, choleric and melancholic combatants show symptoms of psychological trauma more often than sanguine and phlegmatic individuals.

Conclusions: The research shows that police officers–combatants with choleric and melancholic temperament types are more likely to be traumatized than sanguine and phlegmatic police officers. The findings should be taken into account when planning psychological rehabilitation measures for police officers with signs of combat trauma.

KEY WORDS: temperament, police officers, war, combatants, psychological trauma, individual psychological features

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INTRODUCTION

In the context of the russian-Ukrainian war, combatants and civilians in Ukraine are under the negative influence of events that are stressful for everyone. However, they are traumatic for those who are particularly vulnerable to them due to their individual psychological features. Individual psychological features affect a person's behavior, activities, and life and are manifested in temperament, character, and orientation, which are interrelated [1]. Individual psychological features determine whether a psychological trauma will occur or whether a traumatic event will lead to disorders in a person's mental, physical, and social health [2].

Experts [3] note that a psychologically traumatic event is an extremely significant event for a person that threatens life or health, causes intense fear and a sense of helplessness, and causes substantial emotional stress and further negative experiences. Any stressful event that destroys mental boundaries can become psychologically traumatic [4]. This can be combat, serious bodily injury, death or injury of a loved one, amputation of a limb, etc. [5].

According to scientists [6], the peculiarities of the psychological nature of psychological trauma include: discrepancy between the threatening factors of the situation and individual capabilities to overcome them; unconscious mental process that seeks to re-live the situation and respond to it; psychological significance of the traumatic event for the individual (subjectivity of perception); violation of the integrity of the psyche; psycho-traumatic action is concentrated within the individual; unfinished physiological process; blocking the process of experiencing a traumatic event by defense mechanisms.

The consequences of a traumatic event can be the following stressful states, which depend on the time after the event: acute stress response (up to 48 hours after the event), which may resolve on its own; acute stress disorder (up to 1 month after the event); PTSD as a prolonged or delayed response to traumatic stress,

diagnosed in a month, sometimes it can develop a year, several years or many years after the event (delayed PTSD); complex repetitive trauma syndrome (complicated or complex PTSD) [7].

According to experts [8], psychological trauma is usually manifested by symptoms such as anger, irritability, mood swings, feelings of guilt, shame, self-blame, difficulty concentrating, anxiety and fear, separation from others, insomnia or nightmares, fright for no reason, palpitations, pain, fatigue, nervousness, muscle tension, etc. The response to a psycho-traumatic event depends on a combination of many factors [9]: external – peculiarities of the traumatic event (intensity, suddenness, duration); social – peculiarities of the social situation, in particular the presence or absence of social support; biological – genetic predisposition; individual psychological – temperament, character traits, psychological defense mechanisms, coping strategies, characteristics of the emotional sphere, etc.; risk factors, such as gender, age, history of mental disorders and traumatic events, low socioeconomic status, etc. However, the main indirect factors in the manifestation of psychological trauma symptoms are individual psychological features of the personality [10].

AIM

The aim is to investigate the peculiarities of the manifestation of psychological trauma symptoms in police officers–combatants depending on their individual psychological features.

Tasks: 1) to determine the individual psychological features (characteristics) of the personality, which may affect the occurrence of psychological trauma; 2) to study the frequency of manifestation of symptoms of psychological trauma in police officers–combatants depending on their type of temperament.

MATERIALS AND METHODS

The research, conducted in 2023-2024, involved 138 police officers–combatants in the russian-Ukrainian war (men) from the city of Odesa and Odesa oblast with different types of temperament: choleric (Group C, n = 38), sanguine (Group S, n = 34), phlegmatic (Group P, n = 35) and melancholic (Group M, n = 31). The type of police temperament was determined by the method of G. Aizenko [11]. Police officers' experience in combat operations ranged from 6 to 12 months. Special ranks, positions, age, length of service, education, and marital status of the study participants were not considered. The main criterion for selecting participants for the study was the presence of combat experience during the full-scale russian invasion of Ukraine for at least 6 months. The research was organized, and the results were processed by the Department of Psychology and Pedagogy of the Odesa State University of Internal Affairs (OSUIA, Odesa, Ukraine).

Research methods: bibliosemantic, systematic analysis and generalization, survey, statistical. The bibliosemantic method was used to conduct an analytical review of scientific sources on the outlined range of issues (20 sources from PubMed, Scopus, Web of Science, Index Copernicus and other databases were analyzed). The method of systematic analysis and generalization was used to summarize scientific information about the main individual psychological features (temperament, character, orientation), which determine the occurrence and frequency of symptoms of psychological trauma in combatants. The questionnaire method involved surveying policemen using a questionnaire developed by the author's team. The questionnaire contains 10 questions, that are aimed at studying the frequency of manifestation of psychological trauma symptoms in police officers–combatants depending on the type of their temperament. The questionnaire was anonymous, which helped to increase the accuracy of the formulated conclusions. The questionnaire was assessed by the experts in this field (2 professori and 3 associate professors) and was approved by the Academic Council of OSUIA (Protocol No. 05 dated 20.11.2023). Consent to voluntary participation in the survey was obtained from all respondents involved in the study.

Statistical methods. The significance of the difference between the indicators of the frequency of symptoms of psychological trauma in groups of police officers–combatants was determined by Pearson's Chi-square (χ^2) criterion. The significance of the difference was set at $p<0.05$. All statistical analyses were performed using STATISTICA 10.0 statistical package.

The procedure for organizing the study and the topic of the article were previously agreed with the committee on compliance with Academic Integrity and Ethics of the OSUIA. Also this study followed the regulations of the World Medical Association Declaration of Helsinki. Informed consent was received from all participants who took part in this study.

RESULTS

To solve the first task of the research, we examined the main individual psychological features (characteristics) of a personality that can be factors of psychological trauma. Psychological trauma occurs when the impact of a stressor leads to a disturbance in the psyche (in the affective, cognitive, behavioral, emotional, and

volitional spheres), which depends on the response (experience) of the individual. The speed, pace, and intensity of response depend on the combination of excitement, inhibition, balance, and mobility of the individual's nervous system – the temperament type.

The responses of combatants with different types of temperament to traumatic events were studied: choleric (unbalanced, emotionally excitable, active, anxious extrovert) reacts strongly to influence, has difficulty adapting to new and unexpected situations, in traumatic events shows aggression, irritability, impulsively rushes into battle, cannot find the right solution to a difficult situation, therefore is prone to fussiness, which does not contribute to calming and finding optimal solutions; sanguine (strong type, balanced, low-anxiety extrovert) quickly adapts to new conditions, is stress-resistant, solves problems productively in traumatic situations, cooperates with other people, and can take responsibility if necessary; phlegmatic (strong type, balanced, low-anxiety introvert) has difficulty making quick decisions, orientation in stressful situations, the strength of emotions is not visible behind external manifestations, passively solves problems; melancholic (weak type of nervous system, anxious introvert) is vulnerable to minor influences, reacts painfully to nervous overload and stressful situations, avoids them (depression may be a consequence), in difficult situations is passive, confused, cries, seeks help and support from other people.

Awareness of the key temperamental characteristics of combatants makes it possible to conduct and organize high-quality psychocorrectional and therapeutic work both during military activity and in peacetime. For example, introverted individuals are able to "withdraw into themselves", immerse themselves in a problem, get stuck, etc., which will require greater control and coordination during professional psychological assistance.

Let's consider character as a factor in psychological trauma. Character traits are manifested in the attitude to oneself, to other people, to surrounding events, and to one's activities. Attitude to oneself (positive or negative) is manifested in self-esteem, level of claims, self-criticism, etc.; to other people – in sociability, empathy, trustfulness, etc., or the opposite traits; to one's activities – in hard work, responsibility, initiative, diligence, etc. or the opposite traits; to the surrounding reality – in purposefulness, principle, conviction, etc. Emotional qualities are expressed in sensitivity, anxiety, distrust, aggressiveness or cheerfulness, optimism, flow of spirits, enthusiasm, etc.; volitional qualities – in a person's attitude to difficulties, overcoming obstacles (courage, determination, self-control, self-possession in different situations, perseverance, determination) and general behavior regulation (organization, punctuality, etc.). The character traits that lead to combatant maladjustment include shyness, timidity, indecision, uncertainty in new situations, underdeveloped ability to make volitional and physical efforts, increased need for support, low self-esteem, failure, excessively deep feelings when their interests and needs are not met, nervousness and depression in the face of failure, difficulties, problems, and increased need for support. Soldiers' maladjustment to combat activities and constantly changing situations is also affected by uncommunicativeness, inflexibility, irresponsive behavior, and low sensitivity to external stressors. Signs of problems with adaptation include inadequate response to events, such as aggressiveness, irritability, anxiety, apathy, detachment, and substance abuse to relieve emotional stress.

Let's consider personality orientation as a factor of psychological trauma. The orientation is manifested primarily in attitudes to activity and purposefulness, as well as motivation, which generally determines the proactive attitude and behavior of the individual. Thus, life-oriented attitudes arise, are realized, and become relevant or secondary under the influence of character traits, peculiarities of mental cognitive processes, type of temperament (phlegmatic people have more fixed attitudes, choleric people have unstable ones), etc. An important role is also played by the following: the attitude to supplant psycho-traumatic events, unconscious attitude to survival, satisfaction of needs, subjective norms about oneself, one's self-image, etc. Motivational and value attitudes can influence emotions, the desire to obtain certain benefits, and the satisfaction of one's needs. Values, beliefs, ideals, and worldviews are formed based on orientation. The structure of value orientations can be a factor in the emergence of PTSD in combatants (unformed, dominance of material values).

In continuation, it is worth mentioning some other personal factors that lead to the onset of psychological trauma and PTSD. In particular, they include low resilience, stress resistance, and peculiarities in their coping behavior, which again depend on individual psychological features. With low motivation and low will to overcome difficulties, there is a high probability of psychological trauma. The reasons for low-stress resistance are such individual psychological features as lack of purposefulness, volitional self-regulation, irresponsibility, emotional instability, lack of self-confidence in overcoming problems, low proactive attitude, low normativity of behavior, timidity, low intelligence, unsociability, pessimism, lack of orientation to overcome the issues and to the future, low level of performance, low self-control, etc.

An important personal resource of stress resistance (an internal psychological factor of its formation and de-

Table 1. The frequency of symptoms of psychological trauma in police officers–combatants (n=138), depending on their type of temperament, number of people / %

Symptoms of psychological trauma	Frequency	Group C (n=38)	Group S (n=34)	Group P (n=35)	Group M (n=31)
Sleep disorders	Often	21 / 55.3	8 / 23.5	10 / 28.6	18 / 58.1
	Sometimes	9 / 23.6	14 / 41.2	12 / 34.3	7 / 22.6
	Never	8 / 21.1	12 / 35.3	13 / 37.1	6 / 19.3
Fear, anxiety	Often	22 / 57.9	10 / 29.4	12 / 34.3	20 / 64.5
	Sometimes	14 / 36.8	13 / 38.2	15 / 42.9	10 / 32.3
	Never	2 / 5.3	11 / 32.4	8 / 22.8	1 / 3.2
Increased aggressiveness, proneness to conflict	Often	24 / 63.2	13 / 38.3	9 / 25.7	16 / 51.6
	Sometimes	10 / 26.3	18 / 52.9	24 / 68.6	13 / 41.9
	Never	4 / 10.5	3 / 8.8	2 / 5.7	2 / 6.5
Manifestations of depression, suicidal thoughts	Often	5 / 13.2	2 / 5.9	0 / 0	3 / 9.7
	Sometimes	7 / 18.4	5 / 14.7	6 / 17.1	5 / 16.1
	Never	26 / 68.4	27 / 79.4	29 / 82.9	23 / 74.2
Gloomy mood, dissatisfaction with oneself and others	Often	19 / 50.0	12 / 35.3	15 / 42.9	22 / 70.9
	Sometimes	16 / 42.1	17 / 50.0	14 / 40.0	9 / 29.1
	Never	3 / 7.9	5 / 14.7	6 / 17.1	0 / 0
Cognitive impairment (difficulty concentrating, memorizing, etc.)	Often	20 / 52.6	8 / 23.5	5 / 14.3	14 / 45.2
	Sometimes	10 / 26.3	15 / 44.1	17 / 48.6	10 / 32.3
	Never	8 / 21.1	11 / 32.4	13 / 37.1	7 / 22.5
Physiological reactions (headaches, muscle tension, chest pain, etc.)	Often	8 / 21.1	5 / 14.7	4 / 11.4	6 / 19.3
	Sometimes	10 / 26.3	9 / 26.5	7 / 20.0	8 / 25.8
	Never	20 / 52.6	20 / 58.8	24 / 68.6	17 / 54.9
Alcohol, drug and/or medication abuse	Often	6 / 15.8	3 / 8.8	4 / 11.4	7 / 22.6
	Sometimes	18 / 47.4	13 / 38.3	13 / 37.1	16 / 51.6
	Never	14 / 36.8	18 / 52.9	18 / 51.5	8 / 25.8

Source: compiled by the authors of this study

velopment) is self-control over behavior and situation, which largely determines adaptation to the social environment. Self-control is influenced by temperament (internalization-externality) and character traits. The effectiveness of self-control is determined by the locus of control, the type of which determines the assessment of the situation, the attitude to difficulties, and the success of overcoming them. A person with an internal locus of control is more effective in overcoming stress and demonstrates a higher level of social adaptation and adequate family and other interpersonal relationships than externalizers; the higher the personal control over the situation, the lower the level of stress anxiety. The internal locus of control ensures responsibility in decision-making, a proactive social attitude, emotional stability, and awareness of prospects. Individuals with an external locus of control (low level of subjective control) are characterized by emotional imbalance, high tension, helplessness, low motivation, lack of confidence in their abilities, severe anxiety, suspicion, aggressiveness, decreased proactive attitude, leaving problems unresolved, and stopping in front of obstacles. Thus, the above factors and personality features can determine the course of a police officer's mental activity and their ability to resist stressful phenomena of combat (service) activities.

To address the second task of the research, we have conducted a survey to investigate the frequency of symptoms of psychological trauma in police officers–combatants, depending on their type of temperament (Table 1).

The significance of the difference between the frequency of symptoms of psychological trauma in groups of combatants is presented in Table 2.

Based on the research, it has been found that the most common symptoms of psychological trauma (often manifested) in police officers–combatants, regardless of their temperament type, are: gloomy mood, dissatisfaction with oneself and others; increased aggressiveness, proneness to conflict; fear,

Table 2. The significance of the difference between the frequency of symptoms of psychological trauma in groups of police officers–combatants (n=138)

Symptoms of psychological trauma	χ^2 C-S	χ^2 C-P	χ^2 C-M	χ^2 S-P	χ^2 S-M	χ^2 P-M
Sleep disorders	21.188*	14.872*	0.171	1.174	24.782*	18.061*
Fear, anxiety	28.810*	17.406*	1.167	2.318*	37.564*	25.501*
Increased aggressiveness, proneness to conflict	15.192*	36.095*	5.681*	5.172*	3.589*	15.182*
Manifestations of depression, suicidal thoughts	4.022*	14.322*	0.924	5.859*	1.165	5.859*
Gloomy mood, dissatisfaction with oneself and others	5.256*	3.981*	13.886*	2.031	35.156*	25.708*
Cognitive impairment (difficulty concentrating, memorizing, etc.)	18.014*	32.964*	1.219	2.775*	10.462*	22.907*
Physiological reactions (headaches, muscle tension, chest pain, etc.)	1.490	5.864*	0.134	2.079	0.765*	4.287*
Alcohol, drug and/or medication abuse	5.847*	4.415*	3.315*	0.372	17.364*	14.604*

Notes: * – statistically significant differences between groups
Source: compiled by the authors of this study

anxiety; sleep problems (nightmares, dreams, difficulty falling asleep, intermittent sleep); cognitive disorders (difficulty concentrating, memorizing, mental activity, logical thinking). At the same time, it has been found that choleric and melancholic people show symptoms of psychological trauma more often than sanguine and phlegmatic ones. Thus, increased aggressiveness and proneness to conflict are frequently detected in 63.2 % of choleric people and 51.6 % of melancholic people; gloomy mood, dissatisfaction with oneself and others – in 70.9 % of melancholic and 50.0 % of choleric people; fear and anxiety – in 64.5 % of melancholic and 57.9 % of choleric patients; sleep problems – in 58.1 % of melancholic and 55.3 % of choleric people; cognitive disorders are inherent in 52.6 % of choleric and 45.2 % of melancholic patients. A fairly high number of combatants sometimes abuse alcohol, drugs and/or medications (51.6 % of melancholic, 47.4 % of choleric, 38.3 % of sanguine, and 37.1 % of phlegmatic patients). Frequent manifestations of depression and suicidal thoughts are observed in 13.2 % of choleric, 9.7 % of melancholic, and 5.9 % of sanguine people. Even disorders in the physiological sphere (headaches, muscle tension, chest pain, problems in the cardiovascular system, digestive system, etc.) are most common in choleric and melancholic (21.1 % and 19.3 %, respectively) people. Combatants of sanguine and phlegmatic temperament types most often, but less pronounced than choleric and melancholic types, show symptoms of psychological trauma such as dissatisfaction with themselves and others, gloomy mood, aggressiveness, anxiety, and sleep problems. The results obtained are confirmed by statistical analysis, which showed statistically significant differences between groups S, P and C, M by all indicators (Table 2). All of this indicates that temperament, as one of the main characteristics of individual psychological features, is a factor that determines the occurrence and manifestation of symptoms of psychological trauma. That is, police officers–combatants with choleric and melancholic temperaments are more likely to be traumatized than sanguine and phlegmatic police officers.

DISCUSSION

Study [12] shows that psychological reactions to a stressful event occur in all combatants but may differ in severity and consequences. Scientists [13] point out that the level of influence of a psychological traumatic event on a person depends on the strength of affect and personal reactions to a stressful situation. Stress becomes psychologically traumatic if its level exceeds the defense mechanisms of the psyche, stress resistance, and individual adaptive capabilities. The authors [14] emphasize that a psychologically traumatic event violates the integrity of the functioning of the four spheres of personality (physical, emotional, cognitive, and spiritual), which form an integral system of personality. A psychological traumatic event, having violated the integrity of the psyche, causes traumatic stress, which then turns into psychological trauma [15].

There are several approaches to understanding psychological trauma [16]. In the psychoanalytic approach, the source of trauma is the affective experiences of the individual caused by external stimuli and the inability of the psyche (defense mechanisms) to cope with the shock. The nature of trauma is understood as an unconscious mental process that seeks to re-live the situation and respond to it. In the psychoanalytic approach, trauma is caused not only by external events but also by internal factors: the psyche continues to traumatize itself with obsessive repetitions of memories of the event (intrusions) and experiences (flashbacks), which leads to re-traumatization. In the cognitive approach,

the source of psychological trauma is the individual psychological significance of the traumatic event, its maladaptive meaning that a person attributes to the event (subjectivity of perception), and loss of control over the situation. In the existential approach, events become traumatic due to the horror experienced by a person, as the basic perception of life is destroyed at the time of the event. In the body-oriented approach, the nature of trauma is considered to be an incomplete physiological process (an incomplete bodily reaction to a traumatic event): the energy that was mobilized by the body in a situation of shock has no conditions for use, so it remains (is blocked) in the body. In the Gestalt approach, the nature of trauma is considered to be the result of interruption of the process of experiencing a traumatic event (the effect of an "unfinished action/situation," an unfinished reaction to a traumatic event), accompanied by a complex of mental states.

According to scientists [2], in addition to external and social factors, the main factors that influence the occurrence of psychological trauma are a person's individual psychological features, including temperament, character, and personality orientation.

Behavior and activity – the course of all mental processes and individual stable features of all personality substructures – depend on temperament [17]. Individual peculiarities of responses are manifested in such properties (manifestations) as sensitivity – the least force of influence for the occurrence of a response, the speed of its occurrence; response rate – speed of mind, ingenuity, movements, etc.; responsiveness (the opposite of passivity) in overcoming difficult conditions and situations to achieve the goal (purposefulness, perseverance, etc.); rigidity (the opposite of plasticity) – difficulty in adapting to new and unexpected situations, influences, inertia of behavior, habits, judgments; plasticity – ease of adaptation, flexibility of behavior; extraversion (opposite – introversion) – outward orientation of proactive attitude under the influence of current events; introversion – inward orientation under the influence of past and future feelings, thoughts, images; that is, the behavior of an extrovert depends on the influence of the surrounding situation at a given moment and in a given place of an introvert – on his/her inner world; emotional excitability (the opposite property is emotional stability) – a measure of the force of influence for the emergence of an emotional response, the speed of its occurrence. Therefore, according to the authors [3], each person will react individually to the same traumatic events (influences).

According to experts, the character traits of combatants [18] also play an important role in the response to a traumatic event. First, it is worth talking about overdeveloped character traits (accentuation) that manifest during a traumatic event. Thus, scientists point to the possible development of combatants' negative mental states depending on their character accentuation [19]. In particular, the emotional type, due to excessive emotions, sensitivity, tearfulness, extremely deep and prolonged experiences of any event, is prone to intrusion during a traumatic event; the neurotic (anxious and fearful) type, due to increased fearfulness, anxiety, and low self-esteem, is prone to psychological trauma with phobic experiences, PTSD, and adaptation disorders; cyclothymic type (affective-labile) due to constant instability of mood and emotions has a high probability of PTSD; pedantic type (rigid accentuation) due to the inability to displace traumatic experiences is at increased risk of PTSD. At the same time, the demonstrative type of character accentuation is prone to PTSD and adjustment disorders due to increased feelings of indifference from others and an increased ability to displace negative experiences [20]. Given this, being aware of a combatant's dominant character traits makes it possible to predict how they will act in various service and off-duty situations [9, 12]. Thus, temperament, character traits and accentuations, and personality orientation can influence the choice of a certain protection mechanism from a traumatic event.

The results of our research confirm the above conclusions of many scientific works. Thus, in our study it was found that symptoms of psychological trauma are more common in choleric and melancholic people than in sanguine and phlegmatic people. Thus, increased aggressiveness, conflict, gloomy mood, dissatisfaction with oneself and others, fear and anxiety, sleep problems, cognitive impairment are often found in more than half of choleric and melancholic people. Alcohol abuse, frequent manifestations of depression, suicidal thoughts are also more pronounced in representatives of choleric and melancholic temperaments.

Scientists [10, 20] argue that police officers' response to the impact of combat (service) stressors depends on their significance for a particular person and their ability to control behavior. The degree of influence on the psyche depends on individual characteristics, namely, the type of nervous system, character traits, level of motivation, etc. The results of our research confirm the conclusions of the works of many scientists and complement and expand them. [2, 4, 6, 16].

CONCLUSIONS

The main individual psychological features (characteristics) that are factors in the occurrence of psychological trauma in combatants have been identified: tempera-

ment, character, and personality orientation. It has been found that the emotional experiences of combatants of traumatic events depend on the type of temperament and, therefore, the likelihood of psychological trauma. The frequency of manifestation of symptoms of psychological trauma in police officers–combatants, depending on their type of temperament, has been investigated. It has been established that the most common symptoms of psychological trauma in combatants, regardless of the kind of temperament, are gloomy mood, dissatisfaction with oneself and others, increased aggressiveness, proneness to conflict, fear, anxiety, sleep problems, and cognitive impairment. At the same time, choleric and melancholic combatants show symptoms of psychological trauma more often than sanguine and phlegmatic individuals. Thus, increased aggressiveness and proneness to conflict are frequently detected in 63.2 % of choleric people and 51.6 % of melancholic people; gloomy mood, dissatisfaction with oneself and others – in 70.9 % of melancholic and 50.0 % of choleric people; fear and anxiety – in 64.5 % of melancholic and 57.9 % of choleric patients; sleep problems – in 58.1 % of melancholic and 55.3 % of choleric people; cognitive disorders are inherent in 52.6 % of choleric and 45.2 % of melancholic patients. A fairly high number of combatants sometimes abuse alcohol, drugs and/or medications (51.6 % of melancholic, 47.4 % of choleric, 38.3 % of sanguine, and 37.1 % of phlegmatic patients). Frequent manifestations of depression and suicidal thoughts are observed in 13.2 % of choleric, 9.7 % of melancholic, and 5.9 % of sanguine people. The research shows that police officers–combatants with choleric and melancholic temperament types are more likely to be traumatized than sanguine and phlegmatic police officers. Therefore, individual psychological characteristics influence the choice of the type of psychological defense mechanisms in a stressful situation, which must be taken into account when planning and organizing psychological rehabilitation measures for police officers with signs of combat trauma.

PROSPECTS FOR FURTHER RESEARCH

It is planned to study the frequency of symptoms of psychological trauma manifestation in the civilian population depending on their individual psychological features.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Level of cholecystokinin after cholecystectomy in patients with irritable bowel syndrome and obesity

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ABSTRACT

Aim: To investigate changes in cholecystokinin (CCK) levels after cholecystectomy in patients with IBS and increased on body mass index (BMI).

Materials and Methods: We examined and treated 57 patients. The examined patients were divided into two groups depending on the method of cholecystectomy: group 1 included 35 patients who underwent laparoscopic cholecystectomy (LCE), and group 2 consisted of 22 patients who underwent open cholecystectomy (OCE). The level of CCK was determined in blood serum.

Results: A correlation was established between BMI and the clinical form of IBS, namely, in patients of group 1, overweight correlated with IBS-D ($r=0.86$; $p<0.01$), while in patients of group 2, obesity of the 3rd degree correlated with IBS-constipation (IBS-C) ($r=0.90$; $p<0.01$). A significant increase in the level of CCK in the blood serum of the examined patients with IBS and anorexia was established even before the CE.

Conclusions: In patients with IBS and GSD, an increase in the level of CCK in the blood serum was found, which increases maximally during cholecystectomy, especially its open form. A positive correlation was established between the level of CCK and the form of IBS, namely $r=0.90$; $p<0.01$ for patients with IBS-diarrhoea and $r=0.96$; $p<0.01$ for patients with IBS-constipation after cholecystectomy.

KEY WORDS: gallstone disease, irritable bowel syndrome, cholecystectomy, cholecystokinin, body mass index, obesity

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INTRODUCTION

Gallstone disease (GSD) are a common gastrointestinal disease and one of the main causes of hospitalisation, which is associated with a significant burden on health and the economy. The prevalence and incidence rates are approximately 15.0 % and 0.6 % per year, respectively [1].

Gallstone disease is more common in patients with excess body weight [2]. The main risk factor associated with the development of this disease is central obesity, and given the increasing prevalence of overweight and obesity worldwide, the prevalence of GSD is expected to continue to rise. Gallstone disease has a high medical burden due to its treatment, which is mainly surgical [3].

Obesity is recognised as a major risk factor for gallstone disease, but this disease has also been described in patients who are not obese. Therefore, it should be noted that the development of GSD is determined by a combination of various modifiable risk factors, such as physical inactivity, diet, medication and body mass index (BMI), as well as non-modifiable risk factors, such as age, ethnicity, genetics, gender and number of births. The most common type of stones that form in obese and non-obese patients are cholesterol stones (>90%), which are formed as a result of cholesterol metabolism products and cholesterol oversaturation of bile. However, other processes are also involved in the pathophysiology of this disease [3, 4].

The development of GSD is also associated with an imbalance between the liver, gallbladder and intestine, where cholesterol synthesis occurs. Changes in these processes are caused by an imbalance in the transport of bile lipids, cholesterol esterification enzymes, regulatory signalling pathways, and gallbladder motility [3].

Epidemiological studies have shown that 12–43% of patients with irritable bowel syndrome-diarrhoea (IBS-D) have excessive bile acid excretion, and nearly 25% of patients with IBS-D suffer from bile acid malabsorption [5]. Analysis of bile acid profiles in the faeces of patients with IBS showed that bile acid levels were higher in patients with IBS-D and lower in patients with IBS-constipation (IBS-C) than in healthy control groups

[6]. Therefore, bile acids play an important role in the pathology of IBS [7, 8]. Cholecystectomy has a direct effect on bile acid metabolism [7, 9]. Studies have shown a higher incidence of IBS in patients with cholelithiasis and in those with a history of cholecystectomy [10].

Therefore, research into various biologically active substances that affect gastrointestinal motility in patients with obesity and IBS after cholecystectomy is relevant.

AIM

The aim to investigate changes in cholecystokinin levels after cholecystectomy in patients with IBS and increased BMI.

MATERIALS AND METHODS

We examined and treated 57 patients with GSD who had an increased BMI in combination with various clinical variants of IBS. All patients underwent cholecystectomy (CE) in the surgical department No. 1 of the Municipal Non-Profit Enterprise «St. Martin's Hospital».

Among the examined patients, there were 42 women (73.7%), whose average age was 38.9±5.3 years; there were 15 men (26.3%), whose average age was 42.5±4.4 years. The control group included 20 healthy individuals (15 (75.0%) women and 5 (43.3%) men). The average age of women in the control group was 41.7±5.1 years, and that of men was 39.3±4.7 years.

All examinations were conducted with patient approval (written authorization for relevant diagnostic procedures and therapeutic interventions was secured from every patient and control participant), implementing comprehensive safeguards to maintain confidentiality of collected data. The research approach adhered to the 1975 Helsinki Declaration on Human Rights and its 1983 amendments, the Council of Europe's Convention on Human Rights and Biomedicine, as well as Ukrainian legal requirements.

The exclusion criteria were: age under 18 and over 75; BMI corresponding to normal body weight or reduced body weight; cholangiocellular carcinoma, pancreatic cancer, colon cancer; systemic autoimmune diseases, including primary sclerosing cholangitis; pulmonary tuberculosis; mental illnesses that prevent adequate assessment of the patient's health and signing of informed consent for diagnosis and treatment; pregnancy and lactation.

All examined patients underwent general clinical, anthropometric, instrumental, and laboratory examinations. To confirm the diagnosis, the nature of the complaints and medical history were described in detail.

IBS diagnosis was established according to Rome IV criteria and clinical recommendations from the Ukrainian Gastroenterological Association for irritable bowel syndrome patient management.

The anthropometric assessment involved measuring height, weight, and waist circumference, with subsequent BMI calculation. Based on WHO standards, subjects were classified according to BMI ranges: severe underweight (BMI ≤16.0); underweight (16.0-18.5); normal weight (18.5-24.9); overweight (25.0-29.9); Grade I obesity (30.0-34.9); Grade II obesity (35.0-39.9); and Grade III obesity (BMI ≥40.0).

All participants received abdominal ultrasound scanning following standard protocols, with particular focus on hepatobiliary and biliary tract structures. Measurements of gallbladder and common bile duct dimensions were obtained, along with identification of the presence or absence of gallbladder stones.

The examined patients were divided into two groups depending on the method of cholecystectomy: group 1 included 35 patients who underwent laparoscopic cholecystectomy (LCE), and group 2 consisted of 22 patients who underwent open cholecystectomy (OCE).

The level of cholecystokinin (CCK) was determined in blood serum using an enzyme immunoassay with a test system from „Peninsula Laboratories" (USA).

Patient data analysis and result processing were accomplished using STATISTICA 10.0 statistical software (StatSoft Inc, USA), applying parametric and non-parametric methods for statistical assessment of outcomes.

FRAMEWORK

The study was performed within the framework of the scientific topics "Intra-abdominal hypertension (symptoms, pathogenesis, prevention, treatment) and its impact on the occurrence and development of pathological syndromes and complicated course of surgical diseases" researched by the Department of Surgical Disciplines of State University "Uzhhorod National University".

RESULTS

Before performing CE, the main clinical and anthropometric features of the examined patients were characterized. After analyzing the anthropometric data, patients in both groups were divided according to their BMI (Table 1).

As the results indicate, the vast majority of patients in group 1 who underwent laparoscopic CE were overweight, while among patients in group 2, grade 3

Table 1. Indicators of BMI change in the examined patients

Indicator	Examined patients, Absolute number / %	
	1 group (n= 35)	2 group (n= 22)
Overweight	16 / 45.7 %	_
Obesity – grade 1	10 / 28.6 %	3 / 13.6 %
Obesity – grade 2	9 / 25.7 %	7 / 31.8 %
Obesity – grade 3	_	12 / 54.6 %

Source: compiled by the authors of this study

Table 2. Characteristics of clinical forms of IBS in examined patients

Clinical forms of IBS	Examined patients, Absolute number / %	
	1 group (n= 35)	2 group (n= 22)
IBS-diarrhoea	19 / 54.3 %	5 / 22.7 %
IBS-constipation	9 / 25.7 %	13 / 59.1 %
IBS-mixed	5 / 14.3 %	3 / 13.6 %
IBS-unclassified	2 / 5.7 %	1 / 4.6 %

Source: compiled by the authors of this study

obesity was more common. These arguments served in favor of open HE in patients in group 2.

We also assessed the clinical form of IBS in our patients with GSD against the background of increased BMI (Table 2).

It was found that in group 1 of the examined patients, IBS-D individuals predominated, while in group 2 of patients, IBS-C was more common. A correlation was established between BMI and the clinical form of IBS, namely, in patients of group 1, overweight correlated with IBS-D ($r=0.86$; $p<0.01$), while in patients of group 2, obesity of the 3rd degree correlated with IBS-C ($r=0.90$; $p<0.01$).

The level of CCK in the blood serum of the examined patients was assessed before and after CE (Table 3).

A significant increase in the level of CCK in the blood serum of the examined patients with IBS and anorexia was established even before the CE. As the obtained results indicate, open CE contributes to a more pronounced increase in the level of CCK in these patients (Table 4).

The analysis conducted indicates a relationship between the change in the level of CCK in the blood serum and the clinical form of IBS in the examined patients. At the same time, in patients of group 1, the correlation was established with the predominance of diarrhea, while in patients of group 2 - constipation in the examined patients with IBS and GSD.

Therefore, the study of changes in the level of regulatory gastrointestinal hormone in patients with GSD and IBS may open new perspectives on the development and progression of comorbid pathology.

DISCUSSION

A study by Peng D. et al (2024) highlights the potential increase in the risk of developing IBS after cholecystectomy. The role of many factors in this regard remains unclear, requiring further research to confirm the causal relationship and study the underlying mechanisms [11].

The small intestine hormone CCK is known to be the primary stimulator of gallbladder contraction after a meal. Reduced gallbladder motility may lead to gallstone formation by nucleation of cholesterol into solid cholesterol monohydrate crystals when the cholesterol saturation index in gallbladder bile exceeds the solubility limit. In addition, impaired gallbladder motility has been observed in patients with cholesterol gallstones. These observations have led to the suggestion that reduced plasma CCK levels may contribute to the development of cholelithiasis [12].

On the other hand, Simrén M et al. (2018) found that mucosal and plasma CCK levels were significantly higher in IBS patients than in controls. Abdominal pain was identified as the dominant symptom in IBS patients, and pain scores, pain symptoms, and pain frequency were positively correlated with mucosal and plasma CCK levels [13, 14].

Another study evaluated the sensory impact on serosal and mucosal receptors in IBS (12 patients with IBS underwent a procedure of gradual rectal stretching during infusion of CCK and placebo in random order). Ten other healthy individuals from the control group and ten patients with IBS underwent intermittent stretching during infusion of CCK and placebo in random order. No differences in rectal elasticity during stretching were found between patients with IBS and the control group.

Table 3. Level if CCK in blood serum in examined patients

Examined patients	Level of CCK, ng/ml				
	Before CE	After CE			
		day 1	day 7	day 14	day 21
Control group (n= 35): 0.67±0.04					
1 group (n= 35)	0.98±0.05*	2.86±0.09	2.03±0.07	1.85±0.06	1.44±0.04
2 group (n= 22)	1.05±0.09**	3.41±0.08 +	4.11±0.10 ++	3.46±0.08 ++	2.96±0.07 ++

Note: the difference between the control group and the group examined is significant: * – $p<0.05$; ** – $p<0.01$; the difference between the groups of examined patients is significant: + – $p<0.05$; ++ – $p<0.01$

Source: compiled by the authors of this study

Table 4. Comparison of CCK levels with the clinical variant of IBS in examined patients

Clinical forms of IBS	Level of CCK, ng/ml			
	1 group (n= 35)		2 group (n= 22)	
	Before CE	After CE (day 1)	Before CE	After CE (day 1)
IBS-diarrhoea	r=0.76; p<0.01	r=0.90; p<0.01	_	_
IBS-constipation	_	_	r=0.84; p<0.01	r=0.96; p<0.01

Source: compiled by the authors of this study

CCK did not affect the perception of urge and pain in the control group and patients with IBS. Similar results were obtained during intermittent stretching, but at higher stretching pressures, CCK significantly increased rectal sensitivity in patients with IBS. The authors concluded that infusion of exogenous CCK to plasma levels typically observed in the postprandial state did not affect rectal motor function or sensation during distension, but significantly increased pain sensation in patients with IBS during rapid intermittent distension [15].

Our results also indicate an increase in the level of CCK in patients with IBS, especially in the early stages of the postoperative period in GSD. Therefore, the study of the level of gastrointestinal hormones may open new perspectives for a deeper study of the pathogenetic mechanisms of the course of comorbid pathology.

CONCLUSIONS

1. In patients with IBS and GSD, an increase in the level of CCK in the blood serum was found, which increases maximally during cholecystectomy, especially its open form.
2. A positive correlation was established between the level of CCK and the form of IBS, namely r=0.90; $p<0.01$ for patients with IBS-diarrhoea and r=0.96; $p<0.01$ for patients with IBS-constipation after cholecystectomy.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Exploring the Association of *Campylobacter jejuni* with inflammatory bowel diseases: insight from a clinical study

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ABSTRACT

Aim: To demonstrate a possible association between *Campylobacter jejuni* and inflammatory bowel disease Iraqi patients.

Materials and methods: The study involved two groups. The inflammatory bowel disease patient group consisted of 90 participants. This group was further classified into two subgroups: Crohn's disease (n) and Ulcerative colitis (n). The control group consisted of 90 healthy participants who had no history of inflammatory bowel disease. Age and gender distributions in inflammatory bowel disease patient and control groups were computed. The association between inflammatory bowel disease and *C. jejuni* was determined by identifying the bacterial infection using various detection methods, including culturing, biochemical tests and real-time PCR (Polymerase chain reaction). The association was further assessed by comparing the symptoms of IBD patients and *C. jejuni* positive samples.

Results: Finding of the conventional identification showed 5% prevalence of *C. jejuni* among inflammatory bowel disease patients whereas the real-time Polymerase chain reaction. Results showed significantly higher prevalence. Based on real-time Polymerase chain reaction. Results, a significant association was found between *C. jejuni* infection with inflammatory bowel disease and Crohn's disease patients (P-value=<0.001).

Conclusions: No significant differences observed in detection of inflammatory bowel disease and Crohn's disease using conventional and molecular methods. The study provides valuable insights into a possible association between *C. jejuni* and inflammatory bowel disease.

KEY WORDS: *C. jejuni,* inflammatory bowel disease, culturing, biochemical test

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ABBREVIATIONS

IBD - Inflammatory Bowel Disease
CD - Crohn's Disease
UC - Ulcerative Colitis
PCR - Polymerase Chain Reaction

INTRODUCTION

Inflammatory Bowel Disease (IBD) is a recurring inflammatory disorder that affects the gastrointestinal system. It comprises two main types: Crohn's disease (CD) and ulcerative colitis (UC). IBD is most prevalent in North America and Western Europe, with a rate of 0.3%. However, in recent year, there has been an increase in its occurrence in other regions globally, making it a significant healthcare concern worldwide [1], while IBD can affect individuals of all age groups, it tends to be more commonly diagnosed in late adolescents and young adults [2]. This particular age bracket seems to be more susceptible to developing IBD, although it can still occur at any age. Furthermore, there is a notable gender-based variation in the distribution of IBD within this demographic. Studies have shown that IBD can affect both males and females, but the prevalence and disease patterns may differ between the genders. For instance, males tend to have a higher incidence of Crohn's disease, while females have a higher incidence of ulcerative colitis. The reasons for these gender-based differences are not yet fully understood and require further research [2-6]. Though the specific cause of IBD is still unknown, it is believed to be a consequence of a patient's genetics, microbiome, immunological response, and environment, which generate an excessive and inappropriate immune reaction to commensal flora in genetically predisposed individuals [7-9]. Previous studies have indicated a connection between IBD and microbes in patients with IBD [1, 7]. *C. jejuni* is one of the reported causes of gastroenteritis in humans [7]. The disruptive nature of *C. jejuni* infections on normal gut homeostasis have been unveiled, which, in turn, is believed to contribute to the development of IBD [8]. Despite efforts to implement treatment measures, the prevalence of

C. jejuni infections continues to escalate globally. In Iraq, as in numerous other nations, IBD constitutes a prevailing health concern, posing significant challenges to human well-being [9-10], however, despite its substantial impact, the research landscape concerning the relationship between IBD and the bacterium *C. jejuni* remains notably underexplored. Addressing this research gap in the etiology of IBD is imperative not only for expanding our scientific understanding of its etiology in a specific region but also for potentially developing improved diagnostic and therapeutic strategies that are specifically tailored to the unique healthcare landscape of the country in question. The identification and confirmation of *C. jejuni* in clinical samples involve a multifaceted strategy encompassing several techniques. The process usually initiates with the isolation and culture of the bacteria on selective media. Subsequently, a combination of biochemical and molecular tests is used to confirm the existence of the pathogen [11]. The clinical presentation of IBD is known to appear differently among affected person, with symptoms ranging from abdominal pain and diarrhea to more serious indication, such as weight loss and rectal bleeding [12]. Contrasting the symptom profiles of IBD patients to those individuals carrying positive *C. jejuni* samples can contributes to a more nuanced understanding of the potential effects of bacterial presence on symptomatology. This clinical study aimed to provide insights in to the possible association of *C. jejuni* with IBD through the detection of the bacteria in stool samples of local patients with IBD diseases. Strain typing of *Campylobacter conscious* has previously been done using a variety of typing techniques, including multi-locus sequence typing (MLST) and amplified fragment length polymorphisms (AFLP) 23 S rRNA PCR [13]. Multilocus sequence typing (MLST) is a commonly used typing technique that may identify population patterns and lineages in microorganisms [14]. Research has demonstrated that MLST has a good discriminatory power for *Campylobacter jejun*i, *Campylobacter coli*, and developing *Campylobacter* species. The ease with which sequence data can be shared across laboratories for use in international epidemiological research is one of the benefits of MLST [15]. This includes the identification of the bacteria within the studied groups through conventional and real time PCR techniques, also examined the clinical symptoms of IBD patients in comparison to individuals who had positive *C. jejuni* samples.

MATERIALS AND METHODS

SAMPLES PREPARATION

The study included a total of 180 participants, 90 diagnosed with IBD by specialist in gastroenterology comprising the patient group. The remaining 90 participants were apparently healthy individuals and formed control group. The samples for the study had been collected during a period ranging from December 2022 to the end of March 2023, from Al-Imamain Al-Kadhimain Medical City. The study was conducted in the laboratories of Microbiology Department in the College of Medicine-Al-Nahrain University.

INCLUSION CRITERIA

Inclusion criteria were taking samples from patients with IBD over than 18 years old, while exclusion criteria were avoiding patients have or had previously any type of malignancy, or have other disease such as muscular dystrophy (MD), thyroid disorder, congenital adrenal disease, and renal disorder, any congenital or inherited metabolic disorder. Also, pregnant or lactating women and patient on antibacterial drugs with the last three days were avoided. Small quantities of diarrheal stool samples were collected from all participants by plastic spoon and transferred to pre-labeled clean dry plastic container with locked cup under sterile condition. Samples were then transported to the laboratory in cool box within three hours of collection [16].

ETHICAL APPROVAL

This study was approved by the Institutional Review Board of the College of Medicine; Al-Nahrain University under the number 239 and date 30/7/2019.

BACTERIAL IDENTIFICATION

CONVENTIONAL METHOD

Totally, 5 g of freshly collected faucal sample was pre-enriched by suspending the faeces in 45 ml buffered nutrient broth and incubating the suspension at 42°C for 18 hours in a 50-ml closed culture tube were immediately inoculated onto the chromogenic medium CASA (AES Chemunex, France) proved highly efficient for *C. jejuni* and incubated to 48 hours at 42°C under conditions that were microaerophilic .Gram staining, microscopy examination, and Oxidase, catalase, and indoxyl hydrolysis biochemical tests were used to confirm the identity of the suspected colony. The same colonies were tested for cytochrome oxidase enzyme production by placing a portion of the test colony onto oxidase paper impregnated with NNN'N' tetramethyl-p-phenylene-diamine dihydrochloride (Oxoid, Basingstoke, UK). Purple color change was recorded as positive reaction. Reactive colonies were processed for DNA and a portion stored in skimmed milk at -80°C for further characterization [17]. Following this, a series of biochemical assessments were performed, encompassing glucose fermentation, nitrate reduction, oxidase and catalase tests, as well as hippurate hydrolysis tests [18-20].

DNA PREPARATION

Strains isolated from our hospital were cultured in brain-heart infusion (BHI) broth medium and shaken in an oscil-

Table 1. Primer sequence and PCR product

Gene	Primer	Amplified DNA fragment
CAMP-JEJUNI	F: 5′-TAAAAGCGGTGGATTTGGAC-3› R: 5′-CTTTTTCCTTTTTGCCACCA-3′	167 bp

Source: compiled by the authors of this study

Table 2. PCR reaction solution for detection of CAMP-JEJUNI

Component	Volume [µL]
GoTaq® qPCR Master Mix	10
Forward Primer	0.5
Reverse Primer	0.5
Supplemental CXR Reference Dye	0.2
DNA template	4
Nuclease-Free Water	4.8
Total volume	20

Source: compiled by the authors of this study

Table 3. Real-time PCR protocol

No.	Steps	Temperature	Time	Cycle No.
1	Initial denaturation	94˚C	6 min	1
2	Denaturation	94˚C	40 sec	35 cycles
3	Annealing	56 ˚C	45 sec	
4	Extension	72 ˚C	1 min	
5	Final extra extension	72 ˚C	10 min	1

Source: compiled by the authors of this study

Table 4. Account of IBD patient group and control group based on sex and age

Demographic Data			Study groups		P value
			IBD Patient	Control	
Age	(Mean ±SD) years		34.21±10.16	33.47±11.06	0.778
	(Min-Max)		(19-57)	(18-70)	
Sex	Female	n	36	42	0.215*
		[%]	45.0	52.5	
	Male	n	44	38	
		[%]	55.0	47.5	

*P value less than 0.05 was considered as statistically significant

Source: compiled by the authors of this study

Table 5. Growth of *C. Jejuni* in Skirrow's medium

lator at full temperature of 37°C for 20–24 h. DNA was then extracted using a bacterial genomic DNA extraction kit to prepare for subsequent experiments. DNA extraction .The bacterial genomic DNA extraction kit used in this paper was purchased from Alpha DNA Ltd. (Canada).

REAL TIME PCR METHOD

Extraction of DNA from the entirety of stool samples was executed meticulously, adhering to the comprehensive guidelines provided by the Geneaid Presto Stool DNA Extraction Kit. Using a LAMBDA Bio PLUS, the concentrations and purities of the extracted DNA were calculated from the absorbance at 260 nm (A260) and the A260/A280 ratio. Spectrophotometer (Perkin-Elmer, Norwalk, CT, USA), and sterile deionized water was used to dilute the DNA to the proper quantities. Assessment of DNA concentration and subsequent purification steps were meticulously conducted through employment of the Nano-drop system. Designing of primers was performed using the robust Primer 3plus software. These primers, specifically designed for the V4 region, underwent a meticulous validation process, inclusive

Bacteria culture	n	[%]
No growth	76	95.0
Growth	4	5.0
Total	80	100

Source: compiled by the authors of this study

Table 6. Occurrence of *C. jejuni* in IBD patients according to disease type

C. jejuni		IBD		P value
		Crohn's disease	Ulcerative colitis	
		[%]	[%]	
Bacteria culture	No growth	97.4	92.9	0.347*
	Growth	2.6	7.1	

*P value less than 0.05 was considered as statistically significant
Source: compiled by the authors of this study

Table 7. PCR detection of *C. jejuni* in studied groups

Bacteria		Study Groups		P value
		IBD	Controls	
C. jejuni [copy/m]	Median	796.68	370.34	<0.001*
	Percentile 05	193.22	32.5	
	Percentile 95	5685.9	2340.02	

* P value less than 0.05 was considered as statistically significant
Source: compiled by the authors of this study

of scrutiny under the University Code of Student Conduct (UCSC) programs, alongside alignment with corresponding reference sequences housed within the National Center for Biotechnology Information (NCBI) database. The synthesis and lyophilization of these primers were entrusted to the expertise of Alpha DNA Ltd. (Canada), (Table 1).

A total of 40 µL of the PCR mixture were spun down with a minicentrifuge. PCR protocol (Table 3).

COMPARING CLINICAL SYMPTOMS IN INDIVIDUALS WITH IBD AND C. JEJUNI POSITIVE SAMPLES

The symptom data from the IBD patients were collected and compare with signs exhibited in samples that indicate a positive presence of *C. jejuni* [19].

RESULTS

AVERAGE AGE AND GENDER DISTRIBUTION IN IBD PATIENTS

The average age of individuals in both studied groups was computed, the mean age among individuals diagnosed with IBD is 34.21±10.16 years and among healthy individuals is 33.47 ± 11.06. Examining the gender distribution within this cohort, males accounted for a predominant 55% representation, while females constituted 45%. Statistical analysis indicated that no statistically significant disparities, bearing a P-value of less than 0.05, emerged between the patient cohort and the control group in relation to both age and gender (Table 4).

BACTERIAL DETECTION

CONVENTIONAL IDENTIFICATION OF C. JEJUNI

The outcomes arising from the cultivation of *C. jejuni* showed that the bacterial growth rate stood at approximately 5% within the studied samples. In contrast, the control group presented an entirely negative outcome, with no occurrences of *C. jejuni* (Table 5).

Notably, the prevalence of *C. jejuni* growth exhibited distinct patterns based on the subcategories of the disease. For example, among individuals diagnosed with Crohn's disease, the presence of *C. jejuni* was observed in approximately 2.6% of cases. While the occurrence of *C. jejuni* growth within the subset of individuals afflicted by ulcerative colitis, registered at a relatively higher rate of 7.1%. However, these observed differences were not found to be statistically significant (P-value <0.05) (Table 6).

The dark field or phase contrast preparations showed that all bacterial isolates have a distinctive darting motility. Gram stain preparations revealed Gram negative, curved or spiral rods. All isolates were positive for reduced nitrate, oxidase and catalase, indoxyl acetate and hippurate-tests, while were

Table 8. Molecular detection of *C. jejuni* in IBD types

		IBD		P Value
		Crohn's disease	Ulcerative colitis	
C. jejuni (copy/mL)	Median	834.14	758.52	0.583
	Percentile 05	177.4	198.35	
	Percentile 95	5478.3	5893.5	

Source: compiled by the authors of this study

Table 9. Signs and symptoms of IBD patients in association with C. *jejuni*

Signs and symptoms	Bacteria culture								P value
	No Growth				Growth				
Bloody diarrhea	Present		Absent		Present		Absent		0.408*
	n	[%]	n	%	n	[%]	n	[%]	
	34	97.1	42	93.3	1	2.9	3	6.7	
Smoking	Yes		No		Yes		No		0.647
	n	[%]	n	[%]	n	[%]	n	[%]	
	17	94.4	59	95.2	1	5.6	3	4.8	
Abdominal pain	Present		Absent		Present		Absent		0.608
	n	[%]	n	[%]	n	[%]	n	%	
	25	96.2	51	94.4	1	3.8	3	5.6	
Biology Therapy	Yes		No		Yes		No		0.728
	n	%	n	%	n	%	n	%	
	6	100.0	70	94.6	0	0.0	4	5.4	
Behavior	B1		B2		B1		B2		0.136*
	n	[%]	n	[%]	n	[%]	n	[%]	
	26	100.0	11	91.7	0	0.0	1	8.3	
UC extent	No Growth						Growth						0.280*
	E1: Ulcerative proctitis		E2: Left sided UC		E3: Extensive UC		E1: Ulcerative proctitis		E2: Left sided UC		E3: Extensive UC		
	n	[%]	n	[%]	n	[%]	n	[%]	n	[%]	n	[%]	
	28	93.3	3	75.0	8	100.	2	6.7	1	25.0	0	0.0	
CD location	L1: Ileal		L2: Colonic		L3: Ileocolonic		L1: Ileal		L2: Colonic		L3:Ileocolonic		0.630
	n	[%]	n	[%]	n	[%]	n	[%]	n	[%]	n	[%]	
	8	100.0	10	100.0	19	95.0	0	0.0	0	0.0	1	5.0	

*P value less than 0.05 was considered as statistically significant
Source: compiled by the authors of this study

negative for glucose fermentation. This preliminary diagnosis therefore further designated the bacteria as *C. jejuni,* according [20]. This distribution of *Campylobacter* species is consistent with findings from earlier studies conducted in industrialized and developing nations, including Out of the 580 stool samples that were gathered from 11 Kibera schools, 294(51%) of them fit the suspicious Campylobacter spp. phenotype. Upon subjecting these isolates to PCR Campylobacter spp. were identified in 106(18%) of the isolates by means of genus- and species-specific primers. Of the 106 isolates, 28(4.8%) and 44(7.6%) were of the type *C. jejuni* [21].

QUANTITATIVE PCR ANALYSIS OF C. JEJUNI

The utilization of a quantitative PCR approach in this study provided a precise assessment of the presence of *C. jejuni*. The results revealed a noteworthy disparity between the cohort of individuals diagnosed with IBD and the control group. Upon conducting in-depth analysis, a statistically significant difference in the occurrence of *C. jejuni* between these two groups was observed (P-value <0.05) (Table 7).

Conversely, the statistical analysis of the tabulated data revealed a lack of statistically significant differ-

Table 10. Signs and Symptoms of IBD Patients in association with C. *jejuni*

Signs and symptoms		C. jejuni (copy/mL)		
		Median		Percentile 05-95
Abdominal pain	Absent	809.81	177.40	5893.50
	Present	796.68	198.35	4298.66
	P value		0.906	
Smoking	No	851.00	239.60	5893.50
	Yes	607.33	188.09	4298.66
	P value		0.345*	
Bloody diarrhea	Absent	794.33	198.35	5478.30
	Present	813.31	188.09	5893.50
	P value		0.996	
Biology therapy	No	796.68	188.09	5478.30
	Yes	1199.53	198.35	19372.22
	P value		0.465*	
Behavior	B2: stricturing	857.17	124.36	36987.00
	B1: nonstricturing, nonpenetrating	831.79	277.30	4396.32
	P value		0.792	
UC extent	E1: Ulcerative proctitis	843.97	323.78	14852.30
	E2: Left sided UC	793.19	569.30	1642.04
	E3: Extensive UC	420.99	152.07	960.35
	P value		0.048*	
CD location	L1: Ileal	787.32	319.98	5478.30
	L2: Colonic	696.91	124.36	36987.00
	L3: Ileocolonic	892.28	251.20	3595.20
	P value		0.815	

*P value less than 0.05 was considered as statistically significant
Source: compiled by the authors of this study

ence between the two subgroups, Crohn's Disease (CD) and Ulcerative Colitis (UC) within IBD patients (P-value > 0.05), as shown in Table 8.

A COMPARATIVE ANALYSIS OF CLINICAL MANIFESTATIONS BETWEEN IBD PATIENTS AND *C. JEJUNI* POSITIVE SAMPLES

Based on the conventional method results and the statistical analysis conducted, it was found that there was no significant association between signs and symptoms of IBD with and smoking, ulcerative colitis (UC) extent, bloody diarrhea, abdominal pain, use of biological therapy, behavior, CD (Crohn's disease) location (P-value > 0.05), (Table 9).

However, according to the Real time PCR results, signs and symptoms of IBD with *C jejuni* were not correlated with abdominal pain, smoking, bloody diarrhea, behavior, CD location, while were significantly correlated in UC extent (P-value <0.05), (Table 10).

DISCUSSION

AVERAGE AGE AND GENDER DISTRIBUTION ANALYSIS IN IBD PATIENTS

The exact cause of IBD is not well understood, but it is believed to involve a combination of genetic, environmental, and immune system factors. IBD can affect individuals of any age but often presents itself between the ages of 15-30 years [22]. This is consistent with the findings of our study as the mean age of patient group was 34 years. One of the potential factors could indeed contribute to the increased diagnosis of IBD at older ages is the increased awareness among both patients and healthcare providers. As medical technology and awareness have advanced, more individuals might be seeking medical attention for their gastrointestinal symptoms, which in turn leads to more diagnoses being made across a broader age range [23]. Regarding the distribution between males and females, no statistically significant difference in the number of affected males and females, observed little increase in number of affected males over

females. This was consistent with previous studies, which adds credibility to the present study's findings [24-26]. The fact that there is a an increase in the number of males affected with IBD compared to females raises interesting questions about the potential underlying mechanisms and the factors contributing to this gender distribution. While the reasons for this observation might not be fully understood at this point, several hypotheses can be considered. One of them can be the immune response differences between genders which lead to differences in susceptibility to IBD [27]. Exploring the reasons behind such patterns can be crucial for understanding the disease, developing targeted treatments, and improving overall healthcare outcomes.

INTERPRETING THE CLINICAL SIGNIFICANCE OF CONVENTIONAL AND PCR FINDINGS IN IBD PATIENTS WITH *C. JEJUNI*

The results demonstrated the presence of *C. jejuni* in a subset of participants, with a notably higher prevalence observed using the real-time PCR method compared to conventional culturing methods. This underscores the importance of employing advanced molecular tools for accurate detection, which might otherwise be missed using traditional approaches [28]. According to the real time PCR results, there was a significant difference in the prevalence of *C. jejuni* between IBD patients and controls. Several studies also reported an increase in the distribution of *C. jejuni* in IBD patients comparing with the general healthy population. For example, Kaakoush and colleagues found a substantial correlation between *C. jejuni* infection and Crohn's disease compared to healthy controls in their meta-analysis study [29]. Similarly, the work of Barker *et al.* further supports the argument on a relationship between *C. jejuni* and IBD [30]. Their study demonstrated a higher prevalence of this bacterium in individuals with UC compared to healthy controls. The consistency in results across different studies reinforces the reliability of the observed association between *C. jejuni* and IBD. Several hypotheses in literature have been provided in how *C. jejuni* may contribute to the development or exacerbation of IBD, which reinforce the reliability of the observed association between *C. jejuni* and IBD. For instance, *C. jejuni* infection has been found to break an exaggerated immune response, represented by pro-inflammatory cytokines and activation of different immune cells. This may disrupt the balance of the gut microbiota and lead to chronic inflammation in infected individuals [31]. Additionally, *C. jejuni* exhibits surface structures that resemble some host antigens. This mimicry may trigger an autoimmune response, where the immune system attacks both the bacterium and the host's self-tissues, leading to inflammation and disease [32]. Furthermore, *C. jejuni* possesses the ability to adhere and invade intestinal epithelial cells, compromising the integrity of the intestinal barrier. This defect in the barrier can help bacterial translocation and trigger an immune response, hence chronic inflammation [33]. Interestingly, the study did not show significant variations in the detection of *C. jejuni* bacteria between the two types of IBD, UC and CD. This means that the potential contribution of *C. jejuni* may go beyond specific IBD subtypes, suggesting a wider impact on the disease process itself. According to our study, there is a significant correlation between female sex, shorter exclusive breastfeeding length, lower maternal age, lower maternal education, untreated drinking water, and inadequate sanitation and Campylobacter infections [34]. Although the presence of *Campylobacter* species is linked to growth deficiencies, birth weight for age is just a marginal predictor for these species.1. The prevalence of *Campylobacter* was higher in malnourished children from a case control study in Dhaka (6–23 months old) with weight-for-age z score (WAZ) <−2 than in healthy (control) children [weight-for-age z score (WAZ)>−1], but the adjusted effect size was not statistically significant [35]. Using the well-characterized human clinical *C. jejuni* isolate 81–176, another investigation showed that translocation of noninvasive bacteria is increased in C. jejuni-infected human intestinal epithelium [36]. In [37], we have found campylobacter in 5(5%) of our samples overall, additionally, campylobacter was found throughout Iraq, including in the city of Duhok. *Campylobacter* is one of four individuals with diarrhea who are segregated [38]. In the city of Mosul, an incidence of 6.3% of patients with diarrhea had *Campylobacter*. In the western Iraqi city of Ramadi, secluded *Campylobacter* at an 8.92% rate [39]. It is comparable to the findings of a study where the two researchers isolated Campylobacter from the city of Al-Duwaniyah. Using the technique of direct DNA isolation from feces, 100 infected patients had diarrhea, and the percentage of Campylobacter infection was (5.8%) [40] Conversely, a study conducted in India discovered that just 3.40 percent of the 88 participants had diarrhea, which is similar to our findings [41]. Compared to our investigation, current research in New Zealand discovered a negligible amount of *Campylobacter*, finding only 2.9% of all of her samples (1604) [42]. Our study's findings are different from those of Liang et al., who discovered that the lowest percentage of females (36.31%) and the highest percentage of males 63.69% [43], however, Karikari et al. indicate that 32.4% of men and 67.6% of women experienced diarrhea [44-45].

RELATIONSHIP BETWEEN C. JEJUNI INFECTION AND IBD SYMPTOMS

Current study also reveals a lack of significant correlations between certain signs and symptoms of IBD with *C. jejuni* infection and several features defined by the Montreal classification. This may indicate that *C. jejuni's* effect on IBD is not mainly determined by these particular clinical and categorization parameters. This highlights the multifactorial nature of IBD and the numerous complicated interactions that affect the disease development, progression, and symptoms.

CONCLUSIONS

This study clearly shows how crucial *Campylobacter jejuni* is to gastrointestinal health, especially when it comes to the emergence of post-infectious irritable bowel syndrome (IBS). It draws attention to the bacterium's rising prevalence worldwide, its significant effects on the gut microbiota, and the ensuing medical difficulties. It emphasizes the necessity of better epidemiological surveillance, the creation of targeted therapeutic approaches, and a more thorough comprehension of the host-pathogen relationship's dynamics. Examine how *Campylobacter jejuni* contributes to post-infectious IBS.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Virological and antifibrotic efficacy of antiviral treatment for hepatitis B+C in HIV-positive patients

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ABSTRACT

Aim: To study and compare the efficacy of different regimens of combination antiviral therapy for hepatitis C and B in achieving a sustained virological response in patients with HIV/HBV/HCV coinfection and their impact on the degree of liver fibrosis.

Materials and Methods: We examined 113 HIV-infected adult patients with HBV/HCV coinfection who were registered at the Municipal Non-profit Enterprise "Infectious Diseases Center of the Ivano-Frankivsk Regional Council" between 2017 and 2019. The efficacy of HBV AVT was assessed by the achievement of undetectable HBV DNA levels in plasma at the baseline of the surveillance and again 1 year later. Continuous variables were expressed as a median and interquartile range (Q1-Q3). Categorical variables were described as frequencies. Group differences in categorical variables were assessed with Pearson's Chi-square and post-hoc Pearson's Chi-square tests, and in continuous variables, the Kruskal-Wallis' test was used. Wilcoxon signed-rank test was used to compare two dependent groups.

Results: The treatment regimens with two direct-acting antivirals (sofosbuvir/ledipasvir, sofosbuvir+daclatasvir, sofosbuvir/velpatasvir and sofosbuvir/ledipasvir and ribavirin resulted in 100 % sustained virologic response. The efficacy of the pegylated interferon, sofosbuvir, and ribavirin regimen was lower, with sustained virologic response achieved in 86.2 % of patients. The lowest efficacy was observed with the sofosbuvir and ribavirin regimen – sustained virologic response achieved in 75.0 % of patients. Primary HBV resistance to tenofovir/emtricitabine was identified in 1.76 % of patients in the entire cohort. Among patients in the group treated with combined antiviral therapy for HBV and HCV using tenofovir/emtricitabine and direct-acting antivirals with or without ribavirin, the incidence of liver fibrosis F3-F4, as assessed by point-shear wave elastography, decreased from 20 % to 2.5 % ($p<0,05$) after one year of follow-up.

Conclusions: In patients with HIV/HBV/HCV coinfection, interferon-free regimens that include two direct-acting antivirals, either alone or in combination with ribavirin, demonstrated 100 % virologic efficacy and the most beneficial effects on the liver reducing the degree of liver fibrosis.

KEY WORDS: human immunodeficiency virus, hepatitis B, hepatitis C, antiviral agents, liver fibrosis

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INTRODUCTION

Hepatitis B (HBV) and hepatitis C (HCV) coinfection are common among HIV-positive people due to identical modes of transmission, with a prevalence of 50.3 %. Among these cases, HIV/HCV coinfection accounts for 35.4 %, HIV/HBV for 8.4 %, and HIV/HBV/HCV for 5 % [1]. According to estimates from the pre-war period (2021) *(Volosevich et al., 2021),* 5 % of the total population in Ukraine was infected with HCV (2,107,660 people), while HBV cases were recorded in 1.5 % of the population (632,298). Among people who live with HIV, HIV/HCV coinfection has been reported in 2.4 % of their total population. This includes 82.4 % of injecting drug users (IDUs), 6.4 % of men who have sex with men (MSM), and 4.0 % of HIV-positive pregnant women [2]. Overall, among HIV-positive persons under surveillance, 29,946 were diagnosed with HCV, 5,640 with HBV, and 3,004 with HBV/HCV coinfection [3, 4].

The risk of HCV infection or reinfection in HIV-positive individuals is 6 times higher than in the general population. These patients demonstrate an increased incidence of liver fibrosis, progression of liver failure, and, ultimately, death [5, 6]. End-stage liver disease is most common in patients with HIV/HBV/HCV coinfection, less frequent in those with HIV/HBV and HIV/HCV coinfections, and occasionally observed in HIV mono-infection due to alcohol consumption, non-alcoholic fatty liver disease, and, to some extent, due to the toxicity of certain drugs used in ART [7]. Consequently, the risk of death is 1.6 times higher in patients with HIV/HBV and HIV/HCV coinfections and 2.29 times higher in those with HIV/HBV/HCV coinfection compared to patients with HIV mono-infection [8].

Direct-acting antivirals (DAAs) used in the treatment of HCV have not only enabled the full recovery of patients but also significantly impacted the prevalence

of the disease, potentially leading to the eradication of the pathogen within the population. In this regard, the World Health Organization (WHO) has approved a strategy to combat HCV by 2030 through maximum population coverage with screening and treatment [9]. Drugs from the groups of RNA-dependent polymerase inhibitors (anti-NS5B), protease inhibitors (PI, anti-NS3/4A), and NS5A inhibitors are highly effective in improving patient prognosis both in the short and long-term [10]. New interferon-free regimens achieve a sustained virologic response (SVR) in over 95 % of patients, causing fewer side effects than interferon therapy [11]. DAAs are effective not only in inducing SVR but also in preventing the development of hepatocellular carcinoma, liver cirrhosis, and unfavorable outcomes [12]. Patients with HIV/HCV, HBV/HCV coinfections and decompensated cirrhosis constitute a so-called special group, as their treatment outcomes may differ from the expectations and, therefore, require careful monitoring [13].

According to the updated recommendations of the American Association for the Study of Liver Diseases (AASLD) and the Infectious Diseases Society of America (IDSA), patients who have not previously been treated for HIV and HCV coinfection, regardless of cirrhosis status, are suitable candidates for DAA therapy, as supported by the results of a phase 4 MINMON clinical trial conducted by *Solomon et al.* in 2022 [14, 15].

For individuals with HIV/HBV coinfection, the U.S. Department of Health and Human Services Panel on Antiretroviral Guidelines for Adults and Adolescents recommends prescribing an ART regimen containing the nucleoside reverse transcriptase inhibitors tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) or lamivudine (3TC), regardless of CD4+ T-cell count or HBV-related factors such as HBV DNA levels or elevated alanine aminotransferase activity [16]. The use of combination ART is associated with a reduction in FIB-4 score and a lower incidence of liver fibrosis in patients with HIV/HBV coinfection. Its effect on the reversal of liver fibrosis may persist for up to a decade [17].

A systematic review and meta-analysis indicate that HCV treatment with DAAs is associated with a more significant regression of liver fibrosis compared to interferon-based therapy. Additionally, among patients with initially severe fibrosis and cirrhosis (>9.5 kPa), 47 % achieved a reduction in liver stiffness after treatment (<9.5 kPa) [18]. The study by *Lledó GM et al. (2018)* demonstrates that HCV eradication with DAA therapy leads to a reduction in non-invasive markers of liver fibrosis (APRI, FIB-4, and transient elastography) at the time of achieving SVR. This effect is more pronounced in patients with initially advanced fibrosis and cirrhosis. Additionally, the presence of HIV coinfection does not affect the dynamics of liver fibrosis regression [19].

The results of the above studies demonstrate regression of liver fibrosis after achieving SVR for HCV, as well as the positive effects of ART on the reversal of fibrosis in patients with HIV/HBV coinfection. However, there is insufficient data on the effects of antiviral therapy used simultaneously against C and B viruses in patients with HIV/HBV/HCV coinfection, particularly on viral replication and the features of the liver parenchyma morphology.

AIM

To study and compare the efficacy of different regimens of combination antiviral therapy for hepatitis C and B in achieving a sustained virological response in patients with HIV/HBV/HCV coinfection and their impact on the degree of liver fibrosis.

MATERIALS AND METHODS

RETROSPECTIVE STUDY

We examined 113 HIV-infected adult patients with HBV/HCV coinfection who were registered at the Municipal Non-profit Enterprise "Infectious Diseases Center of the Ivano-Frankivsk Regional Council" between 2017 and 2019. Group 1 consisted of 44 patients who did not receive HCV antiviral therapy (AVT). Other patients, who received a 12-week HCV AVT, were divided into the following groups according to the treatment regimen: Group 2 – 29 patients who received pegylated interferon, sofosbuvir, and ribavirin (Peg IFN+SOF+RBV); Group 3 – 40 patients who received interferon-free DAA regimens with or without ribavirin: sofosbuvir and ribavirin (SOF+RBV) – 8 patients, sofosbuvir/ledipasvir and ribavirin (SOF/LDV+RBV) – 8 patients, combination of 2 DAAs (sofosbuvir/ledipasvir (SOF/LDV), sofosbuvir and daclatasvir (SOF+DCV) and sofosbuvir/velpatasvir (SOF/VEL)) – 24 patients. All patients who received HCV AVT had not been previously exposed to this treatment.

Patients were examined per the unified clinical protocol for primary, secondary (specialized), and tertiary (highly specialized) medical care "HCV infection in adults" (Order of the Ministry of Health of Ukraine No. 729 dated July 18, 2016).

All patients received antiretroviral therapy (ART) with nucleoside reverse transcriptase inhibitors, tenofovir and emtricitabine (TDF/FTC), both of which are active against HBV, and dolutegravir (DTG) – a TLD regimen. (Clinical protocol on the use of antiretroviral drugs for treating and preventing of HIV infection

(Order of the Ministry of Health of Ukraine No. 1292 dated June 5, 2019)).

The viral load (VL) of HCV RNA and HBV DNA in the blood plasma of patients was determined using quantitative real-time polymerase chain reaction (PCR) using the Rotor-Gene 6000 system (Corbett Research Pty Limited, Australia) with a lower limit of quantification (LLOQ) of 150 IU/mL.

The stage of liver fibrosis was determined using two non-invasive methods of investigation: the APRI index and point-shear wave elastography (pSWE) of the liver. The APRI (AST to Platelet Ratio Index) was calculated as AST / upper limit of normal AST (40 U/L) / platelet count in 1 μL of blood) × 100. Liver fibrosis was categorized as absent or minimal if the APRI value was <0.5, moderate or significant if the value was 0.5–1.5, and severe fibrosis or cirrhosis if the value was ≥1.5 [20].

Liver pSWE elastography was performed using an ACUSON S3000 ultrasound system (Siemens, Germany). The stiffness of the liver parenchyma was measured as the shear rate of the ultrasound wave (m/sec) passing through the tissue. The stages of liver fibrosis were classified based on pSWE results as follows: F0 (no fibrosis) – 0.87-1.23 m/sec, F1 (mild) – 1.23-1.37 m/sec, F2 (significant) – 1.37-1.68 m/sec, F3 (advanced) – 1.68-2.0 m/sec, F4 (cirrhosis) – >2.0 m/sec [21].

The APRI and liver pSWE indices in group 1 patients who received ART but not HCV AVT were determined twice with a 1-year interval. For patients in groups 2 and 3 who received both ART and HCV AVT, the study was also conducted twice: once before starting HCV treatment and again 1 year later, i.e., 9 months after completing AVT.

The effectiveness of HCV AVT was evaluated based on the achievement of a rapid virologic response (RVR) at week 4 of treatment and a sustained virologic response (SVR12) at 12 weeks after the AVT completion.

The efficacy of HBV AVT was assessed by the achievement of undetectable HBV DNA levels in plasma at the baseline of the surveillance and again 1 year later.

STATISTICS

Continuous variables were expressed as a median and interquartile range (Q1-Q3). Categorical variables were described as frequencies. Group differences in categorical variables were assessed with Pearson's Chi-square and post-hoc Pearson's Chi-square tests, and in continuous variables, the Kruskal-Wallis' test was used. Wilcoxon signed-rank test was used to compare two dependent groups. The proportion of patients with SVR12 and the two-sided exact 95 % confidence interval (95 % CI) were calculated using the Clopper-Pearson method. Differences were judged as significant if the p-value was less than 0.05. Statistical analysis of data was performed using the Microsoft Excel Statistical Package for Microsoft 365 MSO (setup 2801 of version 16.0.14332.20204) (64-bit version). License ID: EWW_1c997d34-53bc-44a6-89a1-90707b6e2997_a3409c5e83bd296b20.

The study design was approved by the Ethics Committee of the Ivano-Frankivsk National Medical University (Protocol № 109/19 dated May 29, 2019)

RESULTS

The mean age of patients across all three groups was 42.60±0.60 years. In each group, the majority were men – 90 (79.6 %), with a predominant contingent of individuals diagnosed with mental and behavioral disorders due to opioid use – 84 (74.3 %). The routes of HIV infection included parenteral transmission through intravenous drug use – 74.3 % and sexual transmission – 25.7 %. The proportion of patients with stage 1-2 HIV infection was 21.8 %, while those with stage 3-4 accounted for 78.2 %. Patients were categorized according to the degree of immune deficiency as follows: 46.90 % exhibited profound immune deficiency (CD4+ T cells/mm^3 <350), 26.55 % had moderate immune deficiency (CD4+ T cells/mm^3 350-499), and 26.55 % showed no immune deficiency (CD4+ T cells/mm^3> 500) (Table 1).

At the beginning of the study, the average HCV RNA levels in blood plasma were $5.9\times10^6\pm1.5\times10^6$ IU/mL in group 1, $3.8\times10^6\pm1.8\times10^6$ IU/mL in group 2, and $4.1\times10^6\pm1.3\times10^6$ IU/mL in group 3. The HCV genotype was determined in 83 patients: genotype 1 was identified in 37 patients (44.58 %), and genotype 3 in 42 patients (46, 98 %) (Table 1).

The APRI value ≥1.5, indicative of severe fibrosis, was found in 13.64 % of patients in group 1, 6.9 % in group 2, and 7.5 % in group 3, with no statistically significant difference between the groups (p>0.05). The median APRI value was 0.49 in group 1 and 0.45 in groups 2 and 3. The median pSWE value at baseline was 1.53 m/sec in group 1, whereas advanced liver fibrosis corresponding to METAVIR stage F3-F4 was detected in 31.82 % of patients, which was 2.3 times higher than the proportion detected using APRI (13.64 %, p<0.05). In group 2, the median pSWE value was 1.46 m/sec, and F3-F4 liver fibrosis was identified in 17.24 % of patients, which is 2.5 higher than according to APRI (6.9 %, p<0.05). In group 3, the baseline median pSWE value was 1.45 m/sec, and F3-F4 liver fibrosis was found in 20.0 %, which is 2.7 higher than according to APRI (7.5 %, p<0.05).

The regimens containing two DAAs were associated with a high rate of RVR: SOF/LDV – 85.7 %, SOF+DCV – 90.0 %, and SOF/VEL and SOF/LDV+RBV – 100.0 %. These regimens achieved SVR12 in 100.0 % of patients (Table 2).

Table 1. Baseline demographic and clinical characteristics of patients with HIV/HBV/HCV coinfection

	Group 1 No HCV AVT, n=44	Group 2 Peg IFN+SOF+RBV 12 weeks, n=29	Group 3 DAA±RBV 12 weeks, n=40	p
Age, M±m	43.7±1.1	41.0±1.1	42.5±0.9	
Sex: n, %				
Men	32 (72.7)	23 (79.3)	35 (87.5)	p>0.05
Women	12 (27.3)	6 (20.7)	5 (12.5)	
HIV transmission route: n, %				
Sexual	11 (25.0)	8 (27.6)	10 (25.0)	p>0.05
Parenteral	33 (75.0)	21 (72.4)	30 (75.0)	
Stage of HIV infection: n, %				
1	1 (2.3)	3 (10.3)	1 (2.5)	p>0.05
2	5 (11.4)	6 (20.7)	8 (20.0)	
3	6 (13.6)	5 (17.2)	7 (17.5)	
4	32 (72.7)	14 (48.3)	22 (55.0)	
CD4+ T cell count / mm^3, n (%)				
<350	15 (34.10)	13 (44.83)	25 (62.50)	p_1 >0.05 p_2=0.028 p_3 >0.05
350-499	20 (45.45)	5 (17.24)	5 (12.50)	p_1= 0.019 p_2= 0.003 p_3 >0.05
>500	9 (20.45)	11 (37.93)	10 (25.00)	p>0.05
RNA HCV viral load, IU/mL, M±m	$5.9x10^6$±$1.5x10^6$	$3.8x10^6$±$1.8x10^6$	$4.1x10^6$±$1.3x10^6$	p>0.05
HCV genotype, n (%)				
1	14 (31.8)	12 (41.4)	11 (27.5)	p>0.05
2	0 (0.0)	0 (0.0)	5 (12.5)	
3	13 (29.6)	8 (27.6)	18 (45)	
3a	0 (0.0)	3 (10.3)	0 (0.0)	
Unknown	17 (38.6)	6 (20.7)	6 (15)	
DNA HBV qualitative test, n (%)				
detected	3 (6.82)	1 (3.45)	2 (5.00)	p>0.05
DNA HBV viral load, IU/mL, M±m	$2.4x10^7$±$2.3x10^7$	$8.5x10^7$	$1.3*10^2$±$1.2*10^2$	p>0.05

Notes: Group differences in categorical variables were assessed with Pearson's Chi-square and post-hoc Pearson's Chi-square tests; in continuous variables, the Kruskal-Wallis' test was used

p – statistical significance of differences among the comparison groups;

p_1 – statistical significance of differences between the 2nd and 1st group;

p_2 – statistical significance of differences between the 3d and 1st group;

p_3 – statistical significance of differences between the 3d and 2nd group

Source: compiled by the authors of this study

The efficacy of the Peg IFN+SOF+RBV regimen was slightly lower, with RVR and SVR12 achieved in 86.2 % of patients (95 % CI: 68.3-96.1). The lowest efficacy was observed with the SOF+RBV regimen – RVR and SVR12 achieved in 75.0 % of patients (95 % CI: 34.9-96.8) (Table 2). Virologic failure occurred in patients with HCV genotype 1 (3 patients – 10.3 %) and 3 (1 patient – 3.4 %), who exhibited resistance to these treatment regimens. No significant difference in achieving RVR and SVR12 was observed between the groups treated with PegIFN+-SOF+RBV and SOF+RBV (p>0.05). However, when comparing the DAA±RBV and Peg IFN+SOF+RBV groups, a statistically significant difference in achieving SVR12 was identified (100.0 % vs. 86.2 %, respectively, p<0.05).

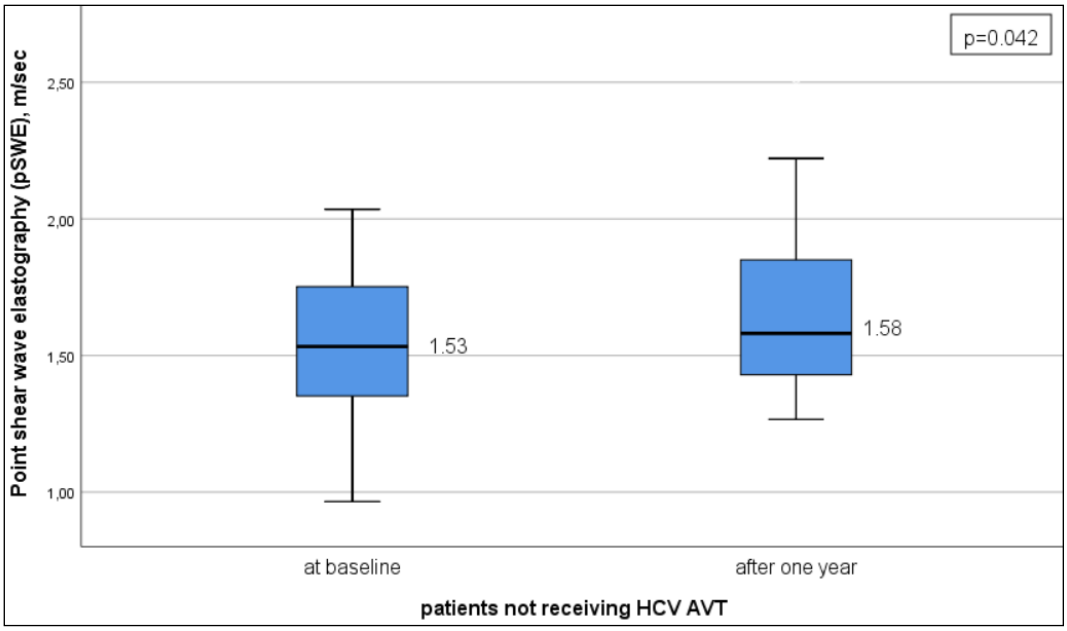


Fig. 1. Dynamics of median values of liver pSWE in patients not receiving HCV AVT
Picture taken by the authors

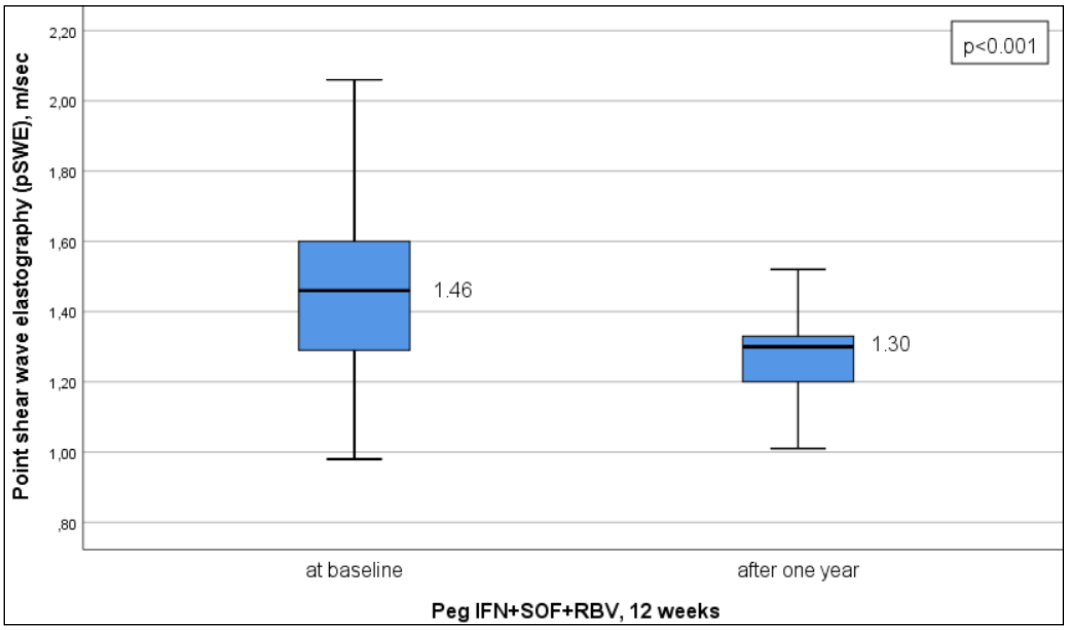


Fig. 2. Dynamics of median values of liver pSWE in patients receiving Peg IFN+SOF+RBV
Picture taken by the authors

All patients received TDF/FTC as part of ART for HBV suppression. However, not all patients reached the integration phase at baseline. HBV DNA remained detectable in 6 (5.3 %) patients: 3 from group 1, 1 from group 2, and 2 from group 3. At the beginning of the study, the average HBV DNA viral load was $2.6\times10^7\pm1.7\times10^7$ IU/mL and was accompanied by severe immune suppression (CD4+ <350 T cells/mm^3) and elevated ALT activity ranging from 49.3 to 60.9 U/L in 5 patients (83.3 %).

After one year of follow-up, HBV replication persisted in four patients (3.5 %) despite regular ART. At the same time, all four patients exhibited minimal to moderate chronic hepatitis activity with ALT levels ranging from 47.5 to 240 U/L and continued to experience severe immune deficiency (CD4+ <350 T cells/mm^3). The causes of replication after one year of follow-up varied among the groups. In group 1 (patients not receiving HCV AVT), three patients had HBV replication at the beginning of the study. After one year, two of these patients achieved HBV suppression, while one patient exhibited viral replication. In this case, primary HBV resistance to TDF/FTC was likely present. Additionally, two patients

Table 2. Rapid virologic response and sustained virologic response when using different regimens of HCV antiviral treatment in patients with HIV/HBV/HCV coinfection

Treatment regimen, n (%)	RVR, n (%)	95% CI	SVR12, n (%)	95% CI	p
Peg IFN+SOF+RBV, n=29	25 (86.2)	68.3-96.1	25 (86.2)	68.3-96.1	> 0.05
SOF+RBV, n=8	6 (75.0)	34.9-96.8	6 (75.0)	34.9-96.8	> 0.05
SOF/LDV+RBV, n=8	8 (100.0)	63.1-100.0	8 (100.0)	63.1-100.0	> 0.05
SOF/LDV, n=7	6 (85.7)	42.1-99.6	7 (100.0)	59.0-100.0	> 0.05
SOF+DCV, n=10	9 (90.0)	55.5-99.7	10 (100.0)	69.2-100.0	> 0.05
SOF/VEL, n=7	7 (100.0)	59.0-100.0	7 (100.0)	59.0-100.0	> 0.05
Total of DAA±RBV, n=32	30 (93.7)	79.0-99.2	32 (100.0)	89.0-100.0	> 0.05

Note: p – statistical significance of the difference between RVR and SVR12
Source: compiled by the authors of this study

in this group (4.54 %) appeared to develop secondary resistance to TDF/FTC, as HBV DNA was undetectable at the beginning of the study but reappeared after one year at levels of 0.6×10^2 IU/mL and 3.0×10^3IU/mL, respectively. At the same time, these patients experienced a significant exacerbation of hepatitis activity, with ALT levels increased to 153.3-240 U/L compared to 54.3-60.9 U/L at baseline.

In group 2, consisting of patients treated with Peg IFN+SOF+RBV, HBV DNA was present in 1 patient both at the beginning of the study and one year later. However, the HBV DNA VL decreased by four orders of magnitude, from 8.5×10^7 IU/mL vs. 1.2×10^3 IU/mL, respectively. This reduction occurred alongside an increase in CD4+ T cell counts, from 183 to 389 T cells/mm^3. Primary viral resistance to TDF/FTC was also observed in this case.

In group 3, consisting of patients receiving DAA±RBV, HBV DNA was detected in 2 patients (with VL of 1.0×10 IU/mL and 2.5×10^2 IU/mL) at the beginning of the study. However, after one year, complete HBV suppression was observed with achieved higher immunity parameters (initial CD4+ count in these two patients were 440 and 97 T cells/mm^3, respectively, increasing to 520 and 348 T cells/mm^3, respectively, after one year).

Therefore, primary HBV resistance to TDF/FTC was observed in two patients across all groups under observation (1.76 %). Secondary resistance was noted in the remaining two patients but only in the group that did not receive HCV AVT (4.54 %).

In groups 2 and 3, where patients received combined HBV and HCV AVT, no HBV reactivation was observed with achieved HCV suppression.

After one year, in patients from group 1 who did not receive HCV AVT, the median pSWE increased to 1.58 m/sec compared to baseline measurements, which showed 1.53 m/sec (p=0.042) (Fig. 1). However, the proportion of patients with F3-F4 remained unchanged (40.91 % vs. 31.82 % at baseline, p>0.05) (Fig. 4). At the same time, the median APRI decreased to 0.40 compared to baseline values of 0.49 (p<0.001). Yet, the proportion of patients with F3-F4 by this indicator also remained unchanged (9.09 % vs. 13.82 % at baseline, p>0.05).

After one year of follow-up and 9 months after completion of HCV AVT, in patients from group 2 receiving Peg IFN+SOF+RBV, the median pSWE value decreased to 1.30 m/sec from 1.46 m/sec at baseline (p<0.001) (Fig. 2). However, the incidence of stages F3-F4 did not change significantly (10.34 % vs. 17.24 % at baseline, p>0.05) (Fig. 4). The median APRI decreased by half (0.23 vs. 0.45 before treatment (p<0.001)), and no patients exhibited advanced fibrosis (F3-F4).

In group 3, consisting of patients who received 2 DAAs or 2 DAAs+RBV, the median pSWE value decreased to 1.31 m/sec at one year of follow-up and 9 months after completion of HCV AVT, compared with 1.45 m/sec before treatment (p<0.001) (Fig. 3). Based on pSWE results, the incidence of advanced F3-F4 liver fibrosis decreased to 2.5 % from 20.0 % before treatment (p<0.05) (Fig. 4). The median APRI also decreased almost twofold to 0.26 compared with the baseline value of 0.45 (p<0.001), with no patient in this group exhibiting APRI ≥1.5, which corresponds to severe fibrosis (0.0 %).

DISCUSSION

According to our study and the literature, pSWE values provide a more accurate reflection of fibrotic changes in the liver compared to APRI [22, 23]. The latter, while showing high specificity but low sensitivity, can only serve as an indicative tool for assessing the degree of fibrosis [24].

The treatment regimens with 2 DAAs (SOF/LDV, SOF/VEL, SOF+DCV) and the regimen, which includes ribavirin (SOF/LDV+RBV), resulted in 100 % SVR12. According to other published studies, such therapy for patients

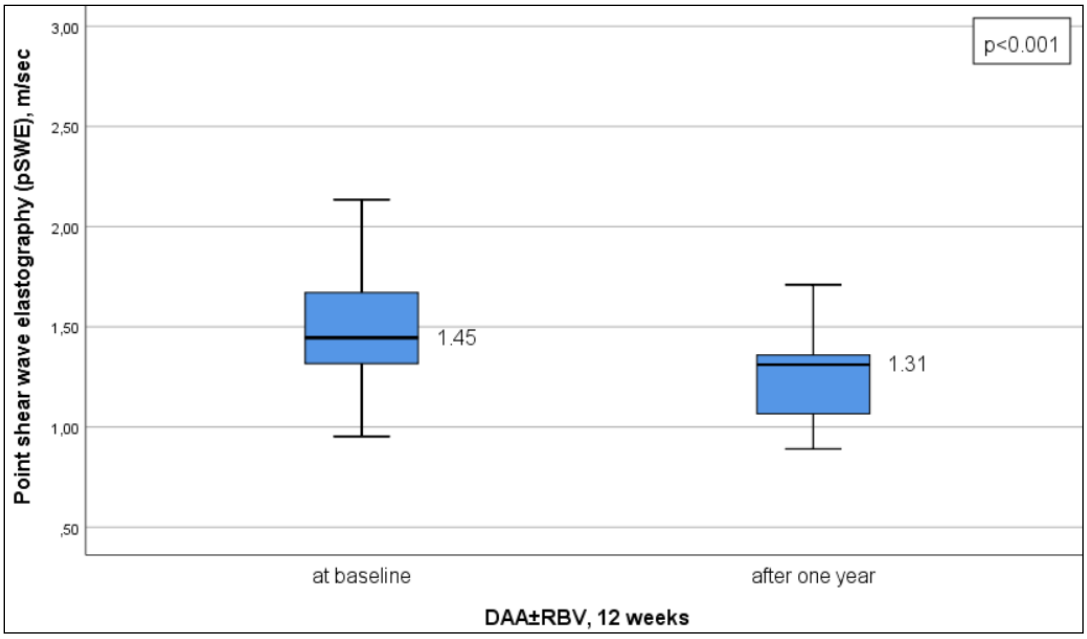


Fig. 3. Dynamics of median values of liver pSWE in patients receiving DAA±RBV
Picture taken by the authors

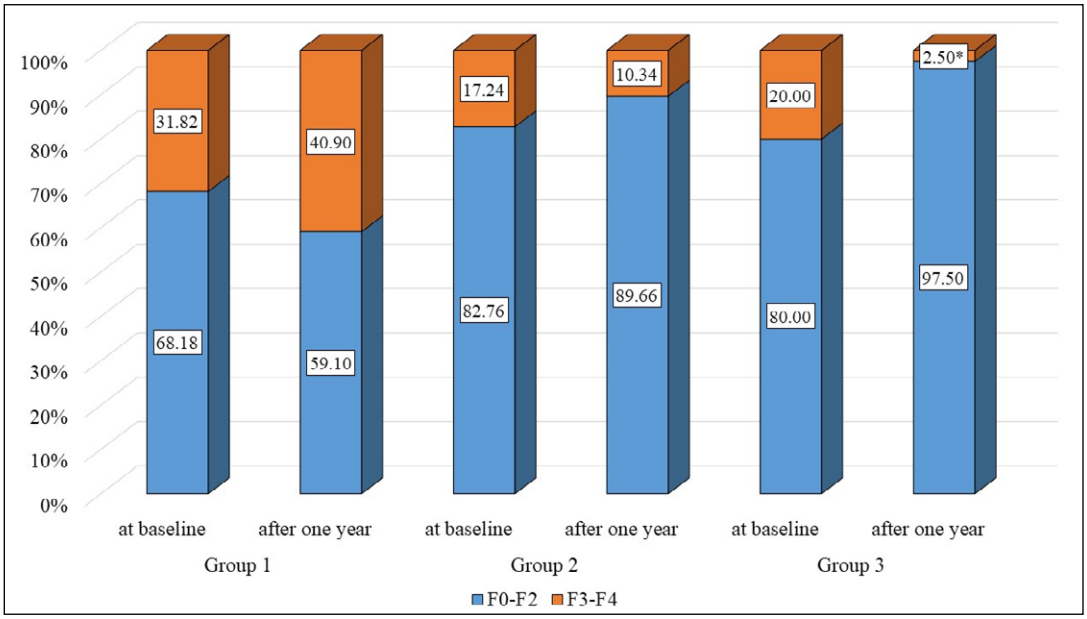


Fig. 4. Dynamics of liver fibrosis stages according to pSWE in patients with HIV/HBV/HCV coinfection
Notes:
Group 1 – patients not receiving HCV AVT;
Group 2 – patients receiving Peg IFN+SOF+RBV for 12 weeks;
Group 3 – patients receiving DAA±RBV for 12 weeks;
* – p <0.05
Picture taken by the authors

with HIV/HCV is effective, and its success rate reaches 93-100 %, depending on the drug regimen [25-27]. Two regimens were not effective enough: sofosbuvir with ribavirin alone (SVR12 was in 75 % of patients) and in combination with pegylated interferons (86.2 %).

At the end of one year of follow-up, HBV replication persisted in 4 (3.5 %) patients across different groups due to HBV resistance to TDF/FTC. Among these, two patients likely exhibited primary HBV resistance, while the other two developed secondary resistance. The latter occurred exclusively in the group receiving ART without concurrent HCV AVT.

While numerous reports in the literature describe HBV reactivation in the setting of HCV suppression, its

incidence in patients with HIV/HBV/HCV coinfection remains poorly understood [28]. In our study, no cases of HBV reactivation were observed in patients receiving combined HBV and HCV AVT. The single instance of HBV replication noted at the beginning and end of the study in the group treated with Peg IFN+SOF+RBV can be attributed to primary resistance to TDF/FTC. The absence of HBV reactivation in the presence of HCV suppression is associated with both the suppressive effect of TDF/FTC on the hepatitis B virus and immune reconstitution when receiving ART, as well as improvement of the functional and morphological state of the liver after combined antiviral therapy.

Interferon-free regimens that include two DAAs, either alone or in combination with ribavirin, demonstrated the most beneficial effects on the liver parenchyma in patients with HIV/HBV/HCV reducing the degree of fibrosis, with a significant decrease in the incidence of advanced fibrosis observed 9 months post-treatment (from 20 % to 2.5 %). Our findings align with those reported by authors who observed a reduction in liver stiffness and improvement in fibrosis indices after successful therapy with DAAs [29, 30].

The distinguished feature of the present study, compared to those previously mentioned, is the focus on HIV-infected individuals with coexisting hepatitis B+C who received combined suppressive therapy for chronic hepatitis B using TDF/FTC and chronic hepatitis C using 2 DAA drugs with or without ribavirin. This treatment is characterized by 100 % suppression of both viruses, effectively preventing hepatitis B virus reactivation except in cases of primary HBV resistance. Consequently, a significant reduction in the degree of liver fibrosis is achieved.

CONCLUSIONS

1. In HIV-infected individuals with chronic hepatitis B+C, HCV AVT regimens SOF/LDV, SOF/VEL, SOF+DCV, SOF/LDV+RBV demonstrated 100 % virologic efficacy. Regimens involving Peg IFN+SOF+RBV (86.2 %) and SOF+RBV (75.0 %) were comparatively less effective.
2. Among patients in the group treated with combined AVT for HBV and HCV using TDF/FTC and DAA±RBV, the incidence of liver fibrosis F3-F4, as assessed by pSWE, decreased from 20 % to 2.5 % ($p<0,05$) after one year of follow-up.
3. Primary HBV resistance to TDF/FTC was identified in 1.76 % of patients in the entire cohort. Secondary HBV resistance was observed in 4.54 % of patients who did not receive HCV AVT.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Possible role of the *TGFB1* polymorphism rs1800470, trophoblastic growth factor β1 (TGF-β1) and connective tissue growth factor (CTGF) in the progression of diabetic retinopathy in type 2 diabetes mellitus

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ABSTRACT

Aim: To determine the significance of growth factors (TGF-β1 and CTGF) in the development and progression of diabetic retinopathy (DR) in carriers of different genotypes of the *TGFB1* rs1800470 polymorphism in type 2 diabetes mellitus (T2DM).

Materials and Methods: 102 individuals with T2DM and DR were examined, who were divided into 3 groups: 1st – with non-proliferative DR (NPDR, 35 individuals), 2nd – with preproliferative (PPDR, 34 individuals) and 3rd – with proliferative (PDR, 33 individuals); 61 individuals were included in the control group. The patients underwent standard ophthalmological examinations. Determination of TGF-β1 in blood serum and intraocular fluid (IOF) and CTGF in IOP was performed by enzyme-linked immunosorbent assay (Invitrogen Thermo Fisher Sci., USA). Alleles rs1800470 (*T869C*) were determined by polymerase chain reaction (TaqMan Mutation Detection Assays Life-Technology test system, USA). For statistical studies, the MedStat and MedCalc v.15.1 software packages (MedCalc Software bvba) were used.

Results: Rs1800470 *A/A* carriers had worse visual acuity ($p=0.016$) and higher central retinal thickness and volume ($p<0.001$) compared to *G/G* carriers. In DR the content of TGF-β1 in the blood and IOF and CTGF in IOF significantly exceeded that in controls (by 1.5-5.2 times; $p<0.05$). The highest content of both factors in DR compared to controls was determined in *G/A* and *A/A* rs1800470 carriers. By stages of DR, a significant increase in the content of TGF-β1 in the blood and IOF and CTGF in IOF was established with maximum values in PDR. A higher content of both factors was established in *G/A* and *A/A* carriers compared to *G/G* carriers. In PDR, the blood content of TGF-β1 in *A/A* carriers was 1.2 times higher ($p<0.05$) than in *G/G* carriers. The increase in CTGF content in the IOF when comparing NPDR and PPDR was characteristic only for *A/A* carriers compared to *G/A* and *G/G* carriers (1.2-1.5 times; $p<0.05$). In PDR, the content of this factor was equally high in *G/A* and *A/A* carriers compared to *G/G* rs1800470 carriers (1.3 times; $p<0.05$).

Conclusions: Thus, the content of both TGF-β1 and CTGF was higher in carriers of the *G/A* and *A/A* genotypes compared with the ancestral *G/G* genotype. Therefore, the clinically worse course of DR in carriers of the SNP rs1800470 of the *TGFB1* gene could be associated with a higher concentration of TGF-β1 and CTGF in the IOF of such patients.

KEY WORDS: diabetic retinopathy, intraocular fluid, rs1800470, *TGFB1*, TGF-β1, CTGF

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INTRODUCTION

Diabetes mellitus (DM) is an extraordinary medical and social problem of the modern world, which is among the ten leading causes of death among the adult population [1, 2]. The global prevalence of DM was 9.3% (463 million) in 2019 and will increase to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 [2].

Among the microvascular complications of DM, one of the most common is diabetic retinopathy (DR), which accompanies the course of the disease in 26-35% of patients [3]. Almost all patients with type 1 DM develop DR within 20 years after diagnosis; for type 2 DM, the probability of DR during this period is 80% [4]. About half of patients with untreated proliferative DR (PDR) are likely to become blind within 5 years of diagnosis [4].

By the mechanisms of development, DR is a progressive multifactorial neurovascular lesion of the retina, which specifically develops in diabetes and involves damage to neurons, glia and microvascular vessels [5]. The pathogenesis of DR includes disorders of glucose metabolism, insulin reception and some molecular factors that play a role in inflammation, primarily vascular endothelial growth factor (VEGF), as well as other growth factors and molecules, including transforming growth factor-β (TGF-β) [6].

An obligatory component of retinal neovascularization is fibrovascular proliferation, which occurs as a result of hypoxia [7]. The initiator of this process, first of all, is TGF-β. Notch and TGF-β signaling pathways have been shown to be two intracellular mechanisms that control fibrosis in general and play an important role in retinal fibrosis in DR [8]. Members of the TGF-β family play an important role in the pathogenesis of DR by inducing endothelial proliferation, local inflammation, and apoptosis of neurons and Müller cells [9]. Impaired TGF-β reception and signaling is accompanied by pathological angiogenesis, impaired blood-ophthalmic barrier permeability, inflammatory reactions, and tissue fibrosis [10]. Analysis of the content of TGF-β1, TGF-β2, and TGF-β3 in the serum and aqueous humor of patients with different stages of DR showed a 5.5-fold increase in TGF-β1 compared to the control group [11].

Single nucleotide polymorphisms (SNPs) in the TGF-β1 gene (*TGFB1*) have shown a role for this gene in tissue fibrosis and are associated with DR [12]. *TGFB1* SNPs are involved in the development of other complications of diabetes and diabetes-associated diseases [13]. Their direct relationship with the development of PDR has been shown [14].

Connective tissue growth factor (CTGF) initiates fibrotic reactions in DR at all stages [15]. It modulates the actions of many growth factors and extracellular matrix proteins, including VEGF and TGF-β1, leading to tissue reorganization, thickening of the basal lamina, pericyte apoptosis, angiogenesis, and fibrosis [15].

Thus, it can be assumed that growth factors, including TGF-β1 and CTGF, play an important role in the development of DR, which has its own perspective for their possible use as biomarkers of DR and its progression. It is likely that the effect of TGF-β1 and CTGF in DR may vary depending on the polymorphic state of the *TGFB1*, which justifies this study.

AIM

To determine the significance of growth factors (TGF-β1 and CTGF) in the development and progression of diabetic retinopathy in carriers of different genotypes of the *TGFB1* rs1800470 polymorphism in type 2 diabetes.

MATERIALS AND METHODS

DESIGN AND STUDY GROUPS

The study was conducted at the Department of Ophthalmology of the Danylo Halytsky Lviv National Medical University. All studies were conducted in compliance with the basic provisions of the Council of Europe Convention on Human Rights and Biomedicine, the Declaration of Helsinki of the World Medical Association on the ethical principles of conducting scientific medical research involving human subjects (1964, with subsequent amendments, including the 2000 version), Order of the Ministry of Health of Ukraine No. 690 of September 23, 2009 and in accordance conclusion of the extract from protocol No. 12 of the meeting of the Ethics Commission on Scientific Research, Experimental Developments, and Scientific Works of the Danylo Halytsky Lviv National Medical University, November 20, 2023.

The study was prospective, cohort, case-control. Patients who were involved in the study provided informed consent.

In total, the study included the results of the examination of 102 people with type 2 diabetes, aged 65.9±0.84 years, 33 men (32.4%), 69 women (67.6%). The diagnosis of DR was made according to the E. Kohner & M. Porta classification, approved by the WHO in 1991, which distinguishes three DR stages – non-proliferative DR (NPDR), pre-proliferative (PPDR) and PDR. According to the diagnosis, the patients were divided into three groups: the 1st group consisted of 35 patients with NPDR, the 2nd – 34 patients diagnosed with PDDR and the 3rd – 33 patients with PDR. The control group consisted of 61 people who did not have diabetes and DR and underwent surgical treatment for age-related cataract.

OPHTHALMOLOGICAL STUDIES

Ophthalmological studies included visometry, Goldmann tonometry, slit lamp biomicroscopy Haag-Streit BQ 900, (Switzerland), gonioscopy, ophthalmoscopy using contact and non-contact lenses (Volk Optical, USA), spectral optical coherence tomography (OCT) on Optovue RTVue, Optovue, (USA). Maximum corrected visual acuity (MCVA, units), intraocular pressure (IOP, mm Hg) were determined; central retinal thickness (CRT, μm) and retinal volume (CRV, mm3) were determined using OCT.

LABORATORY STUDIES

Blood sampling was performed in the morning on an empty stomach in an amount of 3 ml from the cubital vein, immediately after sampling the blood was centrifuged to obtain serum. Intraocular fluid (IOF) was collected through anterior chamber paracentesis before surgery or cataract phacoemulsification by aspirating 0.05-0.1 ml through a 1.0 ml disposable syringe (Hemoplast, Etalon +, Ukraine).

Table 1. Content of TGF-β1 in blood and intraocular fluid and CTGF in intraocular fluid in carriers of different rs1800470 genotypes

Indicator	rs1800470 genotypes			p
	G/G	*G/A*	*A/A*	
Control group				
Blood TGF-β1, pg/ml	396±85.8	404.8±92.4	394.3±95.4	0.918
IOF TGF-β1, pg/ml	129 (119-143)	131 (120-142)	130 (122-150)	0.939
IOF CTGF, ng/ml	1.55±0.46	1.74±0.36	1.65±0.46	0.356
1-st group (NPDR)				
Blood TGF-β1, pg/ml	470±90.1	590±120.1[4]	586.7±124[4]	0.094
IOF TGF-β1, pg/ml	143.5 (125-159)	215 (146.75-261.5)[4]	187.5 (155-239.5)[4]	0.090
IOF CTGF, ng/ml	2.09±0.49[3]	2.47±0.55[3]	3.15±0.37[1.2.4]	<0.001
2-nd group (PPDR)				
Blood TGF-β1, pg/ml	485 (400-580)[2.3]	680 (542.5-742.5)[1.4]	580 (542.5-755)[1.4]	0.132
IOF TGF-β1, pg/ml	215(180-230)[2.3.4]	316 (247.75-347)[1.4]	266 (255-382.5)[1.4]	0.012
IOF CTGF, ng/ml	3.53±0.71[3.4]	3.39±0.85[3.4]	4.22±0.35[1.2.4]	0.016
3-rd group (PDR)				
Blood TGF-β1, pg/ml	560±66.7[2.3]	668.2±76.1[1.4]	669.1±86[1.4]	0.027
IOF TGF-β1, pg/ml	537.8±61.48[2.3.4]	658±69.13[1.4]	681.91±35.64[1.4]	<0.001
IOF CTGF, ng/ml	4.22±0.62[2.3.4]	5.42±0.46[1.4]	5.49±0.50[1.4]	<0.001

Notes: In the case of a normal distribution, the data are presented as M±SD, in the case of a difference in the distribution from normal – as Me (QI-QIII); ANOVA (in the case of a normal distribution) and the Kruskal-Wallis test (in the case of a distribution different from normal) were used to compare data between groups; posterior comparisons were performed using the Scheffe or Dunn test, respectively:
1 – difference from patients in group 1 is statistically significant, $p<0.05$;
2 – difference from patients in group 2 is statistically significant, $p<0.05$;
3 – difference from patients in group 3 is statistically significant, $p<0.05$;
4 – difference from the control group is statistically significant, $p<0.05$
Source: compiled by the authors of this study

Carbohydrate metabolism disorders were determined by fasting venous plasma glucose levels and glycated hemoglobin (HbA1c) levels in the blood. TGF-β1 in serum and IOF and CTGF in IOF were determined by solid-phase enzyme-linked immunosorbent assay using commercial test systems from Invitrogen Thermo Fisher Sci. (USA).

SNP *TGFB1* rs1800470 (*T869C*; L10P) genotypes were determined by real-time polymerase chain reaction using the Gene Amp® PCR System 7500 amplifier ("Applied Biosystems", USA). Genomic DNA was isolated from venous blood (PureLink® Genomic DNA Kit For Purification of Genomic DNA; "INVITROGEN"; USA). For genetic analysis, TaqMan Mutation Detection Assays Life-Technology (USA) test systems were used.

STATISTICAL PROCEDURES

For statistical studies, the software packages MedStat and MedCalc v.15.1 (MedCalc Software bvba) were used. In the case of a normal distribution law, the data are presented as M±SD, in the case of a difference in the distribution law from normal – as Me (QI-QIII). To compare data between groups, ANOVA (in the case of a normal distribution law) and the Kruskal-Wallis test (in the case of a distribution law different from normal) were used.

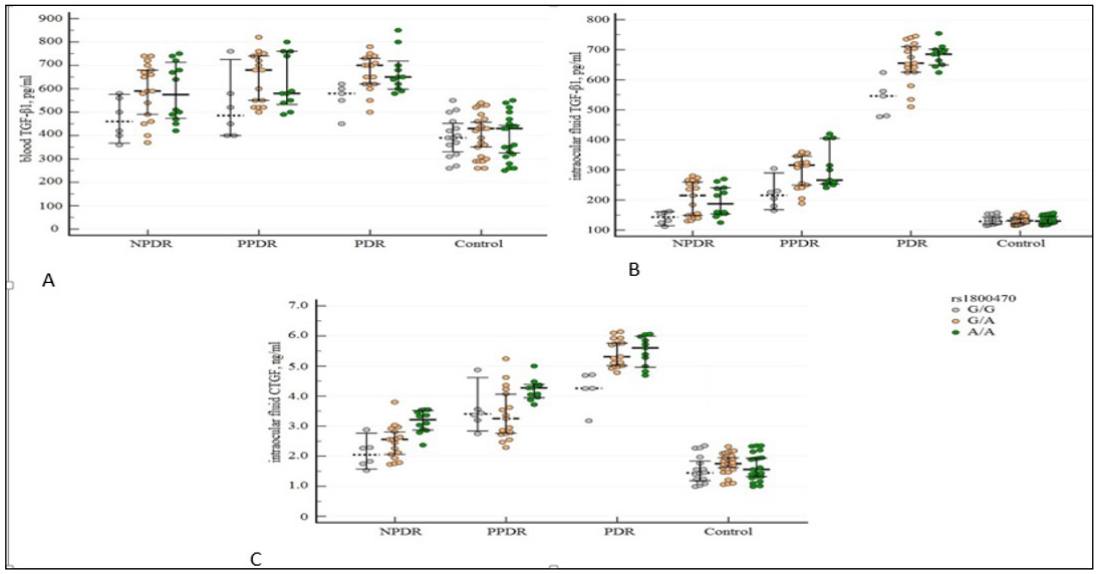


Fig. 1. TGF-β1 content in blood and intraocular fluid and CTGF in intraocular fluid in carriers of different rs1800470 genotypes: A) Blood TGF-βI, pg\ml; B) Intraocular fluid TGF-βI, pg\ml; C) Intraocular fluid CTGF, ng\ml
Picture taken by the authors

Posterior pairwise comparisons were performed using the Scheffe or Dunn test, respectively. The significance of differences between groups was determined using the χ-square test with Bonferroni correction.

RESULTS

A comparison of clinical and ophthalmological indicators in the groups of DR patients showed no difference in the level of hyperglycemia and glycated hemoglobin. Instead, visual acuity deteriorated according to the stage of DR (the median was from 0.9 in NPDR to 0.3 in PDR; p<0.001). The CRT and CRV indicators tended to increase according to the stages of DR (compared to the control, they were 1.2 times higher in PPDR, and 1.4 times higher in PDR; p<0.001).

The content of TGF-β1 in the blood in DR was higher than in the control group (1.4-1.55 times; p<0.001), but no significant difference was found between the groups of DR patients. The content of TGF-β1 in the IOF increased significantly more than in the blood (in NPDR it exceeded the control by 1.2 times, in PPDR – by 2.2 times and in PDR – by 5.0 times; p<0.001 for all cases). The content of CTGF in the IOF also increased significantly: it exceeded the control by 1.6 times in NPDR, by 2.2 times in PPDR and by 3.2 times in PDR (p<0.001 for all cases). The intergroup differences in the IOF content of TGF-β1 and CTGF were also statistically significant (p<0.05).

In this study, the main task was to compare the content of the studied growth factors in patients with DR depending on rs1800470 genotype. Analysis of the distribution of rs1800470 genotypes showed no differences between the control group and patients with DR (χ^2=2.788; p=0.835). However, a certain difference in the phenotype of DR patients was found – mutant genotype rs1800470 *A/A* carriers had worse visual acuity (p=0.016) and higher CRT and CRV rates compared to carriers of the ancestral genotype *G/G* (p<0.001). Therefore, it could be assumed that DR had a worse course in *TGFB1* rs1800470 carriers.

Based on the data obtained, an assumption was made about the possible connection of *TGFB1* rs1800470 with the DR phenotype, which could be realized through the effect on the content of growth factors in the blood and IOF. Table 1 shows the content of the studied factors in carriers of different rs1800470 genotypes.

In the control group, no correlation was found between the content of growth factors in the blood and IOF and the carrier of different rs1800470 genotypes (p>0.05). In DR, the content of both TGF-β1 and CTGF significantly exceeded that in the control (by 1.5-5.2 times; p<0.05). It is characteristic that the highest content of both factors compared to the control was determined in *G/A* and *A/A* genotypes carriers (see Fig. 1).

Also, in patients with DR, a clear tendency was established for a higher content of the studied factors in carriers of the heterozygous and mutant genotype (*G/A* and *A/A* rs1800470) compared to carriers of the ancestral *G/G* genotype. For the content of TGF-β1 in the blood, this tendency became significant for PPDR and PDR (see Fig. 1). Thus, in PDR, the content of TGF-β1 in the blood of *A/A* carriers was 1.2 times higher (p<0.05) than in *G/G* carriers.

The intergroup differences in the content of TGF-β1 in the IOF were more pronounced than in the blood (see Fig. 1). The content of this factor in *A/A* rs1800470 carriers exceeded its level in ancestral homozygote *G/G* carriers by 1.2-1.5 times ($p<0.05$). The highest IOF TGF-β1 content was in patients with PDR – it exceeded that in NPDR and PPDR by 3.0-3.8 times ($p<0.05$).

In contrast, the increase in the IOF CTGF content when comparing NPDR and PPDR (see Fig. 1) was characteristic only for *A/A* rs1800470 carriers in comparison with *G/A* and *G/G* carriers (by 1.2-1.5 times; $p<0.05$). In PDR, the content of this factor was equally high in *G/A* and *A/A* carriers compared to *G/G* carriers (1.3 times; $p<0.05$).

Summarizing the results obtained, it could be said that the content of both TGF-β1 and CTGF was higher in *G/A* and *A/A* carriers compared to the ancestral *G/G* genotype, i.e., under the conditions of carriership of the rs1800470 allele *A*. Comparing these data with the results of determining the phenotype, it could be assumed that the worse visual acuity and higher OCT indicators (CRT and CRV) observed in *A/A* carriers could be due to the higher content of the studied growth factors (TGF-β1 and CTGF) in the IOF.

DISCUSSION

The main function of TGF-β in the immune system is to regulate the proliferation, differentiation and survival of lymphocytes by regulating chemotaxis, activation of lymphocytes, natural killer cells, dendritic cells, macrophages, mast cells and granulocytes [16]. Collectively, it suppresses the development of immunopathology to self or innocuous antigens without compromising the immune response to pathogens.

TGF-β inhibits the differentiation of cytotoxic T lymphocytes (CTL), Th1 and Th2 cells, promotes the generation of peripheral Treg, Th17, Th9 and Tfh cells and the residence of T cells in tissue in response to immune challenges [17]. Similarly, TGF-β controls the proliferation, survival, activation and differentiation of B cells, as well as the development and function of innate cells, including natural killer (NK) cells, macrophages, dendritic cells and granulocytes [17].

Serum TGF-β1 levels may be an additional parameter in predicting the DR development in children with type 1 diabetes [18]. On the other hand, it has been shown that in the retina, TGF-β1 levels can fluctuate independently of changes in its concentration in the blood, which is accompanied by activation of matrix metalloproteinases and remodeling of the cellular matrix [11]. Also, in patients with NPDR, a significant increase in TGF-β1 in the aqueous humor compared with the control group has been shown, confirming its involvement in the development of early vascular changes in DR [19].

In our study, TGF-β1 levels increased in both blood and IOF, but in the latter case the increase was more significant. At the same time, the highest concentrations of TGF-β1 in the IOF were found in patients with PDR, which directly confirmed the importance of this cytokine for its development. The latter may justify the identification of TGF-β1 as a target for targeted therapy [20].

There is evidence that the progression of NPDR to PDR occurs due to the so-called "angiofibrotic switch", which is carried out with the help of CTGF and VEGF [21]. The ratio of CTGF/VEGF in the aqueous humor of the eye was positively correlated with the PDR severity [22]. According to our data, the IOF CTGF content also increased significantly, which was maximally manifested in PDR. This supported the idea of the key importance of the balance of growth factors (VEGF, TGF-β1, CTGF) in the initiation of the angiofibrotic transition and the PDR development. Accordingly, CTGF can also be identified as a possible therapeutic target in the treatment of PDR.

Our study also confirmed numerous results on the significance of SNP *TGFB1* rs1800470 for the development and progression of DR [12-14, 23]. In diabetes, mutations of the *TGFB1* gene are considered an important pathogenetic factor of angiogenesis, endothelial cell proliferation, adhesion and deposition of the extracellular matrix of the retina [12].

However, studies of the association of *TGFB1* SNPs with DR have also had contradictory results. Thus, the absence of associations between rs1800470 genotypes and clinical characteristics or risk factors for DR has been shown [24].

Our study established a worse clinical course of DR in carriers of the mutant genotype *A/A* rs1800470 (worse visual acuity and greater retinal thickness), which was accompanied by higher IOF concentrations of TGF-β1 and CTGF in such patients. The latter was especially true for patients with PDR. This result directly confirmed the pathological role of an increase in the IOF content of growth factors for the DR progression.

This observation also justified the need to study *TGFB1* SNPs to identify a high-risk group, to which, according to our data, mutant allele *A* rs1800470 carriers (genotypes *G/A* and *A/A*) could be attributed. Accordingly, it became obvious that planning of targeted therapy, which should be aimed at inhibiting the TGF-β1/CTGF pathway, should be carried out taking into account the genotypes of *TGFB1* SNPs. In DR, the highest level of TGF-β1 was observed in *A/A* carriers, and the maximum level of CTGF was observed in *G/A* and *A/A* rs1800470 carriers.

CONCLUSIONS

1. In DR, the content of TGF-β1 in blood and IOF and CTGF in IOF significantly exceeded that in controls (by 1.5-5.2 times; $p<0.05$). The highest content of both factors compared to controls was determined in *G/A* and *A/A* rs1800470 carriers.
2. When stratifying patients, a significant increase in the content of TGF-β1 in blood and IOF and CTGF in IOF was found by stages of DR with maximum values in PDR. A clear trend towards a higher content of factors in *G/A* and *A/A* carriers compared to *G/G* was found. Thus, in PDR, the blood content of TGF-β1 in *A/A* rs1800470 carriers was 1.2 times higher ($p<0.05$) than in *G/G* carriers.
3. The increase in IOF CTGF content when comparing NPDR and PPDR was characteristic only for *A/A* rs1800470 carriers compared to *G/A* and *G/G* carriers (1.2-1.5 times; $p<0.05$). In PDR, the content of this factor was equally high in *G/A* and *A/A* carriers compared to *G/G* carriers of (1.3 times; $p<0.05$).
4. Worse visual acuity and greater CRT and CRV observed in *A/A* s1800470 carriers could be due to the higher IOF content of TGF-β1 and CTGF.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Hyperhomocysteinemia – an early marker of retinal damage in patients with type 2 diabetes mellitus

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ABSTRACT

Aim: To investigate the features of changes in serum homocysteine (Hcy) levels in patients with T2DM depending on the presence or absence of retinal damage.

Materials and Methods: We observed 77 patients with T2DM. Patients were divided into two groups: group 1 included 45 patients with T2DM and diabetic retinopathy (DR), group 2 included 32 patients with T2DM without DR. The level of Hcy concentration and B vitamins (vitamin B1, B6, B9, B12) was determined in blood serum.

Results: Proliferative and preproliferative stages of DR were more frequently detected in the examined patients with T2DM (in 44.4% and 26.7% of patients, respectively). Proliferative retinopathy with complications was diagnosed in 17.8% of patients, while nonproliferative retinopathy with maculopathy was diagnosed only 11.1% of cases. In patients of both groups, a decrease in the levels of B vitamins in the blood serum was detected, which was detected against the background of an increase in the level of homocysteine in the blood serum in these patients.

Conclusions: In patients with type 2 diabetes and DR, a decrease in the level of B vitamins was found against the background of an increased concentration of homocysteine (43.5±0.9 mkmol/l – $p<0.001$) in the blood serum, which proportionally increases with the progression of retinal damage in these patients.

KEY WORDS: diabetes mellitus type 2, homocysteine, B vitamins, retinal damage, body mass index, obesity

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INTRODUCTION

The prevalence of type 2 diabetes mellitus (T2DM) continues to rise worldwide. T2DM is a complex disease in terms of pathogenesis, resulting from the interaction of a number of factors, such as genetic or environmental, each of which has a different impact on the onset of the disease. Many cultural, dietary, behavioural, contextual and lifestyle factors are also key determinants of T2DM risk. Given the significant healthcare costs, it is extremely important to identify modifiable factors for diabetes prevention [1]. An estimated 451 million people worldwide had diabetes in 2017, a figure expected to rise to 693 million by 2045 [2].

Diabetic retinopathy (DR) is the leading cause of vision loss in working-age adults in Western countries. It is the most common microvascular complication of diabetes. Diabetic retinopathy can lead to vision-threatening retinal damage and ultimately blindness [3]. Diabetic retinopathy affects people with diagnosed or undiagnosed diabetes. The susceptibility to developing DR is directly proportional to the patient's age and duration of diabetes, as well as poor glycemic control and blood pressure fluctuations [4].

According to epidemiological studies published by the American Academy of Ophthalmology, ninety-three million people worldwide suffer from diabetic retinopathy. The prevalence of DR is 77.3% in patients with type 1 diabetes and 25.1% in patients with T2DM, of whom approximately 25-30% may develop vision-threatening diabetic macular edema. Between 5% and 8% of patients with diabetic retinopathy will require laser treatment, and up to 5% of patients will require vitrectomy surgery [4, 5].

Morphological changes observed in the small retinal vessels in DR include early pericyte loss, thickening of the basement membrane, loss of endothelial cells, increased vascular permeability, platelet aggregation, leukostasis, and capillary atrophy [6, 7]. Diabetic retinopathy affects not only the retinal microvessels but also Müller cells, which are the main glial cells of the retina. The functions of Müller cells are to maintain the structural integrity of the retina, regulate the blood-

retinal barrier and retinal blood flow, absorb and recycle various neurotransmitters, retinoic acid compounds, and ions (such as potassium), and regulate metabolism and nutrient delivery to the retina [8].

Therefore, the assessment of markers that enable the evaluation of endothelial dysfunction and indicate the development of DR is extremely important.

AIM

The aim to investigate the features of changes in serum homocysteine (Hcy) levels in patients with T2DM depending on the presence or absence of retinal damage.

MATERIALS AND METHODS

At the clinical base of the Department of Propaedeutics of Internal Disease, we observed 77 patients with T2DM, who were undergoing inpatient treatment in the endocrinology and gastroenterology departments of the Andriy Novak Transcarpathian Regional Clinical Hospital, as well as undergoing outpatient observation at their place of residence with a family doctor, gastroenterologistor endocrinologist.

The patient cohort consisted of 47 female participants (61.0%) with a mean age of 51.6±5.9 years and 30 male participants (39.0%) with a mean age of 50.9±6.2 years. The control cohort comprised 30 healthy subjects, including 18 women (60.0%) and 12 men (40.0%). Female control participants had a mean age of 50.5±4.7 years, while male controls averaged 52.3±5.1 years.

All clinical assessments were performed following patient consent (written informed consent for applicable diagnostic and treatment procedures was obtained from all patients and control subjects), with comprehensive measures implemented to ensure the confidentiality of all data collected. The study methodology complied with the Helsinki Declaration of 1975 regarding Human Rights and its 1983 revisions, the Council of Europe's Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine, and Ukrainian legislative standards.

The exclusion criteria were: age under 18 and over 75; type 1 diabetes mellitus; systemic autoimmune diseases; open form of pulmonary tuberculosis; mental illnesses that prevent adequate assessment of the patient's health and signing of informed consent for diagnosis and treatment; pregnancy and lactation; cancer, AIDS.

All study participants received thorough evaluation including clinical examination, body measurements, diagnostic instrumentation, and laboratory analysis. Diagnostic verification was achieved through comprehensive recording of presenting complaints and patient history. Standardized abdominal ultrasound examination was performed on all participants, particularly targeting hepatobiliary system and bile duct structures. All patients were consulted by an ophthalmologist.

The diagnosis of type 2 diabetes was established in accordance with the IDF recommendations (2005), as well as taking into account the criteria of the unified clinical protocol (Order of the Ministry of Health of Ukraine dated 21.12.2012 No. 1118) and the criteria of the European Association for the Study of Diabetes (EASD), ADA, 2019. To determine the degree of compensation of carbohydrate metabolism, the level of glycosylated hemoglobin (HbA1c, %) was studied.

Anthropometric evaluation included the measurement of body height, weight, and waist diameter, followed by body mass index (BMI) computation. Participants were categorized using World Health Organization criteria into BMI classifications: extreme underweight (BMI ≤16.0); underweight (16.0-18.5); normal range (18.5-24.9); overweight (25.0-29.9); Class I obesity (30.0-34.9); Class II obesity (35.0-39.9); and Class III obesity (BMI ≥40.0).

Patients with type 2 diabetes were divided into two groups: group 1 included 45 patients with T2DM and diabetic retinopathy, group 2 included 32 patients with T2DM without retinal damage.

All examined patients underwent determination of serum Hcy concentration using the Cobas 8000 test system (Roche Dianostics), as well as B vitamins: vitamin B1 (thiamine) and vitamin B6 (pyridoxine) using high-performance liquid chromatography using test systems (Recipe complet Kit, Germany); vitamin B9 (folic acid) - using the immunochemical method with electrochemiluminescent detection, using the Roche Diagnostics test system (Switzerland), and vitamin B12 (cyanocobalamin) - using immunochemical chemiluminescent detection, using the Abbot Diagnostics test system (USA).

Patient data analysis and result processing were accomplished using STATISTICA 10.0 statistical software (StatSoft Inc, USA), applying parametric and non-parametric methods for statistical assessment of outcomes.

FRAMEWORK

The study was performed within the framework of the scientific topics "Clinical and Pathogenetic Features of Polymorbid Diseases in the Digestive System and Development of Differentiated Therapy Scheme in the Conditions of the COVID-19 Pandemic" (state registration number 0121U110177) researched by the Department of Propedeutics of Internal Diseases of State University "Uzhhorod National University".

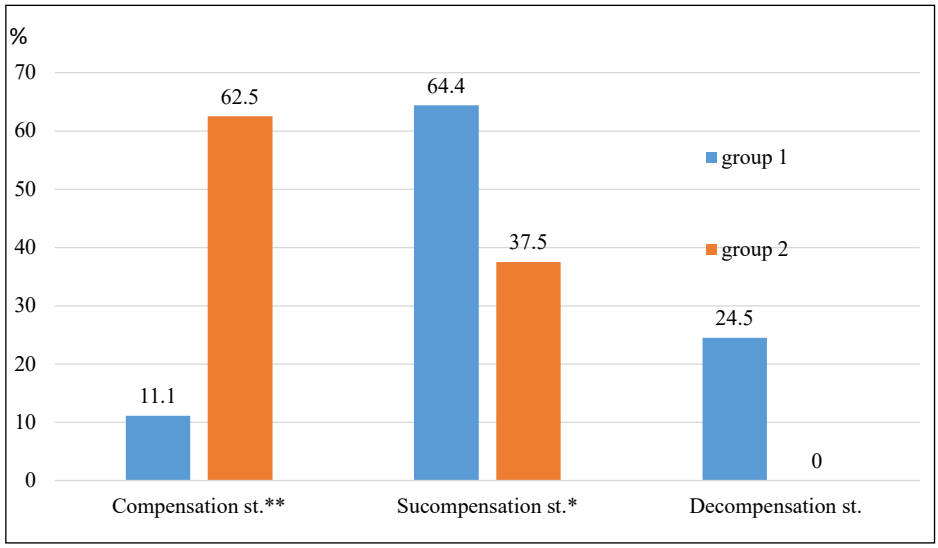


Fig. 1. Severity levels of type 2 diabetes
Note: the difference between the indicators in patients is significant: * - p<0.01; ** - p<0.001
Picture taken by the authors

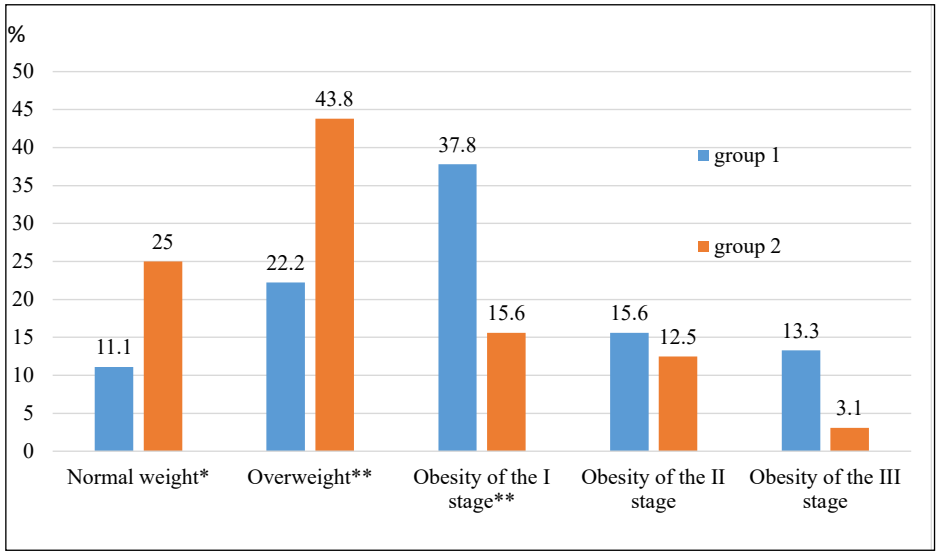


Fig. 2. Changes in BMI in patients with T2DM
Note: the difference between the indicators in patients is significant: * - p<0.05; ** - p<0.01
Picture taken by the authors

RESULTS

Patients with type 2 diabetes are distributed according to the severity of the disease (Fig. 1).

The majority of patients in group 1 had sub- and decompensated stage od diabetes mellitus, while patients in group 2 more often had a compensated stage of the disease.

The distribution of examined patients was carried out depending on the change in BMI (Fig. 2).

Among patients in group 1, people with 1 stage of obesity were more often found (37.8% of cases - p<0.01), while among patients in group 2, people with overweight were more often diagnosed (43.8% of patients, respectively - p<0.01).

Patients in group 1 were divided depending on the severity of retinopathy (Fig. 3).

Proliferative and preproliferative stages of DR were more frequently detected in the examined patients with T2DM (in 44.4% and 26.7% of patients, respectively). Proliferative retinopathy with complications was diagnosed in 17.8% of patients, while nonproliferative retinopathy with maculopathy was diagnosed only 11.1% of cases.

The level of B vitamins and homocysteine in the blood serum of the examined patients with diabetes was assessed (Table 1).

In patients of both groups, a decrease in the levels of B vitamins in the blood serum was detected, which

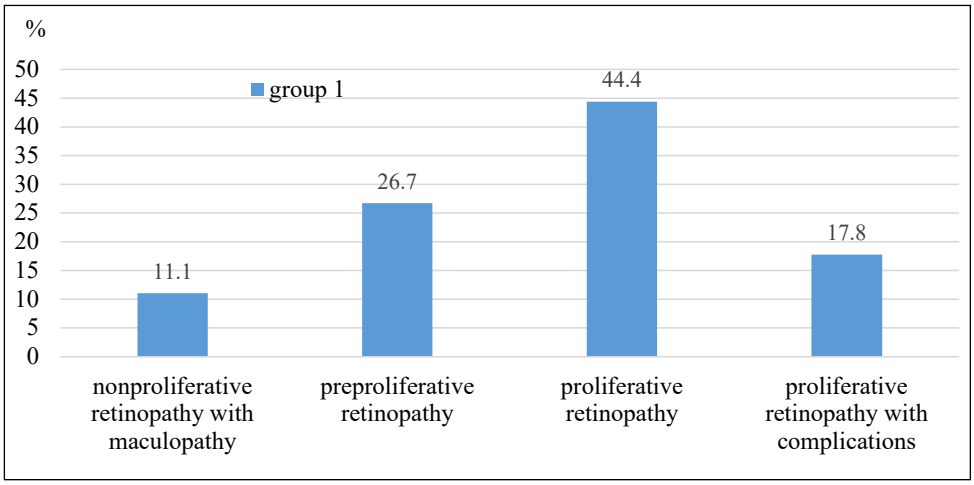


Fig. 3. Stage of DR at patients with T2DM of group 1
Picture taken by the authors

Table 1. Serum levels of B vitamins and homocysteine in the examined persons

Indicator	Reference values	Examined persons		
		Control group (n=30)	group 1 (n= 45)	2 group (n= 32)
Vitamin B1, mkg/l	More than 49.0	89.6±1.3	22.1±1.1++	37.5±0.6+,*
Vitamin B6, mkg/l	8.7-27.2	19.4±0.8	5.4±0.8+	7.6±0.4+,*
Vitamin B9, ng/ml	4.6-18.7	13.1±0,5	1.7±0.3++	3.1±0.6+,**
Vitamin B12, pg/ml	197.0-771.0	463.5±7.7	122.8±4.4++	173.2±5.2+,*
Hcy, mkmoll/l	no more 15.0	8.2±0.4	43.5±0.9++	27.1±0.4+**

Note: the differences between the indicators of the control group and the examined patients are significant: + – $p<0.01$; ++ – $p<0.001$; the difference between the indicators of patients of groups 1 and groups 2 is statistically significant: * – $p<0.05$; ** – $p<0.01$
Source: compiled by the authors of this study

Table 2. Changes in homocysteine levels depending on severity of DR

Stage of retinopathy	Examined persons, group 1 (n= 45)
	Level of Hcy, mkmoll/l
Nonproliferative retinopathy with maculopathy	32.8±1.1
Preproliferative retinopathy	39.9±0.7+
Proliferative retinopathy	51.7±1.5+
Proliferative retinopathy with complications	64.1±2.0+

Note: the differences between the differets stage of DR: + – $p<0.05$
Source: compiled by the authors of this study

was detected against the background of an increase in the level of homocysteine in the blood serum in these patients. It should be noted that more pronounced reliable changes in the examined indicators were mainly in patients of group 1 (patients with T2DM and DR).

For a clearer understanding of the role of homocysteine in the formation of retinal lesions in diabetes, we analyzed its level depending on the phase of DR (Table 2).

The obtained data indicate a progressive increase in serum homocysteine levels depending on the severity and severity of DR of patients with T2DM. It is noteworthy that in patients with nonproliferative retinopathy with maculopathy, the serum homocysteine level is significantly different from that in patients of group 2 without retinal damage ($p<0.05$).

Therefore, the data obtained by us allow us to state the feasibility of assessing serum homocysteine in patients with diabetes for predicting vascular lesions, including DR.

DISCUSSION

Elevated homocysteine (Hcy) levels have a well-established and clear causal relationship with epithelial

damage, leading to ischaemic heart disease. In addition, Hcy is closely related to other components of metabolic syndrome, such as arterial hypertension, which correlates with T2DM [1, 9-11].

DR is becoming an increasingly important cause of visual impairment due to the increasing number of diabetic patients. There is currently no known means to prevent the development of DR. However, there are various known risk factors that influence the progression of the disease. Among the various risk factors known to cause the progression of retinopathy, some are modifiable and others are non-modifiable. Hyperhomocysteinemia is considered a potential risk factor for the development and progression of DR. Homocysteine , an intermediate molecule in methionine metabolism, has attracted considerable interest as a risk factor for cardiovascular disease and other vaso-occlusive diseases, including retinal vascular occlusion. High blood Hcy levels are toxic to the vascular endothelium through the generation of free radicals. Free radicals disrupt the integrity of the endothelium, leading to platelet activation, hypercoagulability, and thrombus formation [12]

Homocysteine and folate metabolism plays an important role in nucleic acid synthesis, amino acid homeostasis, epigenetic maintenance, redox protection, and methylation. This metabolic process is also known as the methionine cycle. When methionine is deficient, Hcy undergoes remethylation. This metabolic pathway requires folate as a methyl group donor for methionine regeneration. The enzyme that plays a key role in this Hcy metabolic pathway is cystathionine β-synthase (CBS), an enzyme that requires vitamin B6 as a cofactor to catalyze the reaction of serine with Hcy to form cystathionine. If the remethylation and/or transsulfuration pathways are impaired, Hcy accumulates in cells and hyperhomocysteinemia develops [13].

Only a few studies have been conducted worldwide to investigate the role of hyperhomocysteinemia in the development of diabetic retinopathy. Vitamin B12 and folate deficiency are associated with increased serum Hcy levels. Therefore, hyperhomocysteinemia may be a potentially modifiable risk factor for the development of diabetic retinopathy [12].

The study by Tawfik A et al. (2019) indicates a correlation between elevated serum level of Hcy and DR in both humans with diabetes and animal models of diabetes. The results obtained by the authors indicate a relationship between elevated serum Hcy levels and increased severity of retinopathy. Therefore, Hcy may be a useful diagnostic marker for screening to predict the incidence and severity of retinal damage in patients with diabetes. In addition, increasing Hcy clearance through pharmacological or genetic manipulations may be a future preventive/therapeutic strategy in the fight against diabetic retinopathy [14].

Our results also indicate high levels of homocysteine in serum in DR at patients with T2DM. At the same time, the analysis conducted indicates the dynamics of homocysteine level depending on the stage of progression and retinal damage in these patients, which allows us to conclude that it is possible to determine the level of homocysteine in these patients to predict the progression or formation of DR for its timely correction.

CONCLUSIONS

In patients with type 2 diabetes and DR, a decrease in the level of B vitamins was found against the background of an increased concentration of homocysteine (43.5±0.9 mkmol/l – $p<0.001$) in the blood serum, which proportionally increases with the progression of retinal damage in these patients.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Protective effects of coenzyme Q10 in renal ischemia reperfusion injury *via* regulation of Notch-1

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ABSTRACT

Aim: To evaluate nephroprotective effects of coenzyme Q10 in rats with renal ischemia -reperfusion injury by action on Notch-1 pathway.

Materials and Methods: Twenty eight Wistar albino male rats were divided randomly into four groups (each group include seven rats): first one named sham group (just laparotomy, no induction of ischemia), second one named control group (30 min. ischemia/ 2 hours reperfusion), third one named olive oil group (rats injected intraperitoneally. with 0.1 ml olive oil once daily for 2 days, 1 hour after the administration of olive oil in 3rd day renal ischemia was induced), fourth one named Coenzyme Q10 group (rats injected with Coenzyme Q10 (10 mg/kg) intraperitoneally. once daily for 2 days, 1 hour after the administration of Coenzyme Q10 in 3rd day renal ischemia was induced). Kidneys were harvested after 2 hours of reperfusion for assessment of kidney injury molecule-1, interleukin 1 β, TNF α, F2 Isoprostane, glutathione, and caspase 3 by ELISA technique, Notch-1, Jagged-1 mRNA level were assessed by reverse transcription-polymerase chain reaction.

Results: Coenzyme Q10 significantly ameliorated renal ischemia -reperfusion injury as evidenced by the significant reduction of kidney injury molecule-1, interleukin 1 β, TNF α, F2 Isoprostane, and caspase 3 in rat kidneys pretreated with Coenzyme Q10, glutathione level was significantly elevated in rat kidneys pretreated with Coenzyme Q10. Notch-1 and Jagged-1 mRNA expression were significantly decreased compared to control group.

Conclusions: Coenzyme Q10 provide nephroprotection against renal ischemia reperfusion injury owing to its antioxidant, anti-inflammatory, and anti-apoptotic properties, those effects may be linked to the downregulation of Notch-1 and Jagged-1.

KEY WORDS: renal ischemia reperfusion injury, COQ10, Notch-1, Jagged-1

Wiad Lek. 2025;78(10):2101-2109. doi: 10.36740/WLek/210071 DOI

ABBREVIATIONS

RIRI - Renal Ischemia Reperfusion Injury
AKI - Acute Kidney Injury
DGF - Delayed Graft Function
ROS - Reactive Oxygen Species
MAM - Mastermind Transcription Facto
NICD - Notch Intracellular Domain
Hes - Hairy and enhancer of split
Hey - Hairy/enhancer-of-split linked with YRPW motif
NF-κB - Nuclear Factor kappa B
ELISA - Enzyme linked immunosorbent assay

INTRODUCTION

Renal ischemia reperfusion injury (RIRI) considered one of the main causes for AKI [1]. During renal transplantation, RIRI is an unavoidable event that causes delayed graft function (DGF), which leads to the loss of essential kidney parenchyma and primes adaptive immune responses that trigger rejection, ultimately culminating in graft loss [2]. The process of reperfusion in an ischemic kidney exacerbates the oxidative and inflammatory condition leading to DNA and protein damage which finally results in necrosis and apoptosis. The production of pro-inflammatory mediators, activation of apoptotic genes, release of reactive oxygen species (ROS), and calcium excess are the underlying pathophysiological mechanisms [3-4]. The Notch signaling pathway is a highly conserved regulatory pathway that exists in all mammalian cells and originated in Drosophila melanogaster. It is critical for a range of functions, including embryonic development and postnatal tissue homeostasis [5]. It is a key regulator of essential developmental processes such lateral inhibition, lineage decision, and border creation, as well as homeostasis and regeneration in adult tissues [6]. The notch receptors are four in mammals (notch1-notch4) with five notch ligands [Jagged-1, Jagged-2, Delta-like 1

(DLL1), DLL3, and DLL4] [7]. Both the receptor as well as its ligands are transmembrane proteins with abundant extracellular domains. Upon ligand binding to the receptor, a conformational change in the receptor occurs which leads to two successive proteolytic cleavages which result in the release of the intracellular domain (NICD) that translocate into the nucleus and binds to c and the coactivator protein Mastermind transcription factor (MAM) to form a ternary transactivation complex which activates the transcription of notch target genes [8]. The two families of transcriptional factors Hairy and enhancer of split (Hes), which includes HES1 and HES5, and Hairy/enhancer-of-split linked with YRPW motif (Hey), which includes HEY one(HEY1) and HEY two(HEY2), which are the main target genes for Notch [9]. Regardless of CSL, NICD has the ability to engage with the mTORC, NF-κB, AKT, PTEN, or Wnt signaling pathways within the cytoplasm and/or nucleus to control the expression of target genes [10-12]. Although Notch signaling is significantly diminished in the adult kidney, both acute and chronic renal injury have been shown to cause Notch to reactivate [13]. In a study of renal IRI induced in rats, Notch2 and hes-1 was found to be upregulated along with NF-κB2, MCP-1 in addition to apoptosis enhancement [14]. Coenzyme Q10 (CoQ10), sometimes known as ubiquinone or ubidecarenone, is a fat-soluble chemical that resembles vitamin A and is necessary for the transfer of electrons during the oxidative phosphorylation of mitochondria. It serves as an electron transporter between the cytochrome systems and the NADH and succinate dehydrogenases [15] CoQ10 functions as a potent antioxidant that scavenges free radicals, prevents lipid peroxidation in cellular membranes, and promotes the regeneration of tocopherol [16]. It is utilized as a dietary supplement and as a co-therapy alongside medication for various diseases such as diabetes, cancer, muscular neurodegenerative disorders, and cardiovascular diseases [17]. The antioxidant and anti-inflammatory effects of CoQ10 was documented in a study of acute nephrotoxicity induced by cisplatin in rats in which CoQ10 decreased the expression of inducible nitric oxide synthase, caspase-3, NF-κB and p53 in renal tissue [18]. In renal IRI induced in rats, CoQ10 pretreatment for seven days at a dose of 10 mg/kg administered intraperitoneally could considerably lower tissue oxidative stress levels as well as histopathology scores and apoptosis [19], furthermore, Liu et al. stated that CoQ10 nanoparticles enveloped with neutrophil membrane (N-NPCoQ10) treatment significantly improved renal function by decreasing oxidative damage, reducing apoptosis of renal cell , and inhibiting inflammatory response in renal I/R injury mouse model [20], so, the effect of COQ10 in renal ischemia reperfusion injury is still to be elucidated in addition to the molecular pathways by which these effects are mediated. The present study aims to investigate the potential nephroprotective effects of CoQ10 in renal ischemia reperfusion injury for rat model in addition to evaluate the possible role of notch pathway in initiating of these effects.

MATERIALS AND METHODS

ANIMAL PREPARATION AND ETHICAL CONSIDERATIONS

The study was conducted on twenty-eight Wistar albino male rats aged (22-24 weeks), weight range 250-300 g. Animals were kept in the animal house at controlled temperature of 25 ± 2ºC and humidity of 60-65% with 12 hrs dark/light cycle, they had free access to water and standard libithium food.

STUDY DESIGN

After two weeks of acclimatization, the rats were allocated into four groups as follows, (seven rats in each group):

Group 1: Sham group in which rats underwent anesthesia and surgery without induction of renal ischemia.

Group 2: control group in which rats underwent 30 minutes of bilateral renal pedicles clumping to induce renal ischemia followed by reperfusion for two hours

Group 3: Olive oil vehicle group in which rats were injected intraperitoneally (i.p) with 0.1 ml olive oil which is a sufficient volume to dissolve the required doses of coq10. Olive oil was administered once daily for 2 days, 1 hour after the administration of olive oil in the third day renal ischemia was induced for 30 minutes followed by two hours of reperfusion.

Group 4: CoQ10 treatment group in which rats were injected with coq10 (10 mg/kg) dissolved in 0.1 ml olive oil i.p. [21] once daily for 2 days, 1 hour after the administration of coq10 in the third day renal ischemia was induced for 30 minutes followed by two hours of reperfusion.

INDUCTION OF BILATERAL RENAL ISCHEMIA REPERFUSION INJURY

An intraperitoneal injection of mixed solution of xylazine 10mg/kg and ketamine 100mg/kg was used to anesthetize the rats [22], then they were kept in cages at constant temperature to avoid hypothermia. When loss of righting reflex is confirmed, the rats were positioned on their back with the limbs, and tail being fixed with

stickers to ensure their stability during surgery following a midline laparotomy incision, the intestine was retracted and both abdominal cavity and renal pedicles were exposed [23]. The renal pedicles were then isolated, and non-traumatic microvascular clamps were placed around the renal pedicles. The kidney color changed from red to dark purple over one to two minutes which confirmed ischemia. After thirty minutes the clamps were removed and renal blood flow was restored to start the reperfusion phase. The abdominal incision was closed by 3/0 silk suture. After two hours of reperfusion, the rats were anesthetized again and both kidneys were removed. The rats were euthanized by an overdose of ketamine. The left kidney was stored in liquid nitrogen for determination of the experiment parameters.

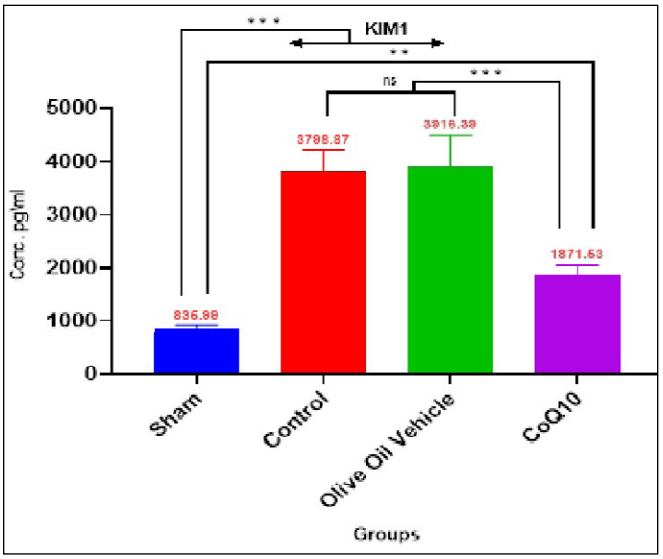


Fig. 1. Error bar chart showing the difference in mean ±SEM values of KIM-1 renal tissue concentration (pg/mL), no. of animals in each group =7, **p< 0.01, ***p< 0.001, non-significant difference
Source: Own materials

ASSESSMENT OF TISSUE KIM-1, IL-1B, TNF A, F2 ISOPROSTANE, GSH, AND CASPASE 3

After the left kidney of each rat was removed, it was washed with cold normal saline to remove any remaining blood clots. Then it was dissected into two halves. One half subjected to homogenization by applying ultrasonic liquid processor of high-intensity in a 1 to 10 (weight by volume) phosphate-buffered saline dilution including a protease inhibitor cocktail and 1% Triton X-100 [24]. Centrifugation at 4°C for 15 minutes at 22,132 × g was then made for this homogenate. The homogenate was then. The supernatant was then collected and divided in Eppendorf tubes for further analysis of KIM-1, IL1β, TNFα, caspase3 and F2 Isoprostane by ELISA technique.

DETECTION OF MRNA EXPRESSION FOR BOTH JAGGED1 AND NOTCH1 BY RT-PCR

To effectively assess the expression level of Notch-1 and Jagged-1 mRNA, we utilized the powerful tech-

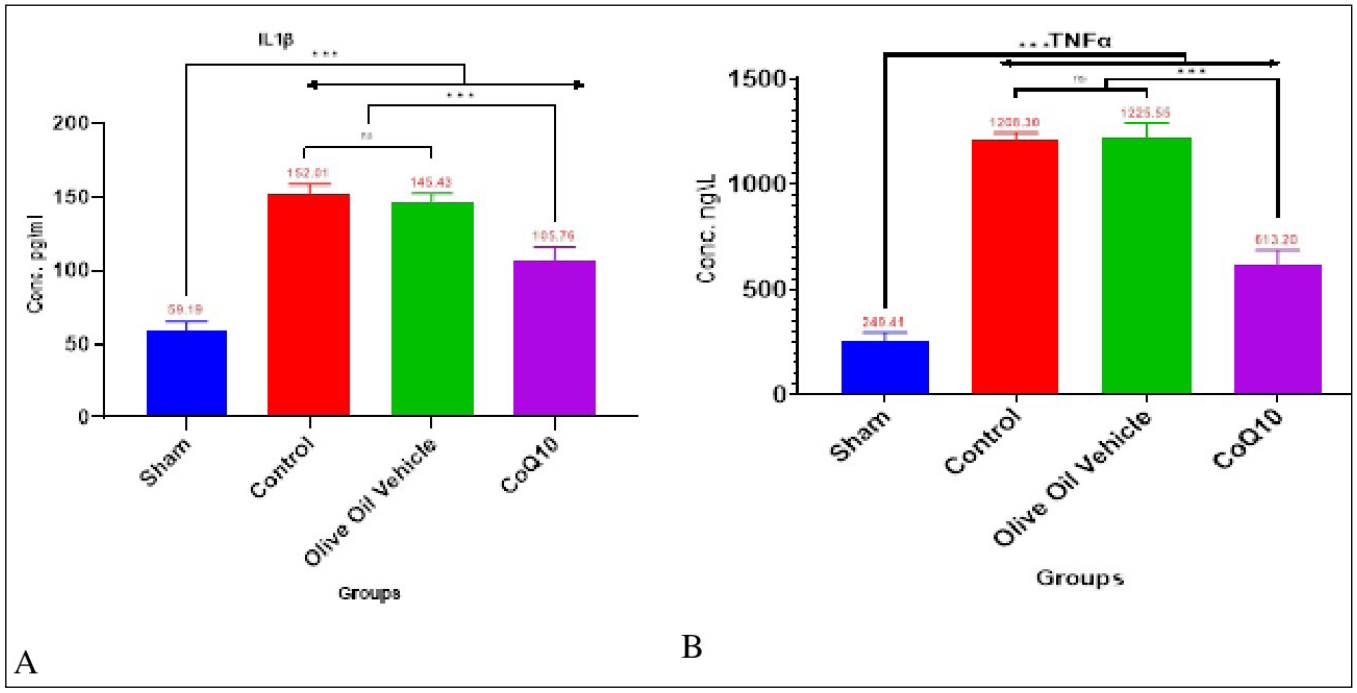


Fig. 2 Error bar chart showing the difference in mean ± SEM values of **(A):** IL1β renal tissue concentration (pg/mL), **(B):** TNFα renal tissue concentration, no. of animals in each group =7, **p<0.01, ***p<0.001.
Source: Own materials

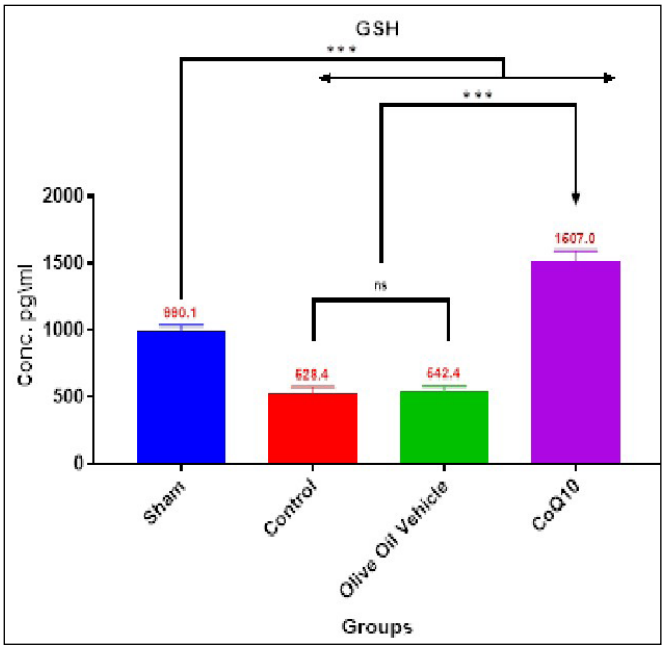


Fig. 3. Error bar chart showing the difference in mean ±SEM values of GSH renal tissue concentration (pg/mL), no. of animals in each group=7, **p<0.01, ***p< 0.001
Source: Own materials

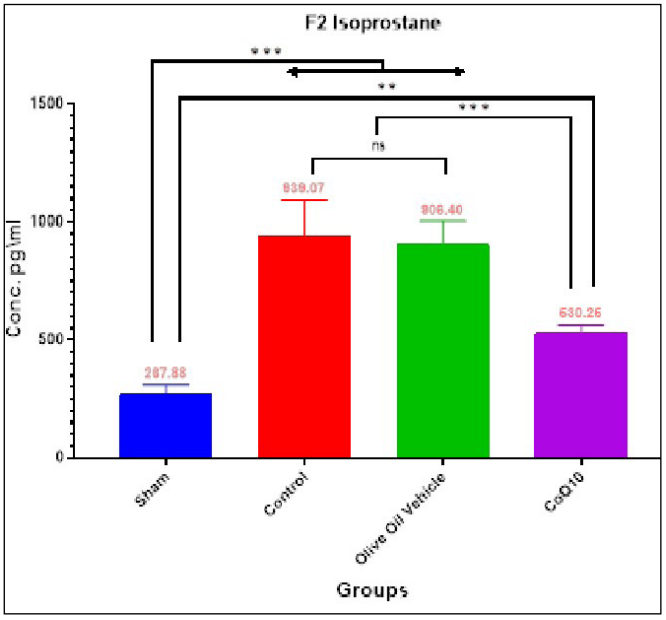


Fig. 4. Error bar chart showing the difference in mean ±SEM values of F2 Isoprostane renal tissue concentration (pg/mL), no. of animals in each group=7, **p<0.01, ***p< 0.001.
Source: Own materials

nique of qRT-PCR. Initially, total RNA was accurately extracted using "Easy-spin™ (DNA free) total RNA extraction Kit". This high-quality total RNA was then expertly reverse-transcribed into cDNA with the help of "AddScript cDNA Synthesis Kit". For the quantitative real-time PCR, we employed the robust GoTaq® RT-qPCR System ensuring the most reliable results. According

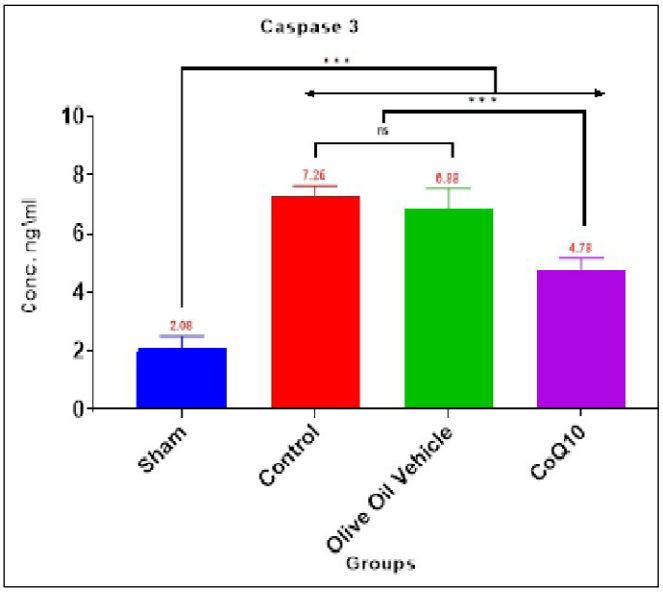


Fig 5. A comparison of Caspase-3 among study groups
**p<0.01, ***p<0.001.
Source: Own materials

to the instruction of GoTaq® RT-qPCR System, Hot-start activation was performed at 95°C for 5 min for 1 cycle, followed by 40 cycles of 10 s of denaturation at 95°C, then 40 cycles of 30 s at 60°C of annealing and 40 cycles of 30 s of extension at 72°C and finally one cycle of 2 min of dissociation at 72°C and specific primers: Notch1 for: CACCCATGACCACTACCCAGTT, Notch-1 rev: CCTCGGACCAATCAGAGATGTT, Jagged1 for: AACTGGTACCGGTGCGAA , Jagged-1 rev: TGATGCAAGATCTCCCTGAAAC [25] and GAPDH for: ATGACTCTACCCACGGCAAG, GAPDH rev. :CTGGAAGATGGTGATGGGTT [26].

STATISTICAL ANALYSIS

To perform the statistical analysis of current study Graph Pad Prism version 8 software was used. Parametric variables were tested using the one-way ANOVA test with multiple comparisons between groups being made by selected Post Hoc test.

RESULTS

COQ10 TREATMENT REDUCES KIM-1 LEVEL IN RAT RENAL TISSUE AFTER RENAL I/R:

KIM-1 renal tissue level was significantly ($p < 0.001$) elevated after renal ischemia /reperfusion (renal I/R) in comparison with sham group. Interestingly, this elevation was attenuated by COQ10 pretreatment in a dose of (10 mg/kg) i.p., however, COQ10 treatment group showed significant ($p < 0.001$) difference when compared to sham group (Fig. 1).

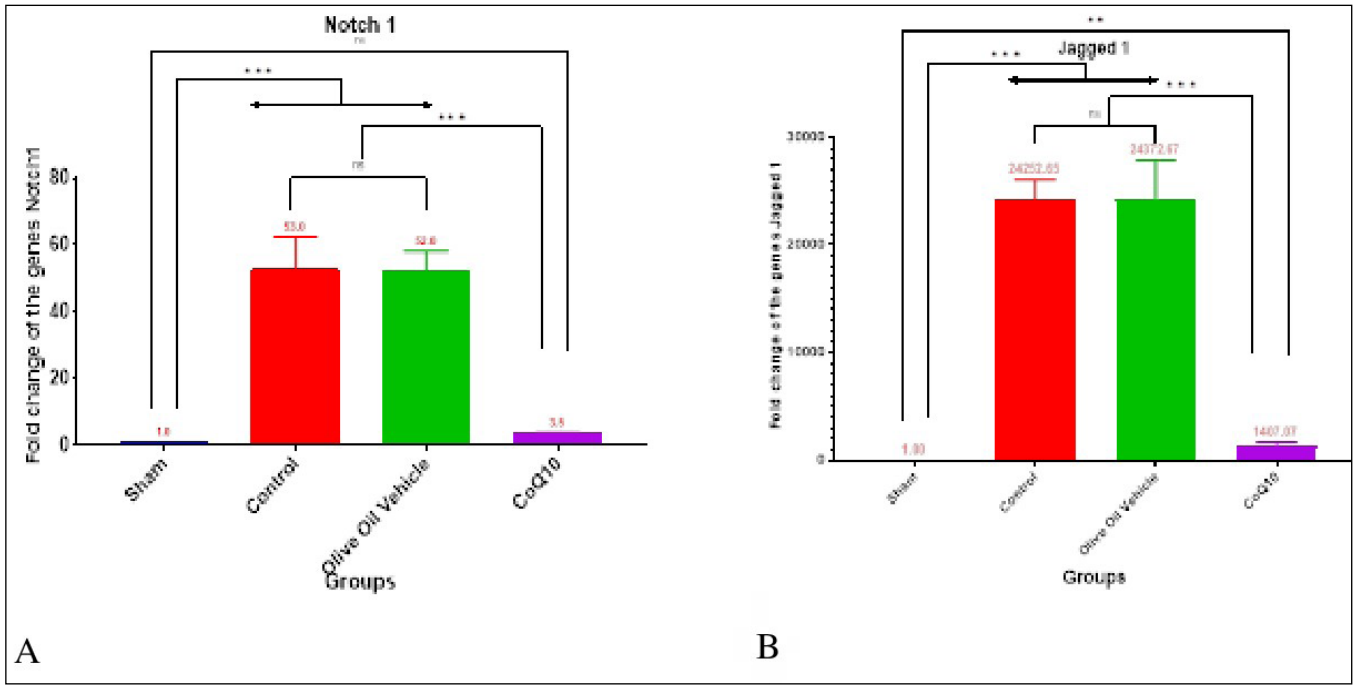


Fig. 6. Error bar chart showing the difference in mean ±SEM values of **A:** Notch-1 mRNA expression level in renal tissue, **B:** Jagged-1 mRNA expression level in renal tissue, no. of animals in each group=7
**p<0.01, ***p<0.001
Source: Own materials

COQ10 TREATMENT REDUCES INFLAMMATORY MARKERS IL-1B AND TNFA IN RAT RENAL TISSUE AFTER RENAL I/R

After renal I/R the renal tissue concentration of both IL-1β and TNFα in rats were significantly (p< 0.001) elevated, COQ10 pretreatment in a dose of (10 mg/kg) i.p. Significantly (p< 0.001) ameliorated the levels of both markers, however, COQ10 treatment group was still significantly (p < 0.001) elevated when compared to sham group (Fig. 2A-B).

COQ10 TREATMENT AMELIORATES GSH LEVELS IN RATS' RENAL TISSUE AFTER RENAL I/R:

Notably, I/R significantly (p< 0.001) reduced GSH level in rat renal tissue relative to sham group. COQ10 pretreatment in a dose of (10 mg/kg) i.p. caused considerable (p< 0.001) elevation in GSH renal tissue concentration in comparison with control group (Fig. 3).

COQ10 TREATMENT REDUCES F2 ISOPROSTANE LEVELS IN RATS' RENAL TISSUE AFTER RENAL I/R

Based on the results of ANOVA as shown in figure 4, the oxidative marker F2 Isoprostane renal tissue concentration was significantly (p<0.001) lower in sham group when compared to control group. However, CoQ10 treatment group was significantly (p<0.001) lower than control group, but it was still significantly (p<0.001) higher, than sham group (Fig. 4).

COQ10 TREATMENT REDUCES CASPASE-3 LEVELS IN RATS' RENAL TISSUE AFTER RENAL I/R

As indicated in figure 5, renal I/R significantly (p<0.001) increased caspase-3 renal tissue concentration while COQ10 pretreatment in a dose of 10 mg/kg i.p. significantly (p<0.001) attenuated this increase, but it was still significantly (p<0.001) higher than sham group (Fig 5).

COQ10 TREATMENT ATTENUATES NOTCH-1 AND JAGGED-1 MRNA EXPRESSION IN RAT'S RENAL TISSUE AFTER RENAL I/R

Both Notch-1 and its ligand Jagged-1 mRNA expression were significantly (p<0.001) elevated in rats renal tissue after I/R, this elevation was significantly (p<0.001) attenuated by COQ10 pretreatment in a dose of 10mg/kg i.p. (Fig. 6A-B).

DISCUSSION

Many molecular pathways have been suggested to mediate the pathogenic processes which ultimately lead to cellular damage that is associated with renal IRI.

In addition, Fundamental research has revealed several targets that could potentially slow the advancement of kidney disease. However, only a limited number of these encouraging findings have been successfully replicated in clinical trials, and they still do not offer a significant alternative to dialysis or transplantation. So, there is an urgent requirement to further elucidate the development of molecular mechanisms underlying renal I/R injury as well as exploring new therapeutic targets that can mitigate the outcomes of this pathologic condition. In this study, we evaluated the nephroprotective effects of COQ10 against renal IRI and the relation of Notch signalling pathway in mediating these effects. The current study revealed significant elevation in renal tissue level of KIM-1 after renal I/R Because KIM-1 is not found in healthy renal tissue and is exclusively expressed in damaged proximal tubules, it serves as a highly sensitive, specific, and precise early indicator of renal tubular injury [27], which confirms the success of IRI model of this study. COQ10 pretreatment caused considerable reduction in KIM-1 renal tissue level in comparison with control group and olive oil vehicle groups which suggests that COQ10 confers protection against renal tissue damage. The decline in KIM-1 level with COQ10 treatment could be due to the antioxidative effects of COQ10 since it can lower the expression of reactive oxygen species (ROS) in damaged cells, decrease mitochondrial depolarization, and improve mitochondrial ATP levels, also, it can boost mitochondrial function, improve cell survival, and decrease apoptosis [28]. IL1β and TNFα were both significantly elevated in control group comparison with sham which confirms the role of the inflammatory response in renal ischemia/reperfusion (I/R) injury. It has become evident that a significant portion of the damage that occurs after renal I/R is due to mitochondrial oxidative stress and the presence of reactive oxygen species (ROS) during reperfusion. These ROS, which include superoxide, peroxynitrite, and hydrogen peroxide (H2O20) trigger the activation of inflammatory cells which result in the release of interleukins (ILs), tumor necrosis factor (TNF-α), and various other inflammatory mediators [29-30] COQ10 pretreatment considerably attenuated the increase in both IL1β and TNFα that is induced by renal I/R. This effect of CoQ10 can be linked to its strong anti-oxidative properties which can block the activation of the NF-B signaling pathway that triggers the expression of the TNF- α and iNOS genes [31]. Glutathione (GSH) which is an intracellular antioxidant has been demonstrated to quickly recognize and interact specifically with hydroxyl radicals (OH•), which are the most reactive types of reactive oxygen species (ROS). Additionally, GSH detoxifies hydrogen peroxide (H_2O_2) and lipid peroxides through the activity of GSH-Px, safeguarding cell membranes from lipid oxidation [32]. The present study showed significant reduction in GSH levels after renal I/R. This reflects the depletion of the tissue antioxidative capacity due the production of huge amounts of ROS during the reperfusion phase as a result of the defect in mitochondrial electron transport chain [33]. The present study comes in line with another study conducted to evaluate the nephroprotective effects of curcumin in renal IRI which have shown the reduction in GSH level in serum after clipping the left renal artery for 45min and then reperfusion for 24h [34]. COQ10 pretreatment on the other hand caused significant elevation in GSH level which affirms its antioxidant properties apart from its role in the respiratory chain. Another marker of oxidative stress was evaluated in this study which is F2-isoprostane. it is generated through a non-enzymatic process, resulting from the powerful peroxidation of arachidonic acid driven by free radicals. In the present study renal tissue concentration of F2-isoprostane was considerably elevated after renal I/R. this elevation was significantly ameliorated by COQ10 pretreatment. This effect could be attributed to the fact that CoQ10 is not only a strong antioxidant but also plays a crucial role in preventing the formation of reactive oxygen species. It helps to eliminate lipid peroxidation byproducts during reactions with free radicals, reduces excessive nitric oxide production, and protects tissues from nitrative stress [35]. Apoptosis, often referred to as programmed cell death, plays an important role in renal ischemia-reperfusion injury (RIRI). Caspase-3 is considered one of the key proteins involved in the apoptotic process which is activated in the final common pathway of both intrinsic and extrinsic apoptosis [36]. our study demonstrated significant increase in caspase-3 renal tissue concentration after renal I/R which come in line with other studies that showed caspase-3 to be upregulated in the early stages of renal I/R injury which strengthen the evidence that apoptosis is activated and might play a role in the pathogenesis of renal I/R injury [37]. COQ10 pretreatment on the other hand resulted in considerable reduction of caspase-3 renal tissue levels confirming the anti-apoptotic effects of COQ10 [19]. To investigate the relation between COQ10 nephroprotective effects and Notch-1 molecular pathway, we evaluated ligand Jagged-1 mRNA expression and Notch-1 in all study groups. Jagged-1 mRNA expression and Notch-1 were noticeably elevated after renal I/R. Notch signaling plays an essential role in the development and segmentation of nephrons and has been associated with kidney disorders [38]. Studies in mouse models have indicated that in adult mice, Notch signaling can be detrimental, resulting in abnormal podocyte differentiation, cell death,

and eventually kidney failure [39]. The upregulation of Notch-1 and its ligand along with the other inflammatory, oxidative and apoptotic parameters in the present study might provide further evidence about the detrimental effects of Notch-1 signaling pathway in renal I/R injury. Interestingly COQ10 pretreatment caused significant amelioration of Notch-1 and Jagged-1 mRNA expression in renal tissues after renal I/R. The present study considered to be novel for assessment the effect of COQ10 pretreatment on Notch-1 and its ligand Jagged-1 m RNA expression after renal I/R in rats showing possible correlation between COQ10 nephroprotective effects and Notch-1 regulation in renal I/R injury. A number of previous studies have shown cross talks between Notch-1 signaling and oxidative stress pathways [40]. By reducing oxidative harm, CoQ10 may help stabilize Notch-1 signaling and avert its disruption in addition to enhancing mitochondrial performance with CoQ10 supplementation could aid in maintaining healthy Notch-1 signaling, especially in high-energy-demand tissues like the kidney.

CONCLUSIONS

The findings of the present study may add further evidence about the role of inflammation, oxidative stress and apoptosis in the pathogenesis of renal I/R injury suggesting the involvement of Notch-1 signaling pathway in mediating these effects. COQ10 may have protective effects in renal ischemia reperfusion injury due to its anti-inflammatory, anti-oxidative and anti-apoptotic effects. In addition, COQ10 protective effects were associated with downregulation of Notch-1 and its ligand Jagged-1.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Effect of infusion solutions on circulating blood volume through different administration routes in animal models of peritonitis

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ABSTRACT

Aim: To compare the effects of different administration routes of various infusion solutions on the dynamics of circulating blood volume (CBV) in an experimental rat model of peritonitis.

Materials and Methods: The experiment involved 100 male white rats, three months old, weighing 200–220 g, born to different dams. Peritonitis was modeled by a single intraperitoneal injection of a 10% suspension of fresh rat feces. During the study, following the development of peritonitis in the animals, the dynamics of neutral red dye concentration in the gastric perfusate were measured. This was done using 0.9% sodium chloride solution and 10% albumin solution, administered via three different routes.

Results: It was determined that intraosseous administration of both 0.9% NaCl solution and 10% albumin solution had a statistically significant impact on CBV in peritonitis. The 10% albumin solution demonstrated a longer hemodynamic effect. Additionally, extraperitoneal administration of the 10% albumin solution was also effective.

Conclusions: 1. Administration of 0.9% sodium chloride and 10% albumin solutions in experimental peritonitis in animals results in an increase in circulating blood volume (CBV) when administered by the intraosseous route, with increases of 30% ($P \leq 0.01$) and 45% ($P \leq 0.001$), respectively. 2. The 10% albumin solution, administered extraperitoneally as a bolus infusion, significantly enhanced the secretion of neutral red dye into the perfusate, which correlated with its effects on CBV and improved microcirculation in the gastric mucosa.

KEY WORDS: albumin, acute peritonitis, circulating blood volume, 0.9% sodium chloride solution

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INTRODUCTION

Peritonitis is an inflammatory reaction of the peritoneum, capable of rapid generalization, leading to sepsis and septic shock, and in complicated cases, resulting in a mortality rate of up to 80% [1]. Literature highlights causes of peritoneal sepsis, including undrained fluid collections, interloop abscesses, inadequate surgical sanitation of the abdominal cavity during operations, immune defects, severe comorbid conditions, and subsequent endothelial dysfunction, enteropathy, and endotoxemia, progressing to multi-organ failure syndrome [2]. Shortly after massive invasion of bacterial agents, mesothelial damage, cytokine release, altered absorptive capacity of the peritoneum, intestinal paralysis, and intra-abdominal hypertension develop [3]. The cytokine cascade results in endothelial dysfunction and microcirculatory blockages [4, 5]. Critical interventions to save patients' lives include adequate volumetric resuscitation to restore oxygen delivery to tissues, normalization of circulating blood volume (CBV) and cardiac output, early antibiotic administration, and combating multi-organ failure [6, 7]. Strategies like the «open abdomen» approach, vacuum systems, and modern sepsis therapy significantly improve survival rates [8].

AIM

The aim of this study was to compare the effects of different administration routes of various infusion solutions on the dynamics of circulating blood volume (CBV) in an experimental rat model of peritonitis.

MATERIALS AND METHODS

The experiment was conducted on 100 male white rats, 3 months old, weighing 200-220 g. All rats were offspring of different dams, born a few days apart. At three months of age, the rats were randomly divided into groups. All animal experiments adhered to international guidelines for biomedical research involving animals, in accordance with the «General Principles of Animal Use», approved by the First National Congress on Bioethics, and aligned with the «European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes» and national legislation [9]. The experiments were conducted in compliance with the European Council Directive 86/609/EEC of November 24, 1986, on the care and use of laboratory animals. The study was approved by the Ethical Committee of the Educational and Scientific Center «Institute of Biology and Medicine», Taras Shevchenko National University of Kyiv, Ukraine, in September 2001. The rats were housed in the vivarium of the «Institute of Biology and Medicine» under standard conditions of temperature (21°C), lighting (12/12-hour light-dark cycle), humidity, and diet (complete laboratory animal feed K-12-4, «Rizan-1», Ukraine), in accordance with the «Standard Rules for the Arrangement, Equipment, and Maintenance of Experimental Biological Clinics (Vivariums)» [10]. Each group was further subdivided into subgroups based on the objectives of the study. To model peritonitis, the rats received a single intraperitoneal injection of a 10% suspension of fresh rat feces. The suspension was administered through a single puncture along the midline in the umbilical region of the abdomen to a depth of 2 mm. It was prepared using isotonic sodium chloride solution and filtered once through a double layer of gauze. A volume of 0.5 ml of the suspension per 100 g of body weight was used to induce peritonitis [11, 12]. Twenty-four hours after peritonitis induction, the rats were enrolled in the experiment. During the study, following the development of peritonitis in the animals, the dynamics of the concentration of neutral red dye in the gastric perfusate were measured. This was done using 0.9% sodium chloride solution and 10% albumin solution, administered via three different routes. The CBV dynamics were assessed using the neutral red dye clearance method through the gastric mucosa. This method relies on the dilution of the dye in circulating blood as blood volume increases (i.e., with a rise in CBV). A corresponding decrease in dye concentration occurs when the dye diffuses through the gastric mucosa, facilitated by hydrochloric acid perfusion of the mucosa [13]. At the 30th minute of the experiment, the animals were divided into three groups based on the routes of administration of 0.9% NaCl solution and 10% albumin solution, with the control group consisting of intact animals. The groups were as follows: Group 1: Intravenous administration. Group 2: Intraosseous administration. Group 3: Extraperitoneal administration. The volume of the administered solutions was 1 ml. Each group was further divided into two subgroups: Subgroup 1: Received 0.9% NaCl solution. Subgroup 2: Received 10% albumin solution. Before the experiment, the rats were weighed using «AXIS AD-50» electronic scales (Poland). Microsurgical instruments and materials from «Surgiwell» (Pakistan) were used for surgical procedures.

For general anesthesia, urethane («Sigma», China) was used at a dose of 1.1 g/kg body weight, administered intraperitoneally. The depth of anesthesia was evaluated by assessing the flexor reflex of the forelimb. Euthanasia was performed by overdose of anesthesia.

The rats were fasted for 12 hours prior to the experiment, with free access to water. During the experiment, tracheotomy and midline laparotomy of the abdominal cavity were performed. The stomach and duodenum were exposed, and a ligature was placed below the pyloric sphincter. An input catheter was introduced into the stomach and secured at the level of the tracheotomy incision. An output catheter was inserted through a duodenal incision into the stomach and fixed above the incision in the duodenum and below the pyloric sphincter. Using a peristaltic pump (MINIPULS GILSON, USA), the perfusion solution was delivered into the stomach through the input catheter at a constant rate (15 ml over 15 minutes). During the first 10 minutes, the stomach was perfused with physiological saline to stabilize blood flow in the gastric mucosa. For the next 15 minutes, an HCl solution with a pH of 2.0 was used. To maintain a constant level of dye in the plasma, a loading dose of neutral red (2 mg per 100 g body weight) was injected into the tail vein of the animals. Every 15 minutes over a 2-hour period, perfusates were collected from the stomach, and the optical density of each perfusate was measured using a «Synergy HT» spectrophotometer at a wavelength of 276 nm. Distilled water was used as the zero reference. The concentration of neutral red in the perfusates was determined using a previously constructed calibration curve.

The statistical analysis of results was performed using methods of variance statistics with the software Statistica 8.0. The Shapiro-Wilk W-test was used to check for normality. The reliability of the identified changes was evaluated using analysis of variance (ANOVA), and the results were presented as M±SD. Differences between groups were considered statistically significant at a significance level of $P < 0.05$ [14]. Graphs and histograms were created using Microsoft Excel.

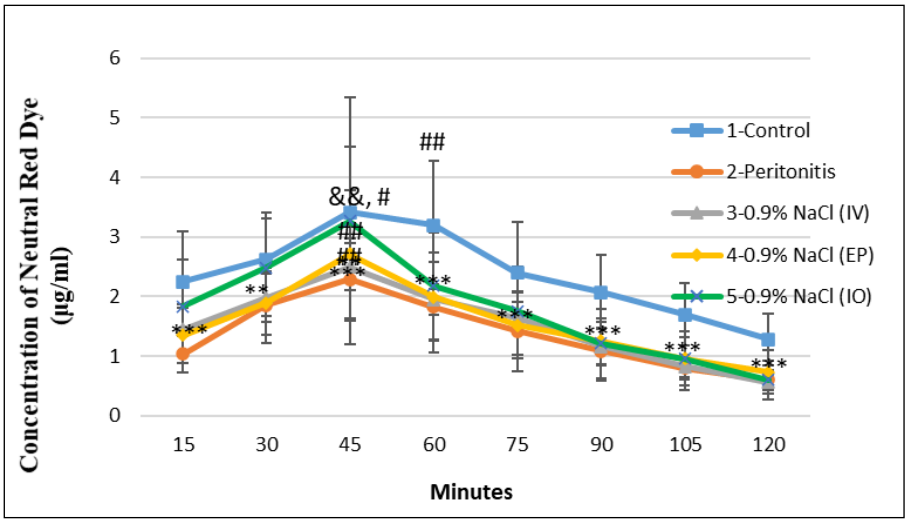


Fig. 1. Concentration of Neutral Red Dye in Gastric Perfusate of Rats After Administration of 0.9% NaCl Solution: 1 – Control, 2 – Peritonitis, 3 – Intravenous Administration (IV), 4 – Extraperitoneal Administration (EP), 5 – Intraosseous Administration (IO). P ≤ 0.01 *P ≤ 0.001 compared to control; #P ≤ 0.05, ##P ≤ 0.01, ###P ≤ 0.001 compared to the 30th minute within each administration group
Picture taken by the authors

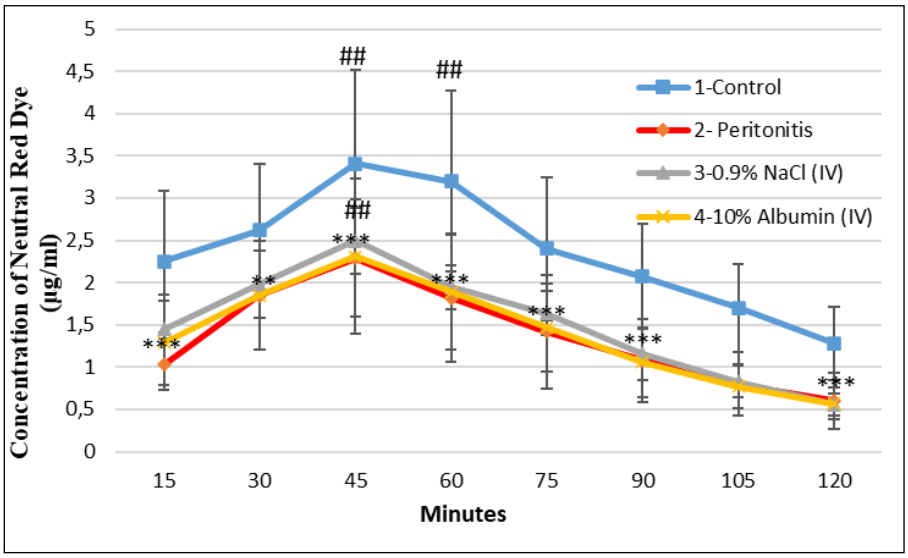


Fig. 2. Concentration of Neutral Red Dye in Gastric Perfusate of Rats After Administration of 10% Albumin Solution: 1 – Control, 2 – Peritonitis, 3 – Intravenous Administration (IV), 4 – Extraperitoneal Administration (EP), 5 – Intraosseous Administration (IO). P ≤ 0.01 *P ≤ 0.001 compared to control; #P ≤ 0.05, ##P ≤ 0.01, ###P ≤ 0.001 compared to the 30th minute within each administration group
Picture taken by the authors

RESULTS

The control group consisted of animals without peritonitis, which received an intravenous injection of neutral red dye at the 15th minute of the study to assess the dynamics of its secretion through the gastric mucosa.

In the second phase, dye secretion by the gastric mucosa was studied in animals with induced peritonitis who did not receive an infusion bolus of 0.9% NaCl solution or 10% albumin. The 10% albumin solution used in the study was the «Albuven» preparation, produced by BIOPHARMA PLASMA LLC, Ukraine. The active ingredient, human albumin, was present in a concentration of 100 g of total protein per 1000 ml of solution, with at least 96% being albumin. The auxiliary substances included sodium caprylate, sodium chloride, sodium hydroxide, and water for injections, with a sodium content not exceeding 160 mmol/L. Based on pathophysiological and pathomorphological data, significant fluid redistribution into inflamed peritoneal layers was anticipated during peritonitis, with subsequent dye saturation in these regions. At the beginning of the study (15th minute), the CBV deficit in peritonitis was 45.78%, likely not due to dye dilution but rather to its intense redistribution into inflamed areas. By the 60th minute, the CBV deficit was 53.4%, and by the 120th minute, the deficit remained at 46.9%.

The third phase involved determining the concentration of neutral red dye in the gastric perfusates after administering a bolus infusion of 0.9% NaCl or 10% albumin in animals with induced peritonitis. When administering 0.9% NaCl physiological solution to animals with modeled peritonitis, the following patterns were established. In the group receiving intravenous administration of 0.9% NaCl, significant differences from the control group were observed from the very beginning of the experiment. Specifically, the concentration of neutral red in the gastric perfusate differed by: 35.6% at the 15th minute, 24.4% at the 30th minute, 26.7% at the 45th minute, 39.1% at the 60th minute, 31.7% at the 75th minute, 43.9% at the 90th minute, 51.2% at the 105th minute, and 56.2% at the 120th minute, respectively.

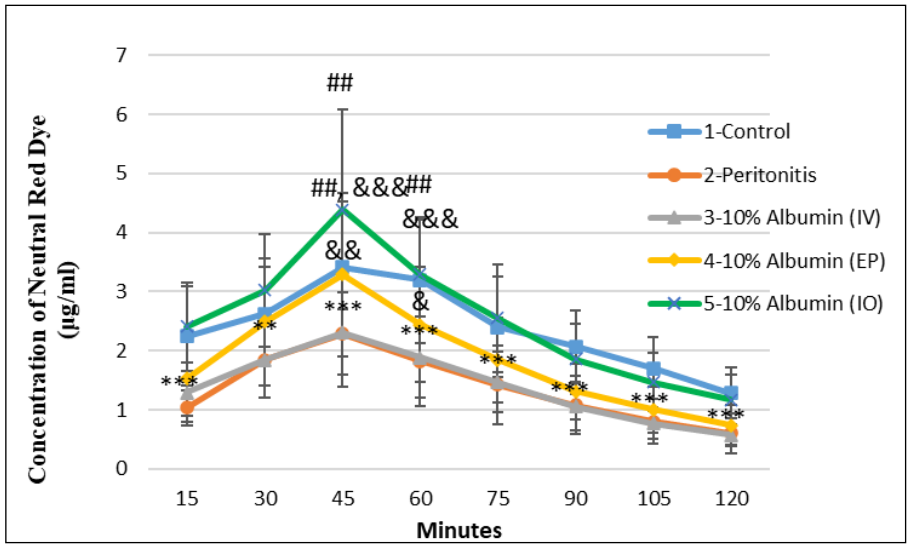


Fig. 3. Concentration of Neutral Red Dye in Gastric Perfusate of Rats After Intravenous Administration of 0.9% NaCl and 10% Albumin Solutions:
1 – Control, 2 – Peritonitis, 3 – 0.9% NaCl Intravenously (IV), 4 – 10% Albumin Intravenously (IV). P ≤ 0.01, *P ≤ 0.001 compared to control; #P ≤ 0.05, ##P ≤ 0.01, ###P ≤ 0.001 compared to the 30th minute within each administration group
Picture taken by the authors

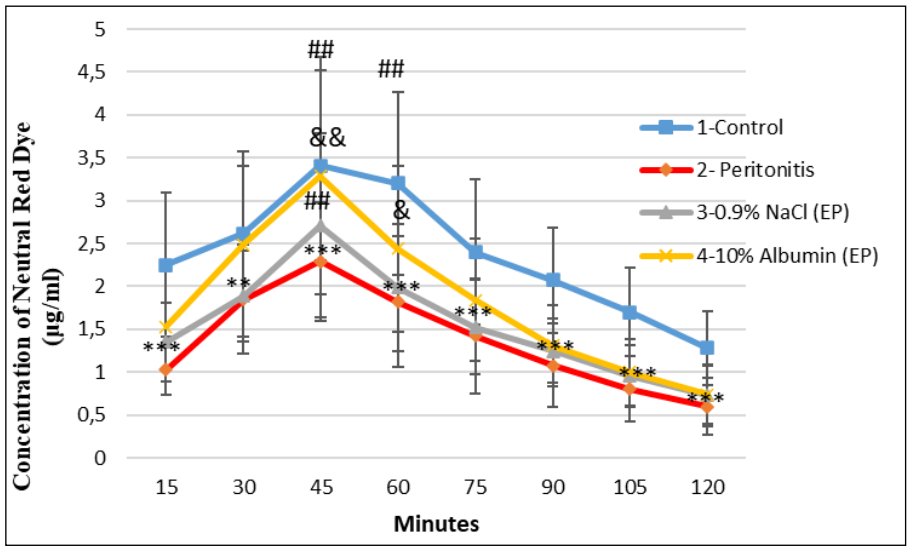


Fig. 4. Concentration of Neutral Red Dye in Gastric Perfusate of Rats After Extraperitoneal Administration of 0.9% NaCl and 10% Albumin Solutions:
1 – Control, 2 – Peritonitis, 3 – 0.9% NaCl Extraperitoneally (EP), 4 – 10% Albumin Extraperitoneally (EP). P ≤ 0.01, *P ≤ 0.001 compared to control; #P ≤ 0.05, ##P ≤ 0.01, ###P ≤ 0.001 compared to the 30th minute within each administration group
Picture taken by the authors

When analyzing the group with extraperitoneal administration of physiological solution in rats with peritonitis, significant differences from the control group were observed as follows: 40% at the 15th minute, 27.9% at the 30th minute, 37.8% at the 60th minute, 36.67% at the 75th minute, 39.6% at the 90th minute, 43.5% at the 105th minute, and 42.9% at the 120th minute, respectively.

When analyzing the group with intraosseous administration of physiological solution in rats with peritonitis, significant differences from the control group were observed at the following time points: 31.9% at the 60th minute, 26.7% at the 75th minute, 41.5% at the 90th minute, 43.5% at the 105th minute, and 53.1% at the 120th minute, respectively. The CBV deficit, compared to the 45th minute (where it was 44%), decreased to 31.9% at the 60th minute. However, by the 120th minute, the CBV deficit increased again to 53.1%.

The summarized data are presented in Fig. 1.

When comparing the effects of 0.9% NaCl solution administered via different infusion routes, notable changes were observed with intraosseous administration, which showed a statistically significant difference at the 45th minute compared to the peritonitis group (29.9%). At the 15th minute, the CBV deficit was 43.7%, and following an intravenous bolus of 0.9% NaCl, the CBV deficit at the 45th minute decreased to 29.9%, a result not observed with other routes (extraperitoneal or intravenous). This finding demonstrates the significant effectiveness of the intraosseous route for infusion therapy.

The second series of experiments analyzed the impact of different administration routes on CBV dynamics using a 10% albumin solution in rats with induced peritonitis. Significant differences in neutral red dye concentrations in gastric perfusates were observed at all time points, similar to the first series of experiments.

For rats with peritonitis, the concentration parameters of perfusates differed from the control group as follows: 15th minute: 54%, 30th minute: 29.4%, 45th minute: 32.8%, 60th minute: 43%, 75th minute: 40.8%, 90th minute: 47.8%, 105th minute: 48.8%, 120th minute:

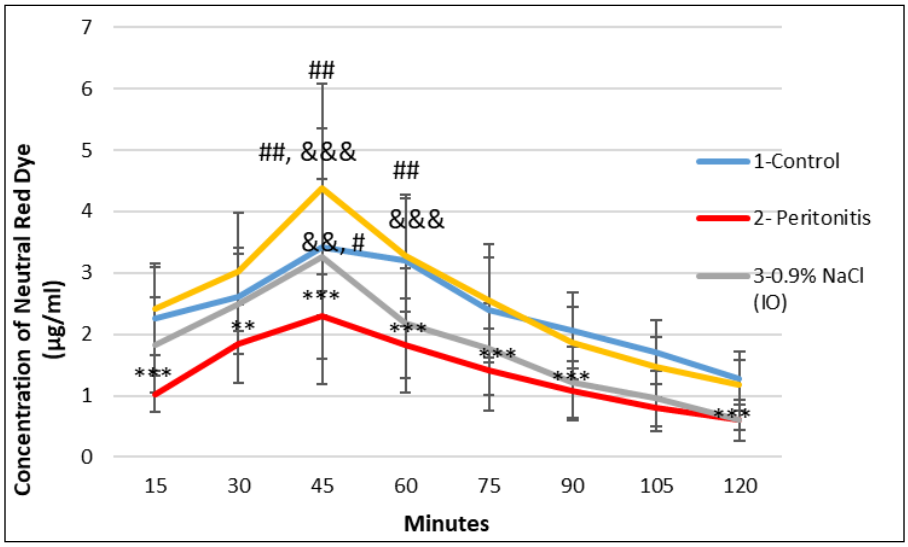


Fig. 5. Concentration of Neutral Red Dye in Gastric Perfusate of Rats After Intraosseous Administration of 0.9% NaCl and 10% Albumin Solutions:
1 – Control, 2 – Peritonitis, 3 – 0.9% NaCl Intraosseously (IO), 4 – 10% Albumin Intraosseously (IO). $P \leq 0.01$
*$P \leq 0.001$ compared to control; #$P \leq 0.05$, ##$P \leq 0.01$, ###$P \leq 0.001$ compared to the 30th minute within each administration group
Picture taken by the authors

53%. The concentration changes in perfusates depended on local hemodynamic changes, gastric mucosal perfusion, its absorptive and secretory capabilities, dye redistribution, and «paralysis» of microcirculation. The results of the second phase of the experiment are presented in Fig. 2.

Following the induction of peritonitis, a 10% albumin solution was administered to the experimental animals via three infusion routes: intravenous, extraperitoneal, and intraosseous. Intravenous administration of albumin solution resulted in significant changes in concentration from the control group, starting at the 15th minute with a 42.67% difference. These significant changes persisted across all measured intervals: 30th minute: 29.4%, 45th minute: 32.3%, 60th minute: 40.9%, 75th minute: 38.7%, 90th minute: 48.8%, 105th minute: 54.7%, 120th minute: 55.5%. The CBV deficit with intravenous administration of 10% albumin in peritonitis was: 42.7% at the 15th minute, 40.9% at the 60th minute, 55.5% at the 120th minute. Intraosseous administration of albumin solution showed significant changes in the concentration of neutral red dye in gastric perfusates. At the 45th minute, there was a 38.7% difference compared to the 30th minute within-group analysis. Compared to the peritonitis group, the differences were 47.8% at the 45th minute and 44.7% at the 60th minute. The 10% albumin solution administered extraperitoneally significantly altered the concentration of neutral red in the perfusates. At the 45th minute, there was a 30.4% difference compared to the peritonitis group. At the 60th minute, the difference was 25.4% compared to the peritonitis group. Additionally, when compared to the control group, significant changes were observed at: 23.7% at the 60th minute, 23.3% at the 75th minute, 36.7% at the 90th minute, 41.2% at the 105th minute, and 42.2% at the 120th minute. These findings indicate the effective impact of 10% albumin solution on CBV dynamics when administered via intraosseous and extraperitoneal infusion routes.

Comparative characteristics of CBV dynamics using identical substances (0.9% NaCl solution and 10% albumin solution) administered via different infusion routes are presented in Fig. 3, Fig. 4, Fig. 5.

In the comparative analysis of neutral red dye concentration curves in gastric perfusates following intravenous administration of 0.9% NaCl and 10% albumin solutions, the dynamics of dye secretion did not significantly differ between the two groups and the peritonitis group. Similarly, as observed in the control group, the peak secretion of neutral red dye occurred between the 45th and 60th minutes.

Following extraperitoneal administration of the studied solutions, a significant dynamic of dye secretion was observed in rats receiving 10% albumin compared to the peritonitis group. At the 45th minute, a 30.4% difference was noted. At the 60th minute, a 25.4% difference was recorded. In contrast, 0.9% NaCl administered via the extraperitoneal route showed no significant effect on CBV.

In a comparative analysis of neutral red dye concentration curves in animals receiving intraosseous bolus administration of 0.9% NaCl and 10% albumin solutions, a significant hemodynamic effect was observed. For 0.9% NaCl, at the 45th minute, there was a 29.9% difference compared to the peritonitis group. For 10% albumin, significant differences were noted at the 45th minute (47.8%) and the 60th minute (44.7%). This indicates CBV changes of 30% at the 45th minute in the 0.9% NaCl group and 48% and 45% at the 45th and 60th minutes, respectively, in the 10% albumin group. These results are reflected in the increased secretion of the dye through the gastric mucosa.

Thus, intraosseous administration of both 0.9% NaCl solution and 10% albumin solution demonstrated significant effects on CBV, with the latter showing a more prolonged hemodynamic effect. The extraperitoneal route of administering 10% albumin also proved effective.

DISCUSSION

The primary goal of fluid therapy is to ensure adequate oxygen delivery to tissues by normalizing cardiac output and restoring microcirculation. CBV deficit, particularly in peritonitis, significantly depends on capillary losses, renal and respiratory function, cumulative fluid balance, and fluid redistribution into the «third space» [15]. Combining infusion fluids with vasopressor therapy to achieve a mean arterial pressure (MAP) of 65 mmHg is essential for addressing these issues. Recommendations for the initial administration of 30 ml/kg crystalloids within the first three hours during septic shock have low evidence quality and are currently under revision. Experts now favor personalized infusion therapy, dynamic fluid balance assessment, and advanced hemodynamic monitoring with dynamic circulatory parameter evaluation [16, 17]. Categorizing patients into «responders» (those with a hemodynamic response to infusion therapy) and «non-responders» helps prevent fluid overload from excessive infusions [18]. Over time, infusion fluids redistribute into the interstitial space, leading to polycompartment syndrome, which primarily affects parenchymal organs (liver, kidneys), myocardium, lungs, and the intestinal wall [19]. Currently, early-stage infusion therapy for peritonitis is considered «liberal» and «massive», but if the patient's condition does not stabilize, the approach should shift toward restriction. These principles are described in the phased shock therapy concept (ROSE): R - Resuscitation, O - Optimization, S - Stabilization, E - Evacuation. Each phase involves specific strategies for fluid therapy [20]. Albumin use for primary stabilization in septic shock resistant to crystalloid therapy has moderate evidence strength, with recommendations favoring the use of 20-25% albumin solutions [21, 22]. While albumin increases MAP, it does not impact mortality compared to crystalloids [23-26]. Crystalloids remain the solutions of choice and should be balanced to avoid excessive chloride ions. Hemodynamic effects similar to those outlined in modern guidelines were observed in this study, with a significant hemodynamic impact demonstrated by the intraosseous administration of 10% albumin solution. The extraperitoneal route, which also proved effective with 10% albumin, requires further research and methodological refinement.

Traditional routes for administering infusion solutions include intravenous (IV) and intraosseous routes [27]. The intraosseous route offers advantages in patients with difficult peripheral venous catheterization, in emergency or battlefield conditions, but requires specialized equipment and skills [28]. However, it is associated with a high rate of infectious complications, such as osteomyelitis, and limited duration of access. Common access points include the tibial tuberosity, humerus, and sternum. The effect of intraosseous infusion is comparable to intravenous infusion in terms of stabilization speed and the range of administered medications. The retroperitoneal route has not been widely studied in intensive care for peritonitis. Nevertheless, some reports indicate its use in conservative treatment of aortic aneurysms, involving prolonged infusion of tetracycline-class antibiotics [29]. This route has been effectively employed for regional blocks, including paranephric, solar plexus, and sympathetic trunk ganglia blockades [30]. Considering the anatomical and physiological features of retroperitoneal lymphatic drainage into the thoracic cavity, the proximity of the sympathetic trunk, and compartmentalization of retroperitoneal structures, this route may be useful for administering certain antibiotics, albumin solutions, and more.

CONCLUSIONS

1. Administration of 0.9% sodium chloride and 10% albumin solutions in experimental peritonitis in animals results in an increase in circulating blood volume (CBV) when administered by the intraosseous route, with increases of 30% ($P \leq 0.01$) and 45% ($P \leq 0.001$), respectively.
2. The 10% albumin solution, administered extraperitoneally as a bolus infusion, significantly enhanced the secretion of neutral red dye into the perfusate, which correlated with its effects on CBV and improved microcirculation in the gastric mucosa.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Clinical and laboratory markers in predicting the probability of alveolitis occurrence

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ABSTRACT

Aim: Our aim was to identify the markers necessary to predict the probability of alveolitis in case of tooth extraction under the conditions of a clinical laboratory and within the workplace of a dental surgeon.

Materials and Methods: 160 patients were under our supervision. Since men and women may undergo tooth extractions throughout their lives, we selected adult patients (from 18 to 75 years old) including 100 men and 60 women. The effectiveness of classical methods was studied and compared according to the treatment protocol and drug treatment regimens developed by us based on a range of laboratory and comprehensive clinical studies. Patients in the main group and the comparison group were identical by age, gender, pathology, and the conducted treatment. Patients who required outpatient tooth extraction were regarded as the inclusion criteria for the research. Depending on the treatment, all patients were divided into three groups, randomized by age, gender, and duration of the disease.

Results: According to the results of our research, OHI indicators, which were conducted for 14 days, showed that the average statistical value of OHI constituted (2.01±0.11) points the day after the extraction in Group II in case of scheduled tooth extraction corresponding to indicators of a satisfactory state of hygiene.

Conclusions: 1. The inclusion of pectin-containing medication in the complex of preventive measures is fully justified in relation to the occurrence of inflammatory complications after tooth extractions in modern conditions of a doctor's work at an outpatient clinic.

2. The wound surface of the socket of the extracted tooth should be covered with a bandage.

3. Under the conditions of a clinical laboratory and within a workplace of a dental surgeon, the markers necessary to predict the probability of alveolitis in case of a tooth extraction are the following: clinical indicators of normalization of the oral cavity hygienic state, a significant decrease in the intensity and prevalence of the inflammatory process in the gums, changes in the oral fluid pH towards the alkaline side.

KEY WORDS: tooth extraction, inflammation, pectin, alveolitis

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INTRODUCTION

The amount of scientific information is large nowadays, therefore doctors are not always able to formulate independently modern pieces of advice and give practical recommendations for patients in case of emergency or scheduled tooth extraction. As a result, a violation of the pre- and postoperative regimen occurs that may lead to alveolitis and its complications. Unfortunately, dentists are often unable to predict them. In the middle of the XX century, the standard method of brushing teeth with toothpaste was recommended before the tooth extraction in order to prevent complications in the preoperative period, and rinsing with a potassium permanganate solution was recommended before the extraction. However, changes in the pharmacopoeia do not allow this method to be used in modern surgical interventions. The scientific literature of recent years does not provide convincing data on the use of effective preventive measures in the preoperative period. Therefore, our previous research was aimed at finding the scientific data comprising pieces of advice for preventing the occurrence of alveolitis and its complications in the pre- and postoperative period of tooth extraction. As a result of the information sources analysis, three groups of factors influencing extraction wounds healing were identified: local, general, and medical [1]. Local factors affecting wound healing after tooth extraction include oral hygiene, periodontal disease, tooth decay and its complications. The presence of constant microflora, most often staphylococci, in foci of chronic odontogenic infection creates conditions for the formation of organism sensitization [2]. According to O.O. Timofeiev (2002), the nature of the postoperative period depends on the degree of the organism sensitization rather than the microbial flora [3]. Untreated tooth decay of adjacent teeth and inflammatory processes in the oral

Table 1. Results of the patients' distribution by age and gender who underwent tooth extraction surgery

Age	Groups and quantity of patients					
	Group I (control) 20 individuals		Study group II 70 patients		Study group III 70 patients	
			Scheduled extraction 36 patients	Urgent extraction 34 patients	Urgent extraction 34 patients	Scheduled extraction 36 patients
	m	w	m	w	m	w
18-25	5	8	6	3	5	4
25-40	2	3	10	8	11	8
40-55	1	1	12	9	11	10
55-75	-	-	12	10	11	10
Total	8	12	40	30	38	32
	20		70		70	

Source: compiled by the authors of this study

cavity also contribute to alveolitis occurrence [4]. A significant percentage of complications during tooth extractions is explained by general economic conditions, which lead to lowering of the scale of living, a decrease in the cultural level and general somatic health of the population, and deterioration of the ecological environment. These are general influencing factors [5]. Rather widespread pieces of advice of some scientists have been presented in the scientific literature for a long time claiming that patients are not recommended to eat spicy, hot, and rough food for 24 hours after the tooth extraction. We consider this problem to be often ignored by both clinicians and patients. This occurs due to the unconvincing advice of the former and the lack of competence of the latter, when recommendations regarding common rinsing of the oral cavity lead to the patient's overactive actions contributing to the clot washing out and exposure of the wound surface with its subsequent infection. We agree with the scientists' opinion that all this affects the development of alveolitis.

Currently, the point of view on the inflammation development, in which free radical processes that accompany changes in the structures of biopolymers play a responsible role, is gaining an increasing support among scientists. However, a problem that precedes these processes still remains. Organic free radicals and highly toxic metabolic products are produced in the course of lipid peroxidation (LP). In particular, a correlation dependence between an increase in the concentration of the action of new conjugates (NC) and malonaldehyde (MA) both in the blood serum system and in the muco-ocular flap was detected in the patients requiring surgical interventions. These indicators could be the markers of the inflammation course in the preoperative period as well as a marker of inflammatory reactions emergence in the early postoperative period. The researchers needed expensive equipment and reagents in order to perform their work, which was difficult for a practicing physician in a daily practice. According to the results of the conducted research, the authors concluded that the inclusion of immunomodulatory and antioxidant drugs in the complex of preventive measures was totally justified in relation to the occurrence of inflammatory complications after the tooth extraction [6]. Dental surgeons rarely take into account this conclusion. Therefore, the problem of developing pharmaceutical drugs that promote or prevent the reduction of the adhesive properties of microorganisms in the area of wound surfaces of extracted teeth is relevant. This was the focus of our previous research which showed the effectiveness of pectin-containing drugs in the prevention of alveolitis. [7].

AIM

Our aim was to identify the markers necessary to predict the probability of alveolitis in case of tooth extraction under the conditions of a clinical laboratory and within the workplace of a dental surgeon.

MATERIALS AND METHODS

Our aim was to identify the markers necessary to predict the probability of alveolitis in case of tooth extraction under the conditions of a clinical laboratory and within the workplace of a dental surgeon. 160 patients were under our supervision. Since men and women may undergo tooth extractions throughout their lives, we selected adult patients (from 18 to 75 years old) including 100 men and 60 women (Table 1). The effectiveness of classical methods was studied and compared according

to the treatment protocol and drug treatment regimens developed by us based on a range of laboratory and comprehensive clinical studies [8]. Patients in the main group and the comparison group were identical by age, gender, pathology, and the conducted treatment.

Patients who required outpatient tooth extraction were regarded as the inclusion criteria for the research. Depending on the treatment, all patients were divided into three groups, randomized by age, gender, and duration of the disease. Group I consisted of apparently healthy people with intact dentition and periodontium. The main group II included patients who underwent tooth extraction, they were divided into two subgroups: 1) Patients with scheduled tooth extraction. They had used "Pectodent", the hygienic and preventive product, for teeth brushing for 7 days before the tooth extraction, and had taken pectin orally for 7 days before the tooth extraction and had rinsed the oral cavity with 1% pectin solution for 7 days. After the extraction, a pectin-containing bandage was applied, the patients used the hygienic and preventive agent "Pectodent" for teeth brushing for 7 days and took pectin orally for 7 days; 2) Patients with urgent tooth extraction who had rinsed their oral cavity with 1% pectin solution before the surgery (immediately before the extraction). After the surgery a pectin-containing bandage was applied, the patients used the hygienic and preventive agent "Pectodent" for teeth brushing for 7 days after the teeth extraction and took pectin orally for 7 days after the teeth extraction. Comparison group III was divided into two subgroups: 1) Patients with scheduled tooth extraction who underwent a traditional tooth extraction. The patients' oral cavity was rinsed with 1:5000 Furacilin solution in the preoperative period. Professional hygiene and sanitary education were conducted; 2) Patients with urgent tooth extraction who underwent traditional tooth extraction. They had their oral cavity rinsed with 1:5000 Furacilin solution before the intervention.

The most recent clinical examinations were performed daily from the day when the patient consulted the doctor for the first time and on the 1st, 3rd, 7th, and 14th days after the extraction. All patients who required tooth extraction underwent target X-ray examinations.

METHODS OF CLINICAL EXAMINATION OF PATIENTS WITHIN THE WORKPLACE OF A DENTAL SURGEON

The patients in Groups II and III were given the following general recommendations after the tooth extraction: not to rinse the oral cavity after the tooth extraction, not to smoke, not to drink alcohol, to avoid heavy physical work, to avoid hot liquids and solid food. They were recommended to brush the teeth being careful in the area of the postoperative wound.

DETERMINATION OF THE HYGIENIC STATE OF THE ORAL CAVITY

The hygienic state of the oral cavity was determined by detecting dental plaque using oral hygiene index (OHI) according to Yu.A. Fedorov and V.V. Volodkina, taking into account the convenience of its use. The labial surface of the lower six front teeth was stained with Lugol's solution and the degree of teeth staining was assessed: 5 points – staining of the entire teeth surface; 4 points – staining of ¾ of the tooth surface; 3 points – staining of ½ of the tooth surface; 2 points – staining of ¼ of the tooth surface; 1 point – no staining. The calculation was conducted according to the formula: OHI= U/6, where U is the sum of the indices. Oral hygiene index was assessed in the following way: 1.1-1.5 points – OHI was good; 1.6-2.0 points – OHI was satisfactory; 2.1-2.5 points – OHI was unsatisfactory; 2.6-3.4 points – OHI was poor; 3.5-5.0 points – OHI was very poor.

DETERMINATION OF THE INTENSITY AND PREVALENCE OF THE INFLAMMATORY PROCESS IN THE GUMS

The intensity and prevalence of the inflammatory process in the gums were determined using the Schiller-Pisarev test. The Schiller-Pysarev (S-P) test was performed based on a well-known fact: glycogen content increases during inflammatory processes in the gums, as evidenced by the intensity of gum staining (from light yellow to dark brown), which is directly proportional to the severity of the inflammatory process. A 5-point testing system from 0 to 4 was used for the convenience of statistical processing.

DETERMINATION OF INDICATORS OF CHANGES IN THE ORAL FLUID PH IN THE TEMPORAL ASPECT

The optimal measurement time was chosen from 10 to 12 a.m. Measurements were performed in fasting state or 2 hours after eating. The measurement of oral fluid pH was determined using a set of litmus paper "Universal Indicator Paper". The method determined the number of hydrogen ions in liquids. The standard paper from the set was immersed in the appropriate solution for 3 seconds, compared with the color scale and the value was calculated. Changes in the pH of oral fluid over time under the influence of various antiseptic

treatments, oral hygiene, and medical dressings were studied [8]. The essence of this method was to study the dynamics of changes in the oral fluid pH values in the patients under the influence of pectin-containing products before their use and on the 1st, 3rd, 7th, and 14th days after their use. The data from all studies were entered into maps and subjected to statistical processing with further analysis.

METHODS OF CYTOLOGICAL STUDIES OF THE MICROBIAL AND CELLULAR STATUS OF THE ORAL CAVITY CONDUCTED IN VIVO

Sampling for examination was conducted in the morning in fasting state before the teeth brushing and oral cavity rinsing. Plaque from the tongue and cheeks was removed with a dental spatula and material from periodontal pockets was removed with a probe. It was applied in a thin layer to a degreased sterile glass and then fixed. The smear was dried at room temperature. Staining was performed according to the Pappenheim-Kryukov method. Reagents: 1. Ready-made May-Grünwald stain consisting of eosin-methylene blue in methyl alcohol. 2. Fresh solution of Romanovsky dye (1-2 drops of dye per 1 ml of distilled water). 3. Neutral distilled water. Combined staining was performed with May-Grünwald stain and Romanovsky dye providing a possibility to differentiate the components of the cells. Bacterioscopic examination of the smear was performed using a Primo-Star Zeiss Plan-Achromat microscope at a magnification of 1000 times. The bacterial and fungal microflora was described in the preparation, as well as its quantitative determination per high power field in different groups of patients before and after the treatment.

Laboratory methods in vivo were used to evaluate the data from bacterioscopic analysis of epithelial cells, microflora from the oral cavity, which was taken in the form of smears from areas of the tongue, cheek, periodontal pockets. Clinical laboratory tests during the treatment were performed at the first visit (before the start of the treatment), on the 7th and 14th days of the treatment process. If a decision to extract teeth was made, the data of the analysis of microflora taken from the cavity of the extracted tooth at the first visit (before the start of the treatment), on the 2nd and 7th days of the study were taken into account.

METHODS OF STATISTICAL ANALYSIS OF THE RESEARCH RESULTS

Statistical processing of the obtained clinical and laboratory studies was conducted using a personal computer based on the Microsoft Excel spreadsheet software application for working with electronic tables. The material was processed by means of paired statistical methods, and the difference method with the use of Student's t-test was also applied using the "Statistika 7.0" package. All results were presented in the form M+m, where M was the average value of the indicator, m was the standard error of the mean. Results were considered reliable at $p \leq 0.05$.

RESULTS

DETERMINATION OF THE HYGIENIC STATE OF THE ORAL CAVITY

According to the results of our research, OHI indicators, which were conducted for 14 days, showed that the average statistical value of OHI constituted (2.01±0.11) points the day after the extraction in Group II in case of scheduled tooth extraction corresponding to indicators of a satisfactory state of hygiene. This index was (2.65±0.14) points in the patients of the same group in case of an urgent tooth extraction indicating a poor state of oral hygiene. OHI constituted (1.49±0.07) points on the third day in case of scheduled extraction corresponding to good hygienic indicators. It should be noted that good OHI indices maintained at the level of (1.12 ± 0.05) points up to day 14. This period was sufficient for the cell to fully heal after the tooth extraction. OHI was (1.91±0.07) points in the patients of the same group on the third day in case of the urgent tooth extraction, that is, it was satisfactory. Our observations indicated good OHI indices with an average statistical result of (1.5±0.07) points on the 7th day. It remained (1.35±0.06) points on day 14. The data we obtained indicated not only the hygienic and preventive effect of the oral hygiene agent, but also the sufficient therapeutic and preventive effect of the hygiene agent.

OHI constituted (2.91±0.12) points the day after the extraction in Group III in case of scheduled tooth extraction corresponding to poor hygiene level. OHI indices deteriorated on the 3rd day and amounted to (2.24±0.13) points corresponding to unsatisfactory hygiene level. However, an average statistical value of OHI constituting (2.04±0.13) points could be observed on the 7th day corresponding to the state of the oral cavity with satisfactory hygiene. OHI remained at this level in this group of patients up to day 14. OHI constituted (3.15±0.22) points in case of urgent tooth extraction the day after the intervention (poor hygiene).

According to the results of our clinical studies, we may conclude that the best results were obtained in Group II in case of the scheduled tooth extraction. The patients in this group were prescribed the hygienic and

preventive agent "Pectodent" and rinsing with 1% solution of apple pectin both before and after the surgery for 7 days. In this group the doctor covered the wound surface with a bandage after the tooth extraction, the formation of which was due to apple pectin. The patient took pectin orally for 7 days at home. The symptoms of the inflammatory process disappeared more quickly in the patients in Group II compared to Group III when the treatment technology was applied in the way we had proposed.

We used apple pectin for our research which was sterilized using the standard ultraviolet irradiation method. The data obtained indicated that 15 minutes were sufficient for apple pectin to be sterile. The shelf life of sterile apple pectin in a kraft bag under the temperature balance conditions of +5-28°C according to the passport data and the manufacturer is 2 years.

DETERMINING THE INTENSITY AND PREVALENCE OF THE INFLAMMATORY PROCESS IN THE GUMS

The dynamics of changes in the S-P test indices signified the anti-inflammatory effect of the hygiene product. They changed significantly towards a decrease in the intensity of inflammation under the influence of the treatment process. Thus, the S-P test indices constituted (1.90±0.09) points in Group II the day after the surgery in case of scheduled tooth extraction, i.e. they decreased by 1.39 times. The indices amounted (1.33±0.06) points on the 3rd day, i.e. a decrease by 1.99 times occurred, the color of the gingival mucosa normalized, and its swelling decreased. (0.83±0.07) points were noted on the 7th day, which was equal to a 3.19-fold decrease in intensity. Minor manifestations of the inflammatory process could be observed in individual patients at the end of the observations on the 14th day of the research, so the average statistical indicator was equal to (0.5±0.05) points and decreased in total by 5.3 times. In the process of performing this scope of work, we obtained reliable data on the reduction in the intensity of the inflammatory process in Group II in case of scheduled tooth extraction, which significantly reduced the alveolitis development. In case of the urgent tooth extraction, the S-P test indices decreased by 1.23 times in Group II the day after the surgery and constituted (2.16±0.12) points. It was (1.78±0.11) points on the 3rd day, i.e. the intensity of the inflammatory process decreased by 1.78 times. It decreased by 1.97 times on the 7th day constituting (1.35±0.09) points and by 2.48 times amounting (1.07±0.04) points on the 14th day. It should be noted that the effectiveness of the influence of pectin-containing agents on the intensity of the inflammatory process decreased compared to the previous subgroup, where scheduled actions were taken in order to conduct surgical interventions with the use of these agents.

The average statistical indices of the S-P test in Group III decreased by 1.1 times the day after the scheduled tooth extraction and amounted to (2.48±0.15) points in digital equivalent. They constituted (2.33±0.14) points on the 3rd day (decreased by 1.18 times), (2.13±0.14) points on the 7th day (decreased by 1.29 times), (2.08±0.14) points on the 14th day (decreased by 1.32 times). A significant slowdown in the disappearance of inflammation signs could be noted in Group III compared to Group II in case of the scheduled tooth extraction increasing the risk of alveolitis.

In case of the urgent tooth extraction in Group III, the S-P test constituted (2.51±0.15) points a day after the extraction (decreased by 1.02 times), (2.36±0.13) points on the 3rd day (decreased by 1.09 times), (2.24±0.11) points on the 7th (decreased by 1.15 times), (2.14±0.10) points on the 14th day (decreased by 1.2 times). Thus, the dynamics of the inflammatory process intensity reduction over 14 days occurred slowly.

DETERMINATION OF INDICATORS OF CHANGES IN THE ORAL FLUID PH IN THE TEMPORAL ASPECT

According to the results of the oral fluid pH determination in the patients under the influence of pectin-containing agents before and on the 1st, 3rd, 5th, and 7th days after their use in the comprehensive treatment of tooth extraction, the changes in the concentration of hydrogen ions in saliva were as follows: pH was slightly alkaline and constituted 7.38-7.42 during all days of observation in the patients of Group I, i.e. apparently healthy individuals. The pH value was 6.2-6.4 before the treatment in Group II in case of the scheduled tooth extraction. Oral fluid pH was 7.38 - 7.40 on the 3rd day after the tooth extraction and after the preventive measures with the use of our developed products were taken. In case of the urgent tooth extraction, the oral fluid pH constituted 5.9-6.1 before the treatment. Digital observation data obtained on the 3rd, 5th, and 7th days after the treatment were 6.8-7.0 pH, which corresponded to an environment with pH close to neutral. This fact was of great importance for preventing the alveolitis occurrence under modern working conditions of a dental surgeon. The pH was 5.8-6.2 and 5.5-5.9 at the 1st observation before the treatment in the patients of Group III in case of the scheduled and urgent tooth extraction, respectively. pH was 6.2-6.5 in case of the scheduled extraction and within 5.5-5.9 in case of the urgent one on the 3rd, 5th, and 7th day after the inter-

vention. Oral fluid in the patients of Group III remained within acidic parameters, slightly approaching the neutral values. This indicated a higher risk of complications in the form of alveolitis in the patients of this group. It should be noted that the oral fluid acquired neutral or slightly alkaline values under the influence of the treatment with the presence of pectin substances. This evidenced that physiological conditions were created under the action of the proposed complex of endo- and exogenous agents in order to prevent alveolitis. The oral fluid environment remained slightly acidic in case of pectin substances absence. This contributed to the deposition of microflora on the unprotected wound surface, since only alkaline environment was able preserve the biogeocenosis of the oral cavity.

METHODS OF CYTOLOGICAL STUDIES OF THE MICROBIAL AND CELLULAR STATUS OF THE ORAL CAVITY CONDUCTED IN VIVO

The bacterioscopic picture was similar before the intervention in both observation groups in case of scheduled and urgent teeth extractions. Clinically, dysbiosis was formed in the oral cavity of the patients with teeth to be extracted, during in vivo dental plaque microscopy. The dynamics changed significantly on the 3rd and 4th day after the extraction. A sharp decrease in microflora of all varieties was observed in Group III. The process of its reduction was gradual in Group II and reached its normalization on the 7th-10th day of the observation. Complications of alveolitis were unlikely in this group. When predicting the probability of alveolitis in Group III after the tooth extraction according to the indicators of in vivo analysis of oral cavity microflora, the main markers should be considered the following: 1. A sharp decrease on day 3-4 and sharp increase on day 7-10 in the number of *Candida* fungi within (2.5 +0.9-26.5+4.4 at (M+m) ($p<0.05$) and yeast-like fungi mycelium (4.5+0.9-22.2+7.7 (M+m) $p<0.05$), respectively. 2. A sharp decrease on day 3-4 and sharp increase on day 7-10 in the number of fusobacteria within (4.5+0.9-8.5+1.1) and Vincent's spirochetes (0.5+0.2-8.5+0.1) at (M+m) ($p<0.05$), respectively. 3. Absence of *L. acidophilus* on day 3-4, and the appearance of their insignificant number on day 7-10 (6.5+4.43-4 at (M+m) $p<0.05$) (Table 2).

Under the influence of treatment with pectin substances during outpatient teeth extractions, the odds ratio (OR) constituted 0.11 (95CI = 0.01-0.95%), indicating a decrease in complications by 9.07 times ($p<0.05$) in case of scheduled extractions, and 0.16 (95CI = 0.03-0.81%) in case of urgent extractions indicating a decrease in complications by 6.2 times ($p<0.05$).

DISCUSSION

Pectin is a natural polysaccharide derived from plant sources such as fruits and vegetables and is widely used in various fields, including pharmaceuticals and dentistry. Its biocompatibility, ability to form hydrogels, and properties for controlled drug release make it a promising candidate for the development of agents aimed at preventing and managing post-extraction complications, particularly alveolitis.

Alveolitis, or dry socket, is an inflammatory condition of the tooth socket following extraction and is among the most common postoperative complications. Since inflammation in the socket can lead to severe pain, infection, and delayed healing, effective management of this condition is a significant concern in clinical dentistry.

THE ROLE OF PECTIN IN POST-EXTRACTION HEALING

Pectin may contribute positively to alveolitis management due to its ability to form hydrogels that promote tissue regeneration. One of pectin's key benefits is its moisture-retention capacity, which helps maintain an optimal healing environment. Additionally, pectin exhibits antimicrobial properties, potentially reducing the risk of infection, which often accompanies dry socket.

When applied locally, pectin can:

1. **Form a protective barrier** over the extraction site, limiting bacterial infiltration and reducing the likelihood of infection.
2. **Provide antioxidant activity**, which helps minimize oxidative stress in tissues and supports overall healing.
3. **Improve local microcirculation**, thereby enhancing tissue regeneration in the socket area.

PECTIN IN THE FORM OF HYDROGELS OR IMPLANTED MATRICES

One of the most promising applications is the development of pectin-based hydrogels for local post-extraction use. These hydrogels can be employed as part of a wound dressing or applied directly into the socket, facilitating a favorable environment for tissue recovery. Furthermore, pectin hydrogels can be combined with other biologically active substances such as antibiotics, anti-inflammatory agents, or growth factors to enhance therapeutic efficacy.

POTENTIAL LIMITATIONS AND CHALLENGES

Despite numerous advantages, the use of pectin for alveolitis prevention still faces several limitations. Firstly, research into pectin's effectiveness in dentistry—particularly in alveolitis

Table 2. Dynamics of quantitative indicators of in vivo microflora from the oral cavity of the patients during tooth extraction under the influence of treatment technologies with a predominance of pectins in Group II and according to treatment protocols in Group III

Groups	Oral cavity microflora (M±m) p*<0.05				
	Research area	Microflora	First visit The number per high power field (HPF)	Day 3 The number per high power field (HPF)	Day 7 The number per high power field (HPF)
Group III	Gums Cheek Tongue	Cocci bacteria **	Densely per high power field	Moderately per high power field	Moderately per high power field
Group II			Densely per high power field	Moderately per high power field	Moderately per high power field
Group III		*L. acidophilus*	-	-	6.5 ±4.4
Group II			-	3.1±2.7	37.1 ±5.7
Group III		*Candida fungi*	Densely per high power field	22.5 ±0.9	26.5 ±4.4
Group II			Densely per high power field	22.5 ±6.9	12.1 ±5.7
Group III		Yeast-like fungi mycelium	Densely per high power field	34.5 ±0.9	22.2 ±7.7
Group II			Densely per high power field	37.5±6.8	18.1±5.3
Group III		Vincent's fuso-bacteria	Densely per ¼ high power field	4.5±0.9	8.5±1.1
Group II			Per 1/4 high power field	0.5±0.2	0.5±0.2
Group III		Vincent's spirochetes	Densely per ¼ high power field	0.5 +0.2	8.5 +0.1
Group II			Densely per ¼ high power field	2.8 +0.6	0.5 +0.2
Group III		Dental ameba	0.5 +0.1	-	-
Group II			0.5 +0.2	-	-
Group III		Oral Trichomonas	8.5 +3.1	4.5±0.2	0.5±0.2
Group II			8.5 +2.2	-	-
Group III		Candida fungi	Densely per high power field	26.5±5.4	10.2 +3.7
Group II			Densely per high power field	-	-
Group III		Candida fungi	Densely per high power field	22.5 +6.9	35.5 +4.7
Group II			Densely per high power field	7.1±0.7	-

Note. * p – the probability of difference in indicators compared to the values obtained on the 7th day of treatment; **identification of coccal microflora was not conducted in this research; ***p – the probability of difference in indicators compared to the values obtained before the treatment
Source: compiled by the authors of this study

prevention—is still in the early stages and requires further clinical trials. Secondly, the efficacy of pectin-based formulations may vary depending on the type and concentration of pectin used, as well as the formulation format (gel, powder, solution).

Another challenge is that pectin may not completely replace traditional methods of alveolitis treatment, such as surgical debridement or antibiotic therapy. Therefore, its use should be viewed as a complementary approach rather than a full substitute for standard care.

FUTURE RESEARCH PERSPECTIVES

Future studies should focus on developing more effective pectin formulations for dental applications, such as

biodegradable pectin membranes or microencapsulated systems for controlled drug delivery. Additionally, it is important to investigate the interaction of pectin with other therapeutic agents to develop combined strategies aimed at reducing the risk of alveolitis and accelerating post-extraction healing.

The results confirmed that the development of alveolitis after tooth extraction is a multifactorial process, in which both clinical factors and laboratory indicators play a leading role. It was found that the presence of traumatic extraction, concomitant systemic diseases (diabetes, blood diseases), as well as smoking significantly increases the risk of developing alveolitis in patients. This coincides with the data of other researchers, who indicate the significance of local and general risk factors [9].

Of the laboratory indicators in our study, an important predictor was elevated levels of leukocytes and ESR, indicating the presence of a systemic inflammatory reaction. Similar results are described in the works of foreign authors, who emphasize the diagnostic value of markers of systemic inflammation for predicting postoperative complications [10]. It was also confirmed that a decrease in local protective factors (the level of secretory IgA in oral fluid) correlates with a higher frequency of alveolitis, which is consistent with the results of studies in recent years [11].

Particular attention should be paid to the comparison of the obtained clinical and laboratory markers with the results of the assessment of the state of the clot in the socket. The presence of its partial or complete lysis in the early postoperative period turned out to be the most reliable local criterion, which coincides with the conclusions of the authors [12].

Thus, the results of our study confirm the feasibility of a comprehensive approach to predicting the risk of alveolitis, which should include the assessment of clinical risk factors, analysis of laboratory indicators of inflammation and the state of local immunity. Further studies with a larger sample will allow us to refine prognostic models and develop personalized strategies for preventing complications after tooth extraction [13].

CONCLUSIONS

1. The inclusion of pectin-containing medication in the complex of preventive measures is fully justified in relation to the occurrence of inflammatory complications after tooth extractions in modern conditions of a doctor's work at an outpatient clinic.
2. The wound surface of the socket of the extracted tooth should be covered with a bandage.
3. Under the conditions of a clinical laboratory and within a workplace of a dental surgeon, the markers necessary to predict the probability of alveolitis in case of a tooth extraction are the following: clinical indicators of normalization of the oral cavity hygienic state, a significant decrease in the intensity and prevalence of the inflammatory process in the gums, changes in the oral fluid pH towards the alkaline side, results of geobiocinosis normalization obtained by cytological studies of the microbial and cellular picture of the oral cavity conducted in vivo.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Prognostic significance of interleukin-6 in patients with acute ischemic stroke undergoing thrombectomy

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ABSTRACT

Aim: To evaluate the prognostic significance of plasma interleukin-6 levels as a predictor of functional outcomes and motor recovery in the acute phase of ischemic stroke.

Materials and Methods: In 2023, 70 patients with acute cerebral vessel occlusion undergoing mechanical thrombectomy were examined. The cohort included 44 (63%) men and 26 (37%) women, aged 38 to 80 years (65.3±9.4). Stroke severity was assessed using the NIHSS (8–23 points), and functional status at discharge was evaluated using the mRS (mRS 0–3: favorable outcome, mRS 4–6: unfavorable outcome). Plasma interleukin-6 concentrations were measured by enzyme-linked immunosorbent assay on days 1 and 7 of the disease.

Results: A statistically significant correlation was found between interleukin-6 levels and the severity of neurological deficits in patients with NIHSS ≤15 and >15 points during the acute phase of ischemic stroke. Interleukin-6 levels were 8.5±0.7 vs. 13.1±0.9 pg/mL on day 1 and 9.8±1.2 vs. 26.4±3.2 pg/mL on day 7 ($p<0.01$). Patients with a negative functional outcome had higher interleukin-6 levels both at admission (9.0±0.7 vs. 13.6±1.0 pg/mL) and over time (9.2±0.6 vs. 31.3±3.6 pg/mL; $p<0.01$). Additionally, higher interleukin-6 levels were observed in patients with hemorrhagic transformation after thrombectomy compared to those without transformation: 15.0±2.1 vs. 27.5±4.4 pg/mL on day 7 ($p<0.01$).

Conclusions: Plasma interleukin-6 levels correlate with the severity of neurological deficits, functional status, and the presence of hemorrhagic transformation, making it a prognostic biomarker for the course and outcome of acute ischemic stroke.

KEY WORDS: interleukin-6, thrombectomy, ischemic stroke, biomarkers, large vessel occlusion, intraoperative neuromonitorin

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INTRODUCTION

Acute ischemic stroke (AIS) is one of the leading causes of mortality and disability. Its occurrence is often due to insufficient cerebral blood supply caused by stenosis or occlusion of the large vessels in the anterior or posterior circulations [1]. Recent advancements in endovascular thrombectomy (TE) have significantly improved reperfusion rates in patients with AIS caused by large vessel occlusion [2]. However, data from five key TE studies indicate that over 50% of patients experience severe impairments, including loss of cognitive, speech, and motor functions, even after successful recanalization of occluded vessels [3]. This outcome may be linked to unsuccessful recanalization and reperfusion injury caused by an inflammatory response in which interleukin-6 (IL-6) — a multifunctional cytokine of immune cells — plays a key role. Elevated serum IL-6 levels are associated with larger infarct volumes and worse clinical outcomes in stroke patients [4].

In recent years, significant attention in the pathogenesis of ischemic stroke has been focused on pathobiochemical changes that play a key role in the processes of early and delayed brain tissue damage [5]. Numerous studies emphasize the critical role of inflammation in stroke pathophysiology, driven by the release of various cytokines and acute-phase proteins. However, the precise function of these cytokines, their impact during the acute and late phases, and their association with treatment outcomes remain subjects of ongoing debate [6]. Since inflammation contributes to edema formation and may trigger apoptosis, inflammatory biomarkers are promising indicators of prognosis and potential targets for neuroprotective therapy [7].

Ischemic damage triggers an inflammatory response in the brain, accompanied by the activation of various cytokines, including IL-6, which participates in numerous inflammatory processes. Experimental models of focal cerebral ischemia demonstrate a

rapid increase in interleukin-6 mRNA levels and its biological activity. However, the pro-inflammatory role of IL-6 in the central nervous system following experimental ischemia remains not fully understood [8]. The relationship between the levels of peripheral inflammatory markers and treatment outcomes in stroke patients is also insufficiently studied. In cases of AIS, some studies indicate a positive correlation between blood IL-6 concentration, infarct volume, stroke severity, or outcomes within six months [9], while others found no such association between serum IL-6 levels and infarct volume or stroke severity after three months [10].

AIM

The aim of our study was to measure the levels of the pro-inflammatory cytokine IL-6 in AIS patients upon admission and during the dynamic acute phase of the stroke, as well as to evaluate its relationship with stroke severity, the development of hemorrhagic transformation, and clinical outcomes.

MATERIALS AND METHODS

A total of 70 patients aged 18 and older with acute ischemic stroke were included in the prospective investigation. The stroke was first-time onset (confirmed by CT/MRI with symptom onset within the previous 24 hours), and mechanical thrombectomy was performed. Patients were included in the study group based on inclusion criteria. Among them, 44 (63%) were men and 26 (37%) were women, with ages ranging from 38 to 80 years (65.3 ± 9.4 years).

Exclusion criteria included: patients with hemorrhagic stroke confirmed by CT/MRI; transient ischemic attack (symptom regression within 24 hours); fever at onset or within a week prior to stroke; rheumatologic disease; autoimmune disease or any acute or chronic infection in history; immunosuppressive therapy (corticosteroids); severe renal or liver dysfunction. A detailed medical history and neurological examination were conducted. The severity of stroke at admission was assessed using the National Institutes of Health Stroke Scale (NIHSS), and patients were divided into groups with moderate (NIHSS ≤15) and severe (NIHSS >15) neurological deficits, comprising 30 and 40 patients, respectively. Functional capacity at discharge was evaluated using the modified Rankin Scale (mRS). Outcomes were classified as favorable (mRS group 0–3) or unfavorable (mRS group 4–6).

The quantitative determination of IL-6 concentration was performed using the enzyme-linked immunosorbent assay (ELISA) method with the DIA-IL-6 kit (DiaProph-Med, Ukraine). Blood samples for serum analysis were collected within the first 24 hours after hospitalization and again on days 7–10 of treatment. Five milliliters of venous blood were drawn into a sterile container and kept at room temperature for 45–60 minutes before centrifugation at 1400 rpm for 15 minutes. Serum was separated and stored at −20°C until further analysis. All samples were thawed only once for laboratory testing.

Symptomatic intracranial hemorrhage (sICH) was defined as any extravascular blood accumulation in the brain parenchyma or subarachnoid space accompanied by clinical deterioration, characterized by an increase of at least 4 points on the NIHSS scale. Hemorrhagic transformation was observed in 24 patients (34.3%) and classified according to the European Cooperative Acute Stroke Study II (ECASS II) scale as HI1 in 10 patients, HI2 in 4, PH1 in 4, and PH2 in 6 patients. Eight patients exhibited asymptomatic hemorrhagic transformation, while sICH was observed in 16 patients. Among the 6 deceased patients, 2 died due to malignant brain infarction, 3 from sICH, and 1 from myocardial infarction.

Statistical calculations were performed using Statistica 7.0 for Windows software (StatSoft Inc., USA). Data were analyzed using arithmetic mean (M) and standard deviation (SD), and the Student's t-test was applied to assess statistical significance between the compared groups. The coefficient of determination (R^2) was used to evaluate the quality of the regression model. Results were considered significant at $p<0.05$.

RESULTS

The serum IL-6 level within the first 24 hours after hospital admission in the group of patients with moderate neurological deficit (NIHSS ≤15) was significantly lower compared to patients with severe neurological deficit (NIHSS >15 at the admission), measuring 8.5 ± 0.7 pg/mL versus 13.1 ± 0.9 pg/mL, respectively ($p < 0.01$).

On day 7, the serum IL-6 level in the NIHSS ≤15 group was 9.8 ± 1.2 pg/mL, while in the NIHSS > 15 group, it reached 26.4 ± 3.2 pg/mL ($p < 0.01$). The differences between the compared groups on day 1 and day 7 were statistically significant. Within-group comparison (based on the paired Student's t-test) showed no statistically significant increase in IL-6 levels in the group with moderate neurological deficit ($p > 0.05$). However, in patients with severe neurological deficit, the IL-6 level significantly increased by day 7 to 26.4 ± 3.2 pg/mL ($p < 0.01$) (Fig. 1).

In the group of patients with a favorable functional outcome at discharge (mRS 0–3), the serum IL-6

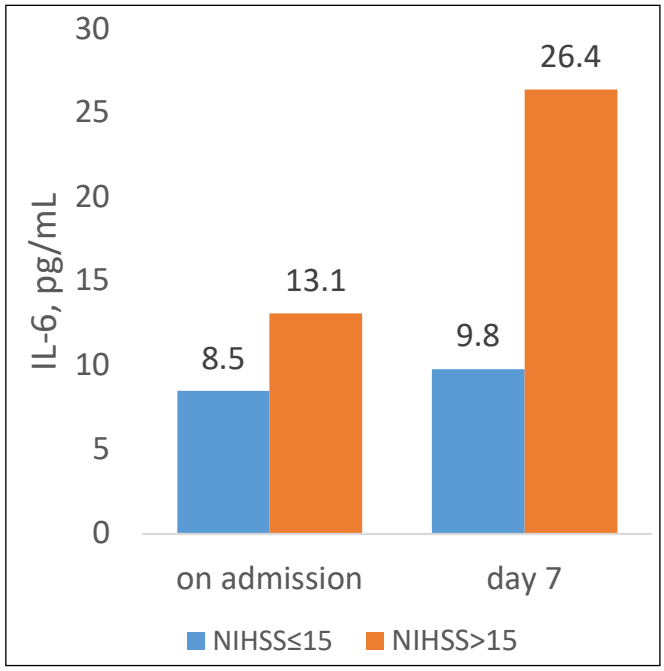


Fig. 1. Correlation between the severity of neurological deficit and IL-6 blood levels
Picture taken by the authors

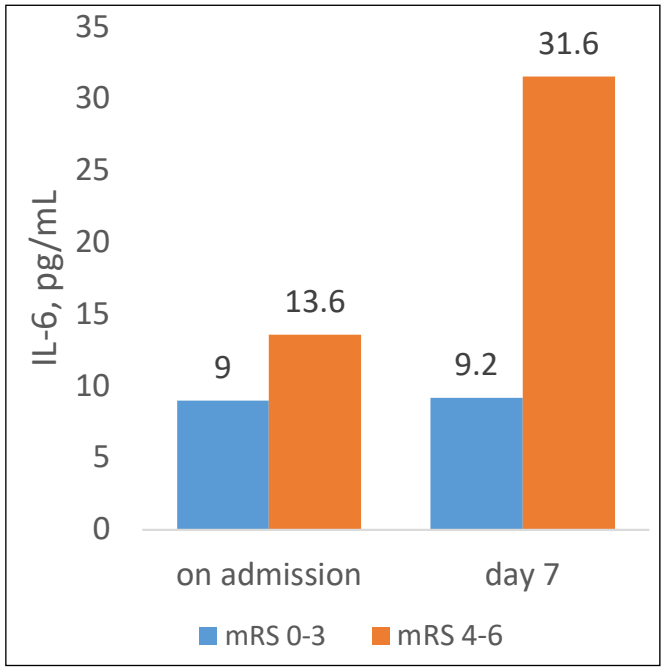


Fig. 2. Correlation between functional independence and IL-6 blood levels
Picture taken by the authors

level within the first 24 hours was significantly lower compared to patients with an unfavorable functional outcome (mRS 4–6), measuring 9.0 ± 0.7 pg/mL versus 13.6 ± 1.0 pg/mL, respectively ($p < 0.01$). A similar trend was observed dynamically: on day 7, IL-6 levels were 9.2 ± 0.6 pg/mL in the favorable outcome group

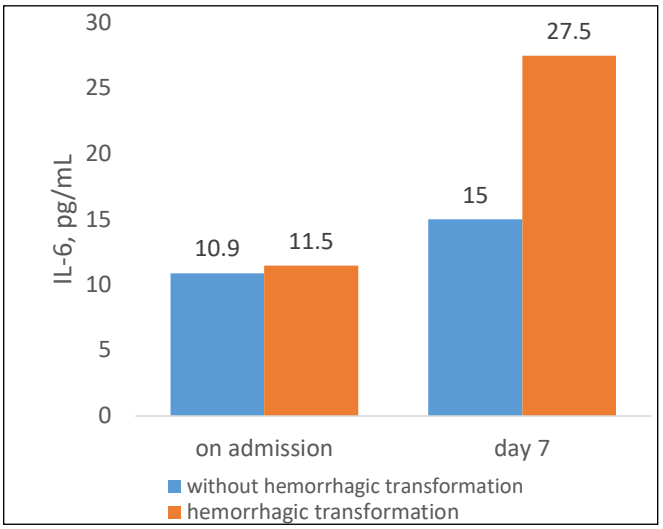


Fig. 3. Correlation between the presence of hemorrhagic transformation and IL-6 blood levels
Picture taken by the authors

versus 31.3 ± 3.6 pg/mL in the unfavorable outcome group ($p < 0.01$). Within-group analysis showed that IL-6 levels significantly increased by day 7 in the group with unfavorable functional outcomes, while no significant changes were observed in the group with favorable outcomes (Fig. 2).

Within the first 24 hours, no significant difference in serum IL-6 levels was observed between patients with hemorrhagic transformation and those without it (11.5 ± 1.0 pg/mL and 10.9 ± 0.8 pg/mL, respectively, $p > 0.05$). However, by day 7, a statistically significant difference was noted between the group with hemorrhagic transformation and the group without it after thrombectomy—15.0 ± 2.1 pg/mL versus 27.5 ± 4.4 pg/mL, respectively ($p < 0.01$). Within-group analysis showed that IL-6 levels increased by day 7 in both groups, but patients with hemorrhagic transformation exhibited a more pronounced rise (Fig. 3).

A weak correlation was identified between IL-6 levels and stroke severity, as assessed by the NIHSS scale. Patients with higher NIHSS scores tended to have elevated IL-6 levels, indicating a greater degree of neuronal damage associated with increased stroke severity. The coefficient of determination (NIHSS = 9,12 + 0,49 × IL-6, R = 0,42, $R^2 = 0,18$, $p < 0,01$) was 0.18, suggesting that 18% of the variation in stroke severity could be attributed to destructive processes accompanied by the release of IL-6 beyond neural cells. This indicates that while IL-6 is not the sole marker, it remains an important indicator of neural tissue damage that correlates with stroke severity (Fig. 4).

A weak correlation was also observed between IL-6 levels and the functional outcomes of patients at discharge, as assessed by the mRS scale. Patients with

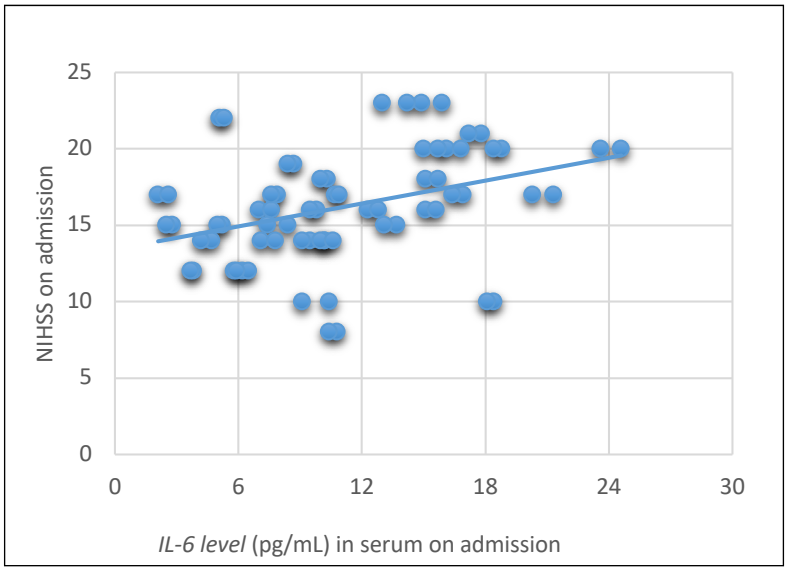


Fig. 4. Scatterplot of the correlation between IL-6 levels on the first day and NIHSS score
Picture taken by the authors

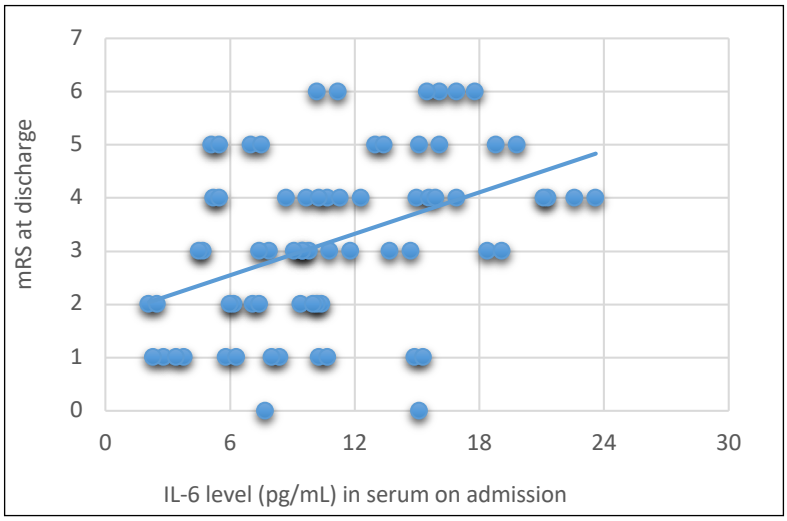


Fig. 5. Scatterplot of the correlation between IL-6 levels on the first day and mRS score
Picture taken by the authors

higher IL-6 levels generally had higher mRS scores, indicating poorer functional outcomes. The coefficient of determination for this correlation (mRS = 2,14 + 0,32 × IL-6, R = 0,46, R^2 = 0,21, p < 0,01) suggests that only 21% of the variation in functional outcomes can be explained by processes leading to increased IL-6 levels. This further highlights the limited role of IL-6 in predicting long-term functional recovery after a stroke (Fig. 5).

DISCUSSION

It has been established that the immuno-inflammatory response plays a key role in the pathogenesis of stroke, influencing its onset, progression, and prognosis. During immunological stress and inflammation, endothelial cells release numerous inflammatory factors, increasing endothelial cell adhesion and promoting the attachment and infiltration of inflammatory cells. This process, in turn, leads to the accumulation of lipids and foam cells, contributing to arterial occlusion and disruption of the blood-brain barrier (BBB), which negatively impacts clinical outcomes [7]. Elevated serum IL-6 levels are associated with more severe neurological deficits and are considered an unfavorable prognostic factor in AIS. IL-6 correlates with radiological indicators such as the mean lesion volume on DWI at admission, perfusion deficit, and final infarct size [11]. Recent studies have identified elevated peripheral blood IL-6 levels as a potential biomarker of ineffective reperfusion in patients undergoing thrombectomy. Low IL-6 levels at baseline have been associated with the «first-pass effect,» defined as achieving complete or near-complete reperfusion after a single thrombectomy attempt, which is a prognostic factor for favorable outcomes in AIS patients [12]. Notably, preclinical studies suggest a dual role of IL-6, demonstrating its ability to promote angiogenesis after stroke while simultaneously reducing ischemic damage [11].

As established in our study, severe neurological deficits are associated with an increased risk of hemorrhagic transformation and poorer treatment outcomes. These

deficits are often accompanied by elevated levels of inflammatory markers at the onset of the disease, consistent with the findings of Gan Y. et al. [13]. It is believed that IL-6 levels are closely linked to the pathogenesis of stroke. Inflammation is now regarded as a promising therapeutic target for reducing stroke risk, as IL-6 is a key mediator of inflammation that promotes leukocyte aggregation and thrombosis, leading to atherosclerosis and, consequently, stroke. Understanding the relationship between IL-6 levels and stroke progression could facilitate the development of novel approaches for stroke prevention and treatment based on the anti-inflammatory properties of cytokines [14].

During reperfusion, inflammation plays a crucial role in exacerbating brain damage. Activated peripheral leukocytes migrate into brain tissue, contributing to BBB disruption, brain edema, and microcirculatory disturbances, ultimately leading to further damage to brain structures [15]. Neutrophils and macrophages are the primary sources of matrix metalloproteinases, which degrade the vascular basement membrane, intensify BBB disruption, provoke hemorrhagic transformation, and exacerbate brain edema [16]. Clinical studies show that serum IL-6 levels increase within the first 24 hours of acute ischemic stroke, a finding consistent with our data, and have a significant association with infarct size and mortality rates [17].

During the inflammatory response, IL-6 not only accelerates lipid deposition and foam cell formation but also stimulates the phosphorylation of vascular endothelial cadherin, leading to vascular damage. IL-6 further activates cytotoxic T-lymphocytes and stimulates antibody production by B-cells, amplifying inflammation [18]. These immune and inflammatory responses directly contribute to the formation and progression of atherosclerotic plaques. The inflammatory process in cerebral vessels is a key mechanism underlying stroke development, highlighting the correlation between IL-6 levels at different stages post-stroke and functional outcomes following thrombectomy [19].

CONCLUSIONS

1. Patients with moderate deficits (NIHSS ≤15 points) at admission demonstrated a statistically significantly lower level of IL-6 compared to patients with severe deficits (NIHSS >15 points). This difference persisted on the 7th day of the acute phase.
2. Patients with favorable functional outcomes (mRS 0–3) had lower IL-6 levels compared to those with unfavorable outcomes (mRS 4–6), with this trend also remaining evident on the 7th day.
3. A significant increase in IL-6 levels in patients with hemorrhagic transformation on the 7th day highlights the prognostic value of this marker as an indicator of complications.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

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CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Examine attitudes and knowledge of the harmful effects of waterpipe tobacco smoking among Thi-Qar university students

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ABSTRACT

Aim: To examines the knowledge, attitudes, and behaviors of nursing students in Iraq about WTS.

Materials and Methods: A cross-sectional study was conducted from February to May 2025 including 410 nursing students at Thi-Qar University, employing a validated English questionnaire. We employed SPSS v26 ($p<0.05$ significant) to analyze the data using chi-square testing.

Results: The prevalence of current WTS was 13.2% (n=54), predominantly male (92.6% of smokers). Smokers had significantly higher mean age (24.2 vs 21.5 years, $p<0.001$) and were more likely to be in later academic years (68.5% vs 36.3%, $p<0.001$). While 81.5% of smokers recognized nicotine content, 68.5% erroneously believed WTS was less addictive than cigarettes. Significant knowledge gaps existed regarding tar content (only 63.0% smokers acknowledged) and toxin filtration (40.7% believed water filters toxins). Attitudinally, smokers were more likely to view WTS as socially advantageous (51.9% believed it increased friendships vs 21.1%, $p<0.001$).

Conclusions: The significant prevalence of waterpipe tobacco smoking (WTS) among nursing students at Thi-Qar University, along with the associated misconceptions, constitutes a critical public health issue, undermining both their health and their effectiveness as patient role models. Our findings indicate the need for the prompt incorporation of a focused educational module into the nursing curriculum that specifically dispels WTS misconceptions and examines its social drivers.

KEY WORDS: waterpipe, nursing students, shisha

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INTRODUCTION

Shisha, or waterpipe tobacco smoking (WTS), is increasingly prevalent globally, particularly in Arab countries where it is socially endorsed and integrated into popular culture [1]. Since 2015, the global prevalence of young individuals who smoke WTS has increased by 40%. Recent meta-analyses reveal that the global rate of youth who smoke WTS is 12% in 2023, but it is much higher in Arab nations. Lebanon has the highest rate at 37.2%, followed by Jordan at 28.6% and Iraq at 26.4% [2].

WTS prevalence is significantly higher among university students in Arab nations compared to other countries. Egypt exhibits a rate of 46.7%, while Kuwait shows a rate of 46% [3]. In the United States, 30.5% of college students with a history of smoking and 8.4% of current smokers reported experiencing WTS [4]. The prevalence of WTS among university students in Saudi Arabia ranged from 36.4% to 36.3% [3]. A study conducted in Palestine, Jordan, and Turkey found that 31.8% of students engage in WTS. The prevalence of WTS was highest in Palestine, with 36.11% of the students engaging in this behavior [5]. A study conducted in Iraqi Kurdistan showed that 28% of university students engage in WTS, with a notable gender disparity: 49% male compared to 10% female [6].

According to previous studies, a significant number of university students believe WTS is less hazardous than cigarette smoking, leading to increased consumption [7–9]. Other studies show that university students often don't know enough about the health dangers of WTS, such lung cancer and respiratory disorders [10–12]. Previous studies showed that many university students also believe it's easy to quit WTS, yet a lot of them have trouble quitting so [8]. Social and cultural factors are very important in initiating and continuing WTS among students [8]. Many people think that WTS is a more social activity, which is a primary reason why it is so popular [9]. Individuals who used WTS in both Arab and Western societies, as well as college students and teenagers, had this perspective [7]. Additionally, numerous individuals believe that public health efforts that attempt to educate students about the harmful effects of WTS have not been enough [8]. A preliminary study shows that nurses lack sufficient knowledge regarding how WTS impacts health [11]. This might make it more difficult for them to carry out duties

Table 1. Socio-demographics of study participates

Characteristics	Waterpipe Smoker			P- value
	Yes (N = 54) n (%)	No (N = 356) n (%)	All (N = 410) n (%)	
Age (Mean + SD)	24.2 + 4.6	21.5 + 3.6	21.9+ 3.9	< 0.001
Age group				
18-19.9	3 (6.5)	110 (30.9)	113 (27.6)	< 0.001
20-24.9	32 (59.3)	201 (56.5)	233 (56.8)	
>25	19 (35.2)	45 (12.6)	64 (15.6)	
Gender				
Male	50 (92.6)	100 (28.1)	150 (36.6)	< 0.001
Female	4 (7.4)	256 (71.9)	260 (63.4)	
Year of study				
1st and 2nd	17 (31.5)	227 (63.8)	244 (59.5)	< 0.001
3rd and 4th	37 (68.5)	129 (36.3)	166 (40.5)	
Marital status				
Single	36 (66.7)	312 (87.6)	348 (84.9)	< 0.001
Married	13 (24.1)	39 (11.0)	52 (12.7)	
Divorced	3 (5.6)	3 (0.8)	6 (1.5)	
Widowed	2 (3.7)	2 (0.6)	4 (1.0)	
Pocket money spent monthly				
<100,000 IQ	18 (33.3)	220 (61.8)	238 (85.0)	< 0.001
100,000-300,000 IQ	19 (35.2)	76 (21.3)	95 (23.2)	
>300,000 IQ	17 (31.5)	60 (16.9)	77 (18.8)	
Place of living				
With family	41 (75.9)	330 (92.7)	371 (90.5)	< 0.001
Own household	9 (16.7)	10 (2.8)	19 (4.6)	
Student Housing	4 (7.4)	12 (3.4)	16 (3.9)	
Others (relative, friends, etc.)	0 (0.0)	4 (1.1)	4 (1.0)	
Residential area				
Rural	42 (77.8)	289 (81.2)	331 (80.7)	0.57
Urban	12 (22.2)	67 (18.8)	79 (19.3)	

Source: compiled by the authors of this study

and make health evaluations. As future healthcare practitioners, nursing students are required to be provided with appropriate knowledge regarding WTS so that they can educate peoples and peers successfully [11]. To overcome misconceptions regarding WTS, nurses are required to be more knowledgeable of them and request assistance that is related to their needs [7].

AIM

This study addresses significant deficiencies in existing research by examining three primary areas: (1) the extent of nursing students' knowledge of the health concerns associated with WTS, (2) individuals' perceptions of utilizing WTS, and (3) the social and cultural influences that may perpetuate its use despite awareness of its detrimental effects.

MATERIALS AND METHODS

A cross-sectional study was performed involving nursing students at the College of Nursing, Thi-Qar University. The College of Nursing is an institution of higher education situated in An-Nasiriyah, Iraq. The institution comprises four departments and has an enrollment of approximately 680 students. A total of 683 students were invited to participate. The final sample consisted of 410 students who completed the survey, yielding a response rate of 60%. Data analysis was performed on the responses from these 410 participants. The sample selection criteria

Table 2. Waterpipe smoking patterns of participants (n=54)

Characteristics [a]	n (%)
Frequency of waterpipe smoking: (During the past 30 days, how many times did you smoke waterpipe/shisha)	
Daily	27 (50.0)
Twice a week	6 (11.1)
Once per week	6 (11.1)
Biweekly	6 (11.1)
Once a month	7 (13.0)
Missing	2 (3.7)
With whom do you usually smoke shisha?	
With friends and/or family members	33 (61.1)
Alone	19 (35.2)
Missing	2 (3.7)
Duration of each waterpipe smoking session: (How long does the waterpipe/shisha smoking session last?)	
Less than 30 min	21 (38.9)
30–60 min	20 (37.0)
>1 hr	11 (20.4)
Missing	2 (3.7)
Place of waterpipe smoking: (Where do you usually smoke waterpipe/shisha?)	
Home	11 (20.4)
Friend's house	7 (13.0)
Relatives' house	1 (1.9)
Public place (cafés/restaurants, someone else's home)	35 (64.8)
When do you usually smoke waterpipe/shisha?	
While drinking tea/coffee	9 (16.7)
After eating	19 (35.2)
When feeling happy	4 (7.4)
When feeling angry or stressed	5 (9.3)
With friends at any time	16 (29.6)
Missing (no smoker)	1 (1.9)
Reason behind waterpipe smoking for the first time	
Curiosity	23 (42.6)
Seeking for pleasure	8 (14.8)
Forming relationships with colleagues	3 (5.6)
Becoming free from community constraints	3 (5.6)
Others	16 (29.6)
Missing (no smoker)	1 (1.9)

Source: compiled by the authors of this study

comprised students of both genders who consented to complete the questionnaire. A validated self-administered questionnaire in English was utilized for the pretest. The questionnaire was developed based on prior studies [8, 13, 14]. The questionnaire consists of four primary sections: demographic data, smoking behavior, attitudes, and knowledge. The survey comprises closed-ended questions. The attitude section comprised nine items measured on a three-point Likert scale (agree, neutral, disagree). The assessment of knowledge was conducted using ten items, with response options of agree, disagree, or don't know. All students at the college have been contacted and asked to participate in the study. The College of Nursing Ethical Committee approved the study. The study occurred between 12 February 2025 and 23 May 2025.

We used SPSS Version 26.0 (IBM Corporation, USA) to do the statistical analysis. Mean ± standard deviation was used to show continuous variables. We demonstrated variables as percentages and numbers. We used

chi-squared tests for categorical variables and Student t-tests for continuous variables to investigate at the relationship between demographics, knowledge, and attitudes about WTS. We used a 95% confidence interval and p-values.

RESULTS

This study included 410 participants, of whom 356 (86.83%) were non-smokers and 54 (13.17%) were current smokers. Remarkably, smokers were older than non-smokers (24.2 years vs. 21.2 years, P < 0.001). Most waterpipe smokers were aged between 20 and 24 years (59.3%). The majority of smokers (92.6%), compared with 28.1% of non-smokers, were males. More than half of the smokers (68.5%), compared with 36.3% of non-smokers, were in the third or fourth year of the study. Only 33.3% of smokers, compared to 61.8% of non-smokers, reported spending less than 100,000 Iraqi dinars per month (Table 1).

Based on a sample of 54 participants, Table 2 indicates the patterns of waterpipe smoking. Most smokers (50.0%) smoked it daily, and approximately 38.9% smoked it each time for less than 30 minutes. In addition, 29.6% of smokers regularly smoked a waterpipe with their friends. More than half (61.1%) smoked it in cafes and restaurants. Furthermore, 35.2 % smoked waterpipes after meals, and 42.6 % had smoked waterpipes for the first time out of curiosity.

When considering the relationship between the various knowledge items and WTS, nine of the ten items were found to be significantly related to smoking waterpipes (p<0.05) (Table3). Smokers were more likely to agree (81.0%) than non-smokers (60.0%) that WTS contains nicotine (p <0.001). Although 76.1% of non-smokers agreed that WTS is addictive, only 72.2% of smokers did the same (p < 0.001). Compared to non-smokers, smokers were more likely to believe WTS is less addictive (68.5% *vs.* 43.5%, p = 0.001). Smokers were more aware of the risks of cardiovascular disease and stroke associated with WTS than non-smokers (92.6% *vs.* 78.9%, p = 0.041). However, they believed incorrectly that water could filter toxins (40.7% *vs*. 20.5%, p = 0.003). Smokers were more likely than non-smokers to correctly identify WTS as containing carbon monoxide (57.4% *vs*. 43.5%, p<0.001) and tar (63.0% vs. 39.0%, p < 0.001). Compared to non-smokers, smokers were considerably more likely to believe that WTS reduced weight (53.7% *vs*. 43.3%, p = 0.003). Most smokers (74.1%) and non-smokers (75.0%) agreed that WTS may cause dental problems (p = 0.037). In terms of total knowledge score, smokers had a slightly higher mean score (0.58 ± 0.14) than non-smokers (0.51 ± 0.22) (p = 0.021).

Participants' attitudes were evaluated on nine items, of which six were found to be significantly related to smoking a waterpipe (Table 4). Only (29.6%) of the waterpipe smokers and (16.0%) of the non-smokers believed that WTS is part of our cultural heritage (p = 0.006). However, non-smokers (15.4%) were less likely than smokers (31.5%) to be convinced that people who smoke waterpipes look "cool" (p = 0.002). In addition, smokers (51.9%) were more likely than non-smokers (21.1%) to believe that people who smoke waterpipes have more friends (p = 0.000). In addition, smokers (48.1%) were more likely than non-smokers (21.1%) to believe that women felt more comfortable smoking waterpipes than cigarettes (p = 0.000). Approximately 44.4% of smokers and 16.0 % of non-smokers perceive that smoking waterpipe is less harmful than smoking cigarettes (p = 0.000). Furthermore, roughly half (53.7%) of smokers reported that smoking waterpipe helps to quit smoking cigarettes (p=0.000).

DISCUSSION

This study is the first examination of the knowledge, attitudes, and behaviors related to waterpipe tobacco smoking (WTS) among nursing students at the University of Thi-Qar in Iraq. The prevalence of waterpipe smoking observed in our sample was 13.1%. This figure is lower than those reported in Palestine (32.9%) by Nazzal et al. [15], in Saudi Arabia (34.0%) by Salih et al.[16] and in Syria (20.2%) by Al-Kubaisy et al. [17]. However, it is higher than the rate documented in Yemen (9.3%) by Nasser & Zhang [3] and is comparable to that found in Qatar (18.1%) by Al-Jayyousi et al.[18].

The current study found a significant relationship between age and WTS, with smokers being considerably older than non-smokers. This finding is consistent with the data gathered by Daradka et al. [10], and may be explained by prolonged exposure to peer dynamics and social influences throughout university life. A significant gender disparity was identified. This aligns with the findings of Khabour et al. [19] in Jordan and corroborates the findings of Hamadeh et al.[20], which indicated that males exhibit a greater tendency to attempt WTS. A study by Othman et al. at Sulaimaniyah University in Iraqi Kurdistan indicates a notable disparity in the prevalence of WTS, recorded at 28%, which was significantly greater among males (49%) than among females (10%) [6]. This suggests that cultural norms render WTS more socially acceptable for males. Furthermore, the prevalence of WTS increased among students in advanced academic years. This pattern is well-documented, as evidenced by research from Al-Rawi et al. and Daradka et al., which consistently shows the highest prevalence rates among senior-year students [10, 21].

Table 3. Associations between socio-demographics and WTS (N = 410)

Knowledge	Yes (N=54) n (%)	No (N=356) n (%)	p-value
Waterpipe contains nicotine			
Agree	44 (81.5)	215 (60.4)	0.000
Disagree	7 (13.0)	13 (3.7)	
Don't Know	3 (5.6)	128 (36.0)	
Waterpipe is addictive			
Agree	39 (72.2)	271 (76.1)	0.000
Disagree	15 (27.8)	28 (7.9)	
Don't Know	0 (0.0)	57 (16.0)	
Waterpipe is less addictive than cigarettes			
Agree	37 (68.5)	155 (43.5)	0.001
Disagree	12 (22.2)	96 (27.0)	
Don't Know	5 (9.3)	105 (29.5)	
Waterpipe smoking can cause lung cancer			
Agree	47 (87.0)	270 (75.8)	0.145
Disagree	3 (5.6)	23 (6.5)	
Don't Know	4 (7.4)	63 (17.7)	
Waterpipe smoking leads to cardiovascular diseases and cerebral stokes			
Agree	50 (92.6)	281 (78.9)	0.041
Disagree	2 (3.7)	16 (4.50	
Don't Know	2 (3.7)	59 (16.6)	
Waterpipe contains carbon monoxide			
Agree	31 (57.4)	155 (43.5)	0.000
Disagree	10 (18.5)	19 (5.3)	
Don't Know	13 (24.1)	182 (51.1)	
Waterpipe smoking leads to dental problems			
Agree	40 (74.1)	267 (75.0)	0.037
Disagree	6 (11.10	13 (3.7)	
Don't Know	8 (14.8)	76 (21.3)	
Waterpipe smoking reduces weight			
Agree	29 (53.7)	154 (43.3)	0.003
Disagree	14 (25.9)	49 (13.8)	
Don't Know	11 (20.4)	153 (43.0)	
Water in waterpipe filters toxins			
Agree	22 (40.7)	73 (20.5)	0.003
Disagree	15 (27.8)	106 (29.8)	
Don't Know	17 (31.5)	177 (49.7)	
Waterpipe contains tar			
Agree	34 (63.0)	141 (39.6)	0.000
Disagree	7 (13.0)	20 (5.6)	
Don't Know	13 (24.1)	195 (54.8)	
Total knowledge (mean+sd)	**0.58 + 0.14**	**0.51 + 0.22**	**0.021**

Source: compiled by the authors of this study

Table 4. Associations between Attitudes and WTS (N = 410)

Waterpipe Smoker			
Attitude	**Yes (N=54) n (%)**	**No (N=356) n (%)**	***p-value***
Having parents who smoke waterpipe make it more likely to smoke waterpipe/shisha			
Agree	39 (72.2)	248 (69.7)	0.330
Neutral	12 (22.2)	65 (18.3)	
Disagree	3 (5.6)	43 (12.1)	
Having friends who smoke waterpipe make it more likely to smoke waterpipe/shisha			
Agree	45 (83.3)	257 (72.2)	0.219
Neutral	5 (9.3)	59 (16.6)	
Disagree	4 (7.4)	40 (11.2)	
Advertisement can encourage waterpipe smoking			
Agree	38 (70.4)	225 (63.2)	0.558
Neutral	8 (14.8)	59 (16.6)	
Disagree	8 (14.8)	72 (20.2)	
Waterpipe smoking is part of our cultural heritage			
Agree	16 (29.6)	57 (16.0)	0.006
Neutral	22 (40.7)	117 (32.9)	
Disagree	16 (29.6)	182 (51.1)	
People smoking waterpipe look cool			
Agree	17 (31.5)	55 (15.4)	0.002
Neutral	21 (38.9)	117 (32.9)	
Disagree	16 (29.6)	184 (51.7)	
People smoking waterpipe have more friends			
Agree	28 (51.9)	75 (21.1)	0.000
Neutral	9 (16.7)	133 (37.4)	
Disagree	17 (31.5)	148 (41.6)	
Girls are more comfortable in smoking waterpipe compared to cigarettes			
Agree	26 (48.1)	72 (20.2)	0.000
Neutral	10 (18.5)	94 (26.4)	
Disagree	18 (33.3)	190 (53.4)	
Smoking waterpipe is less harmful than smoking cigarettes			
Agree	24 (44.4)	72 (20.2%)	0.000
Neutral	12 (22.2)	129 (36.2%)	
Disagree	18 (33.3)	155 (43.5%)	
Smoking waterpipe helps to quit smoking cigarettes			
Agree	29 (53.7)	102 (28.7%)	0.000
Neutral	17 (31.5)	132 (37.1%)	
Disagree	8 (14.8)	122 (34.3%)	

Source: compiled by the authors of this study

Regarding knowledge of WTS, most smokers in our study correctly believed that waterpipes contain nicotine, a finding similar to that reported among dental school students in the United Arab Emirates by Al-Rawi et al. [21]. However, the risk of developing a habit was elevated despite this knowledge. Although a significant proportion of smokers believed that WTS is addictive, they also perceived it as less addictive than cigarettes, a misconception that aligns with the findings of earlier studies by Al-Jayyousi et al. [18], Al-Rawi et al. [21], and others [22, 23]. The majority of smokers were aware of harmful effects such as cardiovascular disease and stroke; only 7.2% reported not knowing about these complications, a level of awareness consistent with

previous research by Al-Jayyousi et al. [18]. A significant number of smokers in this study were also aware that WTS causes dental problems. This finding contrasts with those of Al-Jayyousi et al. [18] and Jackson and Aveyard [22], which showed that smokers were often not aware of the dental health effects of WTS. Our study indicates that smokers hold the belief that WTS contributes to weight reduction, a notion supported by prior research conducted by Raptou and Papastefanou [24] and Stadler et al. [25]. This assumption often relies on the premise that smoking aids in weight loss by increasing metabolism, reducing appetite and caloric intake, and preventing fat storage, as nicotine affects the brain's control of hunger and energy expenditure [24,25]. However, it is essential to acknowledge the paradoxical effect: by impairing respiratory function, cigarette smoking can simultaneously reduce physical activity and potentially result in weight gain, as indicated in the same body of evidence [24, 25]. Smokers in our study were more likely to correctly identify WTS as containing tar and carbon monoxide. This finding supports the earlier research of Abu-Rmeileh et al. [13], who found that students perceived cigarettes as more addictive and containing more nicotine than waterpipes. However, smokers were often incorrect in believing that toxins are effectively filtered by the water, a misconception that was also documented in a previous study by Sutfin et al. [12], which showed participants wrongly believed water filters out all harmful toxins. Collectively, these findings highlight a critical requirement to provide nursing students with targeted health education to correct misconceptions about the health risks of WTS.

Regarding attitudes toward WTS, a small percentage of current smokers in our study viewed WTS as part of their cultural heritage. This finding contrasts with previous research, such as that by Nakkash et al. [26], which indicated that waterpipe smoking is culturally accepted in several Middle Eastern countries. Additionally, in contrast to findings from earlier studies, a minority of current smokers in our cohort perceived waterpipe smoking as 'cool' [6, 18]. The current study found that smokers perceived women to be more comfortable smoking waterpipes than cigarettes. This aligns with previous research, such as that by Al-Jayyousi et al. [18], which has shown that while cigarette smoking is often socially unacceptable for women in Arab nations, waterpipe smoking is quite common and acceptable for women in the Eastern Mediterranean region [18, 27]. Additionally, smokers in our study thought that WTS is less harmful to their health than cigarettes and could even help them quit smoking. This is a common misconception; studies by Akl et al. [28] and Ba et al. [29] have also found that smokers think WTS is less dangerous than cigarettes, and Sutfin et al. [12] found that this belief makes them more likely to smoke waterpipes. These results are in line with the general trend that smokers often don't see themselves as addicts and think they can quit whenever they want. These beliefs may be based on the false idea that water filtration removes toxins or that WTS is less addictive and easier to quit than cigarettes. Our study revealed that smokers perceived waterpipe smoking (WTS) as a popular activity among their peers. This outcome corresponds with the results of Akl et al. [28] and Ba et al. [29]. The study also indicated that a lot of smokers thought that WTS was a fun way to spend time with friends at places like coffee shops and restaurants. This view of WTS as a social activity has been seen in both Arab and Western cultures, especially among college students [28].

Regarding smoking patterns, half of the smokers in our study reported daily waterpipe use, and 38% stated that their typical smoking session lasted less than 30 minutes. These findings differ from those reported by Al-Jayyousi et al. [18], who found that 40% of smokers engaged in WTS only once monthly, and 45% had sessions lasting 30 to 60 minutes. The findings of this study reveal that most smokers preferred cafés for WTS, consistent with the results reported by Obeidat et al. [18]. The desire may create opportunities for social interaction with unfamiliar individuals. The increasing prevalence of waterpipe smoking correlates with greater accessibility of waterpipe products in public venues, including cafés and restaurants, as demonstrated by studies conducted by Al-Jayyousi et al. [18], Al-Rawi et al. [21], and Jradi et al. [30]. Moreover, the majority of participants indicated a preference for smoking waterpipes in social settings with friends and family, a behavior similarly noted among students in the research conducted by Ali and Meer [31]. The research demonstrated that smokers chose WTS after meals, consistent with the results of Al-Jayyousi et al. [16] and Poyrazoglu et al. [32], which suggested that students similarly preferred smoking post-meal.

CONCLUSIONS

The significant prevalence of waterpipe tobacco smoking (WTS) among nursing students at Thi-Qar University, along with the associated misconceptions, constitutes a critical public health issue, undermining both their health and their effectiveness as patient role models. Our findings indicate the need for the prompt incorporation of a focused educational module into the nursing curriculum that specifically dispels WTS misconceptions and examines its social drivers.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

REVIEW ARTICLE

CONTENTS

Vaccination in pregnancy: a systematic review of current evidence

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ABSTRACT

Aim: To summarize current recommendations and the state of knowledge on vaccination of pregnant women against influenza, pertussis, and Covid-19, and to highlight evidence on the efficacy and safety of vaccination during pregnancy.

Materials and Methods: A systematic literature review of studies published between 2014 and 2024 in the PubMed, Science Direct, Google Scholar, and NCBI databases was conducted. Of the total number of 31 studies found, 10 that met our required conditions were included. The inclusion criteria were peer-reviewed articles dealing with vaccination during pregnancy. Data selection and extraction were performed in accordance with PRISMA recommendations.

Conclusions: Vaccination of pregnant women appears to be a safe and effective way to protect mothers and their offspring. Emphasis should be placed on raising awareness and education in clinical practice.

KEY WORDS: pregnancy, vaccination, immunization, vaccine

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INTRODUCTION

Pregnancy is a specific period in a woman's life during which the body is more susceptible to infections that can pose a threat not only to the mother but also to the fetus. Vaccination of pregnant women is a preventive health measure that protects not only the mothers-to-be themselves, but also their unborn children from serious infectious diseases. Immunization during pregnancy reduces the risk of serious complications and also provides passive protection to newborns through transplacental transfer of maternal antibodies. Despite the scientifically proven benefits, there is still uncertainty among the general public about the safety of vaccination during pregnancy, which has a negative impact on vaccination rates.

According to international recommendations, vaccines are permitted during pregnancy in cases where their benefits outweigh the possible risks. Vaccinations during pregnancy are typically divided into routine vaccinations (recommended for all pregnant women), vaccinations indicated for health reasons, and vaccines that are contraindicated during pregnancy, which mainly include live attenuated vaccines [1].

Global health authorities such as the CDC, WHO, and ECDC (European Centre for Disease Prevention and Control) recommend influenza and dTap (diphtheria, tetanus, pertussis) vaccinations as safe, effective, and well tolerated during pregnancy [2,3].

AIM

To summarize current recommendations and the state of knowledge on vaccination of pregnant women against influenza, pertussis, and Covid-19, and to highlight evidence on the efficacy and safety of vaccination during pregnancy.

MATERIALS AND METHODS

A systematic literature review of studies published between 2014 and 2024 in the PubMed, Science Direct, Google Scholar, and NCBI databases was conducted. The paper also analyzes the available literature, recommendations from international health organizations (CDC, WHO, ECDC), as well as national legislative documents issued in Slovakia. Relevant results from epidemiological studies from the H1N1 influenza period (2009-2010) and the Covid-19 pandemic were also included. The keywords were compiled using the terms: "gravidita" and "tehotnosť" (pregnancy), "očkovanie" or

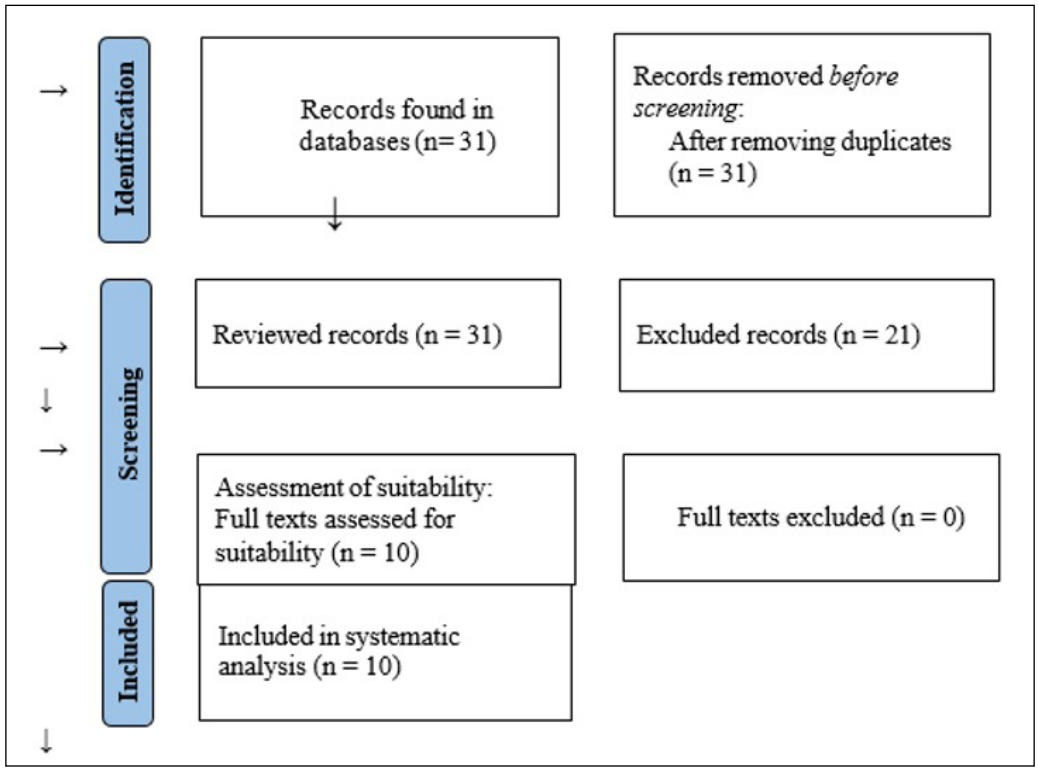


Fig. 1. PRISMA flow diagram
Picture taken by the authors

"vaccination" or "imunizácia" (immunization), and "vakcína" (vaccine). We filtered the studies regardless of their form or language of publication, with the condition that they were published between 2014 and 2024. In total, we found 31 studies, of which 10 met our criteria (Fig. 1 PRISMA flow diagram). Two independent reviewers evaluated the studies, and a third reviewer resolved any disagreements.

REVIEW AND DISCUSSION

An analysis of the studies found points to the safety of vaccination during pregnancy against three infectious diseases: whooping cough (Tdap), influenza, and COVID-19. No evidence of serious adverse effects or adverse pregnancy and perinatal outcomes was found in the vaccine comparison (Table 1).

Vaccination against whooping cough (Tdap) during pregnancy has been evaluated as safe in several studies, including cohort studies and meta-analyses of randomized clinical trials [4,5]. The results showed that administration of the Tdap vaccine in the second and third trimesters is not associated with an increased risk of preeclampsia, premature birth, or low birth weight. In addition, significantly higher levels of pertussis antibodies, which protect infants in the first months of life, were found in newborns of vaccinated mothers. The study by Gomme et al. (2022) confirmed that the highest levels of IgG in umbilical cord blood were achieved when vaccination was given before 31 weeks of gestation, with an optimal interval of at least 7.5 weeks before delivery.

Influenza vaccines have also demonstrated an excellent safety profile in several studies. A randomized controlled study [7] found no serious adverse events (SAEs) after vaccination (such as fetal death, severe preeclampsia, fetal damage), and most local or systemic reactions were mild. The findings of a meta-analysis by Nunes and Madhi (2018) are significant, showing that influenza vaccination of pregnant women reduces the risk of laboratory-confirmed disease in infants by 48% and hospitalizations by up to 72%. A cohort study by Regan et al. (2023) confirms that influenza vaccination does not increase the risk of miscarriage when administered before or during pregnancy.

Due to its recent emergence, vaccination against Covid-19 is still the subject of observational studies and systematic reviews. An analysis [10] involving more than 17 million pregnant women found no association between vaccination and adverse outcomes, regardless of vaccine type or trimester of administration. A cohort study [11] from 2022 showed that the rate of preterm birth was lower in vaccinated (5.5%) compared to unvaccinated (6.2%) pregnant women. Umbilical cord blood samples from mothers who had been vaccinated with mRNA vaccines against SARS-CoV-2 showed effective transplacental transfer of neutralizing antibodies [12], which were still detectable after 12 months.

Table 1. Vaccine safety during pregnancy

Type of vaccine	Side effects in the mother	Impact on pregnancy	Effects on the fetus/newborn	Conclusion on safety
Tdap (whooping cough)	- mild pain at the injection site - low subfebrile temperatures - no serious reactions	- does not increase the risk of preeclampsia, premature birth, miscarriage, or IUGR	- significantly higher IgG levels - no increased risk of SAE	Safe in the 2nd and 3rd trimesters
Influenza	- local reactions (pain, fatigue) - no fever - no systemic complications	- does not increase the risk of miscarriage or complications during pregnancy	- 48% lower incidence of influenza - 72% fewer hospitalizations	Safe throughout pregnancy
Covid-19 (mRNA and others)	Typical post- vaccination symptoms: fatigue, headache, fever (short-term) - Lower risk of severe Covid-19 in mothers	- does not increase the risk of premature birth, miscarriage, preeclampsia, or low birth weight	- effective transplacental transfer of antibodies - long-term protection (neutralizing antibodies ≥ 6–12 months)	Safe, ideally to be administered in the third trimester

Source: compiled by the authors of this study

The studies analyzed showed that vaccination of pregnant women does not pose a risk to either the mother or the child. On the contrary, vaccination has a prophylactic effect, with the mother's immune response providing passive immunity to the newborn and reducing the risk of infection during the first months of life.

In Slovakia, it is recommended to vaccinate with the dTap combination vaccine. This vaccination is recommended during the third trimester of pregnancy, preferably between weeks 28 and 38. It protects the mother from the disease itself and also transfers maternal antibodies to the fetus, which protects the newborn from the disease until it is vaccinated (Decree of the Ministry of Health of the Slovak Republic No. 585/2008 Coll.) [13]. Every pregnant woman should receive the Tdap vaccine regardless of her previous medical history during each pregnancy. Women who have not previously been vaccinated with Tdap should be vaccinated immediately after giving birth [14]. These recommendations are based on evidence of the high efficacy and safety of the vaccine and are consistent with the practice in more than 40 countries worldwide, where vaccination in the third trimester is standard [15].

In connection with influenza, it is important to recall the experience of the influenza A (H1N1) pandemic in 2009/2010, when it became apparent that pregnant women were one of the most at-risk groups. The WHO therefore included them among the priority groups for vaccination, along with children, seniors over 65, and the chronically ill. Statistics showed an increased risk of hospitalization, acute respiratory distress syndrome (ARDS), and mortality in unvaccinated pregnant women compared to those who were not pregnant. Based on these data, the CDC recommends flu vaccination in the fall months regardless of gestational age, including in the first trimester [2,13]. Furthermore, the results of studies [7,8] show that influenza vaccines are safe for both the mother and the fetus and reduce the risk of serious perinatal complications.

At the European level, there is some heterogeneity in the approach to vaccinating pregnant women (Table 2). Some countries allow vaccination in all trimesters, while others recommend vaccination only in the second or third trimester. These differences highlight the need to harmonize recommendations across EU member states. As stated by Aldoosari et al. (2023) [15], the implementation of clear national strategies and a unified communication line on the part of the state contribute to higher immunization rates.

Perceptions of vaccine safety are a key factor in pregnant women's decisions. Studies from 2021 and 2022 have shown that vaccination with the Tdap vaccine during pregnancy is not associated with an increased incidence of adverse effects (e.g., preeclampsia, partus praematurus, or intrauterine growth retardation). On the contrary, children of vaccinated mothers had significantly higher levels of specific antibodies, which supports the effectiveness of transplacental transfer [4, 5].

Similar conclusions can be drawn for vaccination against COVID-19, even though it is a relatively new disease, but available data from large observational and population studies suggest its safety. A meta-analysis [10] involving a large sample of pregnant women showed no increase in the incidence of miscarriage, premature birth, or intrauterine growth retardation. Similar results are confirmed by Dick et al. (2022) [11] in a large cohort study, confirming that vaccination does not lead to adverse obstetric complications. In addition, serological test results show effective transfer of neutralizing antibodies across the placenta, with levels detectable in newborns [12].

The question of why, despite positive evidence regarding the safety and efficacy of vaccines, their

uptake among pregnant women remains suboptimal in many countries is the subject of ongoing research. Woodcock et al. (2023) [16] discuss the statistics of rising and falling influenza vaccination rates in relation to age and ethnicity. Based on a retrospective cohort study, they found that uptake of the seasonal influenza vaccine among pregnant women increased during the decade preceding the Covid-19 pandemic, but remained suboptimal. Adeyanju et al. (2021) [17], in their study, identify the determinants of influenza vaccine hesitancy among pregnant women in Europe. The most commonly reported factors were psychological determinants, such as risk perception, concerns about vaccine safety, insufficient information, anti-vaccination attitudes, and low trust in the healthcare system.

Healthcare professionals play a crucial role in women's decision-making regarding vaccination. The study by Barrett et al. (2018) [18] emphasizes that midwives, gynecologists, and obstetricians are the most important sources of trustworthy information for pregnant women. Their active recommendation of vaccination significantly increases vaccine uptake. Kynčl et al. (2023) [19], in their study from the Czech Republic, note that the availability of the vaccine directly at the facility where a woman receives prenatal care, along with informed consent and reimbursement of the vaccine, are key factors in improving vaccination strategies. In conclusion, currently available evidence supports vaccination of pregnant women against Tdap, influenza, and COVID-19 as a safe and effective preventive measure that reduces morbidity and mortality not only in mothers but also in their newborns. However, to improve vaccination practices in clinical settings, systematic education of healthcare professionals, increased availability of vaccines in prenatal care, and trustworthy communication with pregnant women are essential.

CONCLUSIONS

Based on the available scientific evidence, it can be concluded that vaccination of pregnant women against pertussis (Tdap), influenza, and COVID-19 is safe, effective, and contributes to the protection of both mother and newborn. Studies confirm that administering these vaccines during pregnancy is not associated with an increased risk of adverse pregnancy or neonatal outcomes. On the contrary, it reduces the risk of infections during the early neonatal period.

The findings of this systematic review support the validity of recommendations made by both national and international health authorities. An important factor influencing vaccination uptake remains the awareness of pregnant women and the proactive approach of healthcare personnel. In line with this, we recommend strengthening the education and support of gynecologists in providing reliable and up-to-date information about vaccination during pregnancy.

The systematic review was conducted in accordance with the PRISMA 2020 methodological recommendations, which ensured the transparency, reproducibility, and informative value of the conclusions reached.\

STUDY LIMITATIONS

The systematic analysis faces several limitations. Only a small proportion of the total number of studies searched were included in the review, which may have affected the breadth and representativeness of the results. The included studies differed in design, sample size, and geographical and cultural context, which complicates their comparison. In the case of COVID- 19 vaccination, most of the available data are short-term, and therefore it is not possible to fully assess the long-term effects. We also did not include unpublished data, which may pose a potential risk of publication bias. Despite these limitations, the work provides a valuable overview of current knowledge on the safety of vaccination during pregnancy.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

REVIEW ARTICLE

CONTENTS

The phenomenon of accelerated volume growth and a critical analysis of the interpretation of the mechanism of its occurrence

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ABSTRACT

Aim: To reveal the peculiarities of the phenomenon of accelerated volume growth and to analyze the interpretation of the mechanism of its occurrence.

Materials and Methods: This phenomenon was observed in cases of impaired receptor or root of the auditory nerve, or some central acoustic formations. In case of impaired root, this event occurs in 50 per cent of the patients, and therefore the symptom cannot be regarded as a reliable indication of the cochlear or root lesion.

Conclusions: The phenomenon of accelerated increase in volume as one of the manifestations of irritation symptoms is also most pronounced in peripheral lesions. In tumours of the auditory nerve, the function is impaired, but the irritation is absent. The relatively high frequency of this symptom attendant on peripheral lesion is rooted in the fact that impaired reception manifests itself most conspicuously in the irritation of the auditory nerve, while the central nervous system is not affected, and the patients are able to detect the direction from whence the sound comes.

KEY WORDS: the phenomenon of accelerated increase in volume, auditory nerve, affected receptor, vestibular analyzer, topic-morphological theory

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INTRODUCTION

Sound volume is important in the study of hearing pathology. The recruitment phenomenon is used to differentiate between cochlear and retrocochlear hearing loss. Kok Wooi Teoh, Khurshid Merchant, Josephine Swee Kim Tan, Henry Kun Kiaang Tan, Juin Yee Kong [1-6] drew attention to the fact that this phenomenon is positive when the spiral organ is damaged, and negative when the auditory nerve root is damaged. With a positive FAVG in the affected ear with reduced hearing, there is an increased sensitivity to loud sounds, and this ear acutely perceives the increase in sound intensity.

There are many methods of detecting FAVG. They include the study of loudness balance according to Fowler, the determination of the amount of direct and reverse adaptation, the measurement of differential thresholds of sound power perception according to Luscher, noise audiometry according to Langenbeck, the study of the amplitude of oscillations when recording a curve on an automatic audiometer, the study of the SISI test, and so on.

AIM

The aim of the article is to reveal the peculiarities of the phenomenon of accelerated volume growth and to analyze the interpretation of the mechanism of its occurrence.

MATERIALS AND METHODS

The explanation of PAVG is based on the topical and morphological theory [6], according to which a positive PAVG is associated with a lesion of the receptor apparatus in the cochlea, and a negative PAVG is associated with a retro cochlear lesion. These authors explain the appearance of positive PAVG in tumors of the VIII pair of cranial nerves by the compression of the labyrinth artery, which causes a secondary effect on hair cells.

Y. Matvienko [3] considers the loss of function of the lateral sensitive cells of the spiral organ while the medial cells are preserved as the cause of the appearance of PAVG. Lateral sensory cells perceive weak auditory stimuli, and medial ones – stronger ones.

However, as experience accumulated, there was disappointment in the topical value of this phenomenon, since positive PAVG occurs during overexertion in healthy people with normal hearing and passes after rest. When analyzing a large number of observations with hearing loss of root and central origin Pyatykop V.O., Tovazhnyanska O.L., [4, 5], Matvienko Y.O., Negrych T.I., [3] found in some positive PAVG in some cases. In the process of observation, a positive PAVG can change to normal and even negative [2, 6, 7]. When analyzing a series of

observations of neuron VIII of a pair of cranial nerves with partial preservation of hearing for tumors, a positive PAVG was established in 50% [3, 7]. Therefore, with a root lesion in half of the observations, a positive PAVG is noted, so this symptom cannot be considered a sufficiently reliable differential diagnostic sign of a cochlear or retro cochlear lesion. A significant role in the differentiation of cochlear and retro cochlear hearing loss Andreev O.A. [1] refers to the peculiarities of sound lateralization in Weber's experience: with unilateral cochlear deafness, sound in Weber's experience is lateralized to the better ear, and with retro cochlear deafness and even deafness, sound lateralization in Weber's experience is absent in 80%. This is the main symptom for the differentiation of cochlear and retro cochlear sensorineural deafness.

In contrast to the topical and morphological explanation of PAVG, many authors put forward a physiological interpretation of this phenomenon. Thus, Kok Wooi Teoh, Khurshid Merchant [6] associates a positive PAVG with a special irritation functional state of the VIII pair of cranial nerves, close to hyperacusis, and a phase phenomenon in the auditory analyzer. Some authors believe that a positive PAVG reveals not only the topic of the lesion, but also the functional relationship between excitatory and inhibitory processes. Yes, Andreev O.A. [1], without denying the morphological theory of PAVG, believes that this symptom can be attributed to phase phenomena in the auditory analyzer.

In today's conditions of rapid technical progress, more and more new audio logical threshold tests are offered, which quickly replace each other, but later become disappointed in them.

What is the reason for the restrained attitude of many authors to supra threshold tests? This attitude, in our opinion, is connected with many factors.

The main reason for the inconsistency of the data obtained during supra threshold tests is explained by the fact that these tests are in most cases explained from morphological positions, while, in our opinion, the basis of PAVG lies in peculiar functional changes of the auditory analyzer with a predominance of irritation phenomena close to hyper accuses and phase phenomena in the auditory analyzer, as well as changes in the functional state of the central nervous system.

Different methods of studying PAVG are used, which study different physiological qualities of the auditory analyzer and the central nervous system. In this regard, it is possible to obtain ambiguous and often directly opposite data about PAVG with different research methods. We believe that the most adequate way to study PAVG is the Fowler method, while the most objective study is on an automatic audiometer with the study of the amplitude of oscillations on the audiogram.

To explain PAVG in the literature, only the level of damage to the auditory analyzer is mainly taken into account, and not its functional state in each specific disease (significant prevalence of symptoms of irritation in Meniere's disease, especially in its initial phases) and a clear prevalence of the destruction phenomena of the loss of auditory function, in neuromas VIII pairs of cranial nerves.

When evaluating FAVG, only the state of the auditory analyzer is often taken into account without analyzing the general functional state of the central nervous system. At the same time, all various tests for the study of PAVG are associated with the patients' performance of very fine differentiations, which are ensured only under the condition of high functional activity of the central nervous system.

On the basis of theoretical analysis and observations of studies of PAVG by various methods (by the magnitude of oscillations of the tonal audiogram on an automatic audiometer, the Luscher method, research of the SISI test), we came to the conclusion that a positive PAVG can be observed both with cochlear and with root and central lesions auditory pathways and nuclei. Negative and sharply negative PAVG in the case of disorders in the central nervous system is caused not so much by the defeat of the auditory analyzer as by a change in higher nervous activity in these patients.

Positive PAVG is most often manifested in pathology, when it is dominated by irritation phenomena in the auditory analyzer. As the auditory function fades, irritation phenomena disappear, the positive PAVG turns into normal and even negative.

The high frequency of positive PAVG in peripheral lesions is partially due to the fact that all symptoms of irritation in the auditory and vestibular analyzers are especially clearly manifested in disorders of the receptor apparatus (sharp painful noises, severe dizziness, which can even lead patients to suicide). PAVG as one of the manifestations of irritation symptoms is also most pronounced in peripheral lesions.

In our opinion, PAVG is not so much related to a certain topic as to a special functional state of the cochlear portion of the VIII pair of cranial nerves.

In Ménière's disease, which affects hair cells, this phenomenon is usually positive, and in the case of a root lesion – neurinomas – it is more often negative. However, one cannot ignore the fact that, in addition to the different topic (with Meniere's disease and neurinomas of the VIII pair of cranial nerves), the auditory nerve in these two diseases is in completely different functional states. With neurinomas, the symptoms of loss with slow and gradual fading of the functions of the VIII pair of cranial nerves significantly prevail. Symptoms of irritation are either absent or slightly expressed (hy-

per accuses, dizziness, as a rule, are absent, there is no noise or it is weakly expressed). In Ménière's disease, symptoms of irritation of the vestibular and auditory portions of the VIII pair of cranial nerves (hyper accuses, loud noise, especially during an attack of vestibular vertigo) predominate.

REVIEW AND DISCUSSION

Our observations convincingly indicate that PAVG is associated not only with the level of damage, but also with a certain functional state in the form of hyperacusis.

With hyperacusis, patients perceive even weak and stronger sounds excessively acutely. Most often, hyperacusis occurs during the acute development of the process, and especially often – during Ménière's disease. Some clinicians have previously associated hyperacusis with a specific topic. Our observations showed the fallacy of this opinion. Hyperacusis occurs when the auditory analyzer is damaged at various levels (on the periphery, in the area of the optic tubercle). It is a peculiar functional state of the VIII pair of cranial nerves, which cannot be given only topical significance. In a number of observations, it can obviously be attributed to phase states.

Hyperacusis is of great importance in the appearance of PAVG. It is well known that hyperacusis is particularly common in Ménière's disease. In addition, along with a positive PAVG, the pain threshold often decreases at the same time. All this indicates a peculiar functional state of the VIII pair of cranial nerves with the manifestation of heightened auditory sensitivity.

Theoretical analysis and observations indicate that, in a particularly strongly expressed form, positive PAVG is determined in the case of an irrigative form of damage to the VIII pair of cranial nerves, and over time when the function of the VIII pair of cranial nerves fades; when the phenomena of irritation in the auditory nerve decrease, PAVG may disappear. Our assumption is confirmed by the research of Matvienko Y.O., Negrych T.I., Marienko L.B., Korol H.M. [3], who studied PAVG in the dynamics of Meniere's disease and found that over recruitment could turn into a normal phenomenon, and later it became negative. Yu.O. Matvienko [3] believes that the receptor otoneurological syndrome can sometimes be observed with a negative PAVG. One cannot consider this phenomenon statically, as some authors do. We believe that a positive PAVG in Meniere's disease does not mean that this phenomenon is due to the damage of only hair cells.

Based on the general physiological patterns of the activity of the nervous system, perhaps it should be assumed that this phenomenon can occur with lesions in different areas of the auditory analyzer, but it is most often and clearly manifested in Ménière's disease. Moreover, this manifestation depends not so much on the level of the lesion, but on the extreme peculiarity in the course of Ménière's disease, because in this disease, irritation in the auditory and vestibular analyzers is especially pronounced.

Theoretical analysis of research and our observations show that increased sensitivity to accelerated loudness is easier to detect in peripheral lesions, since in such patients the central parts of the nervous system and higher nervous activity are mostly unchanged, patients give clear responses to changes in sound intensity.

With a central lesion in patients, higher nervous activity is often disturbed, the possibility of accurate and clear differentiation becomes difficult not so much due to local damage to the auditory analyzer, but due to general brain disorders and the predominance of inhibitory processes in the central nervous system.

Our theoretical analysis of literary sources allows us to assert that the explanation of PAVG only from topical positions reflects a fascination with morphology, the desire to assign each symptom to a specific topic, insufficient knowledge of the main physiological patterns in the activity of the nervous system, and the desire to explain everything only by changes in the peripheral part of the analyzer of its role.

Despite the fact that, in our opinion, PAVG is not associated only with the topic of hair cell damage, we believe that this phenomenon is of some importance in the differential diagnosis between neuromas of the auditory nerve and Meniere's disease, since it is observed in Meniere's disease more often However, the basis of the mechanism of this phenomenon is different functional states of the VIII pair of cranial nerves in Ménière's disease and in neurinomas of the VIII pair of cranial nerves.

The increased sensitivity of the VIII pair of cranial nerves, which is inadequate in terms of the strength of the stimulus, leads to an increase in sound intensity and can most likely be considered as a phase phenomenon in the auditory analyzer.

CONCLUSIONS

A positive PAVG is observed when the receptor, the root of the VIII pair of cranial nerves and the central auditory formations are affected. With a root lesion, a positive PAVG is observed in 50%, so this symptom cannot be attributed to reliable differential diagnostic signs of a cochlear or root lesion.

PAVG is associated with functional changes in the auditory analyzer, it is one of the symptoms of irritation, close to hyperacusis and a phase condition in the auditory analyzer, when at a certain intensity, sounds have an overpowering effect.

The frequent detection of positive PAVG in Meniere's disease is not so much related to the topic as to the prevalence of irritation in the auditory and vestibular analyzers, especially in the acute phase of this disease. In tumors of VIII pairs of cranial nerves, the phenomenon of loss of nerve function without irritation phenomena prevails.

The relatively higher frequency of positive PAVG in peripheral lesions is due to the fact that in the case of receptor disorders, the symptoms of irritation in the VIII pair of cranial nerves (loud noises, dizziness) are particularly clearly manifested and, in addition, patients retain the possibility of accurate differentiation in connection with lack of damage to the central nervous system.

Sharply negative PAVG with lesions of the central nervous system in many patients is caused not so much by a violation of the auditory analyzer as by a change in higher nervous activity.

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CONFLICT OF INTEREST

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REVIEW ARTICLE

CONTENTS

Anatomical, physiological and morphological features of the liver in newborns with biliary atresia (based on literature data and personal observations)

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ABSTRACT

Aim: To provide a fundamental understanding of the anatomical and histological structure of the liver in children with BA, which are essential for accurate diagnosis, the development of targeted treatment approaches, and effective surgical correction.

Materials and Methods: A literature review was conducted on embryogenesis, anatomy, and morphology of the disease in newborns and children with biliary atresia (BA). At least 27 scientific sources were analyzed, covering the period from classical descriptions (since 1972) to modern publications (2020s). The selection criteria included studies describing liver embryogenesis, morphological features, and clinical and surgical aspects of BA. The databases PubMed, Medline, Embase, and Google Scholar were searched, covering research areas such as liver anatomy, physiology, and morphology in children, as well as clinical issues related to biliary atresia. In addition, the author's observations were included. These were obtained during diagnostic imaging procedures, intraoperative evaluation of the organ during surgical correction, anatomical studies of 7 deceased children with BA, and histological examination of liver tissue. All patients were under our observation during diagnosis and treatment for BA and demonstrated characteristic clinical, anatomical, physiological, and morphological changes of the liver.

Conclusions: The cause of BA may lie in disruptions or arrest of the embryonic development of the bile ducts and hepatic vascular system. In infants, the liver is in a state of anatomical immaturity and functional insufficiency, and its structure is not fully developed. This must be taken into account when treating children with BA.The anatomical differences in the liver of children with BA have significant practical implications for the surgical management of BA.

KEY WORDS: biliary atresia, infants, anatomy and physiology of liver, treatment

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INTRODUCTION

Biliary atresia (or atresia of the bile ducts) occurs in approximately 1 in 8,000 to 1 in 30,000 newborns, according to various studies [1-3]. In biliary atresia (BA), the affected obliterated ducts create a mechanical obstruction to the normal flow of bile, resulting in its retention in the liver and causing chronic inflammation and hepatocellular damage [4]. BA accounts for up to 39% of all liver-related mortality in children [5,6]. For many years, the condition was considered incurable until 1959, when Kasai M. and Suzuki S. introduced the surgical procedure of hepatic portoenterostomy, which made it possible to transform the "uncorrectable" type of BA into a "correctable" one, thus saving the lives of many children [7,8].

Clinically, the condition presents with persistent jaundice of the skin and mucous membranes, pale stools, and dark urine. Jaundice in BA appears within the first days of life, progresses steadily, and is caused by elevated levels of direct bilirubin. The liver becomes enlarged and firm, and by the age of 5-6 months, biliary cirrhosis of the liver develops. By the age of 2 months, the spleen also enlarges [9,10].

The choice of surgical strategy depends on the type of BA. According to the anatomical classification, there are three types of BA: 1) atresia of the extrahepatic (external) bile ducts with preservation of the ducts at the hepatic hilum and a well-developed gallbladder; 2) atresia of the intrahepatic bile ducts; 3) total atresia of the bile ducts. Atresia of the extrahepatic bile ducts (the "correctable type") at the level of the common bile duct or hepatic duct is considered a favorable form of the defect in terms of successful surgical correction. In such cases, it is generally possible to achieve good bile drainage. In contrast, intrahepatic duct atresia and the total form present significant difficulties for correction, which has led to their classification as the "uncorrectable type" [11-14].

BA is the most common cholestatic liver disease in infants and the leading indication for liver transplantation [15].

Understanding BA involves liver embryogenesis along with its anatomical and morphological features, as these contribute to a complex pattern of pathological changes requiring surgical correction. Successful treatment of children with BA greatly depends on the surgeon's thorough understanding of the disease's nature and mechanisms of development. Without up-to-date knowledge of the disease's etiopathogenesis, as well as the anatomical, functional, and morphological features of the liver, achieving effective treatment outcomes is not possible.

AIM

To provide a fundamental understanding of the anatomical and histological structure of the liver in children with BA, which are essential for accurate diagnosis, the development of targeted treatment approaches, and effective surgical correction.

MATERIALS AND METHODS

A literature review was conducted on embryogenesis, anatomy, and morphology of the disease in newborns and children with biliary atresia (BA). At least 27 scientific sources were analyzed, covering the period from classical descriptions (since 1972) to modern publications (2020s). The selection criteria included studies describing liver embryogenesis, morphological features, and clinical and surgical aspects of BA. The databases PubMed, Medline, Embase, and Google Scholar were searched, covering research areas such as liver anatomy, physiology, and morphology in children, as well as clinical issues related to biliary atresia. In addition, the author's observations were included. These were obtained during diagnostic imaging procedures, intraoperative evaluation of the organ during surgical correction, anatomical studies of 7 deceased children with BA, and histological examination of liver tissue. All patients were under our observation during diagnosis and treatment for BA and demonstrated characteristic clinical, anatomical, physiological, and morphological changes of the liver.

REVIEW AND DISCUSSION

Brief overview of liver embryogenesis. The liver develops from the hepatic diverticulum of the primitive gut during the 4th week of intrauterine development. The central part of this diverticulum gives rise to the liver and hepatic ducts. Hepatic cells grow in the form of cords into the mesenchymal tissue of the transverse septum of the diverticulum, forming the hepatic parenchyma, bile canaliculi, and intrahepatic bile ducts. The caudal portion of the hepatic diverticulum gives rise to the gallbladder and the common bile duct, undergoing a solid proliferative stage followed by vacuolization. By the 7th-12th weeks of embryonic development, the gallbladder becomes a hollow organ, and a lumen appears in the cystic duct.

Simultaneously with the formation of the distal parts of the biliary system, hepatic secretory cords develop, and both extrahepatic and intrahepatic segments of the portal and hepatic veins form. During the embryonic period, liver lobules begin to form, and their final differentiation is completed by the end of the 1st month of life. The vascular system of the liver originates from the vitelline and umbilical veins. As the intestines develop, the biliary segment of the vitelline veins regresses, and the umbilical veins of the umbilical cord and embryo merge. The vessel passing through the hepatic parenchyma (the ductus venosus Arantii), which carries oxygenated blood into the inferior vena cava of the fetus, becomes obliterated after birth. Disruptions or arrest in the embryonic development of the biliary ducts and hepatic vasculature may lead to conditions such as biliary atresia, biliary hypoplasia, choledochal cysts, hepatic cysts, and portal vein anomalies, often resulting in portal hypertension [16,17,18].

The main structural element of the liver is the hepatocyte and the hepatic lobule, which ranges in diameter from 0.7 to 2.0 mm. It consists of a single layer of hepatocytes arranged around a central vein. Irregularly organized cells create interconnected cavities known as lacunae. It is within this histological structure that pathological processes such as necrosis and cirrhosis develop, leading to severe consequences [10].

The liver performs a wide range of functions. Its main roles include: 1-regulating metabolic processes by storing, secreting, and processing carbohydrates, fats, and proteins; 2-synthesizing proteins; 3-producing bile; 4-regulating the blood coagulation system; 5-performing hematopoietic functions; 6-participating in water and electrolyte balance; 7-carrying out detoxification; 8-modulating and supporting the immune system [19].

NORMAL LIVER ANATOMY IN NEWBORNS AND INFANTS

In newborns, the liver is one of the largest organs, accounting for more than 4% of total body weight. It is considered normal for the liver to extend 2-3 cm below the costal margin along the midclavicular line.

Typically, the right and left lobes of the liver in newborns are approximately equal in volume, or the left lobe may be larger than the right. This is due to features of intrauterine development, during which the left lobe is better supplied with blood. This fact should be taken into account during liver resection at this age. In the postnatal period, the liver continues to grow, although its mass increases more slowly than body weight. For example, by 10-11 months of age, liver weight doubles, whereas body weight triples.

The liver is covered with peritoneum on almost all sides (a mesoperitoneal organ), except for the posterior part of the diaphragmatic surface and the porta hepatis. The peritoneal covering of the liver, at the points where it extends to the diaphragm, the porta hepatis, and adjacent organs, forms the ligamentous apparatus of the liver. In addition to providing support, these ligaments serve as conduits for blood and lymphatic vessels and nerves. From a surgical standpoint, the most important ligaments are:

1) The coronary ligament, located in the frontal plane. At its lateral edges lie the left and right triangular ligaments. These are subject to surgical division during liver mobilization, cyst dissection, liver resection, or diaphragmatic hernia repair.

2) The fourth ligament – the falciform ligament, also known as the suspensory ligament, which is located in the sagittal plane. At the anterior edge of the liver, it transitions into the round ligament, which contains the umbilical vessels.

The hepatoduodenal ligament extends from the liver to the duodenum and contains vital anatomical structures: the portal vein, the hepatic artery, and the common bile duct. Its fusion with the hepatogastric ligament and the left gastrophrenic ligament forms the anterior wall of the lesser omentum, and the hepatoduodenal ligament itself forms the anterior border of the omental (epiploic) foramen. Within the hepatoduodenal ligament, the common and cystic bile ducts lie superficially (ventrally), the hepatic artery trunk lies slightly deeper and to the left, and the wide portal vein trunk is located dorsally behind them. These anatomical relationships are crucial and must be taken into account during surgical procedures at the porta hepatis.

During liver mobilization, surgical intervention involves the division of hepatic ligaments. The anatomy of these structures must be considered during resective operations. For example, during mobilization of the left lobe of the liver, the left triangular ligament is divided up to the beginning of the falciform ligament. During mobilization of the right lobe, the right triangular ligament, hepatorenal ligament, round ligament, and falciform ligament are divided.

The liver is divided into right and left lobes, separated by the falciform ligament or the left sagittal fissure, in the anterior part of which lies the round ligament of the liver, and in the posterior part – the obliterated ductus venosus (Arantius' duct).

The right sagittal fissure on the inferior surface of the liver runs from the gallbladder bed to the inferior vena cava. Between the sagittal fissures, anterior to the transverse fissure, lies the quadrate lobe, and posteriorly – the caudate lobe.

Couinaud's classification divides the right and left lobes of the liver into 5 sectors and 8 segments, each with its own autonomous blood supply, bile drainage, innervation, and lymphatic circulation. In the right lobe, two sectors are distinguished: lateral (segments 6 and 7) and paramedian (segments 5 and 8). In the left lobe, three sectors are distinguished: lateral (segment 2), paramedian (segments 3 and 4 – i.e., the quadrate lobe and most of the left lobe), and dorsal (segment 1 or the caudate lobe). These variants of dividing the liver into lobes, sectors, and segments are anatomically justified, as they reflect the liver internal structure. They are diagnostically useful for localizing pathological processes using ultrasound, CT, MRI, and angiography, and are widely applied in resective and alternative liver surgery [19].

The liver is the only organ with two systems of supplying vessels – the hepatic artery and the portal vein. The common hepatic artery originates from the celiac trunk. Within the hepatoduodenal ligament, it gives rise to the gastroduodenal, right gastric, and cystic arteries. In nearly half of cases, anomalies in hepatic artery development are found, including atypical branching and additional vascular branches, unusual origins of the hepatic artery (e.g., from the superior mesenteric artery rather than the celiac trunk), and independent origins of the left and right hepatic branches. The hepatic artery provides 10-35% of the liver blood flow and supplies 50% of its oxygen needs, while 65% of hepatic blood flow is provided by the portal vein.

The portal vein is formed by the confluence of the superior mesenteric and splenic veins. Its tributaries include the inferior mesenteric vein and the coronary vein of the stomach, which typically drain into the splenic vein. The portal vein is 3-8 cm long and 0.5-1.5 cm in diameter, but its size may vary depending on normal anatomical variants or pathological changes, such as those seen in portal hypertension. Pathological changes in the portal vein system are identified and considered during splenoportography and when selecting a surgical method for correcting portal hypertension. Venous outflow from the liver is through three hepatic veins: the right, middle, and left. The latter two may merge into a



Fig. 1. Jaundice in patient K., 1.5 months old, with BA
Picture taken by the authors



Fig. 2. Macroscopic view of the liver in BA (intraoperative picture)
Picture taken by the authors

single trunk draining into the inferior vena cava at the point where it enters the liver. Obstruction of venous outflow due to thrombosis or the presence of an embryonic membrane at the junction of the inferior vena cava and the right atrium causes severe liver dysfunction.

Lymph drainage from the liver occurs in two directions: from the superior surface of the liver, lymphatic vessels pass through the triangular ligaments and diaphragm to enter the thoracic duct [18]; and from the inferior surface of the liver, lymphatic vessels travel to the porta hepatis, to the aortic lymph nodes, and then to the thoracic duct. The porta hepatis contains lymph nodes – the cystic node and nodes of the hepatoduodenal ligament beneath the right edge of the duodenum. These nodes are consistent landmarks used to locate the cystic artery and the common bile duct [12].

Parasympathetic innervation is provided by the right vagus nerve, and sympathetic innervation comes from branches of the right splanchnic nerve. Together, branches of the vagus and sympathetic nerves form the hepatic and cystic plexuses around the structures of the porta hepatis.

CLINICAL AND ANATOMICAL OBSERVATIONS IN CASES OF BA

A notable feature in newborns is that the right and left liver lobes are approximately equal in volume, and the intrahepatic bile ducts, although formed, exhibit impaired patency due to obliteration or underdevel-

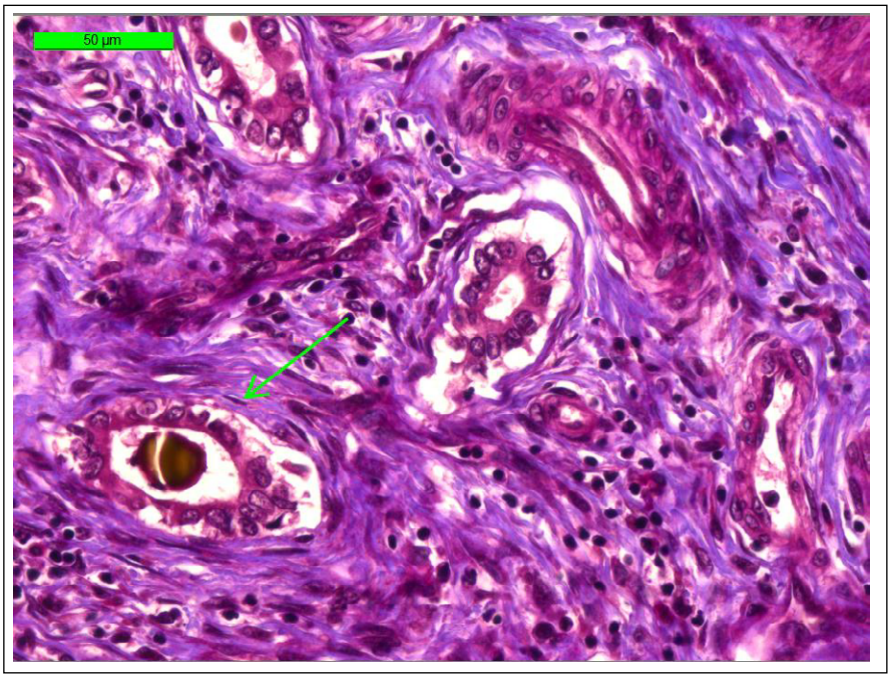


Fig. 3. Gallbladder hypoplasia in BA (intraoperative picture)
Picture taken by the authors

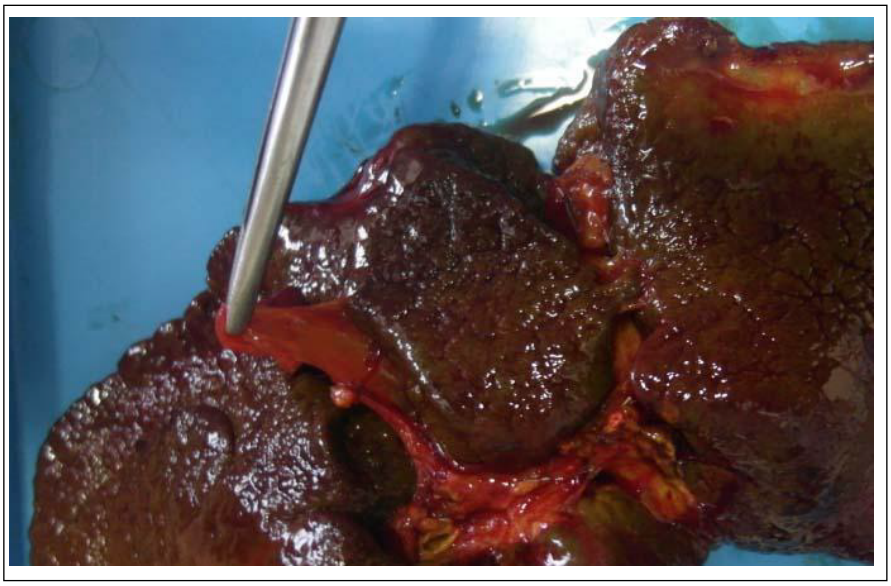


Fig. 4. Proliferation of bile ducts with edema and dystrophic changes in cholangiocytes in BA
Picture taken by the authors

opment. One possible cause of ductal obliteration is neonatal hepatitis with obstructive cholangiopathy, suggesting that atresia can develop after birth and may regress following surgery. BA is not found in embryos, stillbirths, or premature infants – it occurs only in the postnatal period [20].

Currently, several hypotheses have been proposed regarding the origin of BA, including: a viral infection hypothesis, ischemia of bile ducts due to impaired blood supply, disrupted bile acid metabolism, pancreatobiliary maljunction theory, etc.

The symptoms of BA include obstructive jaundice and acholic stools. These may not be noticeable in the first days or weeks of life because bile production begins only after birth, and clinical signs of hyperbilirubinemia require time to develop due to bilirubin accumulation in the serum. Furthermore, physiological jaundice is common and short-lived in newborns. Jaundice in BA typically appears on the 3rd-4th day after birth, does not resolve within 2-3 weeks, and progressively worsens. The direct (conjugated) bilirubin fraction in the serum may reach 100-300 µmol/L. Stools remain acholic, although in the first few days they may be yellow due to partial duct patency and the release of bile pigments from intestinal glands. Urine is dark-colored, resembling the color of dark beer (Fig. 1).

By the end of the first month, the liver begins to enlarge due to the development of cholestatic hepatitis. Intraoperatively, it appears greenish in color and is firm to the touch (Fig. 2). The liver has a yellow-green color, its surface and parenchyma are finely granular, cystic cavities near the porta hepatis contain thick, paste-like bile.

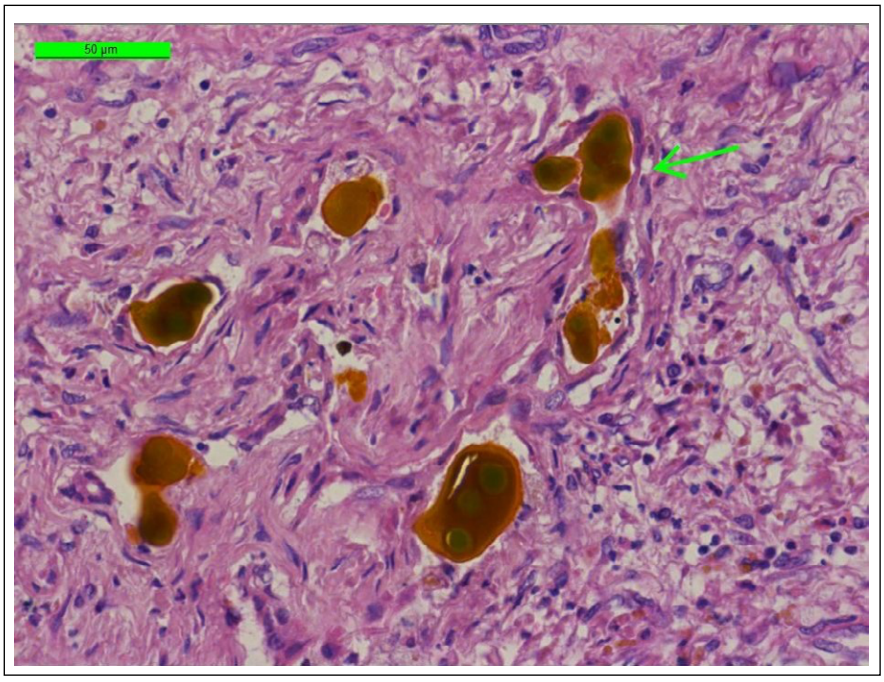


Fig. 5. Bile stasis in the bile ducts in BA
Picture taken by the authors

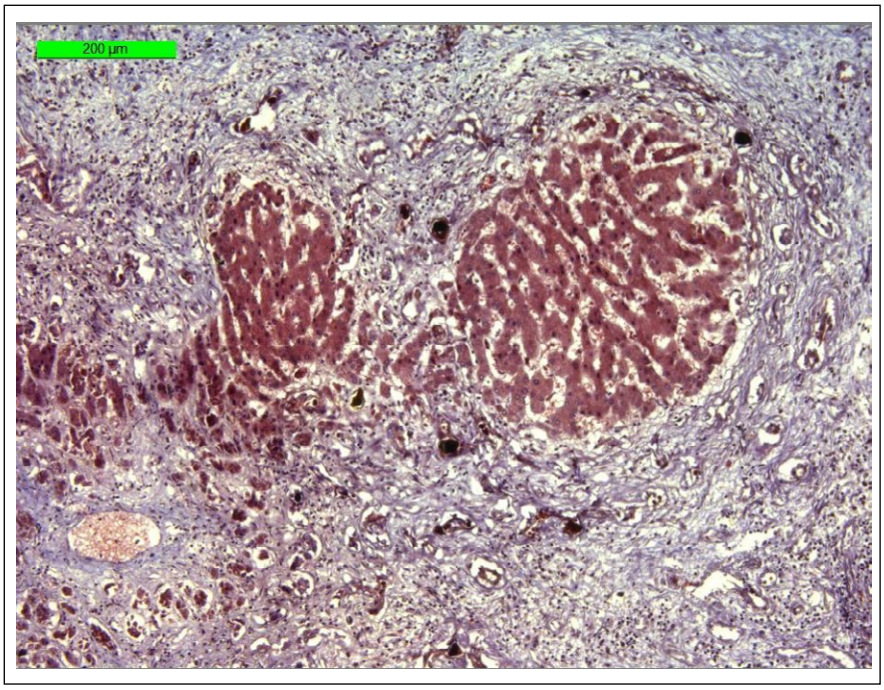


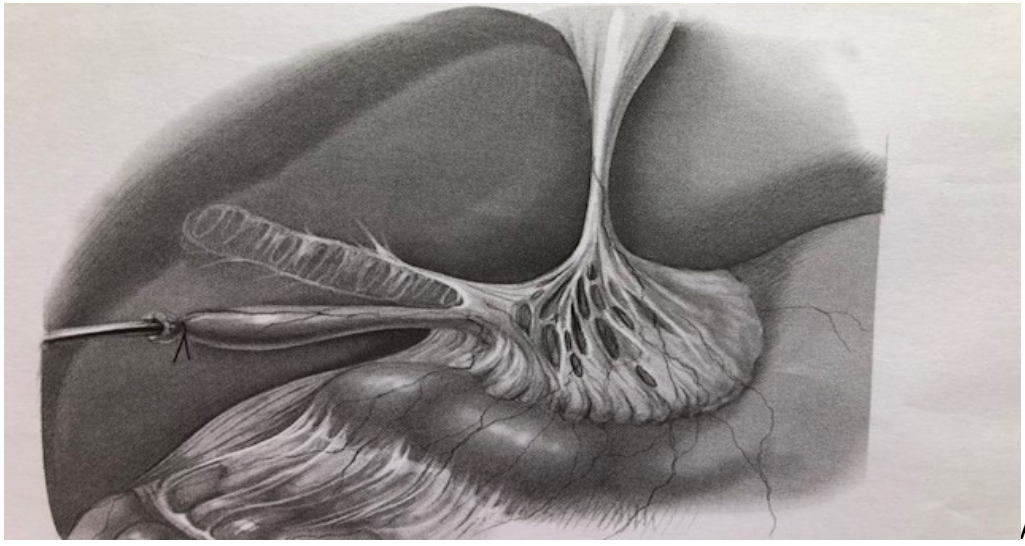
Fig. 6. Liver cirrhosis in an 8-month-old infant with BA
Picture taken by the authors

Over time, portal hypertension develops. Increased pressure in the portal vein leads to its dilation, the development of collateral circulation through the esophageal veins, and the appearance of esophageal varices. As a result of impaired blood flow through the portal vein, the spleen enlarges, and a subcutaneous venous network becomes visible on the anterior abdominal wall. Abdominal distension occurs initially due to flatulence caused by impaired digestion (due to the absence of bile), and later due to ascites from portal hypertension. The average life expectancy is 18 months. Children die from progressive liver failure and gastrointestinal bleeding due to ruptured esophageal varices [21].

Every infant with jaundice should undergo an urgent ultrasound examination. During liver ultrasound, special attention is given to the gallbladder. Since intrahepatic and total BA are more common, the gallbladder is often absent or hypoplastic, appearing as a rudimentary structure. Ultrasound may reveal a triangular cord sign at the porta hepatis, which represents fibrotic remnants of the common bile ducts [22,23] (Fig.3).

The small gallbladder does not change in size after feeding. Gallbladder contraction after feeding excludes BA.

Therefore, the presence of the following signs – persistent jaundice lasting more than one month with elevated direct bilirubin levels that do not show



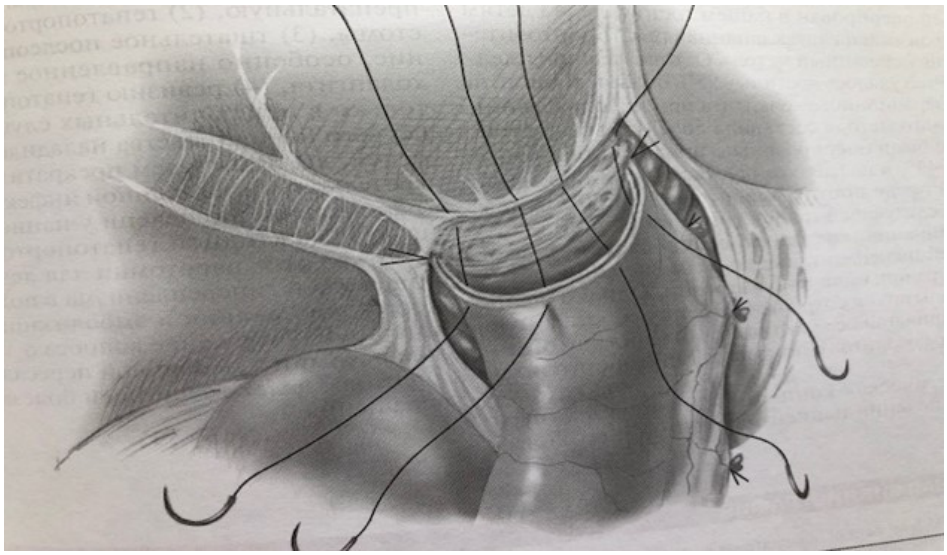


Fig. 7. Schematic representation of the Kasai procedure: a) removal of obliterated bile ducts and exposure of the fibrous cone at the porta hepatis with remnants of bile ducts; b) creation of an anastomosis between the fibrous cone and the end of the jejunum *Picture taken by the authors*

a decreasing trend, acholic (pale) stools, absence of the gallbladder on ultrasound, or a lack of response to conservative "hepatitis" treatment – constitutes an indication for diagnostic laparotomy and intraoperative evaluation of the porta hepatis to determine the type of BA and assess the possibility of surgical correction.

Liver biopsy in cases of BA can be up to 95% accurate, depending on the pathologist's qualifications [24-26]. Histological confirmation of BA is based on typical findings, among which the most characteristic are: bile duct proliferation, bile stasis in the ducts, portal edema, fibrosis, and liver cirrhosis. These histological features were also observed in the analyzed cases (Fig. 4, Fig.5, Fig.6).

The success of treatment largely depends on establishing the diagnosis within the first month of life. A diagnosis of BA made after 3-4 months of age offers almost no hope for successful treatment due to the development of cholestatic hepatitis, fibrosis, and biliary liver cirrhosis. Therefore, the 60-day threshold is considered critical worldwide for achieving a good surgical outcome. Beyond this period, surgery is typically unsuccessful. Many surgical options have been proposed, although some are now of primarily historical interest. The most widely recognized and commonly performed procedures are hepatoportoenterostomy with a Y-shaped Roux-en-Y jejunal loop according to the Kasai technique (with various modifications) and liver transplantation. The former is available in many specialized centers and is most effective when performed on time (within the first 60 days of life). It can also serve as a bridge to subsequent liver transplantation (Fig. 7).

The Kasai procedure is most effective for the treatment of BA when the following five key principles are observed:

1) Timing: the operation must be performed within the first 60 days of life. Under these conditions, bile flow can be achieved in 80% of cases. When surgery is

performed after 90 days of life, bile flow is restored in only 41% of cases.

2) Surgical technique: a) excise the fibrous cone to the appropriate depth (not reaching the liver); b) at the correct anatomical location (posterior wall of the bifurcation of the portal vein); c) use microsurgical magnification devices (surgical loupes); d) form the anastomosis with absorbable sutures, anchoring the bowel to the adventitia of the portal vein posteriorly and to the liver anteriorly.

3) Postoperative management: rational postoperative care includes long-term antibiotics, corticosteroids, and hepatoprotective agents.

4) Timely revision surgery: if bile flow stops postoperatively, a second surgery (revision of the hepatoportoenterostomy with removal of granulation tissue at the porta hepatis) should be performed no later than two months after the initial procedure.

5) Anti-reflux procedures: These should complement the hepatoportoenterostomy to prevent ascending postoperative cholangitis.

Regarding resectional procedures, in children under 1 year of age, the most extensive permissible operation is left-sided hemihepatectomy, although even this is associated with a high risk of liver failure. Notably, in newborns, the right and left liver lobes are approximately equal in volume, which must be taken into account when performing liver resections at this age. An important factor in liver transplantation is the anatomical configuration of the donor segment's blood supply and its size. The volume of the donor's remaining liver must not be less than 40% of the total liver volume [27].

CONCLUSIONS

1. BA is one of the most severe surgical conditions in newborns and infants. Without a thorough understanding of embryogenesis and the anatomical and functional features of the liver and biliary tract, effective treatment outcomes are impossible.
2. The cause of BA may lie in disruptions or arrest of the embryonic development of the bile ducts and hepatic vascular system.
3. In infants, the liver is in a state of anatomical immaturity and functional insufficiency, and its structure is not fully developed. This must be taken into account when treating children with BA.
4. The anatomical differences in the liver of children with BA have significant practical implications for the surgical management of BA.

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CONFLICT OF INTEREST

The Author declare no conflict of interest

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A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

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REVIEW ARTICLE

CONTENTS

Rabies: known and unknown historical aspects

Konstantinos Laios, Spyros N. Michaleas, Miltiadis Perdikakis, Konstantinos Karamouzis, Ioannis Nikolakakis
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ABSTRACT

Aim: To provide a comprehensive historical overview of rabies, with a focus on its early recognition, cultural significance, and advancements in treatment from antiquity to the modern era.

Materials and Methods: A literature review was conducted using electronic databases such as Scopus and PubMed to gather data on rabies incidence, transmission patterns, and public health interventions across various Latin American countries. The search covered publications from 1950 to 2025. The following keywords and their combinations were used: rabies, hydrophobia, Lyssa, Lyssavirus, history of rabies, rabies vaccine, Louis Pasteur, ancient medicine rabies, rabies Greece, canine rabies, public health interventions.

Conclusions: Although significant progress has been made in reducing rabies cases in Latin America through widespread vaccination campaigns and animal control strategies, the disease continues to pose a public health concern in certain areas. This persistence is particularly attributed to challenges in rural and remote regions, bat-mediated transmission, and deficiencies in disease surveillance. Continued investment in preventive measures, public education, and targeted interventions—especially among vulnerable populations—is crucial for achieving the complete elimination of rabies in the region.

KEY WORDS: bite, deity, madness, Lyssa, hydrophobia, Lyssavirus

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INTRODUCTION

Rabies, a viral disease affecting the nervous system, has been known since antiquity, with references dating back thousands of years. The disease progresses through several stages, each marked by specific pathological changes. In its advanced stages, symptoms such as hydrophobia (fear of water) and aerophobia (fear of drafts) frequently appear. These symptoms are often accompanied by severe spasms of the muscles involved in swallowing and breathing. [1]

In antiquity, rabies was primarily associated with infected dogs and the transmission of the disease through their bites. Although other animals were capable of transmitting rabies, this concept was not widely accepted by physicians of the time. Some, such as Caelius Aurelianus (c. 5th century AD), acknowledged that humans could also contract rabies from wolves, bears, leopards, horses, and donkeys. Nonetheless, due to the high incidence of rabies in dogs and their close interactions with humans, canines were viewed as the primary source of transmission. This close contact made rabies a particularly troubling condition for many ancient medical practitioners.[1]

Rabies was often categorized as a form of animal poisoning, akin to envenomation caused by reptiles and insects, with a strong focus on the mode of transmission. Despite this classification, rabies occupied a distinct place in ancient medical literature due to its unique effects on the human mind and body. The disease's mental and neurological symptoms—such as agitation, paranoia, and especially hydrophobia—led to the metaphorical use of the term "lyssa" in ancient Greek to denote delirium and madness. [1]

AIM

To provide a comprehensive historical overview of rabies, with a focus on its early recognition, cultural significance, and advancements in treatment from antiquity to the modern era.

MATERIALS AND METHODS

A comprehensive literature review was conducted using major electronic databases, including PubMed, Scopus, and Google Scholar, supplemented by targeted search-

es in JSTOR, ProQuest, and specialized repositories in the libraries of the National and Kapodistrian University of Athens.

The search covered publications from 1950 to 2025 for public health studies, while no chronological restriction was applied for historical and classical sources (ancient, Byzantine, and medieval texts).

The following keywords and their combinations were used: rabies, hydrophobia, Lyssa, Lyssavirus, history of rabies, rabies vaccine, Louis Pasteur, ancient medicine rabies, rabies Greece, canine rabies, public health interventions.

In total, around 60 references were initially identified. After screening titles and abstracts, approximately 30 were excluded due to irrelevance, duplication, or lack of accessible full text. The final review was based on 30 sources, including both primary historical texts and secondary peer-reviewed articles.

Inclusion criteria were:

a. Historical sources referring to rabies in antiquity, Byzantium, and later European traditions.

b. Peer-reviewed public health studies in English, Greek, Latin, or French.

c. Publications providing data on rabies incidence, transmission, vaccination, or medical perceptions across different periods.

Exclusion criteria and limitations:

a. Non-scientific or anecdotal reports without verifiable historical or medical content.

b. Articles without accessible full text.

REVIEW AND DISCUSSION

ANCIENT GREEK TIMES

The term rabies originates from the Latin verb rabere, meaning "to rage", which may itself derive from the Sanskrit word rabhas, meaning "to do violence". Acute, progressive, and fatal encephalomyelitis is caused by neurotropic viruses of the genus Lyssavirus, a name derived after the Greek word lyssa, meaning "frenzy" or "madness". [1]

Lyssa (also spelled Lytta) was the Greek goddess of rage and frenzied fury. She was closely associated with the Maniai, the daemons of madness and insanity, and had dominion over rabies in animals. Rather than being one of the Olympic gods, Lyssa was a primordial spirit (daimon), believed to have existed before the rise of the Olympian pantheon. At the command of the gods, she could provoke blind rage in both mortals and deities, although she was often reluctant to do so. Known also as Madness, she was considered a dark deity and was commonly referred to as a daughter of Nyx (Night) and Ouranos (Sky). This parentage was attribute to her by Euripides (480-406 BC), although the Roman author Hyginus (64 BC-17 AD) later described her as a daughter of Gaia (Earth) and Aether (Air). [2-4]

As a goddess, Lyssa wielded power over humanity but remained subservient to more powerful deities, particularly those on Mount Olympus. This hierarchy is evident in the myth of Heracles, as dramatized in Euripides' *"The Madness of Heracles"*. When Heracles (Hercules) was married to Megara, daughter of Creon, Hera sought vengeance upon him, the illegitimate son of her husband, Zeus. She sent the messenger goddess Iris to command Lyssa to drive Heracles mad. Though Iris brought Lyssa to the city of Heracles, Lyssa hesitated, declaring that such a deed was wicked. Nevertheless, Iris overruled her, and Lyssa ultimately afflictedHeracles with uncontrollable rage, leading him to murder his wife and children. His rampage was halted only through the intervention of the goddess Athena. [2]

Lyssa also appears in other Greek myths, often indirectly through the consequences of her influence. For instance, some ancient sources attribute to her the madness that caused Actaeon's hunting dogs to devour their master after he saw the goddess Artemis naked. Dionysus is also said to have invoked Lyssa's power to drive the daughters of Minyas mad, resulting in the dismemberment of King Pentheus. Similarly, Lyssa was blamed for the madness that led the daughters of the Athenian king Cecrops to leap to their deaths from the Acropolis. [4]

In ancient Greece, early descriptions of rabies date as far back as the 9th and 10th centuries BC, often appearing in literary texts through metaphor. For example, in Homer's Iliad, the expression "raging dog" is used for Hector, and hydrophobia is metaphorically represented in the eternal torment of Tantalus in Hades. The philosopher Aristotle (4th century BC) observed that dogs were susceptible to rabies, a condition that could be transmitted to other animals through biting. Both Democritus (460–370 BC) and Hippocrates (460–370 BC) described symptoms consistent with rabies. Although Hippocrates did not name the disease, he described patients as *"persons in a frenzy drink very little, are disturbed and frightened, tremble at the least noise, or are seized with convulsions"*. [5]

ROMAN PERIOD

The Romans were keen observers of disease, and their understanding of rabies, though limited by modern standards, was remarkably advanced for their time. Much of their knowledge stemmed from careful observation of infected animals—primarily dogs—and

the subsequent transmission of the disease to humans. Rabies was a recognized affliction in Roman times, and both Roman literature and medical texts offer valuable insights into how the disease was perceived and managed.

One of the most notable Roman sources on rabies is Pliny the Elder (23/24–79 AD), a Roman author and naturalist. In his encyclopedic work Naturalis Historia, Pliny describes the symptoms of rabies and highlights the link between the bites of rabid dogs and the onset of hydrophobia—a hallmark symptom in human rabies cases, marked by an extreme fear of water. [6]

Another significant Roman figure, Aulus Cornelius Celsus (25–50 AD), a medical writer of the early 1st century AD, provided one of the earliest systematic medical accounts of rabies in his seminal work De Medicina. Celsus detailed the manifestations of the disease in both animals and humans, and proposed treatment strategies based on the prevailing medical knowledge of the time. He described the progression of rabies with remarkable accuracy, noting initial symptoms such as fever, anxiety, and hallucinations, followed by hydrophobia and, ultimately, death. His observations underscored the futility of treatment once clinical symptoms appeared, as rabies was almost universally fatal. [7]

Galen, (129-216 AD), the prominent Greek physician, also described the signs of hydrophobia in detail:

"Hydrophobic individuals fear any liquid so intensely that even the mere mention of it causes them to lose control, crying out, trembling, sweating with fear, and chattering their teeth. As the condition worsens, they exhibit all these symptoms even in the absence of water. They become alienated and mutter to themselves, fearing the sound of water, growing heavy and emitting sudden cries. Their trembling is not constant but occurs during hallucinations. Their extremities turn cold, their pulse quickens, and they become weak. If someone offers them a drink, they recoil, scream, and breathe in sobs like children about to be thrown into deep water, unable to swim. As the condition progresses, sobs intensify, the voice weakens, and their speech takes on a barking quality. Some, due to the severity of their agitation, die in convulsions while resisting the illness". [8]

Furthermore, Galen emphasized the seriousness and complexity of rabies, clearly distinguishing it from common wounds. He observed that while ordinary wounds could be treated with simple remedies—such as vinegar-soaked sponges—bites from rabid dogs required aggressive intervention. This included cauterization, potent pharmaceuticals, and long-term care. Galen warned of the grave consequences of untreated rabies, including spasms, delirium, hydrophobia, and ultimately death:

"The Methodists claim it is useless to know the causes of diseases. Yet they study the usefulness of Theriac, a medicine that often cures hydrophobia—among the worst of diseases—and remarkably counters the onset of such evils. Victims experience not only convulsions and extreme internal fever due to dryness but also mental insanity. They fear water; though they crave fluids, they refuse to drink due to madness. I marvel at the Methodists, who say that knowledge of causes is irrelevant to treatment. How can they cure such cases, when the bite seems no different from that of a non-rabid dog?". [9]

Galen's insights reflect the depth of medical understanding in antiquity regarding rabies and its outcomes: *"For common diseases, especially febrile ones, we must ask essential questions. If the wound is from a dog, we must determine whether the dog was rabid—this makes a great difference. For a simple wound, a blood-based remedy or a sponge soaked in vinegar may suffice. But for a rabid dog's bite, even a very small wound must be cauterized. Strong medicines and prolonged care are required. Effective treatments include wormwood, Aristolochia, lycium, boiled water from river crabs, garlic, parsley, and the root known as gentian. Cleansing with hellebore is also beneficial. Without such treatment, spasms, madness, hydrophobia, and death may result".* [8]

The Romans also recognized the importance of preventive measures. They understood that rabies was transmitted through the bite of infected animals and took practical steps to reduce its spread. In rural areas, where encounters with wild or stray animals were more frequent, efforts were made to control stray dog populations and to avoid contact with potentially rabid animals. Columella (4–70 AD), a Roman agricultural writer, advised in De Re Rustica that farmers should exercise caution and kill rabid dogs to prevent disease transmission. [10]

Despite their efforts and observations, the Romans had limited means to control or treat rabies effectively. The absence of knowledge about the viral nature of the disease and the lack of vaccines or post-exposure prophylaxis meant that rabies remained a feared and frequently fatal condition. However, Roman medicine did make use of various folk remedies and medicinal plants believed to possess protective or healing properties. For instance, Pliny the Elder (23/24–79 AD) mentioned the plant Dictamnus (Dittany of Crete), which was thought to have curative effects for wounds and bites. [6]

Additional detailed descriptions of rabies symptoms in animals, particularly dogs, are found in the works of the Stoic philosopher Epictetus (1st century AD) and the physician Galen (2nd century AD). Both noted that rabies frequently affected dogs and that hydrophobia was a defining symptom. Other ancient physicians, in-

cluding Philumenus and Dioscorides (both 2nd century AD), provided in-depth accounts of the disease's manifestations in canines, noting their aggressive behavior and fear of water. They also described early therapeutic attempts, such as a mixture of crab ash and gentian dissolved in aged wine. In the 5th century AD, Caelius Aurelianus reported similar diseases occurring in Crete, with consistent symptoms including hydrophobia. [5]

These records suggest that rabies was present in Ancient and pre-Byzantine Greece as early as 2,000 to 3,000 years ago, particularly among dogs, which acted as reservoirs for the virus. However, it is also possible—though unlikely—that some ancient references to rabies were describing other illnesses with similar neurological or behavioral symptoms. Moreover, fatalities resulting from dog bites may, in some cases, have been attributed to general sepsis rather than specifically to rabies. [5]

BYZANTINE PERIOD

The existence and recognition of rabies during the Byzantine period are well-documented through various scholarly and medical works. The term lyssodi (meaning "rabid") was frequently used by theological authors such as Gregorius Nysenus (4th century AD) and Michael Psellus (11th century AD) to describe aggressive behavior or erotic passion, reflecting an understanding of the disease's symptoms beyond a purely medical context. [5]

Prominent Byzantine physicians like Aetius Amidenus (5th–6th century AD) described a condition in dogs known as lyssa (rabies). Aetius observed that the severity of the disease worsened under extreme weather conditions. He noted a range of clinical symptoms in affected animals: loss of voice and recognition, hydrophobia despite thirst, protruding tongue, foaming saliva, drooping ears and tail, sluggish gait, somnolence, and fear of reflections, which often triggered aggressive responses. Aetius also described similar symptoms in humans bitten by rabid dogs, especially hydrophobia. [5, 11]

Other significant contributors to rabies literature in this period include Apsyrte (a veterinary surgeon under Emperor Constantine), Eumelos Pelagonius, Vegetius (4th century AD), and Paul of Aegina (7th century AD). Apsyrte and Eumelos offered early observations on equine rabies, while others contributed to the general understanding and attempted treatment of the disease in both animals and humans. [11]

The most comprehensive discussion of rabies in antiquity comes from Caelius Aurelianus (c. 5th century AD), who drew heavily on Soranus of Ephesus (1st–2nd century AD). Aurelianus described rabies patients with considerable detail, emphasizing the paradoxical intense thirst coupled with an irrational fear of water. He differentiated this fear from rational avoidance of water due to poisoning concerns. [12]

Aurelianus also categorized symptoms by disease progression: early stages included unjustified worry, irritability, insomnia, and mental and physical weakness. Later stages presented with acute hydrophobia, fear triggered by the sound or mention of water, and hypersensitivity to weather changes. Attempting a differential diagnosis, Aurelianus compared rabies with mental illnesses such as mania and phrenitis, rejecting Eudemus's view that rabies resembled melancholy, since the latter was chronic while rabies was acute. [12]

He also addressed claims that hydrophobia resembled aerophobia seen in phrenitis, arguing that hydrophobia was more specific and consistent, unlike the more generalized fears in mental illness. These distinctions reflect the broader struggle of ancient physicians to classify rabies as either a physical or mental illness. Some suggested that the irrational behaviors pointed to a mental disorder, while Aurelianus firmly attributed the disease to physical causes, particularly the bite of a rabid animal. [13]

META-BYZANTINE PERIOD

In the meta-Byzantine period (10th–14th centuries), physicians such as Theophanes Nonnos (c. 10th century), Nicholas Myrepsos (c. 13th century), Demetrios Pepagomenos (c. 1200–1300), and Joannes Actuarios (c. 1275–1328) discussed rabies and proposed treatments based on natural remedies. Pepagomenos notably suggested surgical removal of parts of a dog's tongue as a preventive measure—an idea that persisted until the 19th century. Much of their work echoed earlier classical Greek medical knowledge. [5]

In Western Europe, Arnaldo de Villanova (1235–1313)—a physician, alchemist, and astrologer—believed that rabies in animals was caused by ingestion of carcasses. Cauterization remained a widely used treatment, supplemented by plant-based poultices. [5, 14]

During the Middle Ages, despite the persistence of Hippocratic principles, diseases were frequently attributed to supernatural or religious causes. Pagan traditions blended with Christian teachings, often linking illness to sin. Treatment frequently involved prayers, penance, and saintly intervention. Saint Hubert (656–727) became known as a patron against rabies. Rituals invoking his protection included branding bitten dogs with the "keys of Saint Hubert", the use of iron rings as amulets, and placing threads from the saint's stole under the skin of bite victims. These practices sparked

debate between religious authorities and early scientific institutions like the Sorbonne. [14]

RENAISSANCE TO 18TH CENTURY

The Renaissance marked a transition toward modern science and public health. As medieval thought gave way to empirical investigation, rabies treatment evolved. Though knowledge remained limited, practices like cauterizing wounds, cleaning bite sites, and using substances such as mercury gained popularity. While not scientifically precise, these methods reflect a rudimentary understanding of the need to disinfect and inhibit viral spread. [14]

During the Ottoman Empire, historical data on rabies is sparse. By 1500, rabies cases in dogs were reported in Spain, and by the late 16th century, outbreaks had reached Austria, Hungary, Flanders, and Turkey. By 1604, rabies was endemic in Paris, and by the early 1700s, wildlife-transmitted rabies had spread across Central Europe. [5]

19TH AND 20TH CENTURIES

The 19th century brought major breakthroughs in the understanding and treatment of rabies. At that time, rabies remained highly feared due to its near-universal fatality once symptoms appeared. Transmission via the bite of infected animals, particularly dogs, was well understood, and hydrophobia was recognized as a hallmark symptom. [15, 16]

A transformative moment occurred in 1885 with the work of Louis Pasteur (1822–1895), who developed the first effective rabies vaccine. His method involved attenuating the virus by passage through rabbits and preparing vaccines from dried spinal cords. The first successful human application was in July 1885, when Joseph Meister, a nine-year-old boy bitten by a rabid dog, was treated and saved. This success laid the foundation for modern vaccination and post-exposure prophylaxis. [14, 15, 17]

Although comprehensive epidemiological data on rabies in 19th-century Greece are lacking, the disease was known to be both widespread and deadly. During the period of British rule in the Ionian Islands (1815–1864), rigorous preventive regulations were established to control its spread. These included several strict measures: a) all dogs were to be kept tethered within residences, and hunting or herding dogs had to be constantly supervised by their owners; b) suburban dogs were required to remain tied from sunrise until two hours after sunset; c) stray dogs—whether truly homeless or simply wandering—were to be killed immediately; and d) guard dogs had to be restrained from early morning until 8 p.m. Furthermore, in cases where a dog displayed symptoms of rabies, behaved abnormally, or was seen being bitten by another dog, its owner had the legal obligation to report the incident to the local police authorities without delay. [18]

In August 1894, Panagiotis Pampoukis (1858–1956), a pioneering Greek microbiologist trained at the Pasteur Institute, established the "Lyssiatrion" (Anti-Rabies Clinic) in Athens. The clinic addressed rabies outbreaks in both rural and urban areas. Pampoukis secured a 15-year state funding agreement to cover treatment costs for indigent patients. Rabies in Greece at the time was transmitted by dogs, cats, foxes, and wolves. [18]

The early 20th century saw the expansion of dog vaccination programs, drastically reducing rabies cases in many regions. In the United States, national efforts included pet and wildlife vaccination, resulting in a significant drop in human infections. [19, 20] Diagnostic progress included the introduction of the direct fluorescent antibody (DFA) test in the 1950s, which enabled rapid and reliable identification of rabies in brain tissue—a gold standard still in use. [21, 22]

Despite such advances, rabies persisted in developing countries, where vaccine access and medical care were limited. Global health bodies like the World Health Organization (WHO) launched widespread campaigns for dog vaccination, public awareness, and improved access to post-exposure prophylaxis. [23]

Later in the 20th century, safer and more effective cell-culture-based vaccines—such as the human diploid cell vaccine (HDCV) and the purified chick embryo cell vaccine (PCECV)—replaced older nerve-tissue formulations, significantly reducing side effects and improving immunity. [24-28]

CONCLUSIONS

Rabies, a disease documented since ancient times, has remained a persistent threat to both human and animal health. Although enormous strides have been made in understanding, preventing, and treating rabies—particularly through vaccination, public education, and global collaboration—challenges remain, especially in rural and low-resource settings. The history of rabies is not only a medical narrative but also a testament to humanity's persistent pursuit of scientific advancement and collective action in confronting one of the oldest known and most feared diseases.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

REVIEW ARTICLE

CONTENTS

The impact of intermittent fasting on cognitive function and neuroprotection: A literature review

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ABSTRACT

Aim: To summarise current knowledge on the effects of intermittent fasting on cognitive functions and neuroprotective mechanisms, with particular attention to Alzheimer's disease and Parkinson's disease.

Materials and Methods: A narrative review based on twelve peer-reviewed publications on the effects of intermittent fasting on cognitive function, neuroprotection, and circadian rhythms.

Preclinical data and selected clinical studies indicate that intermittent fasting improves memory, attention, and executive functions, which is associated with activation of autophagy, reduction of oxidative stress, improved mitochondrial function, and increased levels of brain-derived neurotrophic factor. In Parkinson's disease, intermittent fasting limits alpha-synuclein aggregation and protects dopaminergic neurons, whereas in Alzheimer's disease it reduces beta-amyloid deposition and enhances synaptic plasticity. Intermittent fasting also influences the gut–brain axis and circadian rhythm alignment, which may further support neuroprotection.

Conclusions: Intermittent fasting is a promising adjunct strategy in the management of neurodegenerative diseases. However, well-designed, randomised clinical trials are needed to confirm its effectiveness and safety.

KEY WORDS: Alzheimer's disease, Parkinson's disease, intermittent fasting, cognitive function, neuroprotection.

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INTRODUCTION

Neurodegenerative diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD) represent a growing public health challenge in aging populations. Current treatment options remain limited in efficacy, prompting increased interest in preventive and supportive therapeutic strategies, including lifestyle modifications and dietary interventions. One such promising approach is intermittent fasting (IF), which has gained attention for its potential benefits on cognitive performance and neuroprotection.

Evidence from both preclinical and some clinical studies indicates that IF may improve memory, attention, and executive function. These cognitive improvements are thought to be mediated by multiple biological mechanisms, including the activation of autophagy, upregulation of brain-derived neurotrophic factor (BDNF), reduction in oxidative stress and neuroinflammation, and enhanced mitochondrial efficiency. In animal models, IF has been associated with reduced alpha-synuclein aggregation and preservation of dopaminergic neurons, suggesting therapeutic potential in PD. In the context of AD, IF has shown benefits in promoting synaptic plasticity and decreasing amyloid-beta deposition.

The aim of this literature review is to assess the current evidence on the effects of intermittent fasting on cognitive function and neuroprotective mechanisms, with a particular focus on its role in Parkinson's and Alzheimer's diseases.

MATERIALS AND METHODS

This narrative literature review aimed to identify and synthesize evidence on the effects of intermittent fasting (IF) on cognitive function and neuroprotective mechanisms, with a focus on Alzheimer's disease (AD) and Parkinson's disease (PD).

The search strategy utilized keywords such as "intermittent fasting,""cognitive function,""neuroprotection," "Parkinson's disease," and "Alzheimer's disease".

Twelve eligible peer-reviewed articles were included in the synthesis.

REVIEW AND DISCUSSION

EFFECTS OF INTERMITTENT FASTING ON COGNITIVE PERFORMANCE

Intermittent fasting (IF) refers to a dietary pattern characterized by alternating periods of energy restriction and normal feeding. The most common protocols include alternate-day fasting, time-restricted eating (TRE), and the 5:2 regimen, in which calorie intake is significantly reduced on two non-consecutive days per week [4, 10, 11]. Unlike chronic caloric restriction, IF aims to induce metabolic and cellular adaptations without continuous energy deficit [6].

Several studies have examined the impact of IF on cognitive performance across different populations and experimental models. In a three-year longitudinal study involving older adults with mild cognitive impairment (MCI), Ooi et al. observed that an IF-based nutritional intervention led to significant improvements in global cognition, memory recall, and executive functioning compared to the control group [5]. Similarly, Alkurd et al. reported in their systematic review that intermittent fasting regimens were associated with increased levels of brain-derived neurotrophic factor (BDNF) and improved cognitive outcomes in both healthy individuals and those at risk of neurodegeneration [6].

In preclinical studies, IF has demonstrated the ability to reverse diet-induced cognitive deficits. Lee et al. showed that mice fed a high-fat diet exhibited impaired spatial memory and increased neuroinflammatory markers, both of which were ameliorated by alternate-day fasting. The intervention downregulated proinflammatory proteins such as lipocalin-2 and galectin-3, suggesting a mechanistic link between IF and reduced neuroinflammation [3].

Although evidence from human trials remains limited, current findings suggest that IF may exert beneficial effects on attention, learning, and memory, particularly in aging populations or individuals with early cognitive decline. Further large-scale randomized controlled trials are needed to validate these findings and to determine optimal fasting protocols for cognitive enhancement.

Beyond short-term improvements in cognitive domains, some studies suggest that IF may exert long-term effects on brain plasticity and resilience to age-related decline. Elias et al. [4] reported in their scoping review that IF was consistently associated with enhanced learning, better processing speed, and attention in both healthy and cognitively impaired populations. These effects were hypothesized to arise from modulations in insulin sensitivity, increased synaptic activity, and enhanced neuronal survival pathways.

Interestingly, Mayor [9] highlighted the neurotrophic effects of intermittent fasting, calorie restriction, and exercise, suggesting that different interventions may exert varying influences on cognition and mood. However, specific comparisons between TRE and the 5:2 protocol remain limited. Although mechanistic explanations remain under investigation, these findings point to differential neural adaptations triggered by specific fasting regimens.

Importantly, cognitive benefits of IF may also be mediated by better circadian alignment, reduced systemic inflammation, and modulation of the gut–brain axis, which has been implicated in regulating mood and cognition. These multidimensional effects highlight the potential of IF not only as a metabolic strategy, but also as a holistic approach to preserving cognitive function across the lifespan [10].

NEUROPROTECTIVE MECHANISMS OF INTERMITTENT FASTING

The neuroprotective potential of intermittent fasting (IF) has been increasingly recognized across multiple models of aging and neurodegeneration. Several studies have highlighted the biological pathways through which IF exerts its beneficial effects on the central nervous system (CNS), including autophagy activation, modulation of oxidative stress, mitochondrial biogenesis, neurotrophic factor upregulation, and gut–brain axis regulation.

One of the most consistently reported mechanisms is the stimulation of autophagy, a cellular recycling process essential for the clearance of damaged proteins and organelles. Dong et al. observed that IF significantly enhanced autophagic activity in aged rodent brains, which correlated with improved neuronal survival and synaptic integrity [7]. This finding is particularly relevant in the context of Alzheimer's and Parkinson's diseases, where defective autophagy contributes to protein aggregation and neuronal death.

Oxidative stress reduction is another critical neuroprotective mechanism associated with IF. Hein et al. reported that preclinical studies indicate that intermittent energy restriction modulates the activity of antioxidant enzymes, including superoxide dismutase and catalase, thereby lowering reactive oxygen species (ROS) levels in the brain [10]. These effects may counteract age-re-

lated mitochondrial dysfunction and support redox homeostasis.

IF has also been shown to improve mitochondrial function and stimulate mitochondrial biogenesis via pathways involving AMP-activated protein kinase (AMPK) and sirtuin-1 (SIRT1). These enzymes not only support energy homeostasis but also enhance neuronal plasticity and delay the progression of neurodegenerative pathology [10].

Neurotrophic factor regulation plays a complementary role in the neuroprotective profile of IF. Multiple studies have demonstrated that IF increases brain-derived neurotrophic factor (BDNF) levels, which promote neurogenesis, dendritic growth, and synaptic remodeling [6, 9]. The upregulation of BDNF is believed to underlie improvements in learning and memory observed in both human and animal models.

An emerging area of interest is the interaction between intermittent fasting and the gut–brain axis. Hein et al. suggested that IF influences the composition of gut microbiota, which in turn modulates systemic inflammation, short-chain fatty acid (SCFA) production, and microglial activation in the CNS [10]. These changes may support brain health and reduce neuroinflammatory responses implicated in Alzheimer's and Parkinson's diseases.

Finally, fasting-induced elevation of ketone bodies - particularly beta-hydroxybutyrate (BHB) - has been shown to provide alternative energy substrates for neurons and to exhibit signaling properties that promote neuronal resilience. Szegő et al. reported that BHB supplementation mimicked several beneficial effects of IF in a Parkinson's disease mouse model, including improved synaptic function and reduced alpha-synuclein aggregation [1].

Collectively, these mechanisms suggest that intermittent fasting exerts a multimodal neuroprotective effect, targeting metabolic, inflammatory, and regenerative pathways. Understanding these mechanisms may help develop dietary strategies tailored to prevent or slow neurodegeneration.

INTERMITTENT FASTING AND PARKINSON'S DISEASE

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by dopaminergic neuronal loss in the substantia nigra, aggregation of alpha-synuclein (α-syn) into Lewy bodies, and a broad spectrum of motor and non-motor symptoms. Although dopaminergic therapies remain the standard of care, they primarily provide symptomatic relief, and effective disease-modifying treatments are lacking. Recent experimental studies have suggested that intermittent fasting (IF) may offer neuroprotective benefits through metabolic, anti-inflammatory, and regenerative mechanisms [2].

In a pivotal study by Szegő et al. [1], alternate-day fasting was applied in a transgenic mouse model overexpressing human alpha-synuclein, which mimics key pathological features of PD, including dopaminergic neuronal loss and motor dysfunction. IF significantly reduced α-syn pathology, preserved substantia nigra neurons, and improved striatal synaptic activity. Behavioral analysis also demonstrated enhanced motor coordination and exploratory behavior. Mechanistically, these outcomes were linked to increased autophagic flux and upregulation of genes involved in synaptic plasticity and mitochondrial dynamics.

A review by Neth et al. [2] highlighted several converging mechanisms by which IF may exert neuroprotective effects in PD models. These include reduced oxidative stress, enhanced mitochondrial biogenesis via AMPK/SIRT1 signaling, and increased expression of brain-derived neurotrophic factor (BDNF), all of which promote neuronal survival and delay dopaminergic degeneration. The authors also emphasized IF's potential to modulate brain glucose metabolism and improve synaptic resilience.

Lei and Chen [8] further discussed the relevance of gut-brain axis dysfunction in PD pathogenesis and how IF may act to restore intestinal microbiota, reduce systemic inflammation, and prevent aberrant α-syn propagation from the gut to the brain. This emerging area supports the hypothesis that IF may interrupt early, peripheral drivers of neurodegeneration.

Another mechanism of interest is the elevation of ketone bodies during fasting, particularly beta-hydroxybutyrate (BHB). BHB serves not only as an energy substrate but also as an epigenetic regulator and anti-inflammatory molecule. In the study by Szegő et al. [1], exogenous BHB supplementation in a PD mouse model mimicked several benefits of intermittent fasting, including improved synaptic function and reduced α-synuclein aggregation.

While preclinical findings are promising, clinical evidence remains sparse. To date, no large-scale randomized controlled trials have evaluated the efficacy of IF in patients with Parkinson's disease. The potential clinical utility of IF in PD is therefore hypothetical, and future studies are needed to assess feasibility, safety, and possible interactions with pharmacological treatment regimens [1, 2, 8, 10].

INTERMITTENT FASTING AND ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder globally, characterized by

progressive memory loss, cognitive decline, and behavioral changes. The neuropathological hallmarks of AD include extracellular deposition of amyloid-beta (Aβ) plaques, intracellular neurofibrillary tangles of hyperphosphorylated tau protein, synaptic loss, and widespread neuroinflammation. Despite decades of research, therapeutic strategies capable of halting or reversing AD progression remain elusive. Recent literature suggests that intermittent fasting (IF) may offer neuroprotective benefits by modulating several of the key pathophysiological processes underlying AD [4-6, 10].

Elias et al. [4], in their scoping review, summarized evidence indicating that IF can enhance cognitive performance and delay neurodegenerative progression in individuals at risk of or diagnosed with AD. Animal studies have shown that IF attenuates Aβ plaque accumulation, improves hippocampal synaptic plasticity, and reduces markers of oxidative stress. Similar effects were observed in preclinical models with time-restricted feeding (TRF), which promoted spatial memory retention and long-term potentiation.

Ooi et al. [5], in a progressive three-year study involving older adults with mild cognitive impairment (MCI), reported that IF-based interventions improved memory recall and executive functioning. The authors hypothesized that improved metabolic flexibility and enhanced insulin sensitivity could contribute to reduced central insulin resistance, a condition increasingly recognized in the pathogenesis of AD.

The neuroprotective mechanisms of IF in AD appear to overlap with those identified in Parkinson's disease and include enhanced autophagy, reduction of oxidative and inflammatory damage, increased expression of brain-derived neurotrophic factor (BDNF), and improved mitochondrial function [6,10]. Additionally, IF-induced ketone production, particularly beta-hydroxybutyrate (BHB), provides alternative energy substrates to glucose-hypometabolic regions of the AD brain, thereby preserving neuronal activity and reducing excitotoxicity.

A growing body of evidence supports the role of central insulin resistance in the pathogenesis of AD, often referred to as "type 3 diabetes." Impaired insulin signaling in the brain leads to reduced glucose uptake, mitochondrial dysfunction, and tau hyperphosphorylation. In their longitudinal study, Ooi et al. [5] hypothesized that the cognitive benefits of IF in older adults with MCI may be partly explained by restored insulin sensitivity in the hippocampus and cerebral cortex. Similarly, Hein et al. [10] suggested that intermittent fasting modulates insulin and IGF-1 pathways in the brain, improving neuronal glucose utilization and enhancing energy efficiency under fasting conditions.

Emerging evidence also highlights the role of IF in regulating the gut–brain axis in AD, modulating the intestinal microbiota and decreasing systemic inflammation [10]. Dysbiosis and elevated peripheral inflammation have been linked to amyloid deposition and cognitive dysfunction. IF may counteract these processes by restoring microbial diversity and reinforcing intestinal barrier integrity.

Although promising, most evidence supporting the beneficial effects of IF in AD originates from animal studies and observational data. High-quality randomized controlled trials in patients with early-stage or mild-to-moderate AD are still lacking. Future clinical studies should evaluate the long-term cognitive and functional outcomes of IF and assess its feasibility and safety in vulnerable populations.

CHRONONUTRITION AND CIRCADIAN ALIGNMENT IN INTERMITTENT FASTING

Emerging research highlights the pivotal role of circadian rhythms in mediating the neuroprotective effects of intermittent fasting (IF). The circadian system, governed by a central clock in the suprachiasmatic nucleus and peripheral clocks in various tissues, orchestrates numerous physiological processes, including metabolism, hormone secretion, sleep-wake cycles, and neuronal activity. Disruptions in circadian alignment have been associated with increased risk of cognitive decline, neurodegeneration, and metabolic dysregulation.

Intermittent fasting, particularly time-restricted eating (TRE), has been shown to restore circadian synchrony by aligning feeding-fasting cycles with endogenous biological rhythms. Santos et al. [11] reported that early TRE (eTRE; e.g., 8 a.m. – 4 p.m.) improves insulin sensitivity and reduces oxidative stress, consistent with better circadian alignment.

The gut–brain axis represents a critical mediator of these chronobiological effects. Daas and de Roos [12] reported that IF induces diurnal oscillations in gut microbiota composition and function, notably increasing the abundance of short-chain fatty acid (SCFA) - producing bacteria during the fasting period. These microbial shifts enhance SCFA signaling, improve blood–brain barrier integrity, and modulate microglial activity, thereby promoting anti-inflammatory and neuroprotective responses. Moreover, bile acid metabolism - tightly linked to feeding rhythms - was also shown to be restored under IF regimens, contributing to circadian alignment.

Importantly, circadian misalignment - common in aging populations and neurodegenerative diseases

such as Alzheimer's - has been associated with impaired clearance of neurotoxic proteins during sleep and increased neuroinflammation. By promoting synchrony between central and peripheral clocks, IF may support glymphatic clearance, mitochondrial efficiency, and synaptic homeostasis, thereby protecting against cognitive deterioration [10].

Although clinical data remain limited, existing preclinical and translational studies support the hypothesis that IF may exert cognitive benefits not solely through caloric restriction, but by restoring temporal order to metabolic and neural pathways. The integration of chrononutrition principles into fasting regimens may therefore enhance their neuroprotective potential, particularly in individuals at risk of circadian dysregulation.

LIMITATIONS OF CURRENT EVIDENCE

Although the potential cognitive and neuroprotective benefits of intermittent fasting (IF) have been extensively explored in preclinical models and several observational studies, the current body of evidence remains limited in several key aspects. Most of the available data are derived from animal studies, which, although mechanistically informative, may not fully replicate the complexity of human neurodegenerative diseases or dietary adherence in real-world settings [1, 4, 6].

Human trials investigating IF in cognitive decline are relatively few and often involve small sample sizes, short intervention periods, and limited follow-up [5, 10]. Furthermore, the heterogeneity in IF protocols—ranging from alternate-day fasting to time-restricted eating—complicates cross-study comparisons and hampers the identification of an optimal regimen. Many studies also lack proper blinding, standardized neurocognitive assessments, or control for confounding lifestyle variables such as sleep, stress, and physical activity [6, 9].

Another critical gap is the absence of large-scale randomized controlled trials (RCTs) specifically targeting populations with Alzheimer's disease (AD), Parkinson's disease (PD), or mild cognitive impairment (MCI). While promising trends have been noted in early-stage cognitive decline, the generalizability of findings to patients with established neurodegenerative pathology remains uncertain [4, 10].

Additionally, adherence to IF in older adults or those with neurodegenerative conditions may pose practical challenges, including risks of malnutrition, medication timing issues (especially in PD), and potential exacerbation of frailty. Ethical and logistical considerations often preclude long-term fasting interventions in vulnerable populations, further limiting data availability [2, 5].

Taken together, these limitations underscore the need for rigorously designed, adequately powered RCTs to evaluate the cognitive and neuroprotective efficacy of IF. Standardization of protocols, comprehensive outcome measures, and long-term follow-up will be essential to establish clinical guidelines and ensure safe implementation.

CONCLUSIONS

Intermittent fasting (IF) has emerged as a promising non-pharmacological intervention with potential cognitive and neuroprotective benefits. Preclinical and early clinical evidence suggests that IF may attenuate key pathological processes involved in Alzheimer's disease and Parkinson's disease, including oxidative stress, mitochondrial dysfunction, neuroinflammation, and impaired autophagy. Furthermore, IF improves insulin sensitivity in the central nervous system and modulates gut-brain axis signaling - both increasingly recognized as relevant in neurodegenerative disorders.

Recent findings also highlight the importance of circadian alignment as a mediator of the beneficial effects of IF. By restoring synchrony between feeding patterns and endogenous biological rhythms, IF may enhance neuroplasticity, circadian alignment, and metabolic resilience in aging populations.

Although the majority of current evidence is derived from animal models and observational studies, selected human trials - particularly in individuals with mild cognitive impairment - suggest improvements in memory, executive function, and metabolic health. Compared to other dietary strategies, IF appears to exert its effects through dynamic metabolic switching, chrononutritional entrainment, and cellular stress adaptation, which may uniquely enhance neuronal resilience.

Nevertheless, critical gaps remain in the literature. High-quality randomized controlled trials are urgently needed to assess the long-term cognitive and functional outcomes of IF, its optimal implementation protocols, and its safety in older and vulnerable populations. Future research should also explore its synergistic effects with pharmacological therapies and other lifestyle interventions.

In conclusion, IF represents an encouraging avenue for neuroprotection and cognitive preservation. With growing interest in lifestyle-based strategies for brain health, intermittent fasting holds the potential to complement existing approaches in the prevention and management of neurodegenerative disorders [4–6, 10–12].

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

The right to health care in the system of constitutional human rights: Theoretical and legal analysis of content and structure

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ABSTRACT

Aim: Comprehensive analysis of the constitutional and legal content of the right to health care, determination of its structural elements, and formation of a scientifically grounded classification of powers that constitute this fundamental human right.

Materials and Methods: The methodological basis of the study was the system-structural, comparative-legal, formal-logical methods and legal modeling. The empirical base consisted of 156 sources for the period 2000-2025: constitutional norms of Ukraine and foreign countries (15 documents), legislative acts in the field of health care (28 acts), international legal documents on human rights (12 conventions and declarations), scientific works of domestic and foreign researchers (101 publications). The search was carried out in the following databases: Google Scholar, ResearchGate, V.I. Vernadsky National Library of Ukraine.

Conclusions: The research established that the right to health care represents a complex legal formation with three structural components requiring not only normative consolidation but also effective implementation mechanisms under contemporary challenges.

KEY WORDS: health care, constitutional rights, rights and freedoms of a person and a citizen, legal protection

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INTRODUCTION

The issue of guaranteeing the constitutional and legal status of individuals and citizens occupies a leading position in contemporary theoretical studies of constitutional law and the practical application of constitutional norms. The concept of the constitutional and legal position of human beings and citizens forms the fundamental principles of interaction between individuals, state institutions, and society, defining the powers and mutual obligations of all parties to these legal relations. The right to health care holds particular significance in the system of constitutional human rights, occupying a central place among social rights and serving as a necessary prerequisite for the realization of other constitutional rights and freedoms.

The relevance of studying the right to health care is determined by several factors. First, health as an intangible good is the foundation of human existence and a prerequisite for the full functioning of personality in society. Second, contemporary challenges related to the COVID-19 pandemic, environmental threats, and socio-economic crises have actualized the issue of the effectiveness of legal provision of health care. Third, there is a need to improve the theoretical foundations of understanding the content and structure of the right to health care in accordance with modern international standards and national features of Ukraine's legal system.

AIM

Comprehensive analysis of the constitutional and legal content of the right to health care, determination of its structural elements, and formation of a scientifically grounded classification of powers that constitute this fundamental human right.

MATERIALS AND METHODS

The methodological foundation of the study was a complex of general scientific and special legal methods of cognition. The systemic-structural method was applied to analyze the right to health care as a complex legal formation and to identify its structural components. The comparative legal method was used to compare different theoretical approaches to understanding the

legal status of individuals and the content of the right to health care. The formal-logical method allowed for the formulation of an author's definition of the right to health care and classification of corresponding powers. The method of legal modeling was applied to create a structural model of the right to health care. The empirical base of the study consisted of provisions of the Constitution, legislative acts in the field of health care, international legal documents on human rights, as well as scientific works of domestic and foreign researchers in the field of constitutional and medical law.

The authors conducted a descriptive analytical study of the constitutional and legal content of the right to health care using a complex of general scientific and special legal methods of cognition.

Criteria for inclusion of materials: for regulatory legal acts (current constitutions and basic laws of Ukraine and EU countries), legislative acts in the field of health care adopted after 2010 (international conventions and declarations on human rights ratified by Ukraine); for scientific publications: publications in peer-reviewed publications for 2000-2025, research on constitutional and medical law; works devoted to the structure and content of the right to health care, publications in Ukrainian, Russian and English.

Data sources: Google Scholar, ResearchGate, electronic catalog of the V.I. Vernadsky National Library of Ukraine, official websites of international organizations (WHO, Council of Europe, UN).

The volume of analyzed materials: total number of sources: 156, constitutional and basic legislative acts: 15 (Constitution of Ukraine, constitutions of 8 EU countries, 6 profile laws), special legislation in the field of health care: 28 acts, international legal documents: 12 (including the Universal Declaration of Human Rights, the European Convention on Human Rights, the International Covenant on Economic, Social and Cultural Rights), scientific publications: 101 (of which 67 are domestic and 34 are foreign).

The study was conducted during 2023-2025, while sources for the period from 2000 to 2025 were analyzed in order to ensure the relevance and completeness of the study.

REVIEW AND DISCUSSION

As a result of the conducted research, an author's definition of the right to health care has been formulated as a fundamental human right that consists in the ability to use all available social goods to preserve health in an optimal state and obtain qualified medical assistance when needed. A structural model of the right to health care has been developed, which includes three main components: the right to health preservation, the right to health restoration, and the right to protection and compensation for health damage. A key problem of legal provision has been identified, which lies in the gap between normative consolidation of rights and the creation of effective mechanisms for their practical implementation.

The issue of guaranteeing the constitutional and legal position of individuals and citizens occupies a central place both in theoretical studies of constitutional law and in the practical application of constitutional norms. The reason for such significance lies in the fact that the concept of "constitutional and legal position of human beings and citizens" establishes fundamental principles of relations between individuals, state institutions, and society as a whole. Within its framework, the powers and mutual obligations of parties are defined, and a mechanism for ensuring, guaranteeing, and protecting the rights of all subjects of these legal relations is formed [1].

Legal theorists distinguish four conceptual approaches to understanding the legal status of individuals. According to the first, legal status is reduced to legal capacity. The second approach emphasizes the normative character of status through the consolidation of rights and obligations in legal acts. The third concept integrates legal capacity with normatively defined rights and obligations. The fourth, broadest approach, interprets legal status as a comprehensive system of legally formalized and state-protected rights, freedoms, obligations, and responsibilities that serves as a guideline for the social behavior of legally capable individuals [2].

Thus, central concepts in characterizing the legal status of individuals are subjective rights, legal obligations, and legal capacity. Legal capacity encompasses three key qualities of a legal subject: the ability to possess rights and obligations (legal capacity), the ability to personally acquire and implement them (legal competence), and readiness to bear responsibility for violation of obligations (delictual capacity). Legal science interprets the nature of delictual capacity differently: some scholars see it as an element of legal capacity, others as a component of legal competence, and still others distinguish it as an autonomous component of legal capacity [3, p. 61].

Constitutional and legal science has developed a wide spectrum of definitions of the constitutional and legal status of human beings. Systematizing various scientific views on its nature, it is appropriate to present concepts that reflect the essence of two key theoretical directions. For example, scholar O. Sovhyria characterizes the constitutional and legal status of human beings and citizens as a constitutionally and legislatively

established complex of legal indicators that regulates the actual place of human beings and citizens in the system of social, political, economic, legal, and other connections. Such interpretation emphasizes that legal status is the legally formalized position of an individual within the state and social system [4].

The conceptual foundation of the right to health care is such an intangible good as health. Today, legal and medical sciences offer numerous definitions of health. The substantive content of this concept depends on the specific subject with which it is associated. Scientific discourse includes the concepts of "public health," "national health," "population health," "community health," and "personal health." Common to all these categories is the basic concept of health, but they differ in the level of generalization: increasing scale (increasing the number of individuals in a group) is accompanied by complication of the system of factors that determine its formation [5,].

At the same time, health determinants are understood as a multi-component system of individual, socio-economic, and environmental factors that shape the health of both individual persons and entire populations. This system encompasses a wide spectrum of parameters: behavioral models and lifestyle, economic status and income level, educational potential, working conditions and their safety, housing quality, employment stability, accessibility and quality of medical services, and the ecological situation in the region of residence. An important feature of health determinants is their systemic and synergetic character of influence [6]. Considering the multiplicity, diversity, and variability of external determinants, it is worth supporting the position of scholar O. Shamych, according to which health constitutes the highest possible and optimal physical and mental state of the human organism, necessary to ensure biological functioning, the possibility of long active life, and the birth of healthy offspring. Thus, health represents an ideal state of the human organism, a standard to achieve and a kind of basic guideline [7].

For our research, it is important to understand individual health as a personal intangible good. Personal non-property goods are goods without monetary value that are inseparable from human beings, recognized in society, and protected by law. Most such goods, particularly health, are innate human qualities regulated by civil law. At the same time, the right to health belongs to personal non-property rights that ensure protection of personal inviolability [8].

Health is defined as a state of absolute physical, mental, and social well-being that goes beyond the mere absence of pathologies and physical anomalies. However, the category of "well-being" has a subjective-evaluative nature. Lexicographic sources interpret this concept through the related evaluative category of happiness or characterize it as a harmonious life course without crisis moments and shocks, economic security and material prosperity, standard state of an individual without deviations from the norm and negative manifestations [9].

Physical health is characterized as optimal functioning of the human organism at all structural levels - from cellular to systemic, which presupposes proper functioning of organs and tissues and is determined through a complex of various indicators [10].

Mental health is characterized as a state of brain activity that ensures adequate emotional, intellectual, and conscious-volitional interaction with the surrounding world. Structurally, mental health includes intellectual, emotional, and characterological components. A person is in a state of mental well-being when they possess a developed emotional sphere, high levels of attention, perception, thinking, and memory, and are able to be guided by their own consciousness, character, and will for adequate manifestation of these qualities in various life circumstances [10].

Social health is defined as a complex of social conditions and interpersonal relations of a person in social space that harmonize with natural laws and create favorable prerequisites for the full development of life activity and human activity. It is precisely the social component of health that most clearly demonstrates the conditionality of individual health by external determinants of various nature - both objective and subjective factors generated in the process of social interaction of personality with society as a collective of individualities, each characterized by unique personal qualities [10].

Let us proceed to analyze the content of the second component of the research subject, namely, establish a precise definition of the concept of protection. In contemporary jurisprudence, there is no consolidated theoretical approach to defining the essence of protection - this concept remains insufficiently developed both at the level of individual legal branches and within general theoretical jurisprudence.

The Academic Dictionary of the Ukrainian language also does not offer an independent definition of the concept "protection," using a similar methodology for its explanation, that is, interpretation through the verb "to protect," which has the following meanings: 1) to defend someone or something from danger, ensure security from the threat of attack, encroachment, etc.; 2) to be on guard near someone or something; to stand watch, guard; 3) to guarantee, ensure the inviolability of someone or something; 4) to protect from destruction,

annihilation, causing harm, etc.; 5) to safeguard from something [11].

Researchers provide different definitions of health care, but essentially their approaches to understanding its nature are similar. In particular, I. Seniuta interprets health care as a general obligation and one of the key tasks of the state and society, as one of the priority vectors of state policy oriented toward forming an effective system of social protection, improving population health, preventing demographic crisis, enhancing quality of life and environmental conditions, as well as modernizing the health care system at the current stage of Ukraine's development [12].

In turn, S. Buletsa interprets health care as identification, prevention, and elimination of diseases, early diagnosis, therapeutic measures, improvement of patient condition, prevention of deterioration through preventive examinations and treatment, qualified care, medical rehabilitation, provision of patients with medications and other therapeutic means; a complex of state and public measures aimed at protecting health, preventing and treating diseases, and ensuring human longevity [13].

The state develops health care policy and guarantees its implementation. State policy in the field of health care is oriented toward practical implementation of constitutional norms and legislative provisions of Ukraine regarding guaranteeing accessible quality medical assistance for every citizen of Ukraine, implementing new effective mechanisms of financing and management in health care, creating prerequisites for cultivating a healthy lifestyle.

O. Punda provides a definition of the health care institution in an objective sense and proposes to interpret it as a complex of legal norms that regulate relations regarding preservation, maintenance, and strengthening of health [14].

Based on the legislatively fixed definition of the concept of health care, within the framework of these legal relations, two main types can be distinguished: legal relations concerning health preservation and legal relations related to its restoration. In this regard, for example, V. Moskalenko, T. Hruzieva, and H. Inshakova note that the concept "right to health care" in its content includes rights and a number of conditions, particularly socio-economic ones, without which ensuring health is impossible [15].

N. Korobtsova characterizes the right to health care as a legislatively fixed and state-guaranteed possibility for every person to freely possess, use, and dispose of their own health as a personal good, as well as to engage state and other resources for health preservation and protection [16].

In connection with this, there arises a need to analyze another concept developed within legal doctrine - the concept of human rights in the sphere of medical activity. We note that human rights in the sphere of medical activity represent normative provisions fixed in international and domestic legal acts that guarantee individuals health care and provision of medical assistance upon the onset of disease [17]. The predominant part of subjective rights in the field of medical activity arises in a person precisely from the moment of seeking medical assistance, therefore these rights represent an additional aspect of the right to health care and are included in its substantive content.

One of the key problems of legal support for implementing the right to health care is the state of development of legislation in the medical sphere. The quality of normative-legal regulation of human rights in the field of health care largely determines the successful and progressive development of both medical law in particular and the state in general. The experience of many contemporary states shows that one can have quite developed and exemplary legislation in the field of health care, but this does not guarantee that every person can actually use the rights granted to them. There can be many reasons for this, but one of the main ones is unresolved issues of ensuring human rights in the medical sphere. Indeed, the most perfect legal norms in the field of health care, but not supported by a system of provision, turn into declarations, that is, norms not accompanied by creating a mechanism for their implementation, protection, and defense [18].

Taking into account the entire complex of characteristics of the right to health care considered above, we propose the following definition: "The right to health care represents one of the fundamental human rights that is ensured at the level of international legal documents and national legislation of Ukraine and consists in the possibility for a person to use all available social goods to preserve health in a state as close to ideal as possible, considering individual possibilities of the person regarding maintaining the proper level of physical, mental, and social health determinants, as well as, when needed, obtaining qualified medical assistance and applying other means of health restoration and protection."

Research on the right to health care cannot be considered complete without determining its substantive content. Thus, in particular, every citizen possesses the right to health care, which includes: a) standard of living that encompasses nutrition, clothing, housing, medical care, and social protection necessary to maintain personal health; b) ecologically safe environment; c) sanitary-epidemiological well-being of territory and place of resi-

dence; d) safe and healthy conditions of work, education, daily life, and recreation; e) quality medical assistance, including the right to free choice of doctor, treatment methods according to their recommendations, and medical institution; f) objective and prompt information about their own health condition and population health, including data on existing and potential risk factors; g) participation in discussing draft laws and making proposals regarding state policy in the field of health care; h) participation in managing the health care system and conducting public expertise according to legislation; i) possibility of creating public organizations to promote health care; j) legal protection from illegal discrimination based on health status; k) compensation for health damage; l) appealing unlawful decisions of medical workers and institutions; m) possibility of independent medical expertise when disagreeing with official conclusions or when applying compulsory treatment; n) right of hospitalized patients to visits by medical workers, relatives, guardians, notary, lawyer, and clergy.

Based on the formulated definition of the right to health care, all its essential characteristics considered above, and legislatively established provisions, let us determine the content of the right to health care through forming a complete list of powers that constitute it. Based on the content of the normatively established definition of the concept of health care, we propose to classify all existing rights in the field of health care according to the nature of legal relations arising in this sphere into the following categories: the right to health preservation and the right to health restoration. Additionally, as noted earlier, an inseparable component of every subjective right is the right to protection, therefore we consider that the third category is formed by powers that in content are encompassed by the right to protection and compensation for health damage.

Thus, we propose to distinguish in the architectonics of the right to health care: the right to health preservation, the right to health restoration, as well as the right to protection and compensation for health damage. Each of these components includes certain powers. In particular, the right to health preservation encompasses such powers as: the right to an adequate standard of living, including nutrition, clothing, housing, medical care, and social protection necessary to maintain human health; the right to an ecologically safe natural environment; the right to sanitary-epidemiological well-being of territory and place of residence; the right to safe and healthy conditions of work, education, daily life, and recreation; the right to objective and prompt information about one's own health condition and population health, including existing and potential risk factors and their level.

The right to health restoration in content includes the right to qualified medical assistance and, accordingly, the entire complex of patients' rights, particularly the right of hospitalized patients to access by other medical specialists, family members, guardian, caretaker, notary and lawyer, as well as clergy for conducting worship and religious rites.

The right to protection and compensation for health damage encompasses: legal protection from any illegal forms of discrimination based on health status; the right to participate in discussing draft laws and making proposals regarding formation of state policy in the field of health care; the right to participate in managing the health care system and conducting public control according to legislation; the right to create public organizations to promote health care; the right to independent medical expertise when disagreeing with conclusions of state expertise, application of compulsory treatment, and in other cases of human rights violations by actions of medical workers; the right to appeal unlawful decisions and actions of medical workers, institutions, and health care bodies; the right to compensation for health damage; the right to medical insurance.

The inclusion of the constitutional right to medical insurance requires separate argumentation. In support of this position, legislative norms testify that insurance is characterized as a type of civil legal relations regarding protection of property interests of physical and legal persons upon occurrence of defined events (insurance cases) established by insurance contract or current legislation, at the expense of financial funds created through payment by physical and legal persons of insurance payments (insurance contributions, insurance premiums) and profits from investing funds of these funds.

CONCLUSIONS

The conducted research has allowed formulation of a comprehensive understanding of the constitutional and legal content of the right to health care as one of the fundamental human rights. It has been established that the right to health care represents a complex legal formation that is ensured at the level of international legal documents and national legislation and consists in the possibility for a person to use all available social goods to preserve health in a state as close to ideal as possible, considering individual possibilities of the person regarding maintaining the proper level of physical, mental, and social health determinants, as well as, when needed, obtaining qualified medical assistance and applying other means of health restoration and protection.

Analysis of the content of the right to health care has allowed identification of three main structural components: the right to health preservation, the right to health restoration, and the right to protection and compensation for health damage. The right to health preservation encompasses powers regarding ensuring adequate standard of living, ecologically safe environment, sanitary-epidemiological well-being, safe working and living conditions, as well as access to reliable information about health status. The right to health restoration includes the right to qualified medical assistance and the entire complex of patients' rights. The right to protection and compensation for damage ensures legal protection from discrimination, participation in forming health care policy, possibility of appealing unlawful actions of medical workers, and compensation for damage caused.

The issue of practical implementation of the right to health care becomes of particular significance, since even the most perfect legal norms without an effective system of their provision remain declarative. Therefore, further research should be directed toward developing mechanisms for guaranteeing and protecting the right to health care, improving the medical insurance system as a component of the right to health protection, as well as adapting national legislation to contemporary challenges in the field of health care. The proposed structuring of the right to health care can serve as a theoretical foundation for further development of medical law and improvement of constitutional and legal regulation of relations in the field of health care in Ukraine.

The research results confirm the necessity of a comprehensive approach to understanding the right to health care as a multi-aspect constitutional right that requires not only normative consolidation but also creation of effective mechanisms for its implementation, protection, and defense under conditions of contemporary socio-economic and environmental challenges.

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REVIEW ARTICLE

CONTENTS

Advancements in diagnostic medicine: A comprehensive review of emerging technologies and techniques

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ABSTRACT

Aim: The integration of newly developing technologies is driving a transformative shift in diagnostic medicine by enhancing the speed, accuracy, and personalization of disease diagnosis. Traditional diagnostic methods, such as radiography, histology, and biochemical testing, often face limitations in efficiency, sensitivity, and specificity. This review aims to explore recent advancements in diagnostic tools and techniques.

Materials and Methods: This review examines the latest developments in diagnostic medicine, including digital pathology, molecular diagnostics, and wearable technologies. Digital pathology enables the digitization of slides and the application of Artificial Intelligence (AI) for precise and timely diagnoses, particularly in oncology. Advances in molecular diagnostics, such as genomic and proteomic profiling, are evaluated for their role in improving disease detection and monitoring. The study also highlights emerging trends in telemedicine, personalized medicine, and precision medicine.

Conclusions: Recent technological innovations are revolutionizing diagnostic medicine, offering unprecedented opportunities to enhance healthcare delivery. Digital pathology and molecular diagnostics are setting new standards for accuracy and efficiency, while wearable technologies and telemedicine are expanding access to personalized care. These advancements underscore the transformative potential of integrating cutting-edge technologies into diagnostic practices, paving the way for a future of more effective and patient-centered healthcare.

KEY WORDS: molecular diagnostics, digital pathology, emerging technologies, artificial intelligence.

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INTRODUCTION

The landscape of diagnostic medicine is evolving rapidly, driven by advances in technology and an increasing demand for more accurate, efficient, and personalized healthcare solutions. Diagnostic medicine plays a pivotal role in the healthcare system, serving as the cornerstone for disease detection, management, and monitoring. Traditionally, diagnostic techniques such as histopathology, radiology, and biochemical tests have been the mainstays of clinical diagnostics. These methods, while invaluable, have inherent limitations in terms of sensitivity, specificity, and the time required to obtain results. As the complexity of diseases increases and the emphasis on personalized medicine grows, the need for innovative diagnostic approaches has become increasingly apparent. Histopathology and cytology, long considered the gold standard for diagnosing various conditions, including cancer, involve the microscopic examination of tissue samples [1]. These techniques, which include modalities like computed tomography (CT), magnetic resonance imaging (MRI), and X-ray, offer non-invasive visualization of internal structures and have revolutionized the ability to detect and monitor a wide range of conditions, but they are also time-consuming and heavily dependent on the subjective interpretation of pathologists, which can lead to variability in diagnoses. These imaging methods do have certain drawbacks, though, such as the possibility of false positives or negatives and the occasional exposure to ionizing radiation. Furthermore, the sensitivity and specificity required for an early diagnosis or the detection of pathogens are sometimes lacking from biochemical assays and microbiological diagnostics, which analyze blood and other physiological fluids to discover biomarkers or infections [2].

A surge of cutting-edge technologies is revolutionizing diagnostic medicine in response, opening up new possibilities for more accurate, quick, and customized diagnosis. One example of a game-changer is digital pathology, which makes it possible to digitize pathology slides and integrate them with artificial intelligence (AI) for remote analysis and improved diagnostic accuracy.

The development of AI algorithms that may help detect patterns and anomalies that the human eye might overlook is made easier by this technology, which also simplifies the workflow for pathologists. Artificial intelligence (AI) is being used more and more in medical imaging to help diagnose and characterize diseases like cancer, cardiovascular disease, and neurological problems. Its applications in diagnostic medicine go beyond digital pathology [3].

The field of molecular diagnostics has advanced by making it possible to identify diseases at the genetic and molecular levels. Genetic alterations are being identified, illness development is being tracked, and more individualized treatment choices are being made thanks to methods like polymerase chain reaction (PCR), next-generation sequencing (NGS), and CRISPR-based diagnostics [4]. In oncology, where targeted therapy response may be predicted by identifying specific genetic alterations through molecular diagnostics, these developments are especially noteworthy. The advent of point-of-care testing (POCT) has brought diagnostic capabilities closer to the patient, facilitating quick testing and instantaneous results—important in emergency situations and the management of long-term conditions. Another frontier in diagnostic medicine is nanotechnology, which provides extremely precise and sensitive diagnostic instruments. By identifying biomarkers at incredibly low concentrations, nano-biosensors and nanoparticles are being developed for the early diagnosis of illnesses, including cancer. These technologies allow for non-invasive testing and real-time tracking of the course of disease in addition to increasing diagnosis accuracy [5].

These new technologies have a lot of potential, but there are a few obstacles to overcome before they can be fully incorporated into clinical practice. It is necessary to address ethical issues including data privacy and the possibility of bias in AI systems. Regulations must also change to keep up with technology developments in order to guarantee that newly developed diagnostic instruments are secure and useful for patient care. Another important factor to consider is the expense of putting these technologies into practice and making sure they are accessible, especially in areas with limited resources [6]. By facilitating earlier detection, more precise diagnoses, and individualized treatment plans, advances in diagnostic medicine have the potential to significantly improve patient outcomes. These technologies will surely be crucial in determining the direction of healthcare in the future as they develop, providing new avenues for enhancing the standard and efficiency of diagnostic services.

AIM

The purpose of this review is to provide a concise and structured overview of the transformative impact of emerging technologies on diagnostic medicine. It aims to highlight the limitations of traditional diagnostic methods and emphasize the advancements offered by innovations such as digital pathology, molecular diagnostics, and wearable technologies.

MATERIALS AND METHODS

This review was conducted through a comprehensive analysis of recent advancements in diagnostic medicine, focusing on peer-reviewed literature, clinical studies, and technological reports published between 2015 and 2024. The primary areas of investigation included:

digital pathology, molecular diagnostics, and wearable technologies. Digital pathology enables the digitization of slides and the application of Artificial Intelligence (AI) for precise and timely diagnoses, particularly in oncology. Advances in molecular diagnostics, such as genomic and proteomic profiling, are evaluated for their role in improving disease detection and monitoring. The study also highlights emerging trends in telemedicine, personalized medicine, and precision medicine.

CONVENTIONAL DIAGNOSTIC TECHNIQUES: A SYNOPSIS

Traditional techniques like histology, cytology, radiography, blood testing, and microbiological diagnostics have long been a part of diagnostic medicine. These methods have been the foundation of medical diagnostics, offering vital data for the identification, diagnosis, and treatment of a wide range of illnesses. These techniques do, however, have several drawbacks that may compromise their overall accuracy, sensitivity, and specificity despite their widespread application and proven effectiveness. An outline of these conventional diagnostic techniques is given in this section, together with an emphasis on their advantages and disadvantages.

HISTOPATHOLOGY AND CYTOLOGY

The microscopic analysis of tissues to investigate disease symptoms is known as histopathology. It is regarded as the gold standard for identifying many different diseases, including malignancies. On the other hand, histopathology studies individual cells or groups of cells by processing, staining, and microscopic examination of a surgical or biopsy specimen in order to find cellular abnormalities, tissue architecture, and other pathological

traits suggestive of illnesses. Pap smears are frequently used in cytology to screen for cervical cancer, and fine needle aspiration cytology (FNAC) is a common method for analysing lumps and abnormalities in different tissues. Cytology can be utilized for early disease identification and screening, and it is less invasive than histopathology. Although cytology and histopathology are effective diagnostic methods, they have drawbacks. The pathologist's experience and the sample's quality have a major impact on these procedures' accuracy. Misdiagnosis can result from sampling problems, such as collecting an inadequate or non-representative specimen. Furthermore, these methods take a lot of time because they include processing tissue, staining, and microscopic inspection, which can take hours or even days. Additionally adding to the heterogeneity is the subjective interpretation, which could compromise the consistency of the diagnosis [7].

RADIOLOGY (X-RAY, CT, MRI)

A variety of non-invasive imaging techniques, including as CT, magnetic resonance imaging, and X-rays, are included in the field of radiology. These techniques enable the observation of internal structures. X-rays are frequently used to find tumors, infections, and fractures. CT scans help diagnose complex illnesses such internal bleeding, tumors, and cardiovascular diseases by providing more comprehensive cross-sectional images of the body. MRI is a powerful diagnostic tool for neurological problems, musculoskeletal conditions, and certain malignancies due to its superior soft-tissue contrast. The primary benefit of using them is their capacity to offer non-invasive, real-time insights into the interior architecture of the body, which aids in early diagnosis and directs treatment choices. These techniques do have certain drawbacks, though. Ionizing radiation, which is exposed to during X-rays and CT scans, is normally minimal but has the potential to cause cancer if exposure is sustained or repeated. Even though MRIs are less radiation-intensive, they are costly, time-consuming, and shouldn't be done on those who have specific implants or claustrophobia. Furthermore, radiological imaging occasionally produces false positives or negatives, which can cause misunderstandings. For instance, benign lesions could be misdiagnosed as malignant tumors, or small, early-stage malignancies could go undiscovered, particularly if inadequate contrast or image resolution makes it difficult to see them [8, 9].

BLOOD TESTS AND BIOCHEMICAL ANALYSIS

Among the most widely used diagnostic instruments are blood tests, which offer vital details regarding an individual's general health, organ function, and the existence of disease. Complete blood counts (CBCs), liver and kidney functions, and glucose levels are among the common blood tests. Diagnosing diseases including diabetes, liver disease, and cardiovascular illnesses requires the use of biochemical studies, which count the levels of hormones, lipids, enzymes, and other indicators. Since many biomarkers are non-specific, they might be elevated under a variety of circumstances, making it challenging to determine the precise origin of the aberration. Increased C-reactive protein (CRP) levels, for instance, may be a sign of inflammation, but there are many other possible underlying causes for this, including infections and long-term conditions like rheumatoid arthritis. Furthermore, blood tests frequently only record the body's condition at a specific moment in time, making it difficult to completely understand how a disease progresses or how the body reacts to therapy [10].

MICROBIOLOGICAL DIAGNOSTICS

Identification of pathogens, including bacteria, viruses, fungi, and parasites that cause infectious disorders, is a key component of microbiological diagnosis. Culture, microscopy, serology, and molecular techniques such as polymerase chain reaction (PCR) are among the techniques used. When diagnosing bacterial infections, culture methods where pathogens are cultivated on certain media are regarded as the gold standard. Serological tests identify antigens or antibodies linked to infectious pathogens, indicating past or present illnesses. (11) Microbiological diagnostics can have certain limitations, though. There are diseases that are hard to grow or that need specific conditions and media, which can result in false negatives. Even while serological testing is quicker, it might not be able to distinguish between an ongoing infection and a previous exposure since antibodies might linger long after an infection has healed. Furthermore, even though PCR and other molecular techniques are very sensitive, they can require expensive equipment and knowledge, which makes them inaccessible in environments with limited resources [12].

LIMITATIONS OF TRADITIONAL METHODS

Traditional diagnostic techniques have improved patient outcomes and advanced medical knowledge, but they are not without drawbacks. Timely and accurate diagnosis can be hampered by their reliance on invasive techniques, subjective interpretation, and laborious processes. Furthermore, the inability of many

conventional techniques to identify diseases at the molecular level restricts their capacity to offer individualized therapy options. More sophisticated diagnostic methods that can get beyond these restrictions and provide higher sensitivity, specificity, and speed are therefore becoming more and more necessary [13]. More accurate, timely, and individualized diagnostic options are being made available by the development of digital pathology, molecular diagnostics, and other cutting-edge technology. These new innovations are not only enhancing the accuracy of detection of diseases but also paving the way for the integration of diagnostics into a more personalized and predictive healthcare model.

EMERGING TECHNOLOGIES IN DIAGNOSTIC MEDICINE: DIGITAL PATHOLOGY

OVERVIEW AND BENEFITS

Utilizing state-of-the-art technology, digital pathology is a breakthrough development in diagnostic medicine that improves the effectiveness, precision, and accessibility of pathological examinations. Digital pathology is essentially the process of taking, analysing, and storing high-resolution photographs of tissue samples using digital imaging and computational tools. With the use of digital slide scanning and image analysis, manual microscopy and glass slide processing are replaced by this technology [14]. The main advantages of digital pathology are several. First off, by giving pathologists high-resolution images that can be enlarged and thoroughly examined, it improves diagnostic accuracy. Because of the enhanced visibility, diseased characteristics can be seen more precisely, which could result in more accurate diagnosis. Second, digital pathology makes it easier for medical practitioners to collaborate. Digital image sharing between pathologists and colleagues in different places allows for in-the-moment consultations and second opinions. This ability to work together can enhance the results of diagnosis, especially in complicated circumstances. Digital pathology also increases process productivity. [15] It can take a lot of time to perform traditional microscopy because pathologists have to physically handle and inspect many glass slides. This procedure is streamlined by digital pathology, which provides quick image access for review and diagnosis. Additionally, digitizing slides creates a permanent, readily available record of pathology cases, which is beneficial for research and long-term studies.

The goal of personalized medicine is to adjust medical care to each patient's unique set of traits, including lifestyle, genetic, and environmental influences. This method relies heavily on digital pathology, which offers precise, high-quality images that may be combined with other data sources to improve treatment and diagnosis plans. Pathologists can correlate molecular and genetic data with histopathological findings by using digital pathology. For example, precise digital pictures of tumor samples can be analysed along with genomic information to find particular mutations or biomarkers that guide focused treatments. With this integration, treatment strategies can be more effectively and individually tailored based on the distinct molecular profile of each patient's condition. Furthermore, by facilitating extensive data analysis, digital pathology advances precision medicine. In conventional diagnostic settings, patterns and correlations could go unnoticed. However, high-throughput imaging and data analytics might reveal them. This method can open the door to novel treatments and more accurate illness management techniques by providing fresh insights into the mechanisms underlying disease and how treatments work [16].

Digital pathology is changing as a result of the incorporation of AI and machine learning, which is advancing the field's efficiency and accuracy in diagnosis. Digital pathology photos can be analysed by AI algorithms to find patterns and anomalies that might be signs of a disease. For instance, pathologists can identify and quantify abnormal features more accurately by using machine learning models that have been trained to identify malignant cells or other features with high precision. Workflow automation is further improved by AI and machine learning. Large amounts of data can be processed fast using automated image analysis, which minimizes the human work needed for slide review and interpretation. In addition to speeding up the diagnostic procedure, this automation reduces the possibility of human error, producing more consistent and trustworthy results. Predictive analytics powered by AI can also help personalized medicine by predicting possible treatment outcomes based on past data and image analysis. These prediction models can assist medical professionals in choosing the best courses of action for specific patients, maximizing therapeutic results, and reducing side effects. When combined with AI and machine learning, digital pathology represents a major advancement in diagnostic medicine. It opens the door for more efficient and customized patient care by improving diagnostic accuracy, streamlining workflows, and supporting specific treatment plans [17]. As these technologies develop further, their combination promises to completely transform the area and open up new avenues for the diagnosis and treatment of complicated illnesses.

REVIEW

MOLECULAR DIAGNOSTICS: GENOMIC AND PROTEOMIC PROFILING IN CANCER DIAGNOSTICS

Medicine is being revolutionized by molecular diagnostics, especially in the identification and management of cancer. Through an emphasis on the molecular and genetic basis of diseases, this field provides more accurate, individualized, and successful treatment and diagnostic choices. At the vanguard of these developments are critical technologies like next-generation sequencing (NGS), CRISPR, and genomic and proteomic profiling. These tools enable more individualized treatment strategies and offer comprehensive insights into the molecular causes of cancer.

GENOMIC AND PROTEOMIC PROFILING

Using genomic profiling, one can find genetic mutations, variances, or aberrations linked to cancer by analysing a cell's entire genome. This method makes it possible to pinpoint the precise mutations that fuel the onset, spread, and resistance of cancer to therapy. These genetic alterations can be found throughout the entire genome or just in the sections of the genome that code for proteins, respectively, using methods like whole-genome sequencing (WGS) and whole-exome sequencing (WES). On the other hand, proteomic profiling is concerned with the thorough examination of proteins expressed by an organism, tissue, or cell. Since the majority of biological functions are powered by proteins, a study of the proteome landscape of a malignant cell can offer crucial insights about the behaviour, course, and response to therapy of the disease. Proteomic profiling commonly uses mass spectrometry and protein microarrays to identify and quantify proteins and their changes, which are frequently changed in cancer [18].

Comprehending the intricate biology of cancer requires an understanding of both proteomic and genomic profiling. They make it possible to identify molecules known as biomarkers that can be used to identify cancer, forecast the course of a disease, and track an individual's reaction to treatment. Proteomic analysis has identified proteins like HER2, which is overexpressed in certain types of breast cancer and is targeted by therapies like trastuzumab. Similarly, the identification of mutations in the BRCA1 and BRCA2 genes has led to targeted therapies for breast and ovarian cancers [19].

APPLICATIONS IN CANCER DIAGNOSTICS

The way that tumors are identified and categorized has changed as a result of the incorporation of proteomic and genomic profiling into cancer diagnostics. Conventional diagnostic techniques, like histopathology, frequently depend on the anatomical and morphological features of malignancies. Molecular diagnostics, on the other hand, offers a more thorough and precise categorization according to the genetic and molecular features of the tumor. For instance, more individualized treatment plans have been made possible by the molecular subtyping of breast cancer into subtypes like HER2-positive, hormone receptor-positive, and triple-negative, which has improved patient outcomes. Similar to this, the discovery of certain mutations in lung cancer - like ALK, ROS1, and EGFR rearrangements - has prompted the creation of targeted treatments that, when compared to conventional chemotherapy, greatly increase patient survival. Furthermore, molecular diagnostics makes liquid biopsies easier to use for early cancer diagnosis. By analysing circulating tumor DNA (ctDNA) or other indicators in blood samples, these non-invasive diagnostics make it possible to detect cancer early on, when it is more likely to be treatable. Liquid biopsies have a number of advantages over conventional biopsy techniques, including the ability to track the advancement of a disease and its response to treatment in real-time [20].

CRISPR AND NEXT-GENERATION SEQUENCING

A potent genome editing technology called CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) has been modified for use in diagnostics. With CRISPR, targeted mutations or genome repairs can be made to validate the involvement of particular genes in carcinogenesis and identify them in cancer. With great specificity and sensitivity, CRISPR-based diagnostics, like CRISPR-Cas9, can also identify particular genetic abnormalities linked to cancer. [21] Another ground-breaking tool in molecular diagnostics is next-generation sequencing (NGS). With NGS, millions of DNA fragments may be sequenced simultaneously, giving a complete picture of a tumor's genetic makeup. It is especially useful for figuring out which genomic changes, such as copy number variations and genetic mutations, cause cancer. Comprehensive genomic profiling of cancers has been made possible by NGS, which has allowed for the discovery of therapeutically useful mutations and the creation of individualized treatment regimens. Precision oncology - where treatments are customized to the unique genetic composition of each patient's tumor - is being made possible by the combination of CRISPR and NGS in molecular diagnostics. This strategy lowers the chance of side effects while simultaneously

increasing treatment efficacy since medicines can be tailored to precisely target the genetic changes that the cancer has. At the vanguard of cancer diagnostics is molecular diagnostics, which uses CRISPR, next-generation sequencing, genomic and proteomic profiling, and other techniques. Patient outcomes are greatly improved by these technologies, which make it possible to diagnose and treat cancer in a way that is more precise, individualized, and efficient. With continued development, these instruments could lead to even more breakthroughs in precision medicine, which could eventually result in improved cancer treatment and even possible cures [22].

POINT-OF-CARE TESTING (POCT): ADVANCEMENTS IN RAPID DIAGNOSTICS AND PORTABLE DEVICES

Medical diagnostic testing carried out at or close to the location of patient care is referred to as "point-of-care testing" (POCT). Rapid and accurate test findings are made possible by this method, which enhances patient outcomes and helps clinicians to make decisions more quickly. POCT is becoming more and more popular because of its capacity to offer prompt feedback, which lessens the need for centralized laboratory testing and improves the effectiveness of healthcare delivery. Rapid Diagnostic Tests (RDTs) and portable diagnostic equipment are just two examples of the many technologies that make up POCT, and they are essential for treating both chronic illnesses and infectious infections.

RAPID DIAGNOSTIC TESTS (RDTS)

Quick and dependable results are produced by RDTs, a subset of POCT, frequently in a matter of minutes. In locations with limited access to complete laboratory equipment, like remote or resource-poor areas, these tests are especially helpful. RDTs are frequently utilized in the diagnosis of COVID-19, HIV, and malaria, among other infectious disorders. They usually only require a basic technique that can be done with little training, including using a nose swab or blood drop. By facilitating early identification and treatment, RDTs have completely changed the landscape of infectious illnesses, slowing the spread of illness and enhancing patient outcomes. For instance, RDTs enable the quick detection of malaria cases in malaria-endemic areas, guaranteeing timely treatment and lessening the strain on healthcare systems. Similar to this, during the COVID-19 pandemic, the extensive use of RDTs for antigen detection enabled mass testing and prompt isolation of positive individuals, which significantly aided in containing the virus's spread [23, 24].

ROLE IN INFECTIOUS DISEASES AND CHRONIC CONDITIONS

POCT is useful not only in the treatment of infectious diseases but also in the management of long-term illnesses such diabetes, heart disease, and renal disease. For improved disease control and lower risk of complications, POCT devices such as glucometers enable patients receiving diabetic treatment to check their blood glucose levels at home. In a similar vein, portable blood pressure monitors and cholesterol testing kits are vital resources for cardiovascular disease management since they let patients monitor their status and instantly modify their lifestyle [25]. POCT enhances patient participation and treatment protocol adherence in the setting of chronic illnesses. POCT gives patients the tools they need to actively control their health by giving them prompt feedback. This is especially crucial for the long-term care of chronic illnesses. Additionally, POCT makes it possible for medical professionals to promptly modify treatment strategies in light of real-time data, which eventually improves patient outcomes [26].

PORTABLE DIAGNOSTIC DEVICES

For POCT, the advent of portable diagnostic instruments has changed everything. These gadgets are made to be small, easy to use, and quick to deliver precise results. Wearable biosensors, handheld ultrasonography instruments, and portable ECG monitors are a few examples. These devices are perfect for usage in a range of contexts, such as ambulances, home care, and isolated areas, because of their portability. Additionally important in emergency medicine, when prompt decision-making can save lives, are portable diagnostic tools. For example, trauma victims can be evaluated in ambulances using portable ultrasound equipment, which helps doctors decide on therapy before the patient ever gets to the hospital. Similar to this, portable ECG monitors can offer quick information about a patient's cardiac condition, enabling early intervention in the event of an arrhythmia or heart attack. POCT is transforming healthcare by offering fast and precise diagnostic information through the use of portable diagnostic equipment and RDTs. It plays a vital role in the management of chronic illnesses and infectious infections, providing prompt diagnosis, improved patient outcomes, and improving the effectiveness of healthcare delivery, particularly in environments where traditional laboratory services are scarce. POCT will remain crucial to the provision of healthcare around the world as technology develops [27].

ARTIFICIAL INTELLIGENCE AND MACHINE LEARNING IN DIAGNOSTICS

Medical diagnostics is changing as a result of AI and machine learning (ML), which improve disease detection and prognosis accuracy, efficiency, and speed. These technologies use enormous volumes of data to create complex algorithms that can recognize patterns and make predictions that are more accurate than those of a human. Predictive analytics, AI-powered diagnostic tools, and AI applications in pathology and imaging are leading this transformation and greatly enhancing patient outcomes.

AI-DRIVEN DIAGNOSTIC TOOLS

AI-driven diagnostic technologies are intended to help medical professionals make more accurate disease diagnoses. These tools make use of machine learning algorithms that have been trained on large datasets, such as imaging data, test findings, and medical records. AI may identify minute patterns and connections in this data that human experts might overlook, enabling earlier and more precise diagnosis. AI algorithms are used to analyse retinal images and detect early indicators of diabetic retinopathy, one prominent example of which is this disease. It has been demonstrated that these AI systems can reach accuracy levels that are on par with or even higher than those of human specialists. In a similar vein, AI technologies are being created to diagnose respiratory disorders, cardiovascular ailments, and skin cancer, allowing for a quicker and more accurate diagnosis of these conditions [28].

PREDICTIVE ANALYTICS IN DIAGNOSTICS

With the help of AI and ML, predictive analytics forecasts future health outcomes by utilizing historical data. Predictive analytics in diagnostics enables early intervention and improved condition management by identifying individuals who are susceptible to specific diseases before symptoms manifest. Predictive models, for example, are used in oncology to determine the chance of a cancer recurrence based on patient information such as genetic profiles and treatment histories [29]. By allowing clinicians to more carefully monitor patients and tailor treatment strategies, these models increase the likelihood of positive outcomes. Predictive analytics can identify people who are at risk of complications in chronic diseases like diabetes, allowing for preventative actions to avert serious health problems. The prognosis after a hemorrhagic stroke is typically inaccurate. In order to assist in guiding treatment decisions and assessing patient prognosis, several AI rating measures have been developed to help forecast outcomes for patients who have had intracerebral haemorrhages (ICH) [30]. Furthermore, population health management uses AI-driven predictive analytics to spot patterns and anticipate infectious disease epidemics. AI can assist public health authorities in responding more efficiently to emerging health hazards by analysing data from several sources, such as social media, healthcare records, and environmental data streams.

AI IN IMAGING AND PATHOLOGY

AI has a particularly significant impact on pathology and medical imaging. AI algorithms are employed in imaging to improve the interpretation of CT, MRI, ultrasound, and X-ray images, hence increasing the precision of disease identification. For instance, in radiology, AI can recognize lung nodules in CT scans that might be signs of early-stage lung cancer, even if human vision finds them difficult to discern. Artificial Intelligence is transforming tissue sample analysis in pathology. The physical, subjective, and time-consuming examination of slides under a microscope is the foundation of traditional pathology. Digital pathology solutions with AI capabilities can accurately evaluate whole-slide images, detecting malignant cells, classifying tumors, and forecasting patient outcomes. This technique decreases human error and expedites the diagnosis procedure, resulting in more reliable results. AI is also making it possible to create next-generation instruments, such virtual biopsies, which evaluate imaging data to determine whether disease is present without requiring invasive procedures. The development of AI-driven pathology and imaging is accelerating, improving, and reducing the invasiveness of diagnostic procedures. With the advent of AI-driven technologies, predictive analytics, and enhanced imaging and pathology solutions, machine learning and artificial intelligence are completely changing the diagnostics industry. These innovations are improving the precision of diagnoses, making early detection possible, and customizing patient care. AI will become more and more important in enhancing healthcare outcomes and changing the diagnostic environment as it develops [31].

NANOTECHNOLOGY IN DIAGNOSTICS

With its revolutionary instruments for early disease detection and accurate diagnosis, nanotechnology is being used more and more in the diagnostics industry. Researchers have created nano-biosensors, nanoparticles, and tailored diagnostic methods with previously unheard-of sensitivity and specificity by modifying

materials at the nano-scale. These developments are revolutionizing the diagnosis process by facilitating earlier identification and more precise evaluations, which eventually improve patient outcomes.

NANO-BIOSENSORS AND NANOPARTICLES

Early disease diagnosis is made possible by highly sensitive devices called nano-biosensors, which identify biological substances at the nanoscale. Typically, these sensors use nanoparticles tiny particles with a diameter of one to one hundred nanometers to improve their sensitivity and selectivity. Because of their special qualities, nanoparticles may interact at the molecular level with biological systems, which makes them perfect for identifying biomarkers linked to a variety of disorders. For instance, because of their exceptional conductivity and biocompatibility, gold nanoparticles are frequently utilized in nano-biosensors. In order to target and bind to disease-specific biomarkers, such as proteins or nucleic acids, found in biological fluids like blood or saliva, these nanoparticles can be functionalized with particular antibodies or DNA sequences. Once the biomarker binds to the nanoparticle, the nano-biosensor releases a detectable signal, indicating the presence of the disease at an early stage [32].

APPLICATIONS IN EARLY DETECTION OF DISEASES

One of the most exciting uses of nanotechnology in medicine is its ability to identify diseases, especially cancer, early [33]. Conventional diagnostic techniques frequently identify illnesses after symptoms manifest, which might restrict available treatments and lower survival rates. Long before symptoms appear, diseases can be identified at the molecular level thanks to nanotechnology. For instance, nanoparticles can be designed to target particular cancer cells or tumor markers in the context of cancer diagnosis. After being injected, these nanoparticles travel throughout the body and gather inside the tumor, where they may be seen with fluorescence or magnetic resonance imaging. This method increases the likelihood of a favourable outcome by detecting cancer in its earliest stages. Similarly, nano-biosensors are developed to detect the biomarkers for cardiovascular diseases, neurological disorders, and infectious diseases. These sensors can detect minute concentrations of disease-specific molecules, enabling early diagnosis and prompt intervention of diseases, which is crucial for conditions where early treatment significantly improves outcomes [34].

TARGETED DIAGNOSTIC TECHNIQUES

Targeted diagnostic methods are also made possible by nanotechnology, and they provide more accurate illness diagnosis. Targeted nano-diagnostics are intended to engage selectively with diseased tissues or cells, minimizing injury to healthy tissues, in contrast to standard diagnostic approaches that may involve intrusive procedures or expose patients to high doses of radiation. MRI is one application where magnetic nanoparticles are used. By functionalizing these nanoparticles to target particular tissues or cells, MRI scans can be made more accurate in identifying cancers or other abnormalities by improving contrast. This focused strategy decreases the likelihood of false positives and minimizes the need for repeat imaging in addition to improving diagnostic accuracy. Nanoparticles that have been designed to bind to particular pathogens or toxins can also be used in the diagnosis of infectious disorders. By identifying the infectious agent with greater speed and accuracy, this targeted detection helps to stop the disease from spreading and enables for prompt treatment. Through the development of specialized diagnostic procedures, nanoparticles, and nano-biosensors, nanotechnology is transforming the field of diagnostics. By enabling earlier and more precise illness detection, these advancements enhance patient outcomes and progress the area of customized medicine. Future developments in nanotechnology will have a significant impact on how diagnostic medicine is practiced [35].

The main developments in diagnostic medicine are shown in Figurte 1 along with how they affect patient outcomes. The first ones are AI-Driven Diagnostic Tools, which use predictive analytics to improve diagnostic accuracy and enable early disease detection in imaging and pathology. Advances in nanotechnology, such as tailored diagnostic methods and nano-biosensors, make early disease detection and accurate diagnosis easier. Genomic and proteomic profiling, enables customized treatment regimens and molecular diagnostics. This results in more efficient, specialized care. By concentrating on portable diagnostic tools and remote patient monitoring, telemedicine and remote diagnostics aim to increase access to healthcare, particularly in underprivileged areas. These developments result in earlier and more precise diagnosis, better treatment customisation, and increased accessibility, forming a future that is more effective and efficient.

LIMITATIONS AND DIFFICULTIES OF NEW DIAGNOSTIC TECHNOLOGIES

Emerging diagnostic technologies, like molecular diagnostics, nanotechnology, and artificial intelligence,

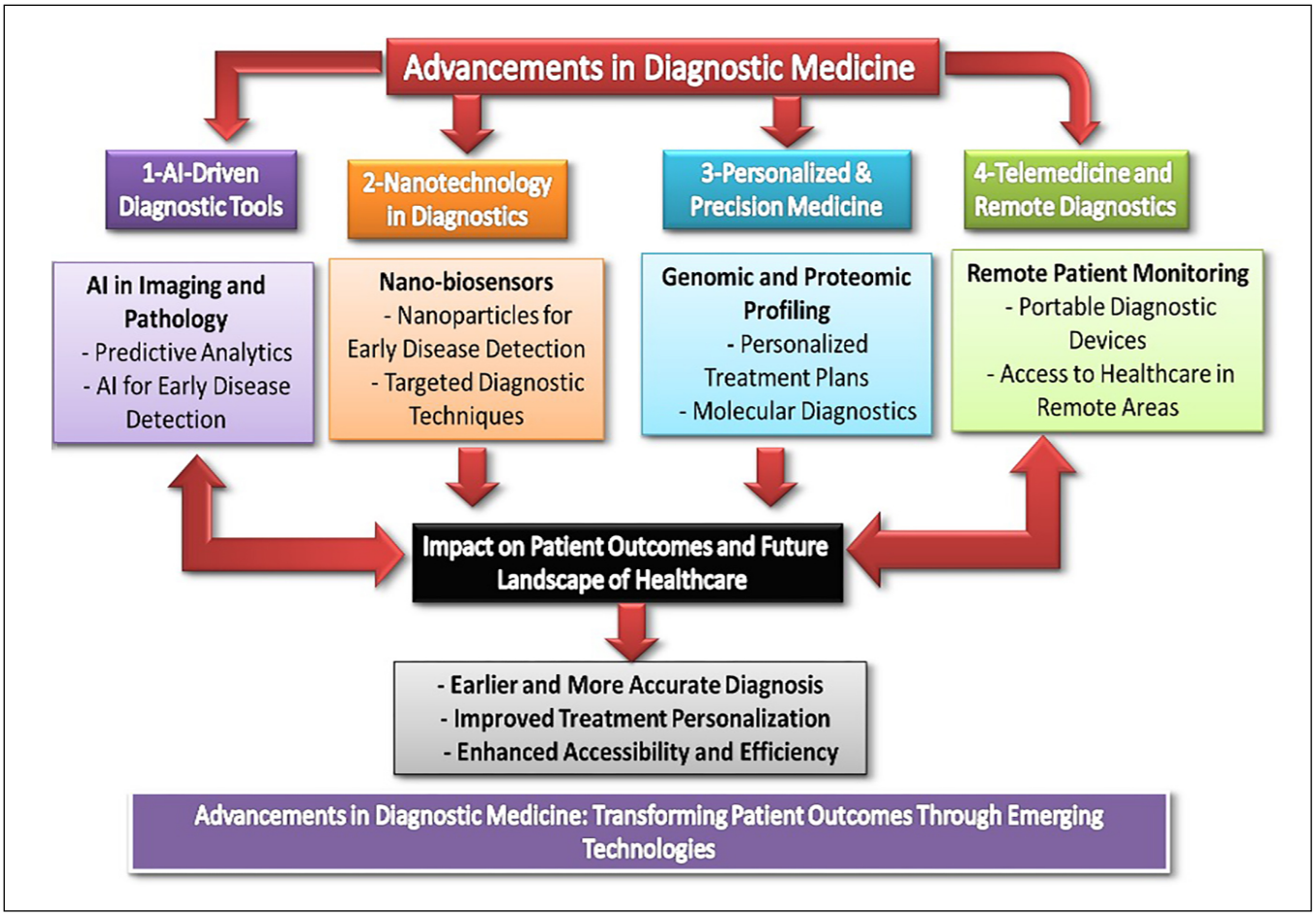


Fig. 1. The main developments in diagnostic medicine
Source: Own materials

present a number of obstacles and constraints as they become more widely used in healthcare. These include issues with data privacy and ethics, obstacles related to regulations, challenges integrating the technology into clinical practice, and queries about accessibility and cost-effectiveness. To guarantee that new technologies can be used in healthcare in a safe and efficient manner, it is imperative that these issues are resolved.

ETHICAL CONCERNS AND DATA PRIVACY

The ethical ramifications of using modern diagnostic technology are one of the main issues they present. For example, AI-driven diagnoses sometimes call for the gathering and examination of enormous volumes of patient data. Concerns around permission, data privacy, and the possible misuse of private health information are brought up by this. The increasing reliance of healthcare systems on digital platforms raises serious concerns about breaches and illegal access to sensitive health data. To preserve patient trust and protect privacy, it is essential to make sure that patient data is stored securely and that strong consent procedures are in place. Additionally, there may be moral conundrums involving decision-making if AI is used in diagnostics. For example, it can be difficult to decide which AI system to believe if its diagnosis differs from a human doctor's. The possibility that AI will reinforce prejudices found in the training data, creating inequities in healthcare results, exacerbates this predicament [36].

REGULATORY CHALLENGES

New diagnostic technologies are developing faster than regulatory agencies can assess and authorize them. One of the regulatory obstacles lies in ensuring that these technologies satisfy strict requirements for quality, safety, and efficacy before to being released onto the market. For example, in order to demonstrate their accuracy and dependability, AI-driven tools and diagnostics based on nanotechnology need to go through extensive testing. The intricacy and originality of these technologies, however, complicate the application of conventional regulatory procedures. Clear and uniform regulatory frameworks that are flexible enough to change with the rapidly developing nature

of these technologies are also necessary. The approval and acceptance of novel diagnostic instruments are frequently delayed by current laws because they are unable to keep up with the rapid pace of invention. This can hinder the timely availability of potentially life-saving technologies to patients [37].

INTEGRATION INTO CLINICAL PRACTICE

There are several obstacles to overcome when incorporating cutting-edge diagnostic technologies into routine clinical practice. It will take time, money, and a change in customs to train healthcare professionals to use these new instruments. Furthermore, because AI and nanotechnology frequently need for new IT infrastructure, equipment, and procedures, incorporating them into current healthcare workflows can be challenging. Healthcare workers who could be wary of using AI-driven technologies or uninitiated with nanotechnology-based diagnostics are similarly resistant to change. Successful integration depends on making sure these technologies are easy to use and that healthcare providers are given the necessary support when implementing them [38].

COST-EFFECTIVENESS AND ACCESSIBILITY

Emerging diagnostic technologies face major obstacles in their widespread adoption, including cost-effectiveness and accessibility. These technologies frequently have substantial development and implementation costs, even though they have the potential to improve patient outcomes and diagnostic accuracy. Because of this, healthcare systems with tight funds may not be able to access them, especially in low- and middle-income nations. Furthermore, patients may not be able to afford these technologies, which could result in unequal access to cutting-edge diagnostics. For these technologies to be widely adopted and to help reduce health disparities, it is imperative that they be both affordable and effective. In order to overcome these financial obstacles and increase the accessibility of these technologies, it may be necessary to investigate different funding options like public-private partnerships or government subsidies. Emerging diagnostic technologies provide a number of intricate and varied problems and constraints. The effective integration of these technologies into clinical practice, cost-effectiveness, regulatory obstacles, and ethical issues must all be addressed. To ensure that these advances can be used safely and effectively to improve patient care, coordination between healthcare professionals, regulators, technology developers, and legislators will be necessary to overcome these challenges [39].

DISCUSSION

The integration of these technologies into diagnostic medicine represents a significant leap forward in healthcare. However, their successful implementation requires addressing key challenges, including cost, accessibility, and ethical considerations related to data privacy. Collaboration between researchers, clinicians, and policymakers will be essential to harness the full potential of these innovations [36]. By bridging the gap between traditional methods and advanced technologies, diagnostic medicine is poised to deliver more precise, efficient, and patient-centered care. This evolution underscores the need for continuous research, investment, and education to prepare healthcare systems for a technology-driven future.

A few crucial areas are set to influence the direction of healthcare in the future as diagnostic technology continues to advance. At the forefront of this transition are advances in wearable diagnostics, telemedicine and remote diagnostics, personalized and precision medicine, and collaborative interdisciplinary approaches that promise to improve patient outcomes. Precision and personalized medicine emphasizes adjusting medical care to each patient's unique needs. More accurate diagnostic instruments that can recognize genetic variants, estimate the risk of disease, and direct specialized treatment regimens are becoming possible because to developments in genomics, proteomics, and bioinformatics. Taking into account each patient's distinct genetic composition not only increases therapeutic efficacy but also minimizes unwanted effects. The delivery of healthcare is being completely transformed by the growth of telemedicine and remote diagnostics. Patients can now obtain medical consultations and diagnostic services from the comfort of their homes thanks to developments in digital health technologies. AI-powered remote diagnostics enable ongoing health state monitoring and early problem detection through connected devices. This is especially helpful for treating chronic illnesses and giving underprivileged or isolated communities access to healthcare [40].

Another exciting area in healthcare is wearable diagnostics. Heart rate, blood sugar, and oxygen saturation are just a few of the many health metrics that can be tracked in real-time by gadgets like smart-watches, fitness trackers, and biosensors. These gadgets offer early warning indicators for possible health problems in addition to continuous health monitoring, allowing for prompt action and better patient outcomes. The use of interdisciplinary and collaborative approaches will also influence the future of diagnostics. It is imperative to include knowledge from domains like bioengineering, data science, and clinical medicine in order to create

novel diagnostic instruments and guarantee their successful application. The translation of state-of-the-art research into useful applications will be fuelled by collaboration between academic institutions, business, and healthcare providers, and will ultimately result in more precise and easily accessible diagnostics [40]. Customized medicine, telemedicine, wearable diagnostics, and collaborative methodologies have the potential to revolutionize diagnostic medicine by enabling more accessible, accurate, and patient-centered therapy in the future. These approaches and technology will become increasingly important as healthcare develops, enhancing patient outcomes and changing the field of medical diagnostics.

CONCLUSIONS

Key developments like molecular diagnostics, nanotechnology, AI-driven technologies, and the fusion of precision and customized medicine are causing a radical change in the field of diagnostic medicine. These developments are improving illness detection's specificity, speed, and accuracy, allowing for earlier diagnosis and more individualized treatment plans. There could be a significant effect on patient outcomes. Healthcare professionals may monitor patients' statuses more successfully, detect diseases at an earlier stage, and tailor treatment regimens based on the unique characteristics of each patient with more precise diagnostics. Through the prevention of illness development and avoidance of needless therapies, this not only raises survival rates and improves quality of life but also lowers healthcare expenditures. Future trends in diagnostic medicine point to a greater uptake of wearable technology, telemedicine, and remote diagnostics as ways to improve patient accessibility and satisfaction. Innovation will continue to be fueled by collaborative and interdisciplinary efforts, guaranteeing that new diagnostic tools are not only cutting edge in terms of technology but also useful and easily available. These developments will have a significant impact on the direction of healthcare in the future as they are incorporated into clinical practice, improving patient outcomes and developing a more effective and efficient diagnostic system.

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REVIEW ARTICLE

CONTENTS

The relationship between metabolic dysfunction-associated fatty liver disease and hormonal disorders in children: A literature review

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ABSTRACT

This narrative review summarizes current evidence regarding hormonal disturbances associated with metabolic dysfunction-associated fatty liver disease in children. Focus was given to thyroid function, insulin sensitivity, gonadal hormones in boys, and vitamin D levels in relation to hepatic and metabolic abnormalities. A comprehensive search of PubMed, Scopus, and Web of Science was conducted for literature published between January 2015 and June 2025. Studies included children aged 0–18 years with confirmed metabolic dysfunction-associated fatty liver disease based on imaging, histological, or biochemical criteria. Eligible articles reported data on at least one hormonal axis and included original research, meta-analyses, or high-quality narrative reviews. Adult-only studies, case series with fewer than ten participants, and articles lacking full text or endocrine data were excluded. Subclinical hypothyroidism occurred in 18–42% of affected children, insulin resistance in over 65%, reduced testosterone and sex hormone-binding globulin in boys, and vitamin D deficiency in 55–70% of cases. These disturbances correlated with liver enzyme elevations and steatosis severity. Weight reduction of 7–10% improved insulin resistance, thyroid and sex hormone parameters, and vitamin D status. Preliminary findings support potential benefits of vitamin D supplementation and levothyroxine therapy, though large-scale trials remain limited. Metabolic dysfunction-associated fatty liver disease in children is a multisystem condition where hormonal dysfunction contributes to disease progression. Comprehensive endocrine evaluation should be part of standard care. Further research is needed, particularly in younger children, girls, and diverse populations.

KEY WORDS: insulin resistance, hypothyroidism, testosterone, vitamin D, gonadal axis.

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INTRODUCTION

Metabolic dysfunction–associated fatty liver disease (MAFLD) has emerged as one of the most prevalent chronic liver conditions in children [17]. While the previous term "nonalcoholic fatty liver disease" (NAFLD) focused on the absence of alcohol as an etiological factor, MAFLD more explicitly emphasizes the metabolic dysfunction that underlies hepatic fat accumulation [1, 17, 21]. Prevalence estimates suggest that MAFLD affects approximately 10–20% of the general pediatric population and over 30% of obese children [20].

Importantly, MAFLD in children manifests as a multisystem disorder: liver injury is only the most visible feature, while endocrine disturbances—affecting thyroid hormones, insulin signaling, sex steroids, and vitamin D metabolism—play critical roles in disease pathophysiology, progression, and long-term outcomes [4, 6, 7, 8, 9]. Pediatric patients with MAFLD often present with subclinical hypothyroidism, marked insulin resistance, lower testosterone and SHBG levels (in boys), and vitamin D deficiency. These alterations can impair somatic growth, delay or disrupt pubertal progression, and heighten lifelong cardiometabolic risk [5, 10, 24].

This narrative review collates peer-reviewed publications from 2015 to June 2025 that address each of these hormonal axes in pediatric MAFLD. Our goal is to synthesize prevalence data, clarify pathophysiological links, and highlight clinical implications—ultimately guiding more effective screening, monitoring, and early intervention strategies.

MATERIALS AND METHODS

To construct this narrative review, we performed a comprehensive search of PubMed, Scopus, and Web

of Science to identify relevant literature published between January 2015 and June 2025. Search terms included combinations of "MAFLD" or "NAFLD" with "children," "pediatric," "hormonal disorders," "thyroid," "insulin resistance," "testosterone," and "vitamin D." No language restrictions were applied, but only English-language articles were retained. We screened titles and abstracts for relevance, focusing on original research, clinical trials, meta-analyses, and high-quality narrative reviews that addressed hormonal parameters in pediatric MAFLD populations. Studies were included if they involved participants aged 0–18 years with confirmed MAFLD or NAFLD diagnosis—based on imaging, histology, or established biochemical criteria—and reported data on at least one endocrine axis (thyroid function, insulin sensitivity, gonadal hormones, or vitamin D status). Exclusion criteria comprised adult-only cohorts, case reports or series with fewer than ten participants, and articles lacking full text or endocrine measurements. After removing duplicates and evaluating full texts, we synthesized findings from the final selection of studies, emphasizing prevalence estimates, correlation analyses, interventional outcomes, and proposed pathophysiological mechanisms. Given the narrative nature of the review, formal quality scoring tools (e.g., NOS or AMSTAR) were not uniformly applied, but methodological rigor and sample size were noted when interpreting individual study results.

REVIEW

Although diagnostic modalities vary (ultrasound, MRI, sometimes biopsy), most reports agree that the metabolic profile of children with MAFLD commonly includes obesity, dyslipidemia, and insulin resistance [4, 8, 16, 20]. The shift from the term "NAFLD" to "MAFLD" in 2019 helped sharpen focus on associated metabolic derangements, not just hepatic fat content [1, 17, 24]. Importantly, MAFLD criteria require evidence of hepatic steatosis plus one of the following: overweight/obesity, type 2 diabetes, or evidence of metabolic dysregulation (e.g., elevated waist circumference, hypertension, hypertriglyceridemia, low HDL, prediabetes, or insulin resistance) [1, 17].

In children, MAFLD prevalence is closely tied to the rising rates of pediatric obesity. Lifestyle factors—high-calorie diets and sedentary behavior—compound genetic predispositions (e.g., PNPLA3, TM6SF2, MBOAT7 polymorphisms) to foster early fat accumulation in the liver [5, 6]. Overall, the burden of MAFLD in childhood sets a trajectory toward adult cardiometabolic disease if left unaddressed.

THYROID AXIS IN PEDIATRIC MAFLD

PREVALENCE AND CORRELATIONS

Multiple observational studies report subclinical hypothyroidism (elevated TSH with normal free T4) in approximately 18–42% of children with MAFLD, compared to 5–10% in age-matched controls [1–6, 12, 13, 15, 29]. Elevated TSH—often within the upper end of the normal range—correlates positively with serum ALT and AST ($r = 0.32–0.47$, $p < 0.05$ in several cohorts), suggesting that even mild thyroid dysfunction exacerbates hepatic inflammation and steatosis [3, 4, 12].

PATHOPHYSIOLOGICAL LINKS

Thyroid hormones are key regulators of lipid and carbohydrate metabolism. In hepatocytes, T3 stimulates lipid mobilization and oxidation; relative T3 deficiency or resistance may impair these pathways, promoting de novo lipogenesis [1, 2, 13]. Animal models show that low thyroid action in the liver upregulates sterol regulatory element-binding protein-1c (SREBP-1c), increasing triglyceride synthesis [1, 15]. In human pediatric cohorts, high TSH is associated with greater severity of steatosis on imaging or histology, even after adjusting for BMI and insulin resistance [3, 4, 12].

CLINICAL IMPLICATIONS

Routine screening of thyroid function (TSH, free T4) in children diagnosed with MAFLD is therefore advised. While overt hypothyroidism is uncommon, early subclinical dysfunction may worsen metabolic control. Some centers advocate for levothyroxine (L-T4) therapy in those with TSH persistently above 4.5 mIU/L, especially if ALT/AST remain elevated despite lifestyle interventions [13, 29]. However, large-scale pediatric trials of L-T4 in MAFLD are lacking, and most evidence comes from small interventional or observational series [6, 12].

INSULIN RESISTANCE AND THE INSULIN AXIS

PREVALENCE AND SEVERITY

Insulin resistance is one of the most consistent findings in pediatric MAFLD. Cross-sectional data indicate that at least 65% of children with MAFLD demonstrate HOMA-IR values > 3, compared to about 15% in non-MAFLD obese or normal-weight peers ($p < 0.001$) [1, 4, 8–11, 14, 16, 20]. HOMA-IR correlates strongly with hepatic fat fraction on MRI and with ALT levels.

MECHANISTIC CONSIDERATIONS

Hepatic steatosis itself contributes to insulin resistance by increasing intrahepatic diacylglycerol content, which activates protein kinase Cε, impairing insulin receptor signaling [4, 8, 10]. Conversely, systemic insulin resistance promotes adipose lipolysis, raising free fatty acids delivered to the liver, perpetuating steatosis—a classic "vicious circle" [1, 4, 8, 20]. Chronic insulin resistance also activates pro-inflammatory cascades via JNK and NF-κB pathways, exacerbating hepatocellular injury [8, 10, 14].

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MPACTS OF WEIGHT REDUCTION

Lifestyle-based studies - emphasizing 7–10% weight reduction over 3–6 months - report a mean decrease in HOMA-IR by 1.5 units ($p < 0.01$) and ALT by approximately 25 U/L [1, 4]. Improvements in insulin sensitivity parallel reductions in hepatic fat on ultrasound or MRI. Some small trials of metformin in adolescents with MAFLD show favorable effects on HOMA-IR and liver enzymes, though findings are mixed and long-term safety data remain limited [10, 11, 16].

GONADAL AXIS: TESTOSTERONE AND SHBG IN BOYS

OBSERVED ALTERATIONS

In MAFLD-affected boys (typically in late preadolescence and adolescence), mean total testosterone declines from approximately 12.5 ng/dL (in age-matched controls) to about 8.7 ng/dL ($p < 0.01$) [7, 24, 25]. Furthermore, sex hormone-binding globulin (SHBG) levels are 20–35% lower in boys with MAFLD, correlating inversely with HOMA-IR ($r = –0.45$, $p < 0.01$) [7].

PATHOPHYSIOLOGICAL LINKS

Lower SHBG - largely produced by hepatocytes - reflects insulin-mediated suppression; hyperinsulinemia inhibits hepatic SHBG production, reducing circulating bound testosterone and raising free estradiol relative to testosterone [7, 24]. This hormonal milieu promotes central fat deposition and may delay the onset or progression of puberty [24]. Experimental data suggest that testosterone modulates hepatic lipid metabolism via PPAR α/γ receptors; low testosterone favors lipogenesis and visceral adiposity [24, 25].

CLINICAL CONSIDERATIONS

Endocrine evaluation in boys with MAFLD should include morning total testosterone and SHBG measurement. Although replacement therapy for hypogonadism is generally reserved for confirmed Leydig cell dysfunction (rather than functional suppression), some endocrinologists consider low-dose testosterone or interventions aimed at improving insulin sensitivity (e.g., metformin) to normalize SHBG and free testosterone levels [7, 25]. Longitudinal studies are needed to determine whether early normalization of testosterone/SHBG mitigates hepatic and metabolic progression.

VITAMIN D AXIS

PREVALENCE OF DEFICIENCY

In multiple cohorts, 55–70% of children with MAFLD have serum 25-hydroxyvitamin D [25(OH)D] levels below 20 ng/mL, compared to 30–40% of age-matched controls ($p < 0.01$) [9, 10, 19, 22]. Low vitamin D is more pronounced among obese MAFLD cases than lean controls, suggesting a synergistic effect of adiposity and hepatic fat on vitamin D sequestration.

MECHANISTIC LINKS

Vitamin D receptors in hepatocytes and adipocytes regulate genes involved in lipid metabolism, inflammation, and fibrogenesis. Deficiency may exacerbate insulin resistance by impairing insulin receptor expression and downstream signaling. It also promotes a pro-inflammatory state by upregulating IL-6 and TNF-α [9, 10, 19]. However, some studies found no direct correlation between 25(OH)D and histologic steatosis grade, suggesting that low vitamin D may be more a marker of overall metabolic health than a direct driver of hepatic fat (negative findings reported in [5, 22, 23, 28]).

INTERVENTIONAL DATA

Randomized controlled trials of vitamin D supplementation (2,000 IU/day for 12 weeks) in vitamin D–deficient MAFLD children show a mean reduction in HOMA-IR by 0.8 units ($p < 0.05$) and a 15% decrease in serum IL-6 ($p < 0.05$) [9, 10]. Improvements in ALT are modest and variable, but insulin sensitivity enhancements suggest a systemic benefit. Longer trials (6–12 months) are needed to assess histological outcomes.

SYNTHESIS OF HORMONAL INTERACTIONS

Pediatric MAFLD epitomizes a multisystem, hormone-driven disorder. Insulin resistance emerges as the central node, promoting hepatic fat deposition, suppressing SHBG, and impairing glucose homeosta-

sis [4, 8, 10]. Subclinical hypothyroidism compounds dyslipidemia and steatosis by reducing hepatic lipid oxidation [1, 2, 13]. Low vitamin D worsens insulin resistance and perpetuates inflammation [9, 10]. In boys, low testosterone and SHBG further drive visceral adiposity and metabolic dysfunction [7, 24, 25].

Figure 1 presents a conceptual model (adapted from Nobili et al. [4] and Di Bonito et al. [20]) illustrating how these hormonal axes interconnect, forming vicious circles that sustain and exacerbate MAFLD.

CONCEPTUAL MODEL OF HORMONAL INTERRELATIONSHIPS IN PEDIATRIC MAFLD.

Insulin resistance → ↑ free fatty acids → steatosis → hepatic insulin resistance

Subclinical hypothyroidism → ↓ lipid oxidation → steatosis

Low SHBG/testosterone (boys) → ↑ visceral fat → ↑ insulin resistance

Vitamin D deficiency → ↓ insulin sensitivity + ↑ inflammation

CLINICAL IMPLICATIONS AND RECOMMENDATIONS

Routine endocrine screening is integral to comprehensive MAFLD management. Baseline evaluation should include TSH and free T4 to detect subclinical hypothyroidism; fasting insulin and glucose for HOMA-IR calculation; morning total testosterone and SHBG in boys aged ten and older; serum 25(OH)D to assess vitamin D status; and imaging (ultrasound as first-line or MRI for quantitation) to evaluate hepatic steatosis. In many centers, transient elastography (FibroScan) estimates fibrosis, though pediatric cutoffs require further validation [4, 8]. Monitoring of these parameters every six to twelve months allows timely detection of evolving hormonal disturbances.

Lifestyle intervention remains the cornerstone of therapy, with a target of seven to ten percent weight reduction through dietary modification and increased physical activity. Such reduction consistently improves insulin sensitivity, lowers transaminases, normalizes TSH and SHBG in many cases, and enhances vitamin D status [1, 4, 7]. For children with persistent TSH above 4.5 mIU/L and elevated liver enzymes despite lifestyle changes, some clinicians initiate levothyroxine (L-T4), noting modest improvements in hepatic indices and lipid profiles [13, 29]. Similarly, vitamin D supplementation (2 000 IU/day for twelve weeks) in deficient patients has been shown to augment insulin sensitivity (HOMA-IR reduction of 0.8) and reduce IL-6 by 15 %, although effects on ALT are variable [9, 10]. Insulin sensitizers such as metformin may benefit those with impaired glucose tolerance or type 2 diabetes, but the data in isolated MAFLD are mixed [10, 11, 16]. In boys with low testosterone and SHBG, weight loss and insulin-sensitizing strategies aiming to restore endogenous hormone production are preferred over exogenous testosterone, which is generally reserved for confirmed hypogonadism [7, 24, 25].

Ethical and cost considerations include the expense of routine endocrine testing and the necessity of parental consent. Early identification and correction of subclinical hypothyroidism or vitamin D deficiency can prevent progression to type 2 diabetes and cardiovascular complications, potentially reducing long-term healthcare expenditures. However, formal cost-effectiveness analyses across diverse healthcare systems remain limited, and equitable access to these evaluations must be ensured in resource-constrained settings.

DISCUSSION

This literature review underscores that pediatric MAFLD cannot be viewed in isolation as a liver-only disease; it reflects a constellation of endocrine disruptions that both drive and result from hepatic steatosis. Across studies from Europe, Asia, and North America, consistent patterns emerge: subclinical hypothyroidism is present in up to 42 % of MAFLD children (versus 5–10 % in controls) [1–6, 12, 13, 15, 29], insulin resistance affects at least two-thirds of cases (HOMA-IR > 3) and improves with seven to ten percent weight loss (HOMA-IR reduction by 1.5, ALT reduction by ~25 U/L) [1, 4, 8–11, 14, 16, 20], and vitamin D deficiency (25(OH)D < 20 ng/mL) occurs in 55–70 % of MAFLD children (versus 30–40 % in controls), with supplementation yielding modest but significant benefits on insulin sensitivity and inflammatory markers [9, 10, 19, 22]. In boys, mean total testosterone falls from 12.5 ng/dL to 8.7 ng/dL ($p < 0.01$), with SHBG levels 20–35 % lower than in non-MAFLD peers, correlating inversely with HOMA-IR ($r = -0.45$, $p < 0.01$); low testosterone likely contributes to visceral adiposity through PPAR α/γ modulation [7, 24, 25].

Heterogeneity in diagnostic criteria - MAFLD *versus* NAFLD - and differing imaging or histologic modalities account for some variability in reported prevalence and steatosis severity (up to 12 % difference) [1, 4, 16, 17]. Nevertheless, the overarching message is that hormonal axes are fundamental to early pathogenesis and represent modifiable targets. Notable gaps include underrepresentation of girls (especially regarding PCOS features), limited studies on children aged three to six, and scarce data from African and Latin American populations, which hinders understanding of ethnic and

epigenetic influences [5, 6, 17]. Interventional trials tend to be small, short-term, and often lack histological endpoints. Further research is required to clarify whether correcting one axis (e.g., vitamin D supplementation or L-T4 for subclinical hypothyroidism) confers meaningful long-term hepatic benefits in larger, more diverse pediatric cohorts.

FUTURE RESEARCH DIRECTIONS

Prospective cohort studies employing uniform MAFLD criteria and standardized imaging—particularly MRI-PDFF—to monitor hormonal changes from early childhood through adolescence are essential. Inclusion of girls, with assessment of PCOS features, androgen profiles, and menstrual function, will elucidate sex-specific interactions. Studies focusing on preschool-aged children (three to six years) may reveal the earliest endocrine alterations, informing primary prevention efforts. Randomized controlled trials of pharmacologic agents—such as PPAR agonists (e.g., pioglitazone), GLP-1 receptor agonists, or novel insulin sensitizers—are imperative to establish safety and efficacy in pediatric MAFLD. Likewise, recruitment of ethnically diverse populations from Africa, Latin America, and underrepresented Asian regions will shed light on genetic (PNPLA3, TM6SF2, MBOAT7) and epigenetic modifiers. Finally, comprehensive cost-effectiveness and ethical analyses of routine endocrine screening and early interventions (vitamin D, levothyroxine) are needed to balance potential benefits against resource allocation, especially in low-income settings.

CONCLUSIONS

Pediatric MAFLD represents a multisystem disorder in which endocrine dysregulation is both a driver and a consequence of hepatic steatosis. High rates of subclinical hypothyroidism, marked insulin resistance, reduced testosterone/SHBG in boys, and widespread vitamin D deficiency underscore the need for an interdisciplinary approach that brings together pediatric hepatology, endocrinology, nutrition, and primary care.

Initial evaluation should include TSH and free T4 to assess thyroid function; fasting insulin and glucose for HOMA-IR calculation; total testosterone and SHBG in boys aged ten and older; and serum 25(OH)D to gauge vitamin D status. Imaging (ultrasound or MRI) remains central to quantifying hepatic steatosis. Monitoring all endocrine parameters every six to twelve months, alongside serial liver imaging, permits early detection of evolving disturbances. Lifestyle interventions aiming for seven to ten percent weight loss should be the first-line therapy, as they improve insulin sensitivity, normalize TSH and SHBG in many cases, and often raise vitamin D levels. Levothyroxine may be considered for persistent subclinical hypothyroidism with ongoing transaminase elevation. Vitamin D supplementation (2 000 IU/day for twelve weeks) in deficient children has demonstrated improvements in insulin sensitivity and reductions in inflammatory markers. Metformin, when indicated for impaired glucose tolerance or type 2 diabetes, may also benefit MAFLD, though evidence in isolated MAFLD remains mixed. For boys with low testosterone and SHBG, emphasis should be placed on weight loss and insulin sensitization rather than exogenous testosterone, which is reserved for confirmed hypogonadism.

Early detection and correction of hormonal disturbances have the potential to slow MAFLD progression, reduce inflammation and fibrosis risk, and improve long-term metabolic outcomes. Collaboration among pediatric hepatologists, endocrinologists, dietitians, and primary care providers is essential for optimizing care. Future research must address existing gaps—particularly in female cohorts, younger age groups, ethnically diverse populations, and rigorous interventional trials—to refine screening guidelines and therapeutic algorithms for pediatric MAFLD.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

Contraceptive options for women with depression, bipolar disorder, schizophrenia, and premenstrual dysphoric disorder

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ABSTRACT

Aim: To select and analyse the scientific literature on the impact of contraception on women with depression, bipolar disorder, schizophrenia, and premenstrual dysphoric disorder.

Material and Methods: A detailed literature analysis was performed using PubMed, WebMD, ScienceDirect, and Google Scholar databases. The articles and books included were written in English and published between 2011 and 2024. A literature review was conducted in the PubMed, WebMD, ScienceDirect, and Google Scholar databases, analysing scientific articles in English from 2011 to 2024. Studies evaluating the relationship between contraceptive use and mental health conditions, including depression, bipolar disorder, schizophrenia, and premenstrual dysphoric disorder (PMDD), were selected. The search combined the following keywords using appropriate Boolean operators (i.e., AND, OR): "contraception", "depression", "bipolar disorder", "schizophrenia", "premenstrual dysphoric disorder", and "mental health".

Conclusions: The impact of hormonal contraception on mental health varies across conditions, making personalized contraceptive counselling essential. Further research is needed to fully understand these complex interactions and develop evidence-based guidelines.

KEY WORDS: bipolar disorder, schizophrenia, depression, contraception, premenstrual dysphoric disorder, mental health

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INTRODUCTION

Hormonal contraceptives are used worldwide by more than 100 million women [1]. An association between oral contraceptive use and the emergence of symptoms of mental disorders has been suggested. Despite their widespread use, the uptake of oral contraceptives has declined in recent years. This decrease is likely due to multiple factors, including more frequent choice of long-acting reversible contraception (LARC) methods, and growing awareness of potential side effects, particularly related to mental health issues [2]. For all reproductive-age women with psychiatric disorders, it is essential to address contraceptive needs and method selection as part of their care plan. Use of inappropriate methods, or non-use, may result in unintended pregnancy, and the sequelae of unplanned pregnancy can be significant, including high-risk pregnancy in the setting of poorly controlled disease or potential exposure to teratogenic medications [3]. Unintended pregnancy is also associated with new episodes of psychiatric disorders, including perinatal depression. Once an episode occurs, a recurrent cycle of risk evolves. The postpartum period is associated with an elevated risk of the emergence of mood disorders in women sensitive to rapid hormonal change. Psychiatric illness instability increases health risks for both the mother and the fetus. That is the reason why adequate information about the methods of contraception should be provided to all women with mental health disorders [4].

Patients and providers may also be concerned about the co-administration of hormonal contraceptives (HCs) with psychotropic medications, given the complex pharmacology of these drugs, so it is crucial to know if there are any interactions [5]. Women with psychiatric disorders often experience difficulties using contraceptive methods, leading to a higher risk of unintended pregnancies. This is partly due to factors such as forgetfulness, cognitive impairments, and the complexities of managing their health conditions alongside contraceptive practices [3]. The best contraceptive method for women with psychiatric disorders will depend on their specific needs and expectations; the goal of this article is to provide clinicians with guidance on choosing the appropriate form of contraception.

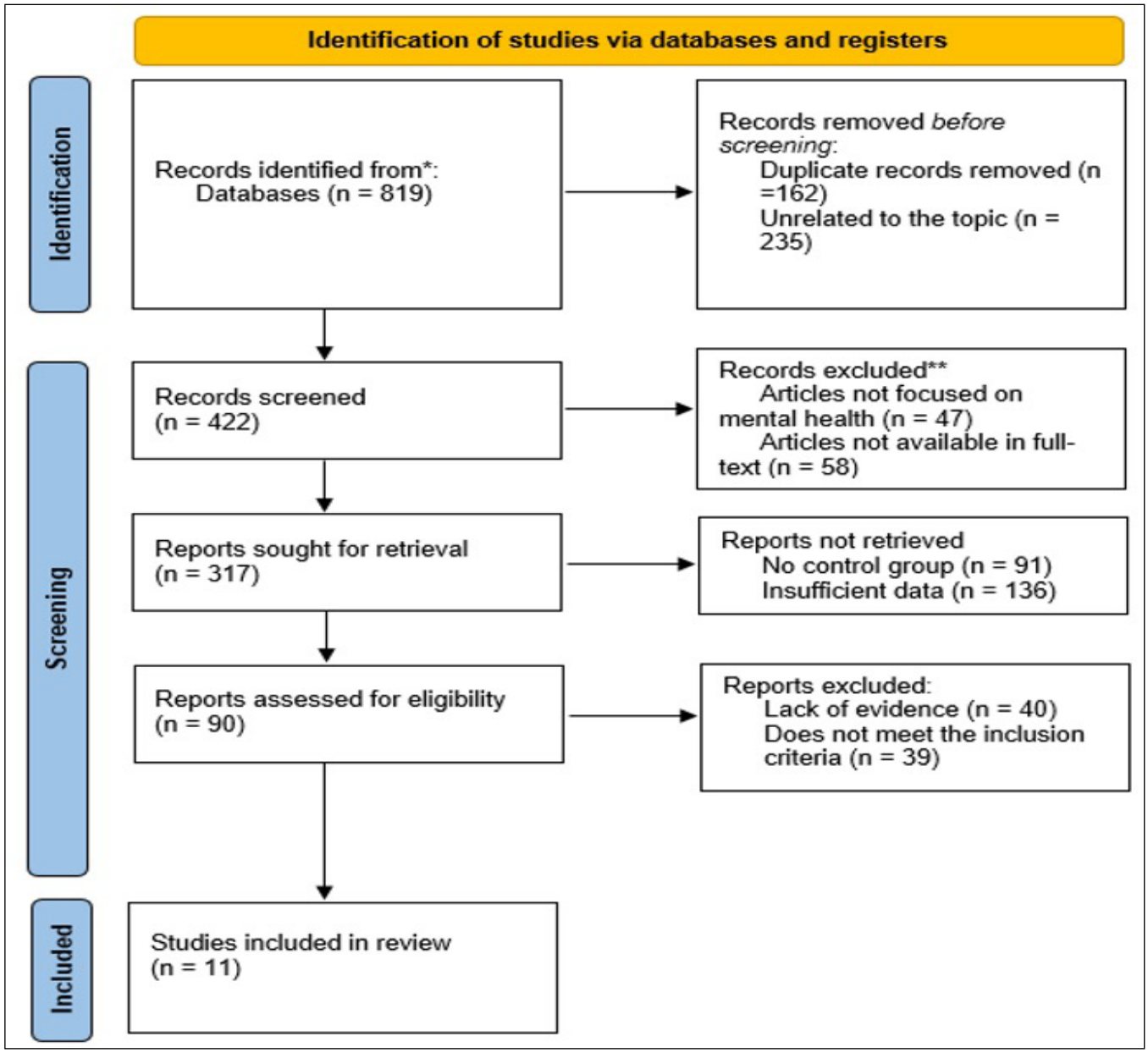


Fig. 1. PRISMA Flow Diagram of Study Selection Process. This figure illustrates the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow chart detailing the systematic review process, identifying and selecting studies examining contraceptive use, safety, and mental health outcomes in women with depression, bipolar disorder, schizophrenia, or premenstrual dysphoric disorder
Picture taken by the authors

AIM

To select and analyse the scientific literature on the impact of contraception on women with depression, bipolar disorder, schizophrenia, and premenstrual dysphoric disorder.

MATERIALS AND METHODS

SEARCH STRATEGY

A literature review was conducted in the PubMed, WebMD, ScienceDirect, and Google Scholar databases, analysing scientific articles in English from 2011 to 2024. Studies evaluating the relationship between contraceptive use and mental health conditions, including depression, bipolar disorder, schizophrenia, and premenstrual dysphoric disorder (PMDD), were selected. The search combined the following keywords using appropriate Boolean operators (i.e., AND, OR): "contraception", "depression", "bipolar disorder", "schizophrenia", "premenstrual dysphoric disorder", and "mental health".

INCLUSION AND EXCLUSION CRITERIA

Studies meeting the following criteria were included in the review:

1. Analysis of contraceptive methods in women diagnosed with mental health conditions, specifically depression, bipolar disorder, schizophrenia, or PMDD, with a focus on method selection, safety, and impact on mental health.
2. Study populations included reproductive-age women diagnosed with one or more of the listed psychiatric disorders, as well as healthy control groups without psychiatric diagnoses.

Studies were excluded if they met any of the following criteria:

1. Conference papers or editorials;
2. Duplicated studies.

DATA ANALYSIS

The studies' results were evaluated using Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

STUDY SELECTION

A total of 819 articles were identified. After removing duplicates and those not meeting the inclusion criteria, a portion was selected for full-text review. 11 studies were determined to meet the inclusion criteria. The results are outlined in the PRISMA diagram (Fig. 1).

REVIEW AND DISCUSSION

CONTRACEPTION AND DEPRESSION

Hormonal contraceptive use, a prevalent method of birth control globally, has been linked to the onset of depressive symptoms in some women, sparking ongoing debates in the scientific community. Several studies have found that hormonal contraception, especially among adolescents, is associated with an increased risk of antidepressant use and depression diagnosis, indicating a potential adverse effect on mental health [1,6]. In particular, younger women are considered the most vulnerable to these risks, with adolescent use of oral contraceptives possibly elevating the likelihood of depression later in life [7]. Furthermore, hormonal contraception has been correlated with a higher risk of suicide attempts, with adolescents showing the highest relative risk [8].

The levonorgestrel-releasing intrauterine system (LNG-IUS) is linked to the onset of depression in a dose-dependent way, with low-, medium-, and high-dose levonorgestrel in the IUS all demonstrating this association. While the observational nature of the study prevents any definitive conclusions about causality, the dose-response pattern adds to the evidence suggesting a connection between levonorgestrel exposure and the risk of developing depression [9].

On the other hand, other research has suggested that hormonal contraceptives do not directly cause depressive disorders, and some studies have found no significant association between contraceptive use and depression [10,11].

Since women in different contraceptive use categories often differ in age, parity, education, exercise habits, health behaviors, smoking status, and various other factors, it can sometimes be challenging to determine whether contraceptives are the direct cause of depression [12]. While oral contraceptive use was not linked to depressive symptoms across all age groups combined, 16-year-old girls reported higher scores for depressive symptoms when using oral contraceptives [13]. Young women appear to be the most vulnerable to the effects of hormonal contraception. Teens should be informed about the potential risks of hormonal contraceptives, including the possibility of a link to depression [14].

A theory suggests that hormonal changes can trigger depressive symptoms, although the exact mechanisms by which this occurs in some women are still unclear. There is also evidence pointing to a genetic predisposition for hormone-induced depression. For example, a twin study identified a genetic basis for depressive symptoms associated with contraceptive use [15]. Additionally, women with depression are more likely to experience unintentional pregnancies, often due to contraceptive errors, which can negatively impact both the parents and the unborn child [16].

Patients with depression may require special consideration due to the potential interactions between psychotropic medications used for depression and hormonal contraception. Some drugs, such as selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs) are safe and can be used together with any contraceptive method. However, medications used for refractory depression, such as tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), and atypical antipsychotics, may interact with hormonal contraception. TCAs and MAOIs can affect steroid metabolism in the liver, potentially decreasing the effectiveness of contraceptive methods like implants, combined oral contraceptive pills, patches, rings, injectables, and progesterone-only pills. In such cases, locally acting IUDs are the preferred choice. Atypical antipsychotics such as risperidone, aripiprazole, and ziprasidone may cause hyperprolactinemia, leading to abnormal menstrual cycles, fertility problems, and galactorrhea. These side effects can be managed with COCPs [17]. Additionally, the herbal remedy St. John's Wort, used to treat mild depression,

induces cytochrome P450 enzymes, particularly CYP3A4, which may speed up the metabolism of both the ethinyl estradiol and progestin components in hormonal contraceptives, lowering their effectiveness and increasing the risk of unintended pregnancy. As a result, locally acting IUDs are considered the preferred alternative [18].

CONTRACEPTION AND BIPOLAR DISORDER

Bipolar disorder is a chronic, recurrent condition characterized by fluctuations in mood and energy, impacting more than 1% of the global population [19]. One of the symptoms of bipolar disorder is impulsivity, which is characterized by a tendency to act fast, without considering the consequences for oneself or others [20] there is mixed evidence as to whether impulsivity is a trait feature of the disorder, present in the euthymic state in the absence of mania. The aim of this systematic review and meta-analysis was to examine whether impulsivity is elevated in euthymic BD in comparison to controls. Electronic databases were searched for papers published until April 2022 reporting data on a self-report or behavioural measure of impulsivity in a euthymic BD group and a healthy control group. In total, 46 studies were identified. Euthymic BD showed significantly higher levels of self-reported impulsivity compared to controls (large effect size. As a result, women with bipolar disorder are more likely to engage in risky sexual behaviors, increasing the likelihood of unwanted pregnancies, which often lead to abortions [20,21]there is mixed evidence as to whether impulsivity is a trait feature of the disorder, present in the euthymic state in the absence of mania. The aim of this systematic review and meta-analysis was to examine whether impulsivity is elevated in euthymic BD in comparison to controls. Electronic databases were searched for papers published until April 2022 reporting data on a self-report or behavioural measure of impulsivity in a euthymic BD group and a healthy control group. In total, 46 studies were identified. Euthymic BD showed significantly higher levels of self-reported impulsivity compared to controls (large effect size. This is linked to challenges in planning their actions, a lack of proper sex education, and insufficient counseling from healthcare providers, which can result in negative obstetric outcomes and an inadequate ability to care for their children [21].

According to a study conducted by Meliha Zengin Eroglu et al. [22], the rate of unplanned pregnancies in women with bipolar disorder was significantly higher compared to women without the disorder, with rates of 49.52% and 15.04%, respectively. Although most of the women with bipolar disorder used contraception, they were more likely to choose less reliable methods (e.g., withdrawal, calendar method). Therefore, it is recommended that all clinicians provide information about effective contraception to women with bipolar disorder to prevent sexually transmitted infections and unplanned pregnancies, thus protecting the health of both the mother and fetus.

Women with bipolar disorder may benefit from the use of COCPs, as they may experience a milder disease course compared to those using nonhormonal methods or no contraception. This may be linked to a pattern where mood swings in women with bipolar disorder align with their menstrual cycle. However, it remains uncertain whether these mood changes are specifically related to bipolar disorder or are also influenced by the presence of co-occurring premenstrual dysphoric disorder, which could also improve with hormonal contraceptives [12].

CONTRACEPTION AND SCHIZOPHRENIA

Hormonal contraceptive use, a prevalent method of birth control globally, has been linked to the onset of depressive symptoms in some women, sparking ongoing debates in the scientific community. Several studies have found that hormonal contraception, especially among adolescents, is associated with an increased risk of antidepressant use and depression diagnosis, indicating a potential adverse effect on mental health [1,6]. In particular, younger women are considered the most vulnerable to these risks, with adolescent use of oral contraceptives possibly elevating the likelihood of depression later in life [7]. Furthermore, hormonal contraception has been correlated with a higher risk of suicide attempts, with adolescents showing the highest relative risk [8].

Women with schizophrenia are more likely to experience unplanned pregnancies compared to the general population. These pregnancies may stem from factors such as social vulnerability, a higher risk of sexual assault, and a greater prevalence of concurrent substance use disorders among women with schizophrenia. Schizophrenia is also associated with impaired cognitive processing, social difficulties, and distorted perception of reality. These challenges can make it hard to plan and consistently use contraception during intimate relationships, even in planned and consensual sexual encounters [26]. These women have complex health needs that require specific reproductive counseling. Women who have multiple sexual partners should receive advice on barrier contraception methods. For women who don't want to have children soon,

long-acting reversible contraception (LARC) methods such as intrauterine devices (IUDs), progesterone depot injections, or implants are reasonable options [27]. Targeted reproductive counseling and support are essential to address the unique health needs of women with schizophrenia, helping them make informed choices about their reproductive health.

THE INTERACTION OF HORMONAL CONTRACEPTION WITH MEDICATIONS USED FOR BIPOLAR DISORDER OR SCHIZOPHRENIA TREATMENT

Bipolar disorder and schizophrenia are distinct, but the overlap in symptoms leads to similarities in treatment approaches. As a result, medications prescribed for both conditions often belong to the same classes, including mood stabilizers (lithium and anticonvulsants), atypical and typical antipsychotics [28]and psychotropic drugs represent cornerstones in the treatment. The primary aim of the review was to summarize the latest evidence with regards to the efficacy and effectiveness of drug treatment of schizophrenia and the manic phases of bipolar disorder. Schizophrenia systematic reviews conclude that antipsychotic drugs are effective in treating overall symptoms of psychosis and in preventing relapse. Some of the newer agents, the second-generation antipsychotics (SGAs.

Hormonal contraceptives may interact with anticonvulsants such as lamotrigine, valproate, carbamazepine, oxcarbazepine, and topiramate. Serum concentrations of lamotrigine and valproate are decreased when ethinyl estradiol is co-administered, probably due to accelerated glucuronidation. Serum concentrations of lamotrigine may be reduced by more than 50%, whereas the effect on valproate concentrations was small and of unclear clinical significance [29]. To prevent the risk of decreased lamotrigine concentrations, women taking lamotrigine should be advised to avoid combined hormonal contraceptives containing ethinyl estradiol, such as combined oral contraceptive pills, patches, or rings. Recommended alternatives include IUDs, implants, injectables, or progesterone-only pills [17].

Carbamazepine, oxcarbazepine, and topiramate induce the cytochrome (CYP) 450 3A4 enzyme. This induction enhances the metabolism of estrogen and progesterone, potentially reducing the contraceptive efficacy of oral contraceptive pills; therefore, an alternative contraceptive option should be chosen [27].

Antipsychotic drugs like risperidone, aripiprazole, and ziprasidone can lead to hyperprolactinemia. In such cases, COCPs are the first choice of treatment [17].

Antipsychotic medications clozapine and chlorpromazine are metabolized by (CYP)3A4, 1A2, and 2C19. These liver enzymes are inhibited by estrogen and progesterone; therefore, if clozapine or chlorpromazine are taken together with COCP, the concentration of antipsychotics increases. For patients using these antipsychotic drugs, alternatives to hormonal contraception should be explored, and patients should be informed about possible interactions. If oral contraceptives are selected, levels of clozapine and chlorpromazine must be regularly checked [27,30]. The antipsychotic ziprasidone, despite being metabolized by the CYP 3A4 enzyme, does not significantly inhibit it at clinically relevant doses [27].

Additionally, it is essential to acknowledge that certain mood stabilizers, particularly valproic acid and lithium, are teratogenic and should be avoided in women of reproductive age. If switching to safer alternatives, such as lamotrigine or certain atypical antipsychotics, is not possible, it is crucial to ensure effective contraception to prevent unplanned pregnancies and potential adverse outcomes [31].

CONTRACEPTION AND PREMENSTRUAL DYSPHORIC DISORDER

Premenstrual symptoms, including both psychological and physical effects, occur cyclically before menstruation and fade after it. While most women experience at least mild discomfort, around 5% of women meet the diagnostic criteria for PMDD. The first-line treatment of PMDD is the use of serotonin reuptake inhibitors; however, it can also be treated with combined oral contraceptive pills (COCP) [32].

A study in the Japanese population conducted by Takashi Takeda et al. [33] investigated the effects of COCPs containing drospirenone 3 mg plus ethinyl estradiol 20 μg taken in a 24/4 regimen on PMDD symptoms. 48 women received the treatment, and by the third and sixth treatment cycles, most premenstrual symptoms had significantly improved.

Meanwhile, progesterone-only contraceptives, such as the mini-pill or the LNG-IUS, may worsen mood symptoms in some women, suggesting that progesterone could have a negative impact on mood for women with PMDD [34]affecting 2-5% of women. Combined oral contraceptive pills (COCPs. Therefore, women with PMDD seeking contraception should be offered combined hormonal contraceptive pills.

The relationship between hormonal contraception and psychiatric conditions, such as depression, bipolar disorder, schizophrenia, and premenstrual dysphoric disorder, presents a multifaceted area of research.

In the case of depression, findings are ambivalent.

Table 1. Contraceptive recommendations for depression, bipolar disorder, schizophrenia, and premenstrual dysphoric disorder

Psychiatric Disorder	Medications	Recommendations
Depression	- TCA/MAOI/St. John's Wort can decrease contraceptive hormone levels, increasing the risk of unintended pregnancy. - SSRIs and SNRIs are safe to use with all types of contraception	-LARCs are recommended for high efficacy and low maintenance when adherence is compromised. - CHCs may worsen mood symptoms, especially in adolescents. Alternative contraception methods are preferred - LNG-IUD may worsen mood symptoms in a dose-dependent manner, individualize based on patient response.
Bipolar Disorder Schizophrenia	- Antiepileptics (carbamazepine, oxcarbazepine, topiramate) decrease contraceptive hormone levels, increasing pregnancy risk. - Antipsychotic medication (clozapine and chlorpromazine) serum levels may increase when using COCPs - Atypical antipsychotics may increase prolactin levels, causing irregular periods, fertility problems, and galactorrhea. Estradiol can help to manage these side effects. - Lamotrigine levels are reduced when combined with CHCs, so mood stability should be monitored.	- LARCs are recommended for high efficacy and low maintenance when adherence is compromised. - Barrier methods advised for STI risk, especially with multiple partners - COCPs may improve mood symptoms related to the menstrual cycle. - Ensure effective contraception for women of reproductive age taking valproate or lithium.
Premenstrual Dysphoric Disorder	-	- COCPs with ethinyl estradiol and drospirenone are effective for both contraception and treating PMDD. - Monitor mood with progesterone-only methods, as they may worsen symptoms.

Source: compiled by the authors based on [7,9,12,17,18,22,27,29-32]

While some studies suggest that there is an association between hormonal contraceptives and the worsening of depressive symptoms, other studies show no significant relationship, especially with the use of progestin-only contraception [1,6,10,11]. The users who seem particularly susceptible to these mood-related side effects are adolescents [7]. That highlights the need for age-specific guidelines and mental health support for young women starting to use or already using hormonal contraceptives. However, given the wide range of studies, the lack of clear causality suggests the possible involvement of other factors, such as genetics and pre-existing mental health conditions, in mediating these effects [12]. Women with depression on SSRIs or SNRIs can use any contraceptive method since they do not interact with hormonal contraception. However, those taking TCAs, MAOIs, and St. John's Wort may benefit more from locally acting IUDs due to potential interactions affecting the efficacy of other hormonal contraceptives [17,18].

For both bipolar disorder and schizophrenia, unplanned pregnancies are a common concern due to a combination of impulsivity, cognitive challenges, and difficulties with consistent contraceptive use [20,21,26] there is mixed evidence as to whether impulsivity is a trait feature of the disorder, present in the euthymic state in the absence of mania. The aim of this systematic review and meta-analysis was to examine whether impulsivity is elevated in euthymic BD in comparison to controls. Electronic databases were searched for papers published until April 2022 reporting data on a self-report or behavioural measure of impulsivity in a euthymic BD group and a healthy control group. In total, 46 studies were identified. Euthymic BD showed significantly higher levels of self-reported impulsivity compared to controls (large effect size. Therefore, LARCs are reliable options that require less frequent adherence, making them particularly suitable for these populations [27]. Additionally, targeted counselling is essential to ensure women with bipolar disorder and schizophrenia understand the most effective contraceptive options and how these choices may intersect with their mental health needs. Medications commonly used to manage these disorders can interact with hormonal contraceptives. The anticonvulsant lamotrigine may have reduced efficacy when combined with hormonal contraceptives containing ethinyl estradiol, necessitating alternative options like non-hormonal IUDs or progesterone-only methods [17,29]. Carbamazepine, oxcarbazepine, and topiramate can reduce the effectiveness of oral contraceptives by accelerating the metabolism of estrogen and progesterone, increasing the risk of unintended pregnancies; therefore, alternative contraceptive methods should

be considered [27]. Clozapine and chlorpromazine can have pharmacodynamic interactions with hormonal contraceptives, which may increase the plasma concentration of antipsychotic medications, requiring careful monitoring or the use of non-hormonal contraception [27,30]. For women taking atypical antipsychotics, such as risperidone, aripiprazole, and ziprasidone, combined oral contraceptive pills may help manage symptoms of hyperprolactinemia [17].

For premenstrual dysphoric disorder, combined oral contraceptives containing drospirenone and ethinyl estradiol have demonstrated promising effects in reducing severe premenstrual symptoms, offering advantages of symptom relief and contraception. This is particularly important, as PMDD greatly affects quality of life, and COCs may provide both effective contraception and symptom management [33].

While hormonal contraception is important for reproductive control, its possible effects on mental health call for personalized, closely monitored approaches. More comprehensive research is needed to explore the long-term mental health impacts of different contraceptive formulations and their interactions with psychotropic medications. Personalized counseling and education on the benefits and risks of hormonal contraception are crucial, especially for adolescents and women with mental health conditions (Table 1).

CONCLUSIONS

The impact of hormonal contraception on mental health is complicated, with differences across different conditions and individuals. Hormonal contraceptives are widely used, but studies suggest they may pose mental health risks, particularly for adolescents, who appear more prone to depression and suicide attempts. COCPs can be an effective option for managing premenstrual dysphoric disorder and may serve as a second-line treatment. These pills might also offer benefits to women with bipolar disorder by alleviating mood fluctuations tied to the menstrual cycle.

There are potential interactions with some of the medications used for depression, bipolar disorder, and schizophrenia. The efficacy of contraception may be reduced when it is taken together with St. John's Wort, TCAs, MAOIs, carbamazepine, oxcarbazepine, and

topiramate, which can potentially lead to unintended pregnancies. Additionally, hormonal contraception can lower the efficacy of lamotrigine, thus reducing its effectiveness, and may increase plasma levels of clozapine and chlorpromazine, which can lead to severe side effects. On the other hand, COCPs may help manage symptoms of hyperprolactinemia for those taking atypical antipsychotics.

Patients with bipolar disorder and schizophrenia are at a higher risk of unplanned pregnancies, so it is important for healthcare professionals to provide essential information on effective methods of contraception and STI prevention, helping them choose the option best suited to their needs.

Given these complexities, individualized contraceptive counseling is essential to ensure safe and effective choices for women with mental health disorders. Further research is needed to confirm the credibility of existing evidence.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

The system of international legal standards in the medical sphere: Universal and regional dimensions

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ABSTRACT

Aim: A comprehensive theoretical and legal analysis of international legal standards in healthcare, determining their content, classification and regulation features at universal and regional levels. Tasks include analyzing the concept of international health law standards, investigating historical formation stages, systematizing international legal acts, determining universal and regional protection features, analyzing specialized standards for certain population categories, and investigating implementation mechanisms at national level.

Materials and Methods: Employ general scientific and special legal methods including dialectical, system-structural, historical-legal, comparative-legal, and formal-legal approaches, along with classification and systematization methods to structure international legal acts and create scientifically based typology. The empirical base consisted of 189 sources for the period 1948-2025: universal international acts of the UN and specialized organizations (23 documents), regional European agreements (15 acts), specialized conventions for individual categories of the population (18 documents), declarations and charters of a non-conventional type (12 acts), scientific publications (121 works). The search was carried out in the following databases: UN Treaty Collection, Council of Europe Treaty Office, WHO Global Health Observatory, Google Scholar, ResearchGate, PubMed.

Conclusions: establish that international healthcare standards constitute a specific category with both general features (obligatory, universal, abstract) and special properties (specificity, scientificity, practicality, reliability). The evolutionary development from 1940s declarative principles to modern detailed norms reflects transition from state-centric to patient-oriented approaches. The multi-level structure includes universal/regional standards, mandatory/recommendatory norms, and general/special provisions, creating flexible legal regulation addressing diverse healthcare subjects' needs.

KEY WORDS: international legal standards, healthcare rights, patient rights, medical law, healthcare regulation, human dignity, medical ethics, legal protection

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INTRODUCTION

The relevance of the study of international legal standards in the field of health care is due to several factors of the modern development of international law and global challenges in the field of health care. In the context of the COVID-19 pandemic, which demonstrated the interdependence of national health care systems and the need for coordination of international efforts, the issues of the effectiveness and efficiency of international legal mechanisms for protecting the right to health have become particularly acute. Modern global challenges, such as the cross-border spread of infectious diseases, inequality in access to medical services, the development of biotechnology and artificial intelligence in medicine, require a review and improvement of existing international standards. In addition, the growing role of non-state actors in the field of health care, including international organizations, NGOs and private providers of medical services, requires a new understanding of the subject composition of international legal relations in this area.

The theoretical and practical significance of the study lies in the need to systematize and analyze international legal standards of health care in the context of their constant expansion and complication. The fragmentation of international health care law due to the presence of numerous universal and regional documents adopted by various international organizations creates problems for law enforcement and requires scientific understanding of their correlation and hierarchy. The issue of implementing international standards at the national level is of particular relevance, in particular in the context of the European integration processes of Ukraine and the need to bring national legislation into line with European health care standards. The study of international legal standards in this area is of practical

importance for improving national health care policy, protecting patients' rights and ensuring the quality of medical services in accordance with international requirements.

AIM

The aim of the study is a comprehensive theoretical and legal analysis of the system of international legal standards in the field of the right to health, determining their content, classification and features of legal regulation at the universal and regional levels. To achieve the goal, it is necessary to solve the following tasks: analyze the concept and essence of international legal standards of the right to health; investigate the historical stages of the formation and development of international standards in the medical and legal sphere; systematize and classify international legal acts regulating the right to health; determine the features of universal and regional standards for the protection of the right to health; analyze specialized international standards for certain categories of persons; investigate the mechanisms for implementing international legal standards at the national level.

MATERIALS AND METHODS

The methodological basis of the study is a set of general scientific and special legal methods of cognition. The dialectical method was applied as a general scientific approach to the study of international legal standards in their development and interconnection. The system-structural method was used to analyze the system of international legal acts and their internal interconnections. The historical-legal method allowed us to trace the evolution of international standards of the right to health from the initial declarative principles to modern detailed norms. The comparative-legal method was used to compare universal and regional standards, as well as to analyze different approaches of international organizations to regulating the right to health. The formal-legal method was used to analyze the content of international legal norms, their structure and legal techniques. Methods of classification and systematization allowed us to structure international legal acts according to various criteria and create their scientifically based typology.

Criteria for inclusion of materials: for international legal acts (universal conventions and pacts ratified by more than 50 states, regional agreements of the Council of Europe and the EU in the field of health care, specialized documents to protect the rights of certain categories of the population (women, children, persons with disabilities), declarations and charters adopted by international organizations after 1948. For scientific publications (articles in peer-reviewed journals on international law and medical law for 1990-2023, monographs and dissertation studies on the issues of international health care standards, publications by leading experts of the WHO, the Council of Europe and other international organizations, works in Ukrainian, English, French and German.

Data sources: international databases (UN Treaty Collection, Council of Europe Treaty Office, WHO Global Health Observatory, EUR-Lex (EU law database), International Labour Organization database), наукові бази даних (Google Scholar, ResearchGate, PubMed, Scopus, Web of Science, HeinOnline Legal Database).

Total number of analyzed sources: 189, including universal international acts: 23 (including the Universal Declaration of Human Rights of 1948, the International Covenants of 1966, UN and ILO conventions), regional European documents: 15 (European Social Charter, Convention on Human Rights and Biomedicine, European Charter of Patients' Rights of 2002), specialized conventions: 18 (conventions on the rights of women, children, persons with disabilities, migrant workers), declarations and charters of a non-conventional type: 12 (Jakarta Declaration, Charter of the Right to Health), scientific publications: 121 (of which 67 are in English, 54 in Ukrainian).

Chronological framework of the analysis: main period: 1948-2023 (from the adoption of the Universal Declaration of Human Rights to the present); key stages: 1940-1960 - the formation of basic principles, 1970-1990 - the development of specialized standards, 2000-2023 - a patient-centered approach.

Time frame of the study: the study was conducted during 2024-2025 with an emphasis on the analysis of the current state of international legal standards and their impact on the national legislation of Ukraine in the context of European integration processes.

REVIEW AND DISCUSSION

Human rights represent the ability of an individual to freely carry out his actions and independently determine the forms and scope of his own behavior to ensure various life needs through the use of public goods within the framework established by regulatory and legal documents. International legal norms in this area were formed over a long period of time through the cooperation of numerous countries to develop common approaches to human rights issues. States orient their policy towards compliance with generally recognized principles, guaranteeing the protection of human rights

by all available means. They are responsible for fulfilling their obligations both to their own citizens and to the world community to ensure the rights and freedoms of the individual. Due to the increased attention of international organizations, about 300 various documents were adopted - declarations, conventions and charters. International legal documents in the field of human rights are usually regarded as international standards, since they are created on the basis of traditional norms that were formed as a result of the recognition by states of the binding nature of the rules of conduct proclaimed by the UN General Assembly through declarations or recommendations [1].

It should be noted that the bulk of international instruments and protection mechanisms emerged in the 1940s of the twentieth century. Human rights institutions contributed to the adoption of both global and regional legal instruments. The establishment of the United Nations and the approval of its Charter marked the beginning of a fundamentally new period in international relations regarding human rights. The UN Charter imposed on countries the obligation to ensure human rights and freedoms, excluding any forms of discrimination. On December 10, 1948, the UN General Assembly adopted the Universal Declaration of Human Rights - one of the most important documents in the field of human rights protection. Its introductory part and thirty articles enshrine civil, political, economic, social and cultural rights of the individual [2].

According to S. Shevchuk, international standards in the field of human rights are universally accepted norms of international law that determine the legal status of an individual at the global level, fix a catalog of fundamental rights and freedoms, establish the obligations of states to observe these rights and freedoms, and also outline the boundaries of their potential or permissible restriction [3]. According to N. Krestovska and L. Matveeva, international legal standards in the field of human rights are legal principles enshrined in international agreements that determine the lowest acceptable standards of behavior of a state in relation to persons staying on its territory [4].

It is worth noting that such an interpretation does not cover the full range of international legal principles that regulate fundamental human rights, since they are formulated not only through international agreements (as noted above - "established in a contractual manner"), but also through documents of a non-contractual type. In addition, it is unnecessary to specify the nature of such principles as "norms of minimally acceptable treatment", since the standard is essentially a "normative minimum", from which the state can deviate only by increasing or detailing it [5]. For his part, Y. Rymarenko offers the following interpretation: international human rights standards are specific criteria for these rights, fixed in international agreements and other international documents, which states should strive to achieve or which they are obliged to ensure [6].

However, from our point of view, the most successful definition that encompasses all the key characteristics of international legal standards of human rights is the formulation of O. Rudneva. The researcher suggests interpreting international legal standards of human rights and freedoms as universal principles and general norms that form exemplary models of consolidation, implementation and (or) protection of human rights, reflect the reference understanding and practical implementation of human rights, their independent and functional significance for the state and society [7].

It should be noted that international legal standards in the field of health care constitute a type of international legal standards. By this concept we mean the principles and provisions enshrined in international legal documents that regulate the rights of the individual in the field of health care, their essence and boundaries. The importance of such standards lies in the fact that they serve as certain legal or moral and political guidelines in the field of health care for state national policy [8].

International legal standards in the field of health care have special characteristics that can be divided into general and specific. General ones include properties inherent in all international standards; these include: determination of the specific scope and content of human rights; mandatory nature; features of the application of sanctions; universality; possibility of various interpretations; high degree of abstraction; historical variability of content. Specific characteristics of international legal standards in the field of health care are: accuracy, clarity and accessibility of formulations; scientificity; timeliness; practicality; reliability [9].

In legal scientific literature, international legal standards in the field of health care are divided into different categories according to certain criteria. In particular, by deontic status they are divided into mandatory - those whose compliance is formally necessary for states and can be ensured by international political and legal sanctions, and recommendatory - formally optional, but also supported by international political sanctions. According to ontic status, international standards are classified into nominal (textual) - these can only be the names of human rights and freedoms used in international documents, and factual - those that contain specific indicators of the content and scope of human rights and freedoms. By the range of subjects, they are divided into general (apply to all categories and groups

of society) and special (relate to the rights of certain groups, social communities - women, children, people with disabilities). Depending on the geographical scope, they are divided into world (also called global or universal) and regional standards. According to the subject of adoption, international legal standards in the field of health care are classified into international standards of the United Nations, the World Health Organization, the International Labor Organization, the Council of Europe, the European Union, etc. [10].

Thus, the way of external expression of international legal standards of the right to health care is international legal acts. That is, international legal acts become a source and correlate with international legal standards as a form and content, respectively.

Universal international agreements are distinguished by the fact that almost all countries of the world community participate in them. In the field of health care, these include: 1) international acts adopted by the UN: a) the International Bill of Human Rights, which consists of the Universal Declaration of Human Rights of 1948, the International Covenant on Economic, Social and Cultural Rights of 1966 and the International Covenant on Civil and Political Rights of 1966; b) international agreements regulating the rights of specific population groups (in particular, the Declaration on the Rights of Persons with Disabilities of December 9, 1975, the Declaration on the Rights of Mentally Retarded Persons of December 20, 1971, the Convention on the Elimination of All Forms of Discrimination against Women of December 18, 1979, etc.); 2) conventions approved by the International Labor Organization (in particular, Convention No. 130 concerning Medical Assistance and Sickness Assistance of 1969) [11].

Part 1 of Art. 25 of the Universal Declaration of Human Rights establishes the following provision: "Everyone has the right to a standard of living adequate for the health and well-being of himself and of his family, including food, clothing, housing and medical care and necessary social services, and the right to security in the event of unemployment, sickness, disability, widowhood, old age or other lack of livelihood in circumstances beyond his control" [12]. In addition, the Universal Declaration of Human Rights establishes other rights that are inseparably linked to the right to health or provide the prerequisites for its exercise (the right to life, the right to physical integrity, the right to privacy, etc.). The right to the highest attainable standard of physical and mental health is regulated by Article 12 of the International Covenant on Economic, Social and Cultural Rights. In order to guarantee the realization of this right, part 2 of the said article of the Covenant obliges States to take the necessary measures to: a) ensure the reduction of stillbirths and infant mortality rates and to promote the healthy development of children; b) improve all aspects of environmental sanitation and industrial hygiene; c) prevent, treat and combat epidemic, endemic, occupational and other diseases; d) create conditions that would guarantee all people medical assistance and medical care in case of illness [13].

The International Covenant on Civil and Political Rights does not contain provisions that directly regulate the right to health care, but it enshrines other rights and prohibitions that mediate the possibility of fully realizing the right to health care, in particular the right to humane treatment and respect for the inherent dignity of the human person (Article 10), the prohibition of torture, cruel, inhuman or degrading treatment or punishment (Article 7), the prohibition of discrimination (Part 1 of Article 2), etc. [14].

As for international treaties regulating the rights of certain categories of persons, the right to health protection is enshrined in them along with other rights. For example, Article 12 of the Convention on the Elimination of All Forms of Discrimination against Women establishes the prohibition of discrimination against women in the field of health protection, Article 14 – the right of women living in rural areas to access appropriate medical care [15]. Article 24 of the Convention on the Rights of the Child establishes the right to the highest attainable standard of physical and mental health [16]. The right to health protection, as well as the rights that mediate its implementation, are regulated in a similar way in other acts of this group.

International legal documents of a non-treaty type contain provisions of a general, declarative nature that proclaim the right to health protection as the highest value (such as the Jakarta Declaration on the Leading Role of Health Promotion in the 21st Century), or enshrine only certain rights in this area (the right to privacy and informed consent - the Charter on the Right to Health). The document that more thoroughly regulates the right to health protection is the Declaration on Patient-Centered Health Care, which enshrines the following principles: respect, choice and empowerment, patient involvement in health policy-making, accessibility and support, and information [17].

It should be noted that the Conventions approved by the International Labor Organization concern the labor activities of children, mothers, persons with disabilities, etc. and are aimed at establishing such labor standards for various categories of workers that will not have a negative impact on the health of each of them [18].

Regional international agreements and documents are characterized by more in-depth regulatory and

legal regulation of relations in the field of health care. An example is the European Social Charter. In particular, Article 11 of the Charter establishes the obligation of the States Parties to the Charter to introduce measures that provide for: 1) the elimination, as far as possible, of factors that impair health; 2) the provision of advisory and educational services aimed at strengthening health and forming a sense of individual responsibility for one's own health; 3) the prevention, as far as possible, of epidemic, endemic and other diseases [19].

Article 13 of the European Social Charter, in order to ensure the effective exercise of the right to social and medical assistance, obliges the Contracting Parties:

to ensure that any person who lacks sufficient means of subsistence and is unable to obtain them either by his own efforts or from other sources is provided with appropriate assistance;

to ensure that persons who benefit from such assistance are not discriminated against on this ground in their political and social rights;

to ensure that everyone, through appropriate public or private services, can receive any advice and any individual assistance which may be required to prevent, eliminate or alleviate personal or family deprivation [20].

Among the instruments adopted at the international level is the Convention on the Rights of Persons with Disabilities of 2006. This instrument enshrines the rights of persons with long-term physical, mental, intellectual or sensory impairments which may hinder their full and equal participation in society. The Convention was adopted to promote, protect and ensure the full and equal enjoyment by all such persons of all the rights and fundamental freedoms due to them as to others, and to maintain respect for their inherent dignity. This international legal instrument contains a significant number of guarantees ensuring the observance of political, civil, social, economic and cultural rights. Thus, in accordance with the provisions of Article 25 of the Convention, States Parties recognize that a person with disabilities has the right to the enjoyment of the highest attainable standard of health without discrimination on the basis of disability. In addition, States Parties must take all measures to ensure access by persons with disabilities to health services, taking into account the gender aspect, especially during the medical rehabilitation of such persons.

Another important international legal document in the field of health care is the Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine of 1997, which also established a number of important human rights in the field of health care, in particular the rights of patients. It emphasizes the need for respect for the individual as an individual and as a member of society and emphasizes the importance of ensuring human dignity. The Convention is binding on the states that have ratified it. This document notes that the European Court of Human Rights may provide advisory opinions on legal issues related to its interpretation [21].

A decisive stage in the development of international standards guaranteeing the protection of the right to health was the adoption in 2002 of the European Charter of Patients' Rights. It was adopted at a meeting of national public organizations of European countries in connection with the failure of EU states to fulfill a number of obligations, which led to a deterioration in the state of ensuring patients' rights. The Charter was drafted by the Active Citizens Network, a European network of civil society organizations for the protection of patients' and consumers' rights. The Charter's significance lies in the fact that it is part of a civil society movement in Europe that has contributed to the involvement of patients in the formation of health policy. The document defines a list of patients' rights, namely: the right to preventive measures; the right to access information; the right to consent; the right to free choice; the right to privacy and confidentiality of information; the right to compliance with quality standards; the right to respect for the patient's time; the right to safety; the right to access to modern achievements; the right to avoid unnecessary pain and suffering; the right to individualized treatment; the right to file a complaint; the right to compensation [21].

CONCLUSIONS

The analysis of international legal standards in the field of health care allows us to formulate a number of important scientific conclusions. First, it was established that international legal standards in health care constitute a specific category of international legal standards, which are characterized not only by general features (obligatory, universal, abstract), but also by special properties - specificity, scientificity, practicality and reliability of formulations. This dual nature emphasizes the complexity of legal regulation of relations in the field of health care at the international level and the need to take into account both the general principles of international law and the specifics of the medical sphere.

Secondly, the study showed the evolutionary nature of the development of international health care standards - from the general declarative principles of the 1940s to the detailed norms of modernity that regulate specific aspects of medical activity and patients' rights. Particularly significant is the transition from a state-centric model to a patient-oriented approach, which was

reflected in the adoption of the European Charter of Patients' Rights in 2002 and other specialized documents. This process indicates a change in the paradigm of international health law towards strengthening the role of the individual as the main subject of legal relations.

Thirdly, the systematization of international legal acts in the field of health demonstrates their multi-level structure, which includes universal (global) and regional standards, mandatory and recommendatory norms, general and special provisions for individual categories of persons. Such differentiation creates a flexible system of legal regulation that allows taking into account the diverse needs of subjects of health law - from general principles of ensuring the right to life to specific guarantees for vulnerable groups of the population (persons with disabilities, children, women).

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

REVIEW ARTICLE

CONTENTS

The role of lipoprotein (a) in the development of cerebral atherosclerosis

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ABSTRACT

Aim: To review information sources on this issue in order to provide up-to-date knowledge on the pathogenesis of this condition

Materials and Methods: The PubMed, Embase, and the Cochrane Library databases were searched for studies from inception to April 16, 2022, without language restrictions. Databases were searched for studies from inception 2010 to December, 2024, without language restrictions. Key words for search: Lipoprotein(a), Lp[a], dyslipidemia, classical vascular risk factors, cardiovascular disease, cardiovascular risk, cardiovascular risk factor, cerebral atherosclerosis. More than 37 sources was analyzed.

Conclusions: Cardiovascular diseases remain the leading cause of disability and mortality globally. While dyslipidemia is a well-established risk factor for coronary atherosclerosis and myocardial infarction, its role in the development of intracranial atherosclerosis is less well characterized. Current evidence suggests that plasma measurement of lipoprotein(a) [Lp(a)] using validated assays is sufficient for cardiovascular risk stratification, obviating the need for genetic testing of Lp(a). Advanced diagnostic methods have demonstrated that elevated Lp(a) levels are associated with increased vascular wall inflammation, reinforcing its causal role in atherogenesis. Intracranial atherosclerosis, a major cause of ischemic stroke, is linked to a heightened risk of recurrent cerebrovascular events and the progression of vascular cognitive impairment. Although Lp(a) is a recognized risk factor for stroke, its predictive value appears to be lower than that for coronary heart disease or composite cardiovascular outcomes. Therefore, the clinical implications of elevated Lp(a) levels in relation to carotid and intracranial atherosclerosis merit further investigation, particularly in the context of stroke prevention and vascular dementia.

KEY WORDS: cerebral atherosclerosis, Lipoprotein (a), cardiovascular risk

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INTRODUCTION

Cardiovascular diseases (CVD) are the primary causes of disability and mortality globally. Over the past 70 years, significant advancements in medicine have allowed the identification of both established and new risk factors for these diseases, which, in turn, have facilitated the development and implementation of numerous pharmacological approaches for primary and secondary prevention [1].

AIM

The aim is to review information resources on this problem for the provision of modern knowledge in the pathogenesis of this pathology.

MATERIALS AND METHODS

The PubMed, Embase, and the Cochrane Library databases were searched for studies from inception to April 16, 2022, without language restrictions. Databases were searched for studies from inception 2010 to December, 2024, without language restrictions.

Key words for search: Lipoprotein(a), Lp[a], dyslipidemia, classical vascular risk factors, cardiovascular disease, cardiovascular risk, cardiovascular risk factor, cerebral atherosclerosis. More than 37 sources was analyzed.

INCLUSION CRITERIA

According to the PICOS (population, intervention, comparison, outcome, and study design), the criteria for considering studies for this review were as follows: types of participants: adult (age > 18 years) general population.

Types of studies: random controlled trails (RCTs), post hoc analyses of RCTs, or observational cohort studies.

EXCLUSION CRITERIA

Cross-sectional studies due to the high risk for bias, studies focusing on children and adolescents; studies not reporting multivariable adjusted outcomes. In addition, articles without sufficient data (reviews, editorials, preclinical studies) and studies not relevant for the purpose of the current meta-analysis were excluded. If the same population was used in multiple studies, we selected the article with the most information or the largest sample size.

REVIEW AND DISCUSSION

THE ROLE OF LIPOPROTEIN (A) AS A CVD RISK FACTOR

Despite substantial progress in CVD treatment, the residual risk of these diseases remains high, even among patients receiving adequate therapy. This is likely due to the still-high prevalence of classical risk factors, such as elevated cholesterol levels, smoking, hypertension, obesity, diabetes, and low physical activity. However, residual risk, which cannot be fully explained by these factors, points to the existence of additional contributors to disease development. Elevated concentrations of lipoprotein (a) [Lp(a)] are present in 10–20% of the population and have long been associated with an increased risk of ischemic heart disease (IHD) and other ischemic cardiovascular conditions. However, Lp(a) is currently not targeted for treatment in CVD prevention.

European and American guidelines, including the 2016 European Guidelines on Dyslipidemias and the 2018 American Heart Association/American College of Cardiology Guidelines, recommend measuring Lp(a) levels to assess risk in high-risk groups [2,3]. These recommendations align with the positions of the European Atherosclerosis Society and the National Lipid Association in the USA, which also advocate screening for elevated Lp(a) levels in individuals at moderate and high CVD risk. Early studies on the composition of Lp(a) suggested that this lipoprotein might contribute to the development of atherosclerosis through its lipid component. Modern techniques, such as fluorodeoxyglucose-positron emission tomography, confirm the presence of increased inflammation in the arterial walls of patients with elevated Lp(a) levels, further supporting its role in atherosclerosis progression [4-7]. A recent study based on data from the UK Biobank, encompassing over 460,000 individuals, became the largest investigation into the risk of atherosclerotic cardiovascular diseases (ASCVD) associated with Lp(a) levels [8]. The findings of this study validated the recommendations of the American College of Cardiology/American Heart Association for using Lp(a) as a marker of elevated risk. This marker may guide the initiation of statin therapy in individuals with a moderate (5%-7.4%) or intermediate (7.5%-19.9%) 10-year predicted risk of ASCVD.

The evidence linking Lp(a) levels to ASCVD is robust, as confirmed by a large population study that demonstrated a consistent risk of ASCVD with progressive increases in Lp(a) levels [9]. Regarding whether the size of apolipoprotein A (apo(A)) might be associated with ASCVD, there is significant interest. However, it is currently believed that measuring the molar concentration of Lp(a) is sufficient for risk assessment, as additional measurement of apo(A) size does not provide significant added value for clinical risk evaluation.

In 2009, two large genetic epidemiological studies were published, confirming Lp(a) as a causal risk factor for ischemic heart disease (IHD) [10]. The first study, conducted using the Mendelian randomization method and including over 40,000 individuals, demonstrated an increased risk of myocardial infarction (MI) associated with high Lp(a) levels and a reduced number of repeats in the LPA gene encoding kringle IV type 2 (KIV2), which is also linked to elevated Lp(a) concentrations. Instrumental analysis revealed that doubling Lp(a) concentration increases MI risk by approximately 20%. The second case-control study included 3,100 IHD cases genotyped for 49,000 gene variants across 2,100 candidate genes, identifying two single nucleotide polymorphisms (SNPs) in LPA (rs10455872 and rs3798220) with the strongest association to IHD risk among all tested SNPs. Together, these studies provided strong genetic evidence of a causal link between Lp(a) and IHD, consistent with earlier research examining Lp(a) phenotypes and their association with IHD risk. Additionally, further studies on the general population cohort in Copenhagen found that high Lp(a) levels and corresponding risk-related genotypes are associated with increased cardiovascular and overall mortality and more frequent recurrences of cardiovascular events, further confirming the genetic link between Lp(a) and CVD. The PROCARDIS study, which included approximately 4,000 patients and a control group of similar size, demonstrated that carriers of certain genetic variants had 39% lower median Lp(a) levels and a 21% reduced risk of IHD, indicating potential therapeutic benefits of lowering Lp(a) levels. Similarly, a large genetic study involving over 100,000 individuals, conducted by Emdin et al., reported a 29% reduction in IHD risk and decreased risks of peripheral arterial disease, heart failure, and stroke with a one standard deviation (SD) reduction in Lp(a) levels. Finally, recent Mendelian randomization studies using genetic data provided compelling evidence of a causal link between reducing Lp(a) levels and lowering the risk of cardiovascular diseases, highlighting the potential of such therapeutic approaches for future risk reduction [11-14].

For predicting CVD risk, measuring Lp(a) plasma levels using validated methods is sufficient, without requiring LPA

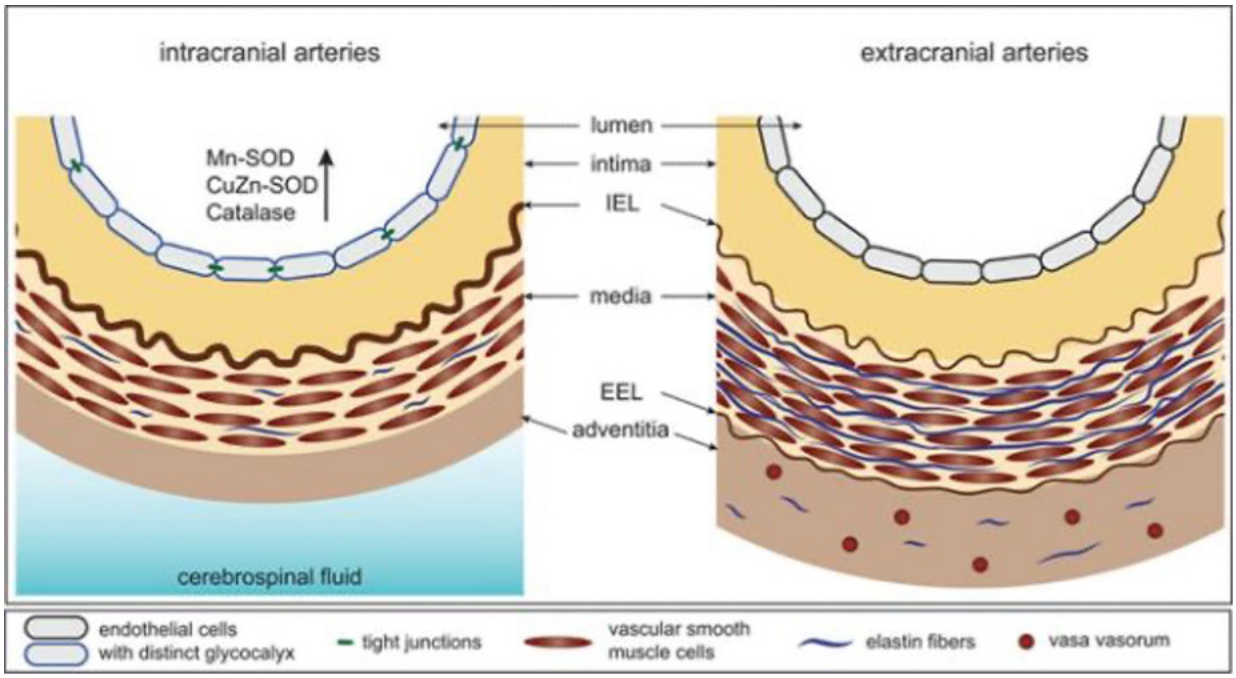


Fig. 1. Key Structural Characteristics of Intracranial and Extracranial Arteries Intracranial arteries are muscular-type arteries that generally have thinner medial and adventitial layers, fewer elastic medial fibers, and a thicker and denser internal elastic lamina (IEL). They lack an external elastic lamina (EEL) and vasa vasorum and are surrounded by cerebrospinal fluid rich in nutrients. Animal studies suggest the presence of tight junctions and a specific glycocalyx. Additionally, intracranial arteries are reported to exhibit enhanced antioxidant responses. CuZn-SOD refers to copper-zinc superoxide dismutase, while Mn-SOD refers to manganese superoxide dismutase.
Source: compiled by the authors based on [16]

genotyping. This is supported by the findings of the Bruneck study, where incorporating Lp(a) concentration data significantly improved risk prediction based on the Framingham and Reynolds risk scores, while adding information about apo(A) isoform size did not provide additional benefits [15].

THE ROLE OF LIPOPROTEIN (A) IN CEREBRAL ATHEROSCLEROSIS

Intracranial atherosclerosis, a leading cause of ischemic stroke, is associated with an increased risk of recurrent strokes and the development of dementia. Recent European studies have identified a much higher prevalence of intracranial atherosclerotic lesions than previously assumed, highlighting the potentially significant role of intracranial atherosclerotic disease as one of the most common causes of ischemic stroke worldwide. Even minor stenosis of intracranial vessels (<50%) can have clinical significance. The role of non-stenotic plaques in ischemic stroke development underscores the need for a deeper understanding of the mechanisms and pathogenesis of intracranial atherosclerosis (Fig. 1) [16].

Hoff (1972) noted that there are no significant differences in the chemical and enzymatic characteristics of plaques between intracranial and extracranial arteries [17]. Dyslipidemia is a well-established risk factor for coronary atherosclerosis and myocardial infarction; however, its role in intracranial atherosclerosis remains insufficiently studied. High levels of low-density lipoprotein cholesterol (LDL-C) are primarily associated with extracranial lesions, whereas a high apo(B) to apo(A) ratio and low apo(A) levels—the main protein of high-density lipoproteins (HDL-C)—correlate with intracranial lesions. Extracranial atherosclerosis may also act as a risk factor for developing intracranial lesions.

Key differences between intracranial and extracranial atherosclerosis include a later onset of disease and a more stable plaque phenotype in intracranial arteries. These differences may be attributed to the anatomical features of intracranial vessels, which may also relate to their role in regulating cerebrovascular resistance.

The progression of atherosclerosis involves stages of fatty streak formation and fibrous plaque development [18]. It has been hypothesized that plaque morphology may be more important for clinical outcomes than the degree of stenosis. The rupture of an unstable plaque, characterized by a large lipid core and a thin fibrous cap with signs of inflammation, can lead to thrombus formation and ischemic stroke in patients with intracranial atherosclerosis.

Studies have shown that elevated serum levels of Lp(a) are associated with a higher risk of cardiovascular diseases (CVD) and cerebrovascular disorders [19]. High Lp(a) levels are an independent risk factor not only for CVD but also for atherosclerotic obliterative diseases.

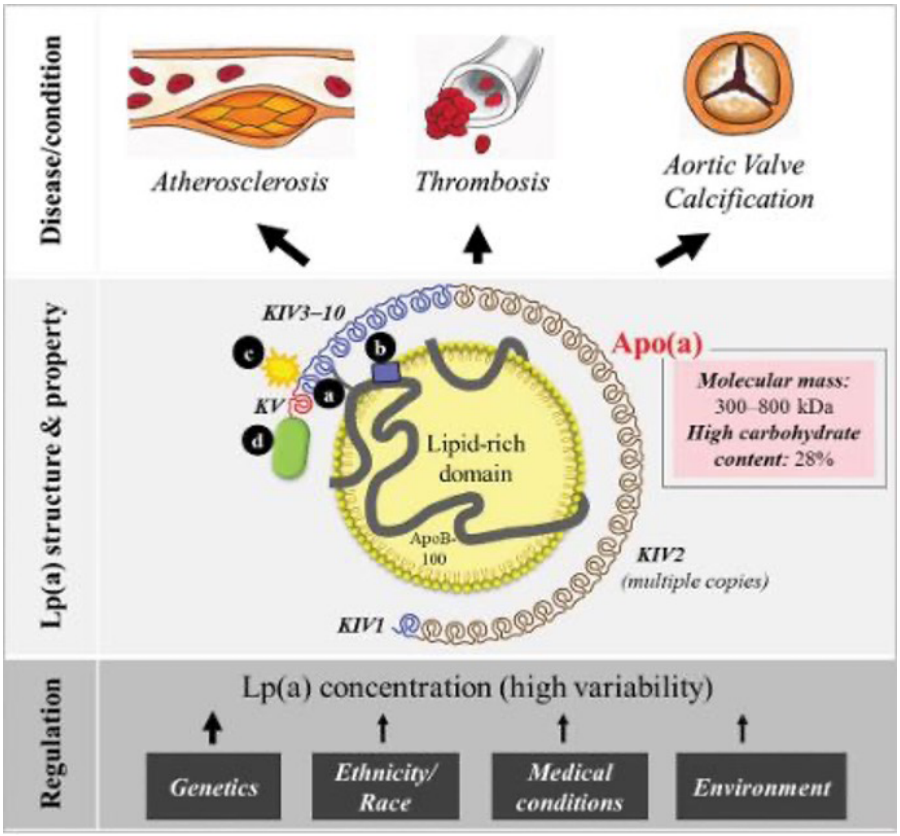


Fig. 2. Lipoprotein (a): Structure, Genetic Regulation, and Correction Options
Source: compiled by the authors based on [27]

However, the findings on its impact on cerebrovascular diseases remain inconclusive, and study data are still contradictory [19]. Moreover, elevated Lp(a) levels are often detected in clinical practice among patients with acute and recurrent strokes during lipid profile analysis.

Liqun et al. (2010) found that Lp(a) levels correlate with the severity of carotid atherosclerosis. Yubao and colleagues (2015) demonstrated that blood lipid levels vary in patients with different types of atherosclerotic plaques in intracranial arteries. However, further investigation is required to determine how specific lipid parameters affect the development of atherosclerotic plaques. In a study by Haitao (2015), the development of carotid atherosclerosis in patients with cerebral infarction was shown to correlate with levels of triglycerides (TG) and LDL-C. These markers were higher in patients with carotid plaques compared to those without such plaques [19 - 21].

LARGE-SCALE POPULATION STUDIES AND LP(A) IN ISCHEMIC STROKE RISK

A large-scale population study demonstrated that high Lp(a) levels are correlated with an increased risk of ischemic stroke. A recent retrospective case-control analysis confirmed that elevated Lp(a) levels positively correlate with ischemic stroke risk. This study also showed that high Lp(a) levels are an independent risk factor for carotid atherosclerotic stenosis. Furthermore, the risk of acute ischemic stroke is heightened in patients with such stenosis if their Lp(a) levels are elevated [22].

Lp(a) levels are relatively stable and are not influenced by factors such as sex, age, diet, smoking, lipid-altering medications, or environmental conditions. Carotid atherosclerosis correlates with Lp(a) and cholesterol levels in patients with acute ischemic stroke. Both Lp(a) and cholesterol serve as independent risk factors for this type of atherosclerosis, underscoring the necessity of developing therapies aimed at reducing Lp(a) levels [22].

The Larsson (2020) study utilized the UK Biobank cohort for Mendelian randomization to evaluate the causal effect of circulating Lp(a) levels on atherosclerotic and cerebrovascular diseases. The study included 367,586 individuals of European ancestry. The results confirmed a moderate association between genetically predicted Lp(a) levels and ischemic stroke risk [23]. A key advantage of this study was its evaluation and comparison of associations between genetically determined Lp(a) levels and atherosclerotic, cerebrovascular, thrombotic, and valvular diseases within a single European population cohort.

UNCERTAINTY IN LP(A) AND STROKE RELATIONSHIPS

Despite consistent evidence linking Lp(a) to ischemic heart disease (IHD), the impact of Lp(a) on ischemic stroke prevalence has been less conclusive in previous

observational studies. Most research focused on the general risk of ischemic stroke, leaving the relationship between Lp(a) levels and stroke subtypes poorly explored [24]. The association of Lp(a) with cerebrovascular diseases and stroke remains less clearly defined. However, recent data from pooled Danish cohorts confirmed that Lp(a) is a risk factor for stroke, albeit less prominently than for coronary artery disease or cardiovascular disease (CVD) [25].

Additionally, a large genetic analysis (N>100,000) identified four single nucleotide polymorphisms (SNPs) in the LPA gene associated with lower Lp(a) levels. These SNPs correlated with a 13% reduced risk of stroke when Lp(a) levels were reduced by one standard deviation (SD) [26].

WHAT IS LIPOPROTEIN (A)?

Lipoprotein (a) [Lp(a)] is a modified form of low-density lipoprotein (LDL) that acts as a genetically determined risk factor for atherosclerosis, IHD, stroke, thrombosis, and aortic stenosis. It comprises an LDL particle linked to apolipoprotein (a) [apo(a)] through a disulfide bond, forming a structure that is primarily synthesized in the liver [39]. Lipoprotein (a) [Lp(a)] is a modified form of low-density lipoprotein (LDL) and a genetically determined risk factor for the development of atherosclerosis, ischemic heart disease (IHD), stroke, thrombosis, and aortic stenosis [27] (Fig. 2). Structurally, Lp(a) is a variant of LDL, where apolipoprotein (a) [apo(a)] binds to apolipoprotein B100, forming a bond stabilized by disulfide bridges. These molecules create structures assembled in the membranes of hepatocytes [28].

Apo(a) is synthesized in the liver, where it binds to apolipoprotein B100 of LDL to form Lp(a). The assembly of Lp(a) is a two-step process: the first step is non-covalent and likely occurs intracellularly, while the second step, involving covalent bond formation, happens extracellularly [29]. Apo(a) has a unique structure, including looped domains stabilized by internal disulfide bonds known as "kringles." These domains are also present in other coagulation factors, such as plasminogen [30]. The relationship between plasma Lp(a) levels and apo(a) variants is inverse. Variations in the number of kringle repeats in the apo(a) structure can explain the variability of Lp(a) levels in populations. Individuals with fewer kringle repeats have smaller Lp(a) particles but higher serum concentrations, while larger apo(a) isoforms lead to protein accumulation in cell structures.

Approximately 20% of individuals have high Lp(a) levels exceeding 42 mg/dL, a characteristic noted in Danish cohorts. Additionally, Lp(a) levels may vary depending on racial or ethnic backgrounds, with higher concentrations typically observed in individuals of African descent compared to those of European or Asian origin [31]. The concentration of Lp(a) is predominantly genetically regulated and linked to the LPA gene locus on chromosome 6, which encodes apo(a). Since Lp(a) levels are genetically determined, there are currently no recommendations for genetic testing of the LPA gene. Measuring Lp(a) levels in blood is considered sufficient for evaluating the impact of genetic variants [32].

MANAGEMENT OF ELEVATED LIPOPROTEIN(A) LEVELS: CURRENT STRATEGIES AND FUTURE DIRECTIONS

For patients with elevated lipoprotein(a) [Lp(a)] levels, current guidelines suggest reducing concentrations to below 50 mg/dL, although specific therapeutic targets remain under debate. Nicotinic acid (niacin) has demonstrated the ability to reduce Lp(a) levels by approximately 20–30%; however, despite its favorable effects on other lipid parameters, clinical trials have not shown a significant reduction in cardiovascular event rates associated with its use [33]. Statins, while foundational in the treatment of hypercholesterolemia, exhibit inconsistent effects on Lp(a). Some studies suggest that statins may increase Lp(a) levels, although their benefits in reducing low-density lipoprotein cholesterol (LDL-C) and atherosclerotic cardiovascular risk remain undisputed. In patients with elevated Lp(a), statins remain a cornerstone of lipid-lowering therapy primarily for their LDL-C–lowering effect and associated reduction in ischemic heart disease (IHD) risk. PCSK9 inhibitors have shown promise, reducing Lp(a) levels by an average of 26% and improving cardiovascular outcomes in high-risk populations. Nevertheless, it remains unclear whether Lp(a) persists as an independent risk factor once LDL-C is effectively controlled with PCSK9-targeted therapy [34]. Lipid apheresis is reserved for patients with severely elevated Lp(a) refractory to pharmacotherapy. While apheresis can acutely reduce Lp(a) concentrations, levels often rebound post-procedure, and its utility is limited by cost and accessibility. Emerging therapies, particularly antisense oligonucleotides (e.g., AKCEA-Apo(a)-LRx) and small interfering RNA (siRNA) agents (e.g., pelacarsen), are currently in clinical development. These agents directly target apo(a) mRNA, leading to substantial reductions in Lp(a) levels in early-phase trials [35]. Their long-term efficacy and safety, particularly with respect to cardiovascular outcomes, require validation in large-scale randomized controlled studies. Hormonal therapies have also been explored. While estrogen may lower Lp(a) levels, it has not been associated with improved cardiovascular outcomes and is not recommended due to its complex risk-benefit profile. Conversely, testosterone replacement therapy (TRT) has shown

modest Lp(a)-lowering effects, but its clinical relevance in cardiovascular prevention remains uncertain and warrants further investigation [36]. L-carnitine supplementation has been linked to reductions in Lp(a) levels; however, it may paradoxically raise trimethylamine N-oxide (TMAO), a metabolite implicated in atherosclerosis development, raising concerns about its overall cardiometabolic safety. Several ongoing clinical trials are evaluating next-generation therapies aimed at inhibiting apo(a) synthesis, with the goal of substantially lowering Lp(a) levels and thereby attenuating cardiovascular risk [37]. The clinical impact of these agents must be established in terms of both biomarker reduction and hard outcomes such as myocardial infarction, stroke, and cardiovascular mortality.

CONCLUSIONS

Today, cardiovascular diseases remain the main cause of disability and mortality worldwide. Despite receiving correct therapy, a number of patients have a residual risk that cannot be explained by classical risk factors. Dyslipidemia is one of the risk factors for the development of atherosclerosis, in particular coronary atherosclerosis and myocardial infarction. It has been proven that by measuring the level of Lp(a) in plasma, the risk of cardiovascular diseases can be predicted. It has been established that Lp(a) can contribute to the development of atherosclerosis through its lipid component, but its specific role in the development of atherosclerosis of the carotid arteries in patients with intracranial atherosclerosis is not well understood. A number of studies in recent years have revealed a higher prevalence of intracranial atherosclerotic lesions than previously thought and, as a consequence, a significant role of intracranial atherosclerotic disease as one of the most common causes of ischemic stroke worldwide. Therefore, the clinical implications of elevated Lp(a) levels in relation to carotid atherosclerosis and intracranial sclerosis deserve further investigation, especially in the context of stroke and vascular dementia prevention.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

REVIEW ARTICLE

CONTENTS

Understanding the risk and therapeutic possibilities for periprosthetic joint infections: a comprehensive review

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ABSTRACT

Aim: To summarize the current evidence on risk factors, microbiological profiles, and treatment complications associated with PJIs. By synthesizing findings from recent studies, this review identifies key challenges and areas for improvement in the prevention, diagnosis, and management of PJIs.

Materials and Methods: A comprehensive literature search was conducted across major academic databases, including PubMed, Embase, and Web of Science, to identify relevant articles published between 2015 and 2025. Studies were selected based on their focus on microbial, biomechanical, and patient-related factors influencing PJI outcomes.

The review highlights the predominance of Gram-positive bacteria, such as *Staphylococcus aureus* and *Staphylococcus epidermidis*, as major pathogens in PJIs, with an increasing role of *Candida* species in complex cases. Key patient-related risk factors include obesity, diabetes, advanced age, and immunosuppression. Two-stage revision arthroplasty remains the gold standard for managing chronic PJIs, though single-stage revisions are gaining attention for specific scenarios. Emerging diagnostic biomarkers, such as calprotectin, offer potential for earlier and more accurate diagnosis, while advancements in biofilm-targeted therapies represent promising directions for treatment.

Conclusions: PJIs represent a multifaceted clinical challenge requiring a multidisciplinary approach. Current knowledge underscores the importance of individualized patient optimization, standardized diagnostic protocols, and targeted antimicrobial strategies. Future research should focus on addressing knowledge gaps in diagnostic biomarkers, biofilm management, and the optimization of surgical approaches to improve patient outcomes and reduce the burden of PJIs.

KEY WORDS: periprosthetic joint infection, total joint arthroplasty, microbial pathogens, two-stage revision, diagnostic biomarkers, infection management

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INTRODUCTION

Periprosthetic joint infection (PJI) is a severe complication following total joint arthroplasty (TJA), occurring in approximately 1–2% of primary hip and knee replacements. PJIs significantly impact patient morbidity, healthcare expenditures, and mortality rates [1]. Although bacterial pathogens, particularly *Staphylococcus aureus* and *Staphylococcus epidermidis*, are predominant causative organisms, fungal PJIs, primarily involving *Candida* species, present unique diagnostic and therapeutic challenges despite their relatively low prevalence (around 1%) [2].

Candida albicans is the most frequently identified fungal pathogen in PJIs; however, non-albicans *Candida* species, such as *Candida parapsilosis* and *Candida tropicalis*, have been increasingly recognized as clinically significant [3–5]. Fungal PJIs often occur in a polymicrobial setting, complicating their diagnosis and management. They are also commonly associated with patient-related risk factors, including diabetes mellitus, immunosuppression, and multiple revision surgeries [6, 7].

The gold-standard treatment for chronic PJIs, including fungal infections, typically involves a two-stage revision arthroplasty - removal of the infected implant, extensive debridement, insertion of an antibiotic-loaded spacer, and subsequent reimplantation [8, 9]. Nevertheless, fungal PJIs generally have poorer clinical outcomes compared to bacterial infections, characterized by higher recurrence rates, longer hospitalization periods, and lower rates of infection-free survival [10]. Additionally, variability in management strategies persists, with some advocating single-stage revision procedures or implant retention in select scenarios [11].

Current literature predominantly focuses on bacterial PJIs, leading to a gap in high-quality evidence specifically addressing differences in clinical outcomes, therapeutic responses, and prognoses between *Candida albicans*

and non-albicans *Candida* species infections [12, 13]. Bridging this gap is critical for the development of improved clinical guidelines and optimized patient care.

AIM

The main aim of this narrative review is to provide a comprehensive overview of periprosthetic joint infections (PJIs), with particular emphasis on fungal infections caused by *Candida albicans* and non-albicans *Candida* species. Specifically, the review aims to: (1) Compare clinical outcomes of PJIs caused *by Candida albicans versus* non-albicans *Candida* strains, focusing on infection-free survival, recurrence rates, and surgical results. (2) Identify and summarize key risk factors associated with fungal PJIs, including patient demographics, comorbidities, and previous surgical history, as well as highlight important contributing factors to PJIs in general, such as obesity, diabetes, and operative conditions. (3) Evaluate current treatment strategies for fungal PJIs, particularly the effectiveness of single-stage versus two-stage revision surgeries and antifungal therapy. (4) Highlight existing knowledge gaps in the management of fungal PJIs and suggest directions for future research, particularly emphasizing the need for development of biofilm-active antifungal agents and standardized clinical guidelines. While the primary focus of this review is on fungal PJIs, additional clinically relevant factors - including bacterial etiologies, surgical factors, and patient-specific risk profiles - are also summarized briefly and systematically to provide comprehensive context.

MATERIALS AND METHODS

SEARCH STRATEGY

The search strategy for this narrative review was designed to comprehensively identify relevant studies on PJIs, specifically fungal PJIs caused by *Candida albicans* and non-albicans *Candida* strains. We searched major academic databases including: PubMed/MEDLINE, Scopus, Embase, Web of Science, and Cochrane Library. A combination of MeSH terms and keywords was used to capture all relevant studies: "Periprosthetic joint infection" OR "PJI" "Fungal infection" OR "*Candida*" "*Candida albicans*" OR "non-albicans *Candida*" "Total hip arthroplasty" OR "Total knee arthroplasty" "Revision surgery" OR "Two-stage revision" OR "Single-stage revision" "Antifungal therapy" "Polymicrobial infection"

QUALIFICATION CRITERIA

Inclusion criteria: studies published in peer-reviewed journals from 2015 to 2024. Articles in English. Studies that examined fungal PJIs in patients undergoing total joint arthroplasty, specifically those infected with *Candida* species. Articles reporting clinical outcomes, risk factors, or treatment strategies for fungal PJIs. Exclusion Criteria: articles focusing solely on bacterial PJIs without discussing fungal infections. Studies involving animal models or in vitro research without clinical application. Case reports with insufficient data or outcome measures.

DATA EXTRACTION

Initial screening was performed by reviewing titles and abstracts. Full-text articles were subsequently reviewed to ensure relevance. Reference lists of relevant articles were also screened to capture any additional studies missed in the primary search. Data was extracted from each article on the study design, patient population, infection type, surgical methods (one-stage *vs.* two-stage), antifungal therapy used, clinical outcomes (e.g., infection-free survival, recurrence rates), and conclusions.

REVIEW

After a comprehensive database search, 23 research trials and 11 review papers and/or meta-analyses were included in this review, all contributing to the understanding of PJIs, particularly those involving fungal pathogens. The selected studies provide valuable insights into the multifactorial risk factors, diagnostic challenges, and treatment outcomes associated with PJIs. Key findings emphasize the predominance of Gram-positive bacteria such as *Staphylococcus aureus*, alongside the increasing recognition of *Candida species* as significant pathogens in complex, polymicrobial infections. Individual risk factors, including advanced age, diabetes, high body mass index (BMI), and chronic illnesses, further exacerbate infection risks, complicating the management of PJIs. Operative factors, such as prosthesis type and intraoperative contamination, also play a crucial role in infection rates, particularly in revision surgeries. Treatment outcomes favor two-stage revision surgeries, which demonstrate higher infection-free survival, although cases involving fungal or polymicrobial infections often result in poorer prognoses and higher recurrence rates.

In this review, the studies have been grouped thematically according to key issues related to periprosthetic infections:

- Risk factors (BMI, diabetes) [17, 18, 21-23, 25, 31, 37, 42, 46],
- Bacterial infections [14, 15-17, 19, 23, 32-35, 39, 43, 45],
- Fungal infections (Candida albicans and non-albicans) [3, 4, 5, 7, 12, 13, 27, 29, 35],
- Surgical revisions and surgical treatment strategies [16, 20, 27, 28, 30, 33, 34, 38, 41].

Table 1. Summary of the research papers on PJI risk factors

No.	Authors, year	Journal	Study type	Study group	Study aim	Risk factors	Results
1	Guo, H. et al., 2020 [14]	*Journal of Infection in Developing Countries*	Case-control	96 PJIs, 192 controls	Explore PJI risk factors	Microbiological, Individual	Gram-positive bacteria common (76.19%), S. aureus in knees
2	Lee, Mak, Wong, 2015 [15]	*Journal of Orthopaedic Surgery*	Case-control	1133 TKAs, 8 PJIs	Incidence, risk factors in TKAs	Microbiological, Individual, Operating	*S. aureus, coagulase-negative staph prevalent*
3	Erivan et al., 2017 [16]	*Orthopaedics & Traumatology: Surgery & Research*	Retrospective	26 PJIs	Manage spacer-related complications	Microbiological, Biomechanical	78% Gram-positive cocci, high spacer complications
4	Papalia et al., 2019 [17]	*European Review for Medical and Pharmacological Sciences*	Case-control	28 PJI, 84 controls	Assess incidence, risk factors	Individual (BMI, diabetes), Microbiological	*S. aureus and polymicrobial infections common*
5	Yilmaz et al., 2020 [18]	*BMC Musculoskeletal Disorders*	Retrospective	57 octogenarians	Outcome of PJI in elderly	Individual (age, BMI)	86% chronic infections; high BMI in cases
6	Ibrahim et al., 2018 [19]	*Bone & Joint Journal*	Comparative	50 culture-positive vs negative	Culture-negative vs positive PJIs	Microbiological, Individual	*S. epidermidis dominant, antibiotics impact*
7	Kingsbury et al., 2022 [20]	*Bone & Joint Journal*	Cross-sectional	568 revisions	Revision routes and symptoms	Individual (age)	Younger patients, better outcomes
8	Velez de Lachica et al., 2022 [21]	*J ISAKOS*	Prospective	83 PJIs	Compare vancomycin-loaded vs control	Individual (BMI, diabetes)	CRP/ESR elevated, PJI higher with high BMI
9	Lenguerrand et al., 2018 [22]	*Lancet Infectious Diseases*	Cohort	623,253 hips	Risk factors for hip revisions	Patient-related, Surgical	High BMI, diabetes, pulmonary disease increased PJI risk
10	Lenguerrand et al., 2019 [23]	*Lancet Infectious Diseases*	Cohort	679,010 knees	Risk factors for knee PJI	Patient-related, Surgical	Obesity, diabetes, prior infection, cemented replacements risk
11	Vasiliadis et al., 2021 [24]	*Cureus*	Case series	4 patients	Effectiveness of DAIR in PJIs	Laboratory, Surgical	High success in treating Acinetobacter PJIs
12	Panula et al., 2021 [25]	*Acta Orthopaedica*	Cohort	33,337 THAs	Risk factors in Finnish registry	Patient, Surgical	High BMI, ASA grade, bleeding >500mL risks
13	Watanabe et al., 2021 [26]	*Journal of Orthopaedic Surgery and Research*	Cross-sectional	109 PJIs	Risk factors for culture-negative PJIs	Laboratory, Microbiological	CRP, ESR levels predictive, S. epidermidis common
14	Gao et al., 2018 [27]25-129 months	*Medical Science Monitor*	Retrospective	18 fungal PJIs	Success rate in fungal PJIs	Microbiological, Laboratory	*Candida species common; 2-stage revision success rates moderate*
15	Singh et al., 2016 [28]	*Canadian Journal of Surgery*	Registry	930 THAs, 734 TKAs	Revision patterns post-registry	Surgical	Registry reduced early revision rates
16	Leitner et al., 2020 [29]	*Journal of Arthroplasty*	Retrospective	135 FA cases	Evaluate site-specific factors	Individual, Microbiological	Nicotine abuse, multiple revisions increased FA risk
17	Newman et al., 2018 [30]	*Journal of Arthroplasty*	Randomized	160 revisions	ciNPWT vs standard care	Microbiological	ciNPWT reduced complications in high-risk revisions

Table 1. Cont.

18	Bae et al., 2022 [31]	*Joint Diseases and Related Surgery*	Retrospective	12,320 TJAs	Incidence and risk factors in TJAs	Individual	Hypertension, UTI, prior corticosteroids risk factors
19	Li et al., 2021 [32]	*BMC Musculoskeletal Diseases*	Cohort	64 polymicrobial, 158 monomicrobial PJIs	Polymicrobial PJI risk factors	Microbiological, Individual	Streptococcus, Enterococcus, Gram-negative species prevalent
20	Steinicke et al., 2023 [33]	*Archives of Orthopaedic and Trauma Surgery*	Retrospective	55 PJIs	Success rates of 2-stage revision	Microbiological, Surgical	High success, but obesity and S. epidermidis linked to failures
21	Metcalf et al., 2023 [34]	*Antibiotics*	Retrospective	470 revisions	Outcomes between one- and two-stage revisions	Microbiological	*S. aureus and MSSA frequent in failed revisions*
22	Wingert et al., 2016 [35]	*Clinical Orthopaedics and Related Research*	Prospective	1,536 TJAs	Accuracy of NSQIP in predicting infections	Post-op	NSQIP effective for predicting 30- and 90-day infections
23	Karczewski et al., 2022 [35]	*International Orthopaedics*	Retrospective	29 Candida PJIs	Candida PJI outcomes	Microbiological, Laboratory	*Candida albicans vs non-albicans PJIs analyzed, moderate success with antifungal therapy*

Abbreviations: BMI, body mass index; THA, total hip arthroplasty; TKA, total knee arthroplasty; PJI, periprosthetic joint infection; ASA, American Society of Anesthesiologists; CRP, C-reactive protein; ERS, erythrocyte sedimentation rate; ciNPWT, Closed-incision negative-pressure wound therapy; UTI, urinary tract infection; methicillin-sensitive Staphylococcus aureus; NSQIP, National Surgical Quality Improvement Program
Source: compiled by the authors of this study

RESEARCH TRIALS

Table 1 shows the research trials on PJI risk factors.

Guo et al. [14] conducted a case-control study among 96 patients who developed PJIs after receiving primary artificial joint replacements and 192 control patients who underwent similar surgeries but did not develop infections. It was demonstrated that older patients and those with compromised immune systems were at higher risk of developing PJIs. Furthermore, differences in infection patterns were observed between hip and knee arthroplasty patients, suggesting that anatomical and procedural factors may influence the risk of infection.

Lee, Mak, and Wong (2015) [15] conducted a case-control study including a total of 1,133 TKA procedures performed between 2000 and 2014, out of which 8 cases (0.71%) developed PJIs. The study suggested that longer operative durations may increase the likelihood of intraoperative contamination, particularly in cases where optimal sterile conditions are difficult to maintain for extended periods. This finding emphasizes the need for meticulous infection control protocols during surgery and highlights the potential benefit of reducing operative time wherever possible.

Erivan et al. (2017) [16] conducted a retrospective study focusing on the management of complications related to the use of antibiotic-loaded spacers in two-stage revision surgeries for chronic PJIs. The study highlighted the need for careful selection and placement of spacers, especially in patients at higher risk of mechanical complications. It also underscored the importance of balancing the mechanical stability of spacers with the need for effective local antibiotic delivery, as complications can lead to delays in infection control and complicate the second-stage reimplantation process.

Papalia et al. (2019) [17]total Hip arthroplasty (THA conducted a case-control study on 28 patients who developed PJIs and 84 control patients who underwent similar procedures but did not develop infections. The study emphasized that the presence of multiple microorganisms in PJI cases complicates both diagnosis and treatment, as managing mixed bacterial populations requires broader-spectrum antibiotic regimens and may lead to prolonged recovery times.

Yilmaz et al. (2020) [18] investigated the outcomes of PJIs in a cohort of 57 octogenarians. The findings underscore the importance of optimizing preoperative risk factors, such as weight management, particularly in elderly populations where these factors exacerbate the complexity of infection management. Yilmaz et al. [18] further emphasize the need for tailored treatment strategies in elderly patients with PJIs, suggesting that more aggressive treatment approaches, such as two-stage revisions, may be necessary to improve infection control in this vulnerable group.

Ibrahim et al. (2018) [19] included 100 patients, 50 of whom had culture-positive PJIs and 50 with cul-

ture-negative PJIs. The study emphasized the importance of optimizing preoperative antibiotic protocols to prevent the emergence of culture-negative PJIs. Additionally, it underscored the need for advanced diagnostic tools, such as molecular techniques, to improve the detection of pathogens in culture-negative cases, which remain challenging to manage effectively.

Kingsbury et al. (2022) [20] conducted a cross-sectional study on 568 revision cases, with a primary focus on understanding how patient age and other individual factors influenced the success of revision surgeries and overall outcomes. This study underscores the importance of considering age and comorbidities when planning revision surgeries for PJIs, as these factors significantly impact the likelihood of success. Tailored approaches that take into account the patient's age, physical condition, and medical history may improve outcomes in revision surgeries for PJIs, particularly in older populations.

Velez de Lachica et al. (2022) [21] conducted a prospective study involving 83 patients undergoing revision procedures for PJIs, with a focus on identifying how patient-specific factors such as BMI and diabetes impacted infection rates and outcomes. The study indicated that obesity and diabetes were important individual risk factors that increased the likelihood of postoperative infections. Patients with a high BMI had a disproportionately higher incidence of PJIs, further supporting the need for targeted infection prevention strategies in this group.

Lenguerrand et al. (2018) [22]surgical, and healthcare factors. Existing evidence is based on short-term follow-up. It does not differentiate between factors associated with early onset caused by the primary intervention from those associated with later onset more likely to result from haematogenous spread. We aimed to assess the overall and time-specific associations of these factors with the risk of revision due to PJI after primary total hip replacement.\nMETHODS: We did a prospective observational cohort study analysing 623 253 primary hip procedures performed between April 1, 2003, and Dec 31, 2013, in England and Wales and recorded the number of procedures revised because of PJI. We investigated the associations between risk factors and risk of revision for PJI across the overall follow-up period using Poisson multilevel models. We reinvestigated the associations by post-operative time periods (0-3 months, 3-6 months, 6-12 months, 12-24 months, >24 months analyzed data from 623,253 hip replacements performed in England and Wales, making it one of the largest datasets ever studied for this purpose. The study suggested that impaired lung function could affect the patient's overall resilience to infection and complicate their recovery following surgery. Surgical factors, including the complexity of the revision procedure and the use of cemented implants, were also associated with higher infection rates. The study highlighted that longer operative times and more complex surgeries increase the risk of intraoperative contamination and postoperative complications, which further contribute to the development of PJIs.

Lenguerrand et al. (2019) [23]surgical, and healthcare system factors. Existing evidence is limited by heterogeneity in populations studied, short follow-up, inadequate power, and does not differentiate early prosthetic joint infection, most likely related to the intervention, from late infection, more likely to occur due to haematogenous bacterial spread. We aimed to assess the overall and time-specific associations of these factors with the risk of revision due to prosthetic joint infection following primary knee replacement.\nMETHODS: In this cohort study, we analysed primary knee replacements done between 2003 and 2013 in England and Wales and the procedures subsequently revised for prosthetic joint infection between 2003 and 2014. Data were obtained from the National Joint Registry linked to the Hospital Episode Statistics data in England and the Patient Episode Database for Wales. Each primary replacement was followed for a minimum of 12 months until the end of the observation period (Dec 31, 2014 conducted a large cohort study and analyzed an extensive dataset comprising 679,010 knee arthroplasty procedures, making it one of the largest studies to assess patient-related and surgical factors contributing to PJIs in TKA. The study suggested that cemented replacements may increase the risk of infection due to the potential for intraoperative contamination and the challenges of achieving optimal cement fixation in certain patients.

Vasiliadis et al. (2021) [24]we aimed to investigate the effectiveness of debridement, antibiotics, irrigation, and implant retention (DAIR, in a case series reported high success in treating these PJIs, with all four patients achieving infection control following DAIR. They demonstrated that DAIR can be a viable option for managing PJIs, particularly in cases where the pathogen is well-characterized and antibiotic therapy can be carefully selected. The study advocates for further research with larger cohorts to validate the findings and assess long-term outcomes of DAIR in PJIs involving multidrug-resistant organisms like *Acinetobacter*.

Panula et al. (2021) [25]we assessed risk factors for PJI after primary total hip arthroplasty (THA utilized data from 33,337 THA procedures performed between 2002 and 2013. The study recommended that surgical teams take measures to minimize blood loss during THA pro-

cedures, such as using advanced hemostatic techniques or considering strategies like intraoperative blood salvage. They emphasized the importance of addressing modifiable risk factors, such as obesity and blood loss, to reduce the incidence of PJIs in THA patients. They also called for individualized care plans based on patient health status, as indicated by ASA grades, to improve outcomes and minimize infection risks.

Watanabe et al. (2021) [26] examined 109 patients with PJIs, comparing culture-negative cases with culture-positive ones to identify specific risk factors and laboratory markers that could aid in the diagnosis of culture-negative PJIs. The authors suggested that many culture-negative PJIs may be due to biofilm-forming bacteria like *S. epidermidis*, which are difficult to detect using conventional culture techniques. They emphasized the need for advanced diagnostic methods, such as molecular testing and next-generation sequencing, to improve pathogen detection in culture-negative PJIs. These techniques could enhance diagnostic accuracy and allow for more effective, targeted treatment in cases where standard cultures are inconclusive.

Gao et al. (2018) [27]25-129 months involved 18 cases of fungal PJIs, with the primary aim of assessing the microbiological profiles and clinical outcomes associated with these challenging infections. They reported moderate success rates for two-stage revision surgeries in treating fungal PJIs. While the two-stage approach, involving the removal of the infected prosthesis, placement of an antibiotic-impregnated spacer, and eventual reimplantation, remains the gold standard for managing PJIs, fungal infections posed additional challenges. The overall success rates were lower compared to bacterial PJIs, reflecting the complexity of treating fungal organisms and the need for prolonged antifungal therapy.

Singh et al. (2016) [28] utilized data from 930 THAs and 734 TKAs, with the goal of assessing how the implementation of joint replacement registries influenced early revision rates in these procedures. From a surgical perspective, the study emphasized that registries serve as a critical tool for improving the quality of arthroplasty procedures. Surgeons were able to use registry data to track patient outcomes and identify specific factors associated with revision, such as implant failure, infection, or technical complications during surgery. This feedback loop allowed for continuous improvement in surgical practices, ultimately lowering the rates of revision surgeries within the first few years postoperatively.

Leitner et al. (2020) [29] analyzed 135 cases of FA to determine site-specific and individual risk factors that contributed to the development of this challenging condition. They demonstrated that nicotine abuse and multiple revision surgeries are key risk factors for FA in joint arthroplasty patients. The findings underscore the importance of preoperative counseling on smoking cessation and careful surgical planning in patients with a history of multiple revisions to reduce the risk of fungal infections.

Newman et al. (2018) [30] involved 160 revision surgeries, with the primary aim of evaluating whether ciNPWT could lower the risk of complications in high-risk patients. They found that ciNPWT was more effective than standard wound care in reducing postoperative complications in high-risk revision surgeries for PJIs. The study supports the use of ciNPWT as a valuable tool for improving outcomes in patients undergoing revision procedures, particularly those with elevated infection risk.

Bae et al. (2022) [31]including demographic features, comorbidities, prior corticosteroid usage, and preoperative laboratory values.\n\nResults\n\nThere were 34 revision TJAs as a consequence of PJI (hip, 16; knee, 18 analyzed data from 12,320 TJA procedures to identify individual patient risk factors that contribute to an increased likelihood of developing PJIs. They concluded that hypertension, UTIs, and prior corticosteroid use were significant risk factors for PJIs in TJA patients. The study emphasized the need for preoperative risk assessment and management of these conditions to reduce the likelihood of infections and improve surgical outcomes.

Li et al. (2021) [32] included 64 cases of polymicrobial PJIs and 158 cases of monomicrobial PJIs, focusing on microbiological profiles and individual patient risk factors that contributed to these infections. I was highlighted the distinct challenges associated with polymicrobial PJIs, particularly due to the prevalence of *Streptococcus, Enterococcus*, and Gram-negative bacteria. The study emphasized the importance of identifying high-risk patients and tailoring antimicrobial therapies to address the complexity of these infections.

Steinicke et al. (2023) [33]management of recurrent infection after a two-stage exchange remains debated and the outcome of a repeat two-stage procedure is unclear. This study investigates the success rates of repeat two-stage exchange arthroplasty and analyzes possible risk factors for failure.\n\nMaterials and methods\nWe retrospectively identified 55 patients (23 hips, 32 knees included 55 patients who underwent two-stage revisions, focusing on microbiological profiles and surgical factors that influenced the outcomes. The study suggested that obesity creates a more challenging environment for infection control, highlighting the need for preoperative weight management strategies to improve surgical success. They demonstrated that while two-stage revision remains an effective approach for managing PJIs, the presence of S. epidermidis and patient factors such as obesity can significantly impact treatment success. The

Table 2. Summary of the reviews and meta-analyses on PJI risk factors

No.	Authors, year	Journal	Design	Participants	Study aim	Key findings
1	Kong et al., 2017 [36]	*International Wound Journal*	Meta-analysis	3,561,446 hips (40 studies)	Identify risk factors for PJIs post-THA	Higher BMI, diabetes, rheumatoid arthritis, and AVN are key risk factors for PJIs after THA.
2	Ren et al., 2021 [37]	*BMC Musculoskeletal Disorders*	Meta-analysis	40 studies (various regions)	Patient-related PJI risk factors in THA	High BMI, diabetes, chronic pulmonary disease, and RA were significant risk factors for PJIs.
3	Mian et al., 2022 [38]	*BMC Musculoskeletal Disorders*	Review	NS	Review of current debridement practices for PJIs	Biofilm eradication is critical for successful PJI debridement; two-stage revision remains the gold standard.
4	Rottier et al., 2023 [39]	*Journal of Bone & Joint Surgery*	Review	NS	Principles of antimicrobial treatment in PJIs	Biofilm-active antibiotics like rifampicin and fluoroquinolones are essential in treating Gram-positive and Gram-negative PJIs.
5	Schindler et al., 2023 [40]	*Journal of Arthroplasty*	Systematic review	19 studies	Novel diagnostic biomarkers for PJIs	Calprotectin shows high sensitivity and specificity for early PJI diagnosis.
6	Zhao et al., 2024 [41]	*Clinical Orthopaedics and Related Research*	Meta-analysis	8,711 revisions (40 studies)	Compare single-stage vs. two-stage revision	No significant difference in reinfection/reoperation rates between single- and two-stage revisions.
7	Rodriguez-Merchan et al., 2022 [42]	*Journal of Clinical Medicine*	Narrative review	NS	Overview of TKA PJI risk factors	An increased risk of PJI is associated with factors such as male sex, younger age, and pre-existing conditions like type II diabetes, class II obesity, rheumatoid arthritis, and hypertension. Procedural and postoperative factors, including prolonged operative time, use of blood transfusions, patellar resurfacing, and prolonged hospital stays, also contribute to higher infection rates.
8	Tsikopoulos et al., 2023 [43]	*Antibiotics*	Narrative review	NS	Review PJI diagnostic markers	Determining the bacterium that caused the infection is essential to implement the antibiotics, long-term treatment, and prognosis for PJIs patients.
9	Almeida et al., 2021 [44] and persistent wound drainage (PWD	*EFORT Open Reviews*	Narrative review	75 articles	Review PWD and risk factors	Smoking, obesity, anemia linked to PWD
10	Dobson, Reed, 2020 [45]	*EFORT Open Reviews*	Review	119 articles	Infection reduction in primary THA/TKA	The risk of SSI and PJI is related to surgical factors and patient factors such as age, BMI, co-morbidities, and lifestyle.
11	Alamanda et al., 2021 [46]	*EFORT Open Reviews*	Review	75 articles	Review PWD and risk factors	Factors minimizing risk of PJIs: better postoperative glycemic control, appropriate treatment of obesity, malnutrition, metabolic syndrome, preoperative anemia and smoking abstinence.

Abbreviations: NS, not specified; BMI, body mass index; THA, total hip arthroplasty; RA, rheumatoid arthritis; PJI, periprosthetic joint infection; PWD, persistent wound drainage; AVN, avascular necrosis; SSI, surgical site infection
Source: compiled by the authors of this study

study emphasized the importance of addressing both microbiological and individual risk factors to improve outcomes in patients undergoing two-stage revisions.

Metcalf et al. (2023) [34]males have a higher rate of revision. The literature lacks studies examining the relationship between sex and outcomes following single and two-stage exchange for periprosthetic joint infection (PJI analyzed 470 revision cases to determine the effectiveness of each approach and identify microbiological factors associated with failed revisions. It was demonstrated that two-stage revisions tend to yield better outcomes for PJIs caused by S. aureus and MSSA. The study emphasized the importance of considering microbiological factors when selecting the appropriate revision strategy to improve success rates and reduce the likelihood of recurrent infections.

Wingert et al. (2016) [35] analyzed data from 1,536 TJA procedures to assess how effectively NSQIP could predict 30- and 90-day postoperative infections. The study highlighted the value of NSQIP not only in infection prediction but also in enhancing overall quality improvement efforts in TJA procedures. In conclusion, NSQIP is an effective tool for predicting 30- and 90-day postoperative infections, including PJIs, in patients undergoing TJA. The study emphasized the importance of using data-driven approaches, such as NSQIP, to improve patient outcomes and reduce postoperative complications.

Karczewski et al. (2022) [35] included 29 cases of Candida PJIs, with the goal of comparing the outcomes of infections caused by *Candida albicans versus* non-albicans species, as well as assessing the success of antifungal therapies. Laboratory findings revealed that elevated CRP and other inflammatory markers were present in most *Candida* PJIs, aiding in the diagnosis of these infections. Antifungal therapy, particularly the use of azoles, demonstrated moderate success in treating *Candida* PJIs. However, the study noted that outcomes were generally better in *Candida albicans* infections compared to non-albicans species, which tend to be more resistant to standard antifungal treatments. They highlighted the complexity of managing *Candida* PJIs, particularly those caused by non-albicans species. While antifungal therapy can be effective, success rates remain moderate, and a combination of surgical debridement and targeted antifungal treatment is often necessary to achieve infection control.

REVIEWS AND META-ANALYSES

Table 2 shows reviews and meta-analyses on PJI risk factors.

Kong et al. (2017) [36] included data from 24 studies, which encompassed a wide range of patient characteristics, surgical factors, and postoperative outcomes. The study highlighted prolonged surgical duration as a major contributor to infection risk. Extended operative times increase the likelihood of intraoperative contamination, as sterile fields become more challenging to maintain over time. Prolonged surgeries also lead to greater tissue trauma and exposure, which further elevates the risk of infection. The authors emphasized the importance of minimizing surgical time without compromising procedural outcomes to reduce the likelihood of PJIs.

Ren et al. (2021) [37] analyzed 40 studies, involving a total of 3.5 million hip arthroplasties, making this one of the largest meta-analyses focused on PJI risk factors in THA. The study identified several modifiable patient-related risk factors that were strongly associated with the occurrence of PJIs. High BMI was a leading risk factor, with obese patients exhibiting a significantly higher likelihood of developing infections. The study suggested that obesity impairs wound healing and promotes inflammation, both of which increase the risk of postoperative infections. Diabetes was another major factor linked to PJIs in THA patients. Poor glycemic control was particularly associated with elevated infection risk, as hyperglycemia can weaken the immune system and delay the healing process.

Mian, Samuel, and Hurley (2022) [38] published a comprehensive review emphasizing the significance of aggressive surgical debridement combined with systemic and local antimicrobial therapy in treating PJIs. One of the key challenges highlighted in the review is the role of biofilms in chronic infections, which necessitates mechanical removal for effective eradication. The gold standard for chronic PJIs, according to the review, remains two-stage revision arthroplasty. This approach involves the removal of the infected prosthesis, the use of an antibiotic-impregnated spacer, and reimplantation after the infection has been cleared.

Rottier et al. (2023) [39] highlighted the role of rifampicin and fluoroquinolones as essential components of PJI treatment regimens. These antibiotics are particularly effective in penetrating biofilms and inhibiting bacterial growth within these protective layers. Rifampicin is highlighted for its potent activity against Staphylococcus species, especially when used in combination with other agents like fluoroquinolones to prevent the development of resistance. The review advocates for ongoing research into novel antimicrobial agents and strategies to combat biofilm-associated infections, which remain a major barrier to successful PJI management.

Schindler et al. (2023) [40] reviewed 19 individual studies that explored the use of emerging diagnostic markers beyond traditional indicators like CRP and ESR. One of the key findings of this review is the potential role of calprotectin as a highly sensitive and specific biomarker for detecting PJIs. Calprotectin is an inflammatory protein released by neutrophils, and its elevated levels in synovial fluid have shown a strong correlation with PJIs. The review demonstrated that calprotectin offers both high sensitivity and specificity, making it a promising tool for early diagnosis, which is critical for timely intervention and better clinical outcomes.

Zhao et al. (2024) [41] included data from 8,711 revision cases across 40 studies, making it one of the most extensive analyses to date on this topic. The study found no significant difference in reinfection or reoperation rates between single-stage and two-stage revision

procedures. Despite the common perception that two-stage revisions provide a more definitive solution to chronic PJIs, particularly in cases with biofilm-producing organisms, the study's findings suggest that single-stage revisions can offer comparable outcomes under certain circumstances. This conclusion has important implications for clinical decision-making, particularly in patients where minimizing surgical interventions is a priority due to comorbidities or other risk factors.

Rodriguez-Merchan et al. (2022) [42] conducted a narrative review focused on patient-related, surgical, and postoperative factors that influence the likelihood of developing PJIs, offering insights into prevention and management strategies. The review identified male gender, diabetes, and obesity as the most significant patient-related risk factors for PJIs following TKA. Male patients, particularly those with comorbid conditions such as diabetes and elevated BMI, were found to have a higher incidence of infections compared to female patients. The metabolic and immunological changes associated with diabetes and obesity were highlighted as contributing factors that impair wound healing and increase susceptibility to infection. Surgical factors also played a key role, with longer operative times, complex procedures, and the use of cemented prostheses being linked to increased infection rates.

Tsikopoulos et al. (2023) [43] synthesized findings from multiple studies to provide a comprehensive overview of the most reliable markers for diagnosing PJIs, which are critical in guiding timely and appropriate treatment. The review highlighted that Staphylococcus aureus and Staphylococcus epidermidis were the most commonly isolated pathogens in PJIs, particularly in infections related to biofilm formation on prosthetic materials. S. aureus was often associated with acute, more aggressive infections, while S. epidermidis, a coagulase-negative Staphylococcus, was linked to chronic, low-grade infections that are harder to detect and treat due to their biofilm-forming capabilities.

Almeida et al. (2021) [44] analyzed 75 articles on the risk factors associated with perioperative wound dehiscence (PWD) in total joint arthroplasty (TJA) patients. The review focused on patient-related, pharmacological, and surgical factors that contribute to an increased risk of PWD, which is a common postoperative complication that can lead to PJIs. The review identified several key patient-related risk factors, including smoking, obesity, and anemia. Smoking was strongly linked to PWD due to its adverse effects on wound healing and tissue oxygenation. Obesity, reflected by a high BMI, was another significant risk factor, as excessive adipose tissue complicates wound closure and healing. Anemia, particularly in the preoperative period, was associated with impaired wound healing and increased susceptibility to wound breakdown.

Dobson and Reed (2020) [45] analyzed 119 articles focused on strategies to reduce infections in primary THA and TKA. The review evaluated operating room practices, microbiological factors, and surgical techniques that influence infection rates in these procedures. The review identified MSSA as the most common pathogen associated with infections in primary THA and TKA. MSSA's prevalence underscores the importance of preoperative screening and decolonization protocols to reduce infection risk. It was emphasized that targeted antibiotic prophylaxis is essential to prevent postoperative infections caused by this pathogen. Operating room factors, particularly the use of perioperative blood transfusions, were also associated with an increased risk of infections. Transfusions can have immunosuppressive effects, making patients more susceptible to infections. Surgical techniques that prolong operative time or increase tissue trauma were similarly linked to higher infection rates, highlighting the need for optimizing surgical protocols.

Alamanda and Springer (2018) [46] highlighted the critical role that patient-related and perioperative factors play in the development of PJIs, with an emphasis on those that can be altered to reduce infection risk. The review identified obesity, diabetes, smoking, and poor oral health as key modifiable risk factors. Obesity was found to be a major contributor to increased PJI rates due to its impact on wound healing and immune function. Patients with a higher BMI face a greater risk of surgical site infections, and this risk grows proportionally with the severity of obesity. Diabetes, particularly in cases where blood glucose levels are not well-controlled, was another major factor, given its adverse effects on immune system efficiency and tissue repair. Smoking, due to its impact on tissue oxygenation and immune response, was also strongly associated with an increased risk of infections.

DISCUSSION

FINDINGS SUMMARY

This narrative review has compiled evidence from 23 studies and 11 reviews reporting various aspects of PJIs, with a focus on microbial, biomechanical, and patient-related risk factors, as well as treatment outcomes. The predominance of Gram-positive bacteria such as Staphylococcus aureus in PJIs is consistent across multiple studies [39], while *Candida* species have emerged as significant pathogens in more com-

plex, polymicrobial infections [14]. Individual factors, including high BMI, diabetes, and advanced age, have been repeatedly identified as critical risk factors for PJIs, which complicates treatment strategies [17, 18]total Hip arthroplasty (THA. Additionally, several studies support the notion that two-stage revision surgeries provide better long-term outcomes in terms of infection-free survival, particularly in chronic or biofilm-associated infections [33, 34]management of recurrent infection after a two-stage exchange remains debated and the outcome of a repeat two-stage procedure is unclear. This study investigates the success rates of repeat two-stage exchange arthroplasty and analyzes possible risk factors for failure.\n\nMaterials and methods\nWe retrospectively identified 55 patients (23 hips, 32 knees. Diagnostic advancements, especially novel biomarkers like calprotectin, offer promising potential for improving the early detection of PJIs [40]"properties":{"formattedCitation":"[40]","plainCitation":"[40]","noteIndex":0},"citationItems":[{"id":28709,"uris":["http://zotero.org/users/local/lSN7m5lZ/items/I7HJKPT7"],"itemData":{"id":28709,"type":"article-journal","abstract":"BACKGROUND: Identifying novel biomarkers that are both specific and sensitive to periprosthetic joint infection (PJI.

KNOWLEDGE GAPS

Despite significant advancements, there are notable gaps in the understanding and management of PJIs. One such gap involves the variability in diagnostic criteria across institutions, particularly in the use of biomarkers like calprotectin or alpha-defensin. While these biomarkers show promise, their clinical utility requires further validation in large, multicenter trials to establish consistent cut-off values and integration into diagnostic protocols [40]the aim of this systematic review is to identify and evaluate the effectiveness of novel biomarkers for the diagnosis of PJI.\nMETHODS: We searched the MEDLINE, EMBASE, PubMed, and Cochrane Library databases from January 1, 2018, to September 30, 2022, using the search terms \"periprosthetic joint infection,\" \"prosthetic joint infection,\" or \"periprosthetic infection\" as the diagnosis of interest and the target index, combined with the term \"marker.\" We excluded articles that mentioned established biomarkers such as CRP, ESR, Interleukin 6, Alpha defensin, PCT (procalcitonin. Another critical gap is the lack of consensus on the best surgical approach for treating PJIs. While two-stage revisions are widely considered the gold standard, the recent findings [41] question this, showing no significant difference in outcomes between one-stage and two-stage revisions in select cases. Further research is needed to determine the patient populations and infection profiles that may benefit from a one-stage approach. Additionally, more research is necessary on how to optimize antimicrobial therapy in the context of biofilm-associated infections, as many current treatments remain inadequate against biofilm-producing organisms.

FUTURE DIRECTIONS

Future research should focus on closing the knowledge gaps identified in this review. First, efforts should be made to establish standardized diagnostic protocols that incorporate novel biomarkers alongside traditional methods like CRP and ESR. This will ensure earlier and more accurate diagnoses, which are critical for improving patient outcomes. Additionally, prospective randomized trials are needed to further evaluate the comparative efficacy of one-stage versus two-stage revision surgeries, especially in cases involving low-virulence organisms or well-characterized infections [41]. Another important avenue for future research is the development of biofilm-targeted therapies. Studies should explore novel antimicrobial agents or delivery systems that can effectively disrupt biofilms on prosthetic surfaces. Finally, exploring the role of patient optimization strategies, such as preoperative weight management and glycemic control, in reducing PJI risk in high-BMI or diabetic patients, would be beneficial [21, 37].

CONCLUSIONS

This narrative review highlights the multifaceted nature of periprosthetic joint infections (PJIs), which remain a major complication in arthroplasty procedures, particularly in high-risk populations such as those with obesity, diabetes, or chronic pulmonary conditions. The predominance of Gram-positive bacteria, particularly *Staphylococcus aureus*, remains a constant, though the increasing emergence of *Candida* species in polymicrobial infections adds to the complexity of management. Advances in diagnostic biomarkers like calprotectin and novel treatment modalities, including biofilm-active antibiotics, represent promising developments but require further clinical validation. Importantly, this review underscores the ongoing debate regarding one-stage *versus* two-stage revisions for PJIs. While two-stage procedures are still considered the gold standard for chronic or biofilm-associated infections, some studies suggest that single-stage revisions may offer comparable outcomes in selected cases, challenging traditional surgical paradigms. The integration of standardized

diagnostic protocols, biofilm-targeted therapies, and a more nuanced understanding of surgical interventions will be crucial for improving outcomes in patients suffering from PJIs. Additionally, optimizing patient health through preoperative interventions such as weight management and diabetes control should be prioritized to minimize infection risks in vulnerable populations. Continued research into these areas will be essential for developing more effective and tailored strategies for PJI prevention and treatment

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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