Wiadomości Lekarskie

Medical Advances

Official journal of the Polish Medical Association
Wiadomości Lekarskie has been published since 1928

Volume LXXVII, Issue 10, OCTOBER 2024 ISSN 0043-5147 E-ISSN 2719-342X

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ALUNA Publishing

Volume LXXVII, Issue 10, OCTOBER 2024

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Wiadomości Lekarskie Medical Advances is abstracted and indexed in:
PUBMED/MEDLINE, SCOPUS, EMBASE, INDEX COPERNICUS,
MINISTRY OF SCIENCE AND HIGHER EDUCATION, POLISH MEDICAL BIBLIOGRAPHY

Distribution and Subscriptions:
Bartosz Guterman prenumerata@wydawnictwo-aluna.pl

Graphic design / production:
Grzegorz Sztank fajne.work

Publisher:
ALUNA Publishing
29 Przesmyckiego st.,
05-510 Konstancin – Jeziorna, Poland
www.wydawnictwo-aluna.pl
www.wiadomoscilekarskie.pl
www.wiadlek.pl

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CONTENTS

ORIGINAL ARTICLES

REVIEW ARTICLES

CASE STUDIES

ORIGINAL ARTICLE

CONTENTS

Prognostic value of ultrasound and ENMG in predicting the results of treatment of tunnel compressive and post-traumatic neuropathies

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ABSTRACT

Aim: Explore how demographics affect neuropathy treatment outcomes; track ultrasound and ENMG changes; compare treatment efficacies; and develop predictive models using ultrasound and ENMG for tunnel compressive and post-traumatic neuropathies.

Material and Methods: A retrospective cohort design. A sample size of 200 patients consisting of 100 with tunnel compressive and 100 post-traumatic neuropathies selected through a convenient sampling technique was used. Data analysis was done using SPSS 25 version.

Results: Current study analyzes demographic, clinical, ultrasound, and electromyography characteristics of 200 patients with tunnel compressive and post-traumatic neuropathies. It found older mean ages and higher hypertension in the tunnel compressive group. Minor differences in nerve ultrasound and electromyography parameters indicated slight variations in nerve function between the groups. Treatment outcomes showed 55% improvement overall, slightly favoring the post-traumatic group. Ultrasound and electromyography parameters like W-CSA (82% sensitivity) and SNAP (85% sensitivity) demonstrated high effectiveness in predicting treatment responses. SCV (95% specificity) and R-CSA (91% specificity) accurately identified patients unlikely to benefit from treatment, with SCV and DSL showing high predictive values (86.7% and 84.2%, respectively), enhancing treatment precision.

Conclusions: Current study compare s tunnel compressive and post-traumatic neuropathies, revealing slight but significant differences in demographics, clinical features, and responses to treatment, aiding in personalized therapeutic strategies.

KEY WORDS: evaluation of ENMG, validation of prognostic models, monitoring of therapeutic intervention, choice of treatment method, surgical treatment of neuropathies

Wiad Lek. 2024;77(9):1833-1841. doi: 10.36740/WLek/195123 DOI

INTRODUCTION

Tunnel compressive and post-traumatic neuropathies encompass a spectrum of debilitating conditions characterized by nerve dysfunction arising from mechanical compression or traumatic injury. Post traumatic peripheral neuropathies are an important cause of long-term morbidity and disability. The prevalence of these lesions occurs in 2-3% of the patients admitted to hospital. As for the traditional TPNL diagnosis, the physician's examination for clinical neurologic features and electrodiagnostic tests have brought a few challenges such as the problematic diagnosis and therapy [1].

The handling of these neuropathies calls for much clinical attention, and the doctor's judgement needs to be very precise about their pathology and prognosis. The current studies have proven the observational value of various ultrasounds and electromyography modalities in determining the treatment course; however, the question regarding prognostic applicability of these methods still demands thorough research. Ultrasound helps diagnose and manage compressive and posttraumatic peripheral nerve entrapment while electromyography helps in the diagnosis and management of neuropathies of the peripheral nerves [2].

Trauma to peripheral nervous system is a less common but potentially disabling disorder among the patients and critically impact the patient's life. Overall incidence rate is estimated to be somewhere between 2 and 3 percent of critically injured patients, treating about 13 to 23 per 100.000 people per Anum. Specific trauma has a higher chance of injury to specific nerves, as radial nerve that is affected to about 10% of the patients in humeral shaft fracture. It is estimated that about 1.5–2% of the patients with a crush injury or joint dislocation will also have PNS injury. The specific trauma mechanism knowledge is mandatory to predict which nerve will be affected [2].

Ultrasound imaging has emerged as a valuable adjunct in the diagnostic armamentarium, offering unparalleled visualization of nerve structures and adjacent tissues. Through high-resolution imaging, ultrasound facilitates precise localization of nerve

compression sites and assessment of morphological alterations, thereby informing therapeutic planning. Additionally, ultrasound enables dynamic evaluation of nerve mobility and vascularity, providing insights into the pathophysiological mechanisms underpinning neuropathic conditions [3].

Alongside the fact of using electromyography as one of the cornerstones, in which the functional evaluation of peripheral nerves is carried out, the integrity of neuromuscular transmission and conduction pathways of the central nervous system is demonstrated. ENMG uses the digitalization of muscle and nerve impulse to provide an objective assessment of nerve function and capture any cave-like alterations in the physiology [4]. ENMG has also made it possible to discover the secondary denervation of muscles and to demonstrate compensations through which earlier prognostic indicators are associated with the treatment response. Furthermore, interpreting the diagnostic relevance of ENMG parameters in both cases of tunnel compressive neuropathy and post-traumatic neuropathy, needs to be further intensified [5].

Ultrasound and ENMG provide valuable prognostic factors to evaluate primarily the treatment outcomes of the tunnel syndromes and the posttraumatic neuropathies; nonetheless, the drawbacks and complexities have to be necessarily addressed and considered as well. Implementing a secure and ethical operating basis will result in more research, education and regulations to prevent cases or surgical mishaps from happening [6]. Therefore, combining ultrasound with ENMG for prognosis of outcome in tunnel compressive and post-traumatic neuropathies adds precision to findings which would warrant the idea of redefining the standard of clinical protocols for treatment of neuropathies [7]. Besides, pre-test ability of ultrasound and EMNG in assessing the effects of tunnel compression and post-traumatic neuropathy can be used to monitor the progression of the patient which will guide decision on treatment and may play a role in improving the prognosis of the patient in neuropathic condition [8,9].

The detailed analyses of ultrasound and ENMG parameters are expected to contribute to our knowledge of prognostic plan and therapeutic precision. Empowerment of clinicians by identifying the rapid diagnostic modalities' prognostic potential is achieved with the aim of helping the professionals to use clear, proof-based means for improving their patients' quality of life and the outcomes of them who are suffering from tunnel compressive and post-traumatic neuropathies [10,11]. Up to 20% of the cases of neurological pathogenesis with tunnel compressive and post-traumatic etiologies are clinically difficult to tackle, which requires immediate intervention to avoid permanent, severe consequences. These ailments are distinguished by nerve compression. For example, carpal tunnel syndrome and ulnar nerve entrapment are relatively more frequent causes of compression neuropathies. The causes of these conditions are the consequences of the pressure on peripheral nerves, which are either influenced by factors like repetitive movements, obesity, pregnancy or the use of casts and splints, all of which affect the susceptibility of peripheral nerves to impairment [12].

When paired with ultrasound, ENMG holds a firm place as a defining element in functional assessment of the neural conduction, and it gives information on how the conduction properties of the peripheral nerves and the integrity of the neural pathways. The capability of ENMG to determine the impairment of nerves and muscles through the measurement of their electrical activity can be effectively utilized to diagnose nerve dysfunction and aid in formulating accurate prognoses and effective treatments. Secondly, the nerve monitoring, nerve therapy follow-up and the efficacy of interventions evaluation in ENMG help to have an integrated assessment of the whole dynamic of the neurophysiological conditions of the affected organ [13].

In spite of the fact that the individual contribution of ultrasound and ENMG are valuable in their own right, the synergistic application may even add to the accuracy of prognostic aspects and improve the treatment outcomes in entrapped nerve and post-traumatic neuropathies. Through integrating of structural as well as functional data, doctors can produce highly-customized disease management plans, which should address ton specific features of any particular patient. In addition to the combination, the monitoring of the nerve recovery through these modalities allows longitudinal tracking of any changes that time makes, necessary therapy adjustment to be done based on the obtained objective neurophysiological measures [14].

Accordingly, this study is focused on clarifying the prognostic meaning of ultrasound and ENMG use as predicators for the success of treatment applications utilized in cases of "tunnel compression" and after trauma neuropathies. A critical analysis of the literature and clinical evidence will be the stone upon which we will build the strong foundation. It will be through this that we will outline the merits, limitations, and the future research prospects of these diagnostic modalities in prognostic assessments. We guide the achievements of crucial predictive ability of ultrasound and ENMG through this because we try to reach a more thorough understanding of the neuropathic pathophysiology and we facilitate a more precise choice-making of the prognoses in clinical practice.

AIM

1. To identify demographic factors of the people like gender and age that influence prognosis and response to treatment in tunnel compressive and post-traumatic neuropathies.
2. To evaluate the changes in ultrasound and electro-myography parameters such as W-CSA, R-CSA, SNAP, and SCV in subjects with these types of neuropathies.
3. To compare the outcome of treatments outcome in tunnel compressive and post-traumatic neuropathies.
4. To determine predictive values using ultrasound and electromyography parameters as indicator for treatment outcomes.

METERIALS AND METHODS

STUDY DESIGN

A retrospective cohort.

SAMPLE SIZE

A sample of 200 patients including 100 patients with tunnel type compressive and 100 post -traumatic neuropathies by way of convenience random sampling.

STUDY POPULATION

Subjects of a study are patients with a tunnel-compression type and post-traumatic neuropathies attending the Hospital.

INCLUSION CRITERIA

Patients with age from 18 years and above based on clinical examination, imaging studies, and electrodiagnostic evaluations.

EXCLUSION CRITERIA

Patients with incomplete medical records or insufficient follow-up data.

DATA COLLECTION

The medical records of qualified patients being retrospectively discussed and information reviewed - demographic, clinical characteristics, the imaging findings from the ultrasound studies, and electromyography reports. Along with that, the records on the type of treatments and their duration explanation are also documented such as surgical procedures, pharmacotherapy, and physical training and occupational therapy. Besides the data collection made during the period of treatment, the follow-up data, including the post-treatment clinical outcome, functional assessments and subjective symptomatology are also being noted.

ULTRASOUND EVALUATION

Ultrasonic images of the affected nerve (s) and its surrounding (anatomical) structure are reviewed by expert radiologists in order to investigate the signs of nerve compression, abnormal morphology, as well as the vascular dynamics. The results of quantification are the following, the womens' cross-sectional areas of wrist, perimeter of wrist, the ratio of the cross-sectional area of wrist to one-third of the distal forearm, the ratio of the perimeter of wrist to one-third of the distal forearm, the difference of cross-sectional area from the wrist to one-third of the distal forearm.

ELECTROMYOGRAPHY (ENMG)

Electro diagnostic studies, are called nerve conduction studies, and they are performed by qualified neurophysiologists, according to the recommended patterns. These parameters are NS parameters such as DML (ms), SMAP (mV), and Motor conduction velocity (m/s), DSL (ms), SNAP (mV) and Sensory conduction velocity (m/s).

OUTCOME ASSESSMENT

The outcome measure is treatment response, categorized as improvement, stabilization, or deterioration based on clinical evaluation and functional assessments.

DATA ANALYSIS

Descriptive statistics are used to summarize demographic characteristics, clinical features, and baseline ultrasound and ENMG findings using SPSS 25 data was presented in table form. Predictivity of Ultrasound and ENMG Parameters for Treatment Outcomes.

ETHICAL CONSIDERATIONS

This study is conducted in accordance with the principles outlined in the Declaration of Helsinki and approved by the Institutional Review Board (IRB) of [Name of the Institution]. Patient confidentiality and data anonymization protocols are strictly adhered to throughout the study process.

Table 1. Demographic and Clinical Characteristics of Study Population

Characteristics	Tunnel Compressive Neuropathies	Post-Traumatic Neuropathies	Total (n=200)
Age (years)	52.78 ± 5.28	38.19 ± 9.32	45.49 ± 6.80
Gender (Male/Female)	30/70	90/10	120/80
Duration of Symptoms (months), Median (IQR)	18(23.25-11)	10.5(24-12)	18(24-12)
Comorbidities (%)			
Hypertension	35(35.00%)	32(32.00%)	67(33.5%)
Diabetes Mellitus	50(50.00%)	49(49.00%)	99(49.5%)
Obesity	15(15.00%)	19(19.00%)	34(17.0%)
Total	100(100%)	100(100%)	200(100%)

Table 2. Ultrasound Findings

Ultrasound Parameters	Mean ± SD	Tunnel Compressive Neuropathies	Post-Traumatic Neuropathies
Cross-sectional area at wrist (mm2)	13.78 ± 2.26	13.80 ± 2.28	13.74 ± 2.24
Perimeter at wrist (mm)	18.33 ± 2.18	18.11 ± 2.20	18.66 ± 2.13
Ratio of cross-sectional area of wrist over one-third distal forearm	2.19 ± 0.64	2.15 ± 0.62	2.26 ± 0.65
Ratio of perimeter of wrist over one-third distal forearm	0.96 ± 0.16	0.95 ± 0.16	0.97 ± 0.16
Changes of cross-sectional area from wrist to one-third distal forearm (mm2)	8.24 ± 1.62	8.20 ± 1.65	8.30 ± 1.58
Changes of perimeter from wrist to one-third distal forearm (mm)	7.38 ± 1.65	7.38 ± 1.76	7.39 ± 1.49

Table 3. Electromyography (NCS) performance Mean

Parameters	Mean ± SD	Tunnel Compressive Neuropathies	Post-Traumatic Neuropathies
Distal ML (ms)	5.54 ± 1.75	5.49 ± 1.72	5.73 ± 1.76
Compound motor action potential (mV)	4.96 ± 1.74	4.97 ± 1.69	4.99 ± 1.75
Motor conduction velocity (m/s)	18.24 ± 2.68	18.24 ± 2.69	18.27 ± 2.69
Distal sensory latency (ms)	5.55 ± 1.75	5.53 ± 1.72	5.57 ± 1.73
Sensory nerve action potential (μV)	9.42 ± 1.24	9.25 ± 1.16	9.38 ± 1.39
Sensory conduction velocity (m/s)	32.81 ± 0.27	32.69 ± 0.35	32.63 ± 0.16

RESULTS

The Table 1 presents demographic and clinical characteristics of a study population divided into two groups including those with tunnel compressive neuropathies (n=100) and those with post-traumatic neuropathies (n=100), with a total sample size of 200 individuals. Regarding age, the mean age for Tunnel Compressive Neuropathies is 52.78 years (±5.28), while for Post-Traumatic Neuropathies, it is 38.19 years (±9.32), with an overall mean of 45.49 years (±6.80). Gender distribution shows 30% male and 70% female in the Tunnel Compressive Neuropathies group, contrasting with 90% male and 10% female in the Post-Traumatic Neuropathies group, resulting in a total of 60% male and 40% female. Median symptom duration is 18 months (IQR: 23.25-11) for Tunnel Compressive Neuropathies and 10.5 months (IQR: 24-12) for Post-Traumatic Neuropathies, with an overall median duration of 18 months (IQR: 24-12). The prevalence of comorbidities differs slightly between groups, with hypertension at 35% and 32%, diabetes mellitus at 50% and 49%, and obesity at 15% and 19% for Tunnel Compressive Neuropathies and Post-Traumatic Neuropathies, respectively, culminating in an overall prevalence of 33.5%, 49.5%, and 17% for each comorbidity, respectively.

Table 2 presents ultrasound findings for nerve cross-sectional areas and related parameters in patients with tunnel

Table 4. Treatment Outcomes

Treatment Response	Total (%)	Tunnel Compressive Neuropathies (%)	Post-Traumatic Neuropathies (%)
Improvement	110(55%)	53	57
Stabilization	66(33%)	37	29
Deterioration	24(12%)	10	14

Table 5. Predictivity of Ultrasound and ENMG Parameters for Treatment Outcomes

Variable	Predictor	TP	FP	TN	FN	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)
Ultrasound Parameters	W-CSA	82	22	78	18	82	78	78.1
	W-P	77	16	84	23	77	84	80.5
	R-CSA	69	9	91	31	69	91	85.3
	R-P	75	20	80	25	75	80	76.9
	CSA	80	13	87	20	80	87	83.3
	P	72	12	88	28	72	88	80.0
ENMG Parameters	DML	78	18	82	22	78	82	79.6
	CMAP	75	12	88	25	75	88	81.5
	MCV	70	10	90	30	70	90	83.3
	DSL	80	15	85	20	80	85	84.2
	SNAP	85	25	75	15	85	75	75.6
	SCV	65	5	95	35	65	95	86.7

compressive neuropathies and post-traumatic neuropathies. The nerve widened cross-sectional area W-CSA' (presumably widened cross-sectional area) shows very slight variation between groups, with tunnel neuropathies at 13.80±2.28 mm^2 and post-traumatic at 13.74±2.24 mm^2. The 'W-P' parameter, potentially a measure of width or pressure, shows a more noticeable difference between groups, at 18.11±2.20 mm^2 in tunnel neuropathies and 18.66±2.13 mm^2 in post-traumatic neuropathies. 'R-CSA' (possibly residual cross-sectional area) and 'R-P' (residual pressure) show minor variations, with R-CSA at 2.15±0.62 mm^2 for tunnel neuropathies versus 2.26±0.65 mm^2 for post-traumatic neuropathies, and R-P values are 0.95±0.16 mm^2 and 0.97±0.16 mm^2, respectively. Lastly, 'CSA' and 'P' are very similar across groups, with CSA values of 8.20±1.65 mm^2 for tunnel neuropathies and 8.30±1.58 mm^2 for post-traumatic neuropathies, and P values closely aligned at 7.38±1.76 mm^2 and 7.39±1.49 mm^2, respectively. The table suggests subtle but potentially clinically significant differences in ultrasound parameters between the two neuropathic conditions**.**

Table 3 presents the mean and standard deviation of various electromyography (NCS) performance parameters across different neuromuscular conditions, specifically comparing tunnel compressive neuropathies and post-traumatic neuropathies. The parameters measured include Distal Motor Latency (DML), Compound Muscle Action Potential (CMAP), Motor Conduction Velocity (MCV), Distal Sensory Latency (DSL), Sensory Nerve Action Potential (SNAP), and Sensory Conduction Velocity (SCV). DML is reported as 5.54±1.75 m/s overall, with slight differences between the neuropathy conditions (5.49±1.72 m/s for tunnel compressive neuropathies and 5.73±1.76 m/s for post-traumatic neuropathies).

CMAP shows minimal variance across conditions, averaging 4.96±1.74 mV overall. MCV and DSL are similarly consistent across groups, with MCV averaging 18.24±2.68 m/s and DSL 5.55±1.75 m/s. SNAP values show a bit more variability (9.42±1.24 mV overall) with slightly lower mean values in tunnel compressive neuropathies (9.25±1.16 mV). SCV exhibits the lowest variability but displays a minor decrease in post-traumatic conditions (32.63±0.16 m/s compared to 32.69±0.35 m/s in tunnel compressive neuropathies). These statistics provide a detailed comparison of nerve conduction parameters in different neuropathic conditions, highlighting subtle differences that may reflect the impact of the specific neuropathy type on neuromuscular function.

Table 4 presents treatment outcomes for 200 patients, divided equally between tunnel compressive and post-traumatic neuropathies. Overall, 55% of the patients (110 individuals) showed improvement following treatment, with 53 suffering from tunnel compressive neuropathies and 57 from post-traumatic neuropathies. Another 33% of the patients (66 individuals) experienced stabilization of their condition, including 37 with tunnel compressive and

29 with post-traumatic neuropathies. Deterioration was observed in 12% of the patients (24 individuals), with 10 cases of tunnel compressive neuropathies and 14 cases of post-traumatic neuropathies.

Table 5 presents the predictive performance of ultrasound and electromyography (ENMG) parameters for treatment outcomes in tunnel compressive and post-traumatic neuropathies. Ultrasound parameters like W-CSA and ENMG parameter SNAP demonstrate high sensitivity (82% and 85% respectively), ensuring effective identification of patients likely to respond positively to treatment. Specificity is notable in parameters such as SCV and R-CSA (95% and 91% respectively), indicating their ability to accurately pinpoint patients who may not benefit from treatment.

However, the parameters (SCV and DSL) having high positive predictive values (86.7% and 84.2% consequently) suggest that these parameters are also a promising way to determine which patients will benefit from treatment and whose results of the positive test will be true. These data tell us that that between ultrasound indicators and ENMG there might be some of them which are really strong markers of the treatment efficiency of these neuropathies and this could help to point clinicians in more exact and efficient therapeutic ways. The main statistical values as correct positives, false positives, correct negatives, false negatives, sensitivity, specificity, and positive predictive value are used in the paper to help provide the reader with a detailed, and therefore good understanding of the predictive utility of each parameter.

DISCUSSION

Current study compares demographic and clinical characteristics of individuals with tunnel compressive neuropathies and post-traumatic neuropathies on a sample size of 200 patients with a mean age of 51.35 years. among the two groups, tunnel compressive neuropathies group has a slightly higher mean age of 52.78 years as compared to the post-traumatic neuropathies group having 49.19 years of age. As far as gender distribution is concerned it shows 90 males and 110 females in the total population. Patients included were having median duration of symptoms of 8 months with 34% hypertension, 49.5%, diabetes mellitus and 16.5% obesity. We should also mention other relevant studies when it comes to the predictive value of ultrasonography and EMG in the treatment of tunnel compressive and post-traumatic neuropathies. In one study ultrasound has emphasized the necessity of this modality in the process of carpal tunnel syndrome diagnostics and nerve revival after surgery [15].

Another study made a link between carpal tunnel pressure and shear wave velocity which brings ultrasound into diagnostic possibilities in the CTS [16]. Moreover, a study designed to determine the cut-off values for stratifying carpal tunnel syndrome patients using ultrasound could also make an impact on treatment outcome forecasting [17]. The studies as a whole revealed details about the demographic and clinical factors influencing Persons with tunnel compressed neuropathy and post-traumatic neuropathy. Nevertheless, it would be beneficial if more studies are conducted that further examine the specific diagnostic and prognostic capabilities of ultrasound in conditions like carpal tunnel syndrome.

According to ultrasound findings, slight differences in the cross-sectional areas of the nerves were found between tunnel and post-traumatic neuropathies. The tunnel neuropathies demonstrate a slight reduction in widened cross-sectional area (W-CSA) and reciprocal increase in width/pressure (W-P) compared to acute neuropathies. On the other hand, both structural forms of neuropathies possess generally same values of cross-sectional area (CSA) and pressure (P) and small deviations in remaining values (R-CSA and R-P). The last point was about early diagnosis and treatment of patients with traumatic neuropathies. This helps in recovering more functions which stresses the importance of early nerve damage determination. [10].

However, some studies have focused upon evaluating the importance of assessing the situation and the type of nerve damage via such approaches as the DNP score in the acute traumatic neuropathy cases so that the suitable treatment can be given, especially severe nerve damage has to be dealt with through the surgical intervention [18,19]. Moreover, another study went into the issues of indications for neuromuscular ultrasound emphasizing the role of medical specialists and experts in identification of lesions, performance of diagnostics, and evaluation of the extent of damage caused to the nerve, this being very important for treatment planning [20]. These studies statistically demonstrate that accuracy of ultrasound and ENMG diagnosis for peripheral nerve injuries is crucial, and it allows clinicians to have well informed opinions on prognosis, which then can help patients to recover faster, better, and at least get to avoid personal nightmare.

While present analysis includes the mean and standard deviation of various parameters of EMG studies for cases involving tunnel compressive neuropathies and post-traumatic neuropathies, these are compared for the two groups as well. The analysis reveals about the slight variances obtained among neuropathic conditions, the post-trauma neuropathies demonstrating slightly higher DML and lower SCV compared to tunnel compressive neuropathies. Such results describe distinctions in nerve parameters which may reflect on the specific against the general effects of the type of neuropathy on functions of neuromuscular.

Other studies concentrate on using ultrasound and ENMG in determining treatment results for neuropathies. The role

of electrodiagnostic testing, which involves nerve conduction studies and needle electromyography, is also discussed in these studies in regard to the prognosis and management of peripheral neuropathy. Foremost, it emphasizes the significance of electrophysiological testing as the diagnostic method for neuropathy in order to pinpoint the causes of neuropathy, and in assessing treatment responses [21].

Another study sheds light on the role played by electromyography and nerve conduction studies in the assessment of patients with neuromuscular disorders and highlights the significance of using the clinical information as well as the symptoms of the patients to augment the findings of these tests, which can be normal in certain cases of neuropathy [22].

Another study outlines the diagnostic process and prognostic evaluation of the patients with the suspected Guillain-Barré syndrome, and it also being used to determine the role of the nerve conduction studies in identifying demyelination and predicting treatment outcomes [23].

Collaboratively, these studies stand out the significance of detailed electrodiagnostic studies in the diagnosis and management of neuropathies and underscore the reality that pattern of the test results and clinical context together with the patient's symptoms should be considered when interpreting findings of electrodiagnostic tests as sometimes test results may be normal in some instances of neuropathies.

While the recent studies reveals that the ultrasound is an effective tool for the diagnosis and treatment of both neuropathies caused by compression in tunnel and post traumatic damages. The result of the treatment was quite positive, with 55% of the 200 patients reported the improvement whereas 53 had syndromes due to Tunnel Compressive Neuropathies and 57 were affected by the Post-Traumatic Neuropathies. Another 33% of the patients experienced stabilization of their condition, including 37 with tunnel compressive and 29 with post-traumatic neuropathies. Deterioration was observed in 12% of the patients, with 10 cases of tunnel compressive neuropathies and 14 cases of post-traumatic neuropathies.

In cases of severe nerve damage, surgical intervention may be required, and ultrasound can help identify the extent of the injury. Similarly, another study reported that early diagnosis and care are vital to enhancing the functional prognosis in patients with peripheral nerve injuries, and that ultrasound can be a valuable tool in this regard and found that among 1-2% of individuals with peripheral nerve injuries associated with central nervous system damage, 60% of cases were spinal injuries, fractures, and dislocation of adjacent bones [24].

Additionally, a review discussed the role of peripheral nerve ultrasound in the diagnosis and management of patients with (suspected) peripheral nerve system trauma and highlighted the ability of ultrasound to detect various pathologies, such as scars, adhesions, neuroma outgrowth, and remodeling, which can guide surgical intervention and pain management [25].

Furthermore, another study found that the prevalence of peripheral neuropathy in patients with systemic sclerosis ranged from 28 to 36.6%, and that compression neuropathies were reported in 26.5% of the studies and emphasized the role of high-resolution ultrasound in the diagnosis of these neuropathies in rheumatological patients [26]. In conclusion, the results presented in the original study are supported by the findings of other relevant studies, which collectively demonstrate the prognostic value of ultrasound in the assessment and management of both tunnel compressive and post-traumatic neuropathies.

The predictive performance of ultrasound and electromyography (EMG) parameters in identifying treatment outcomes for tunnel compressive and post-traumatic neuropathies has been studied extensively in current study and highlighted the sensitivity and specificity of parameters like W-CSA, ENMG SNAP, SCV, R-CSA, and DSL, that are promising in guiding clinicians towards more targeted and efficient treatment approaches. Similarly, one study that supports these findings is the systematic review and meta-analysis conducted that compared the diagnostic accuracy of ultrasound with nerve conduction studies and electromyography in carpal tunnel syndrome and found that ultrasound had comparable sensitivity and slightly higher specificity than NCS and EMG, indicating its potential as an alternative diagnostic test for CTS [27].

Another study that corroborates the predictive value of ultrasound parameters investigated the use of nerve ultrasound in chronic inflammatory demyelinating polyneuropathy and found that nerve ultrasound was a useful tool in facilitating the diagnosis of CIDP, especially when nerves were in excitable on NCS. This demonstration, therefore, underscores the role of ultrasound in diagnosis of some of the most common neuropathies and identifying the treatment course [28].

At the very last, there was a study which tried to compare ultrasonography with electrodiagnosis in detecting ulnar neuropathy at the elbow and the researchers ultimately proved that ultrasonography is very sensitive and specific and that it is a suitable method to diagnose cases of ulnar neuropathy at the elbow in a pinch [29].

As indicated by these studies, ultrasound scan constitutes of the hallmarks during tunnel compressive and post-traumatic neuropathy diagnosis and management. The results from these studies corroborate the predictive performance of ultrasound parameters such as the W-CSA, ENMG SNAP, SCV, R-CSA, and DSL (as indicated in the original results), and show the possibility of the efficient ultrasound applications in clinical practice for guiding the physicians towards more target-oriented and refined therapies implementation.

CONCLUSIONS

Following are the conclusion of the study

1. Mean age gap between two groups indicates that the tunnel compressive neuropathies tend to come later than the post-traumatic neuropathies do toward the end ages.
2. Prevalence of comorbidities such as hypertension and diabetes mellitus were almost equal across both groups demonstrating that those might be either risk factors or happens to be concurrent conditions of neuropathic ones.
3. Ultrasound parameters which were the widened cross-sectional areas versus the residual pressure, showed minimal differences between the groups. That suggests the Ultrasound scanning can as well identify the nerve structure which is different between two kinds of neuropathies.
4. EMGs indicated light deviations in extent of neuromuscular function between the two groups. This could potentially be used to refine diagnostic approaches or tailor interventions more closely to the type of neuropathy.
5. The treatment outcomes indicate a comparable rate of improvement and stabilization between the two groups, though a slightly higher deterioration rate in post-traumatic neuropathies.
6. Ultrasound and ENMG parameters indicated correlations with eventual treatment success (predictive). Pivotal parameters such as W-CSA, SNAP, and SCV demonstrated high sensitivity in prediction of positive responses to therapy while being highly specific for R-CSA in estimation of those not susceptible to treatment. These features reaffirm the relevance of these diagnostic tools in clinical decision-making.

Overall, this comprehensive analysis not only illuminates the differences and similarities between tunnel compressive and post-traumatic neuropathies but also highlights the role of ultrasound as advanced diagnostic tools in predicting treatment outcomes. These insights could lead to more personalized and effective therapeutic strategies, improving patient prognosis and quality of life.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 10.06.2024
ACCEPTED: 28.09.2024

ORIGINAL ARTICLE

CONTENTS

Markers for predicting the severity of acute pancreatitis

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ABSTRACT

Aim: To identify markers for predicting the severity of acute pancreatitis and the possible development of pancreatic necrosis.

Materials and Methods: Prospective analysis of 81 patients with moderate and severe acute pancreatitis while performing correlation analysis, building a logistic regression model.

Results: A direct correlation of medium strength between sFGL2 and the following parameters was found D-dimer (R=0.47 (p<0.001)), C-reactive protein (R=0.3 (p=0.03)), intra-abdominal pressure (R=0.54 (p<0.0001)). It was discovered that such indicators as: BISAP score (OR=1.62 95%CI 0.99-2.66) p=0.05, sFGL2 (OR=1.01 95%CI 1.0-1.05) p=0.007, CRP (OR=1.01 95%CI 1.0-1.01) p=0.02, D-dimer (OR=2.59 95%CI 1.57-4.26) p=0.0002, intra-abdominal pressure (OR=195 95%CI 1, 39-2.72) p=0.0001 allow to predict the progression of necrotic changes in the pancreatic tissue and retroperitoneal space. And such indicators as: BISAP scale (OR=2.19 95%CI 1.33-3.59) p=0.002, sFGL2 (OR=1.01 95%CI 1.0-1.1) p=0.0002, CRP (OR=1.02 95%CI 1.01-1.02) p<0.0001, D-dimer (OR=1.99 95%CI 1.4-2.83) p=0.0001, intra-abdominal pressure (OR=2.36 95%CI 1.59-3.5) p<0.0001) may play a role in predicting worsening of acute pancreatitis.

Conclusions: It has been revealed that elevated levels of sFGL2, D-dimer and intra-abdominal pressure can predict the progression of necrotic changes in the pancreatic tissue and retroperitoneal space. And such indicators as the BISAP score, sFGL2, CRP, D-dimer and intra-abdominal pressure may play a role in predicting the deterioration of acute pancreatitis.

KEY WORDS: severe acute pancreatitis, soluble fibrinogen-like protein 2, D-dimer, intra-abdominal pressure, C-reactive protein

Wiad Lek. 2024;77(9):1842-1851. doi: 10.36740/WLek/195124 DOI

INTRODUCTION

Acute pancreatitis is an acute aseptic inflammation of the pancreas of a demarcation nature, which is based on the processes of pancreatic necrosis and fermental autoaggression with further development of necrosis of the gland and parapancreatic tissue, degeneration of the gland and parapancreatic space and possible addition of secondary infection.

According to the review of global epidemiology, the cumulative incidence of acute pancreatitis is 34 cases per 100,000 people in the general population per year with 1.16 deaths [1]. Mortality among patients with persistent organ failure and pancreatic necrosis can reach 30-40% [2].

Studies have shown that apart from the autodigestion of pancreatic parenchyma by pancreatic enzymes, ischemia, occurring because of pancreatic edema and leading to the development of acute necrotizing pancreatitis, also plays an important role [3]. Moreover, microcirculatory disorders are present in the pancreas and extrapancreatic organs. Clinical studies have revealed that fibrinogen degradation products (FDP) in blood plasma are significantly higher in patients with acute pancreatitis compared to healthy individuals, and higher levels of FDP are associated with disease severity [4]. Furthermore, early complications of severe acute pancreatitis associated with blood supply disorders include portosplenomesentric venous thrombosis, which, according to the literature, occurs in approximately 17.86% of patients [5]. These data suggest the need for prescribing anticoagulant and antithrombotic therapy in the treatment strategy for severe acute pancreatitis, in accordance with the 2019 WSES treatment protocols [6].

A substantial number of biochemical markers that can be predictors of complications of acute severe necrotising pancreatitis are still being studied. More specifically, they are IL-6, IL-8, polymorphonuclear elastase, TNF-alpha, trypsin-alpha-1 protease complex, hepsidin, copeptin, ISAM-1, resistin, presipsin, and others. However, most of them are expensive and their indicators are elevated only in the first 24-48 hours after the onset of the disease, so they are not used in daily clinical practice.

One of the most promising biochemical markers is fibrinogen-like protein 2 (FGL2), which can break

Table 1. Patients characteristics

Characteristic	Total number of patients (n=81)
Age. years	49 (39-68)
Men, n (%)	45 (55,5%)
Women, n (%)	36 (44,5%)
BMI, kg/m2	28,34 (±4,256)
Comorbitities, n (%)	31

down prothrombin to thrombin and results in fibrin deposition. FGL2 leads to histopathological lesions and ischemic damage through 'immune coagulation', fibrin deposition and microthrombosis. Microvascular disorders are caused by microthrombi that are activated and formed by the action of FGL2. FGL2 can be used as a disease biomarker and therapeutic target [7].

AIM

To determine the markers for predicting the severity of acute pancreatitis and possible development of subsequent pancreatic necrosis.

MATERIALS AND METHODS

The study design was a prospective analysis of 81 patients with acute pancreatitis, who underwent enzyme-linked immunosorbent assay of serum for determining FGL2, held at the clinical base of the Department of General Surgery No. 1 of the Bogomolets National Medical University in the period from 2022 to 2024.

The given study was conducted within the framework of the research work "Development and improvement of diagnostic methods, prognosis and surgical treatment of complications of hepatopancreatoduodenal region diseases", 2023-2025 (state registration number 0123U100953). Permission to conduct the study was approved by the expert decision of the Bioethical Commission dated 20.06.2022, protocol № 159. All study procedures were carried out in line with the current legislation of Ukraine on ethics, the principles of Good Clinical Practice (ICH 6CP), and the recommendations of the Helsinki Declaration of 2013.

The sample included 81 patients, with 45 (55.5%) men and 36 (44.5%) women respectfully. The average age of the patients was 49 (39-68 Q1-Q3) years. The average body mass index (BMI) was 28.34 (±4,256) (Table 1).

All patients with severe acute pancreatitis of nutritional etiology received anticoagulant therapy in a standard dosage from the second day of hospitalisation. Patients, requiring endoscopic papillosphincterotomy, had anticoagulant therapy started 12 hours after the intervention

The moderate severity of the disease was determined in 41 (50.6%) patients, while severe acute pancreatitis was specified in 40 (49.3%) patients. Patient characteristics are presented in Table 2. The Revised Atlanta Classification for Acute Pancreatitis 2012 was used to determine the severity of pancreatitis [8]. The presence of pancreatic tissue necrosis was assessed based on computed tomography with intravenous contrast (CTSI Baltazar), intraoperatively and by autopsy. The clinical and morphological classification of acute severe pancreatitis was used to evaluate intraoperative and autopsy materials [9]. Pancreatic necrosis was diagnosed in 32 (39.5%) patients. Among them, there were 4 (4.9%) patients with total transmural necrosis and 28 (34.5%) with superficial subtotal and focal forms of necrosis.

The groups differed statistically significantly in the levels of the following indicators: D-dimer, C-reactive protein, bilirubin, gamma-glutamine transpeptidase, alkaline phosphatase, creatinine, urea, fibrinogen, and intra-abdominal pressure.

Blood samples were taken 15-24 hours after hospitalisation. The obtained serum was evaluated for the presence of lipemia. Repeated sampling was performed 48 hours after hospitalisation.

According to the instructions provided, after serum collection, the latter was stored at -20°C prior to the analysis.

Serum was analysed using an enzyme-linked immunosorbent assay Human FGL-2 ELISA Kit, code EH3064.

Inclusion criteria: patients with moderate to severe acute pancreatitis, with or without pancreatic necrosis, without any medical or social contraindications, patients over 18 years of age, patient consent to participate in the study and subsequent outpatient monitoring.

Non-inclusion criteria: patients with COVID-19 (severe course), chronic fibrotic degenerative pancreatitis in the acute stage (presence of pseudocysts, virsungoectasia and virsungolithiasis), pancreatic surgery; presence of oncological pathology; long-term use of high doses of anticoagulants and antiplatelet agents before the onset of the disease.

Exclusion criteria: patients with mild acute pancreatitis, patient's refusal of diagnosis and treatment at any stage of the study, patient's death not related to the underlying disease, as well as patients with hypertriglyceridemic acute pancreatitis.

Endpoints of the study:

- To identify factors that predict the deterioration of acute pancreatitis and development of pancreatic necrosis.

Table 2. General characteristics of patients with acute pancreatitis in the study groups

Indicator	Group of patients with moderate acute pancreatitis (MAP) (n=41)	Group of patients with severe acute pancreatitis (SAP) (n=40)	P-value
Age	44,5 (38-58)*	53 (43-72)	0,156
Sex (men/women)	16/15	19/11	
BMI (kg/м2)	27,83 (±4,752)	28,83 (±3,708)	0,335
Intraabdominal pressure (mm Hg)	5,3(4,6-6,1)	10,75 (8,8-11,8)	<0,001
Amylase (U/l)	1449,5 (728-2137)	1676,5 (940-2503)	0,405
Lipase (U/l)	1502 (±979,8)	1753 (±1040)	0,36
Bilirubin (μmol/l)	20,1 (15,1-28,7)	26,65 (15-52,8)	0,041
Alanine aminotransferase (U/l)	84,35 (34,9-263,9)	105,3 (73,9-327)	0,349
Aspartate aminotransferase (U/l)	134,5 (53,2-324,8)	180,35 (67-348,2)	0,658
C-reactive protein (mg/l)	105 (37,78-150)	250 (205-303,33)	<0,001
Gamma-glutamine transpeptidase (U/l)	65,5 (30-110)	182 (120-494)	0,018
Alkaline phosphatase (U/l)	88,5 (57-100)	133 (106-180)	0,018
Glucose (mmol/l)	6,97 (6,41-7,7)	6,62 (5,86-9,72)	0,371
Creatinin (μmol/l)	84 (75-95)	101 (92-131)	0,006
Blood urea nitrogen (mmol/l)	5,9 (4,5-7,9)	7,45 (6,3-10,5)	0,022
Prothrombin index (%)	96,4 (90-109,2)	92,2 (85,6-101,9)	0,393
Fibrinogen (g\l)	5,63 (4,5-7)	7,14 (6-12)	0,015
International normalized ratio	1,016 (±0,136)	1,05 (±0,1659)	0,36
D-dimer (pg/ml)	0,5 (0,4-1)	4 (2,88-5)	<0,001
Hemoglobin (g/l)	148,4 (±16,06)	157,9 (±21,27)	0,084
White blood cells (109/l)	11,95 (10,2-14,4)	13,5 (9,4-16,8)	0,109
Platelets (109/l)	189 (141-237)	167,5 (138-212)	0,334

Note:* - median (Q1-Q3

- To conduct a correlation analysis between the level of sFGL2 and other biochemical parameters.

Statistical software used in this study included MedStat, EZR (R-STATISTICS). The Shapiro-Wilk test was used to assess the normality of continuous variables. Categorical data were presented as numbers (percentages). All continuous variables were presented as median (interquartile range [Q1-Q3]) and standard deviation. The Mann-Whitney U test was performed to compare continuous variables in the two given groups. Bivariate correlation was analysed using the Spearman correlation test. A logistic regression model was performed to determine the effect of sFGL2 and other biochemical parameters (C-reactive protein, D-dimer, lipase, fibrinogen) and BISAP on the severity of acute pancreatitis. The independent association was determined by the odds ratio (OR) and 95% confidence interval (CI). A ROC curve was constructed and the optimal cut-off values for serum levels of sFGL 2 were selected, while the corresponding sensitivity and specificity values were obtained. The p-value < 0.05 was considered statistically significant.

RESULTS

The Spearman's rank correlation method revealed the presence of a correlation of average strength R=0.47 (p<0.001) between sFGL2 and D-dimer, whereas an increase in D-dimer was accompanied by an average increase in sFGL2 (Fig 1).

A direct correlation of average strength R=0.3 (p=0.03) was found while analysing the relationship between CRP and sFGL2. On average, an increase in sFGL2 is accompanied by an increase in CRP (Fig. 2).

A direct correlation of average strength R=0.54 (p<0.0001) was found while analysing the relationship between intra-abdominal pressure and sFGL2. On average, an increase in intra-abdominal pressure was accompanied by an increase in sFGL2 (Fig. 3).

There was no correlation revealed between sFGL2 and such indicators as lipase (R=0.07, p=0.62), fibrinogen (R=0.2, p=0.15), BISAP (R=0.2, p=0.15).

The method of building logistic regression models on the total cohort of patients was used in order to analyse the development of pancreatic necrosis and the severity of acute pancreatitis. The univariate model was built

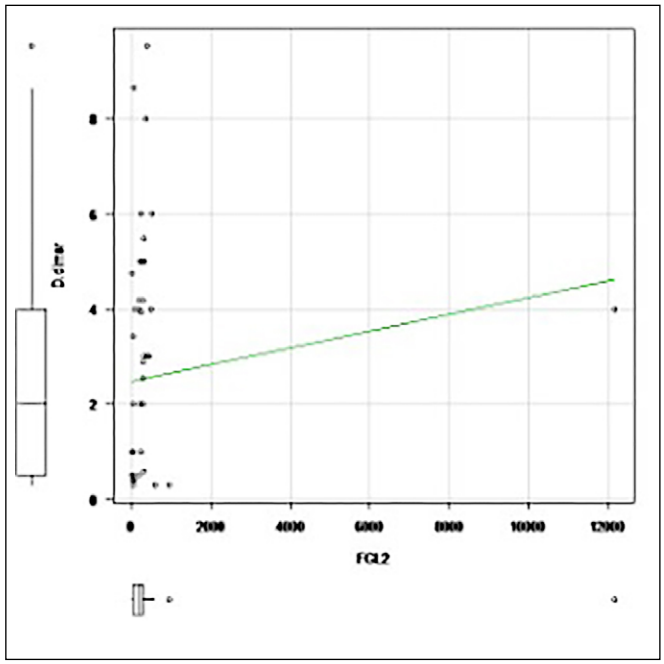

Fig. 1. Correlation analysis of the relationship between sFGL2 and D-dimer in patients with acute pancreatitis.

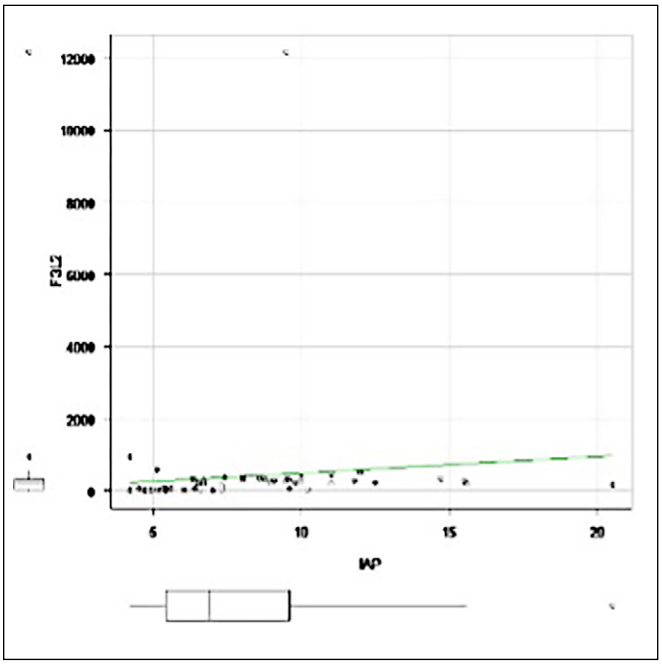

Fig. 3. Correlation analysis of the relationship between sFGL2 and intra-abdominal pressure in patients with acute pancreatitis.

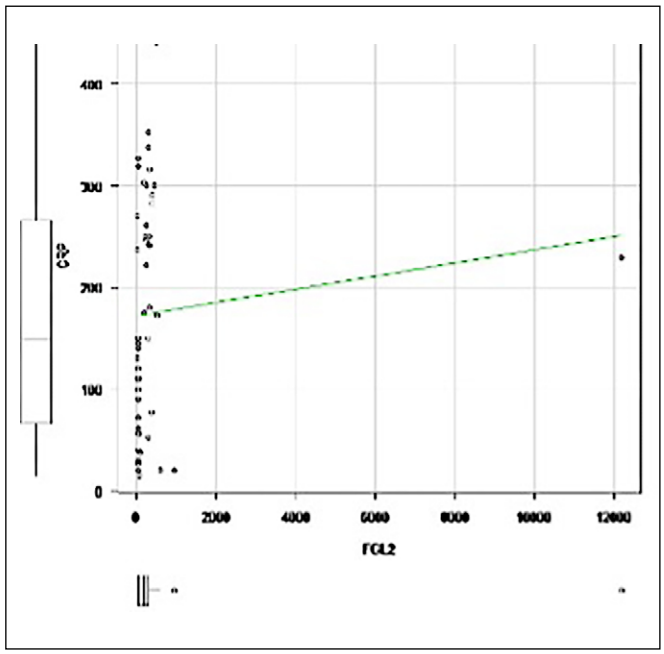

Fig. 2. Correlation analysis of the relationship between sFGL2 and C-reactive protein in patients with acute pancreatitis.

considering the following factors: body mass index, BISAP, intra-abdominal pressure, lipase, C-reactive protein, fibrinogen, soluble fibrinogen-like protein 2, international normalisation ratio, prothrombin index, D-dimer, platelets, leukocytes. The risk of pancreatic necrosis was found to be associated with: BISAP score (OR=1.62 95%CI 0.99-2.66) p=0.05, sFGL2 (OR=1.01 95%CI 1.0-1.05) p=0.007, CRP (OR=1.01 95%CI 1.0-1.01) p=0, 02, D-dimer (OR=2.59 95%CI 1.57-4.26) p=0.0002, intra-abdominal pressure (OR=195 95%CI 1.39-2.72) p=0.0001.

A logistic model for predicting the risk of pancreatic necrosis was built based on the selected factor features (AUC=0.93 95%CI 0.87-1.0) p<0.05, which is evidence of the adequacy and very good quality of the model.

It was found that the risk of pancreatic necrosis increases with increased levels of sFGL2 (OR=1.96 95%CI 1.27-3.03) p=0.002, D-dimer (OR=2.57 95%CI 1.45-4.56) p=0.01 and intra-abdominal pressure (OR=172 95%CI 1.2-2.46) p=0.003.

Using ROC-analysis, the cut-off value of D-dimer, at which pancreatic necrosis was most often diagnosed, was determined with cut-off value = 2.54 μg/ml AUC = 0.90 (95% CI 0.83-0.98) (sensitivity 80.6% (95% CI 64.2%-94.2%), specificity 91.7% (95% CI 62.5%-92.5%), PPV 80% (95% CI 61.4-92.3%) NPV 83.3% (95% CI 65.3%-94.4%)) (Fig. 4).

Using ROC-analysis, the cut-off value of sFGL2, at which pancreatic necrosis was most often diagnosed, was determined with the cut-off value = 80pg/ml AUC = 0.85 (95% CI 0.75-0.95) (sensitivity 69.4% (95% CI 44.9%-70.9%), specificity 100% (95% CI 80.4%-100%), PPV 91.7% (95% CI 81.6-97.2%) NPV 83.3% (95% CI 65.3%-94.4%)) (Fig. 5).

Using ROC-analysis, the threshold value of intra-abdominal pressure at which pancreatic necrosis was most often diagnosed was determined, cut-off value = 8.5 mmHg. AUC = 0.9 (95% CI 0.82-0.98) (sensitivity 83.3% (95% CI 73.4%-92.9%), specificity 87.5% (95% CI 70.8%-97.6%), PPV 80% (95% CI 61.4-92.3%) NPV 90% (95% CI 73.5%-97.9%)) (Fig. 6).

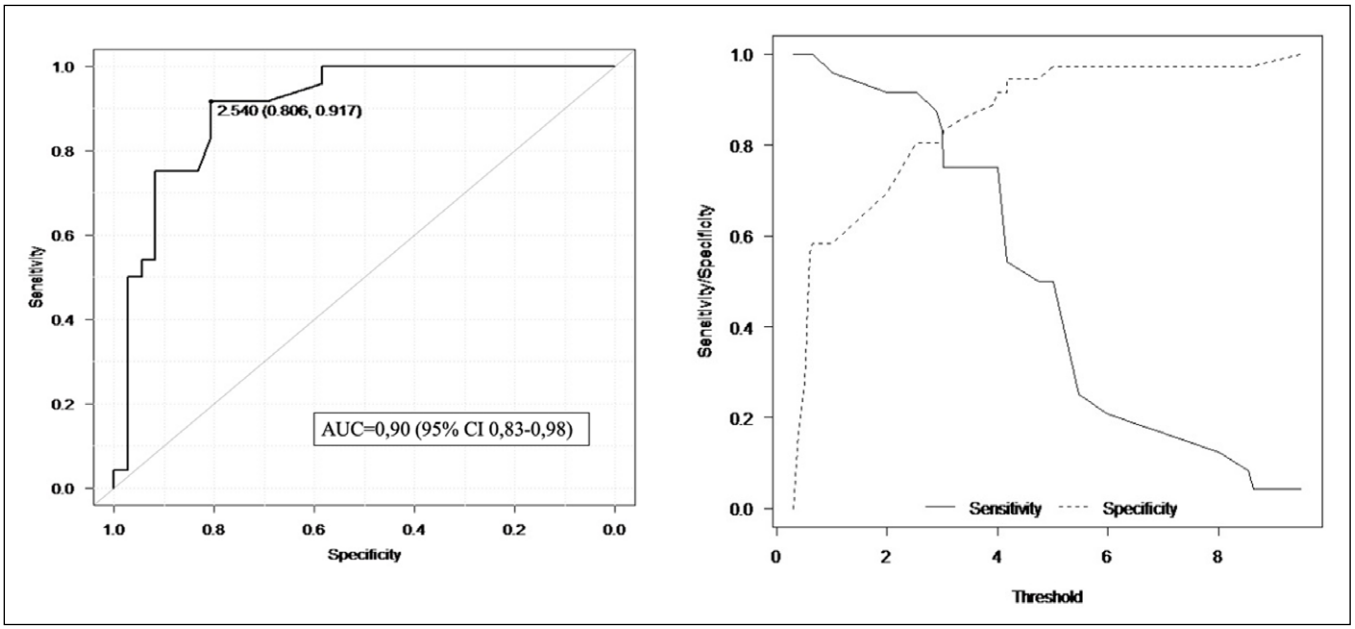

Fig. 4. ROC curve of the test for predicting the risk of necrosis depending on the level of D-dimer.

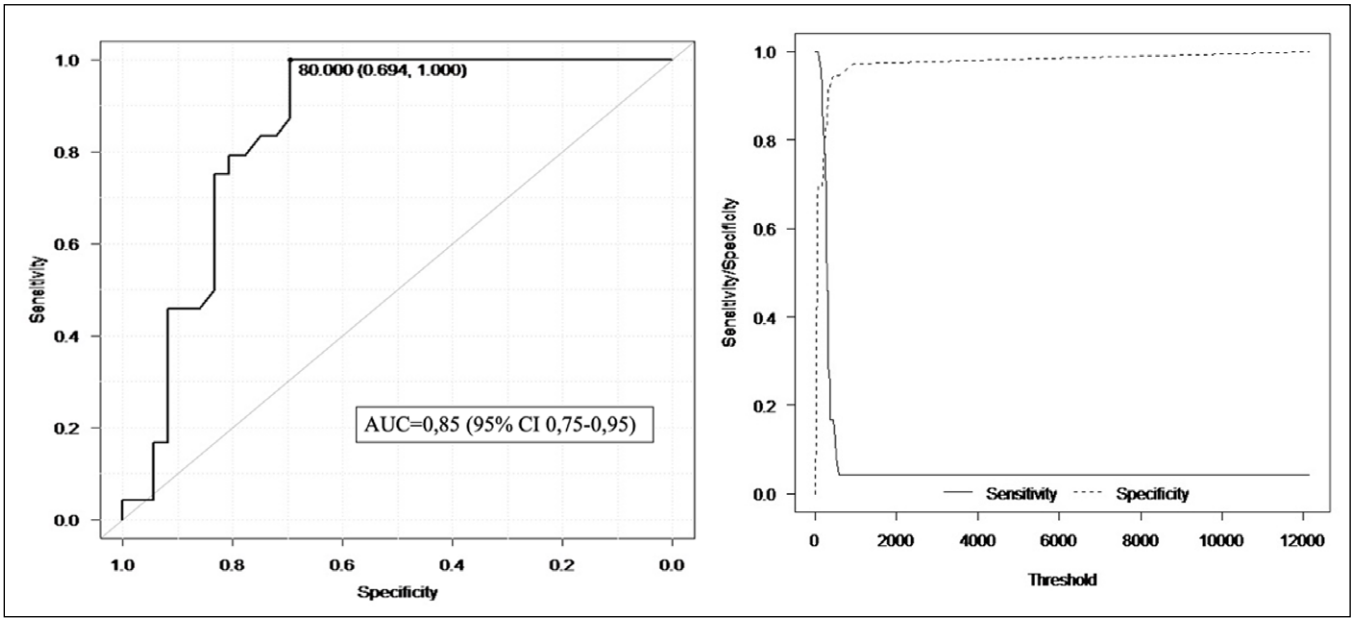

Fig. 5. ROC curve of the test for predicting the risk of necrosis depending on the level of sFGL2.

When building a univariate model, the following factors were considered: body mass index, BISAP, intra-abdominal pressure, lipase, C-reactive protein, fibrinogen, soluble fibrinogen-like protein 2, international normalisation ratio, prothrombin index, D-dimer, platelets, leukocytes. It has been found that the severity of acute pancreatitis is associated with: BISAP scale (OR=2.19 95%CI 1.33-3.59) p=0.002, sFGL2 (OR=1.01 95%CI 1.0-1.1) p=0.0002, CRP (OR=1.02 95%CI 1.01-1.02) p<0, 0001, D-dimer (OR=1.99 95%CI 1.4-2.83) p=0.0001, intra-abdominal pressure (OR=2.36 95%CI 1.59-3.5) p<0.0001.

In multivariate analysis with the evaluation of statistically significant factors, risk factors for severe acute pancreatitis were identified (AUC=0.97 95%CI 0.94-1.0) p<0.05.

It was revealed that the risk of severe acute pancreatitis increases with the elevation of sFGL2 (OR=1.01 95%CI 1, 0-1.01) p=0.006, CRP (OR=1.02 95%CI 1.0-1.03) p=0.005, D-dimer (OR=1.86 95%CI 1.11-3.1) p=0.02, intra-abdominal pressure (OR=1.88 95%CI 1.16-3.05) p=0.01.

Using ROC analysis, we determined the threshold value of D-dimer, at which the risk of severe acute pancreatitis increases. The Cut-off value = 0.65 μg/ml AUC = 0.88 (95% CI 0.79-0.97) (sensitivity 66.7% (95% CI 51.6%-76.9%), specificity 96.7% (95% CI 76.2%-99%),

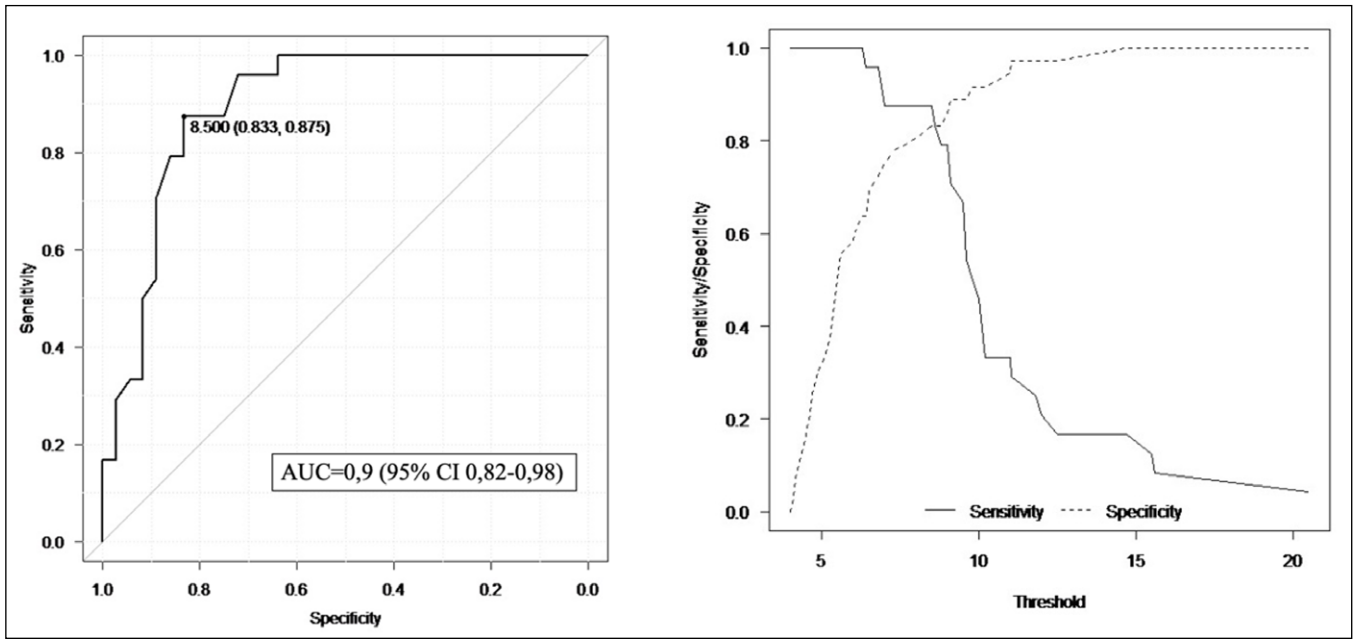

Fig. 6. ROC curve of the test for predicting the risk of necrosis depending on the level of intra-abdominal pressure.

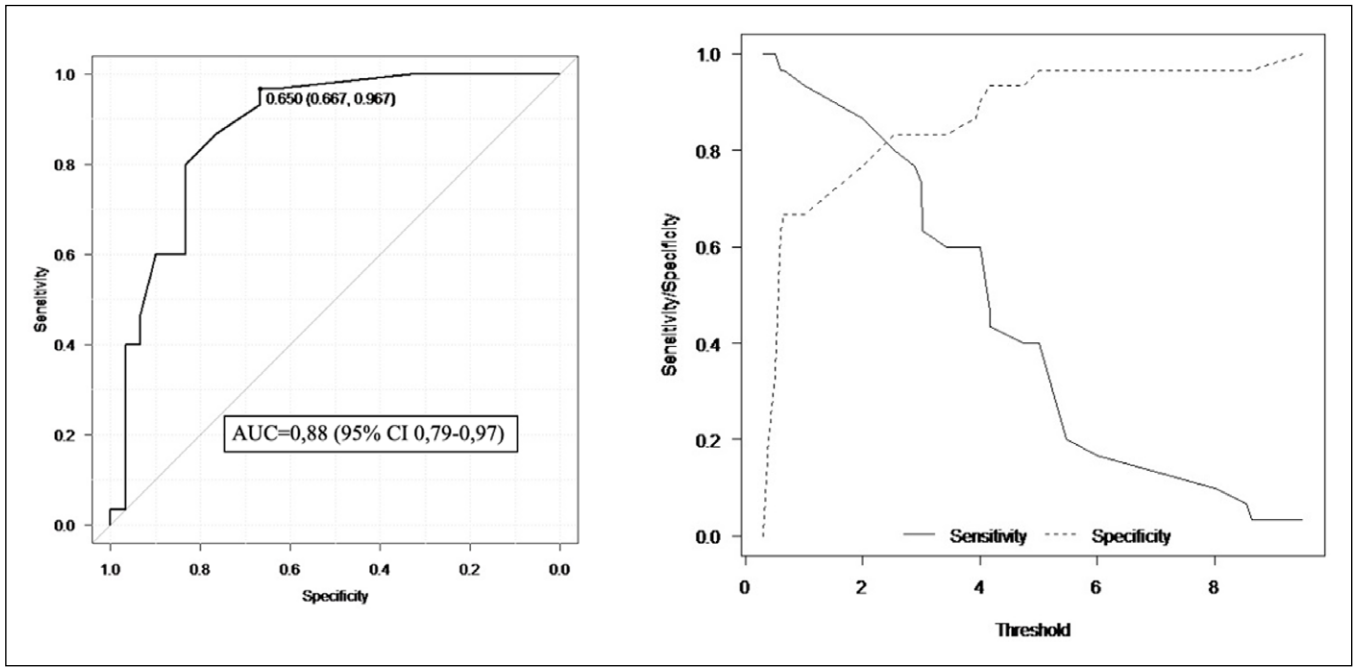

Fig. 7. ROC curve of the test for predicting the severity depending on the level of D-dimer.

PPV 96.7% (95% CI 82.8-100%) NPV 66.7% (95% CI 47.2%-82.7%)) (Fig. 7).

The threshold value of CRP, at which the risk of severe acute pancreatitis increases, was determined using ROC analysis. Cut-off value = 172 mg/l AUC = 0.86 (95% CI 0.77-0.96) (sensitivity 80% (95% CI 61.4%-92.3%), specificity 86.2% (95% CI 69.3%-96.2%), PPV 81.2% (95% CI 63.6-92.8%), NPV 85.7% (95% CI 67.3%-96%)) (Fig. 8).

The threshold value of sFGL2, at which the risk of severe acute pancreatitis increases, was determined Using ROC analysis. Cut-off value= 145 pg/ml AUC=0.91 (95% CI 0.81-1) (sensitivity 86.7% (95% CI 69.3%-96.2%), specificity 100% (95% CI 81%-100%), PPV 93.3% (95% CI 83.8-98.2%), NPV 88.2% (95% CI 72.5%-96.7%)) (Fig. 9).

ROC analysis was used to determine the threshold value of intra-abdominal pressure at which the risk of severe acute pancreatitis increases. Cut-off value = 7.2 mmHg. AUC=0.94 (95% CI 0.88-1) (sensitivity 86.7% (95% CI 69.3%-96.2%), specificity 100% (95% CI 83.3%-100%), PPV 88.2% (95% CI 72.5-96.7%), NPV 93.3% (95% CI 83.8%-98.2%)) (Fig. 10).

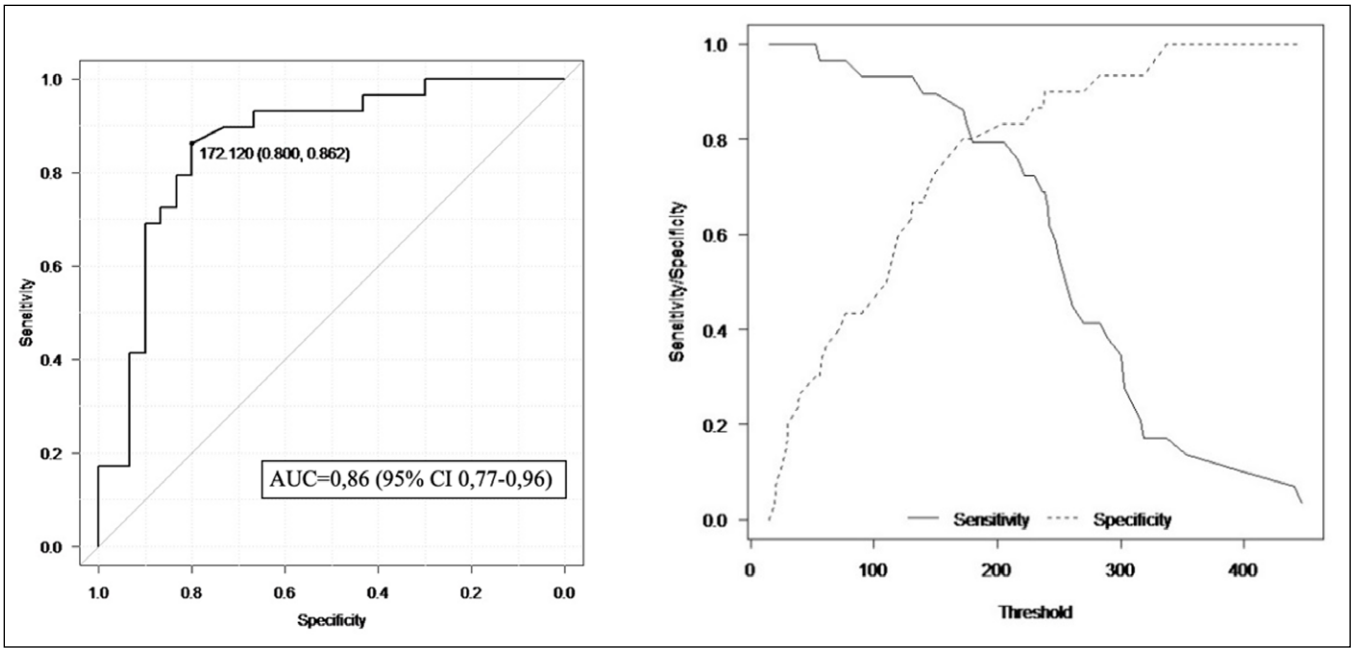

Fig. 8. ROC curve of the test for predicting the degree of severity depending on the level of C-reactive protein.

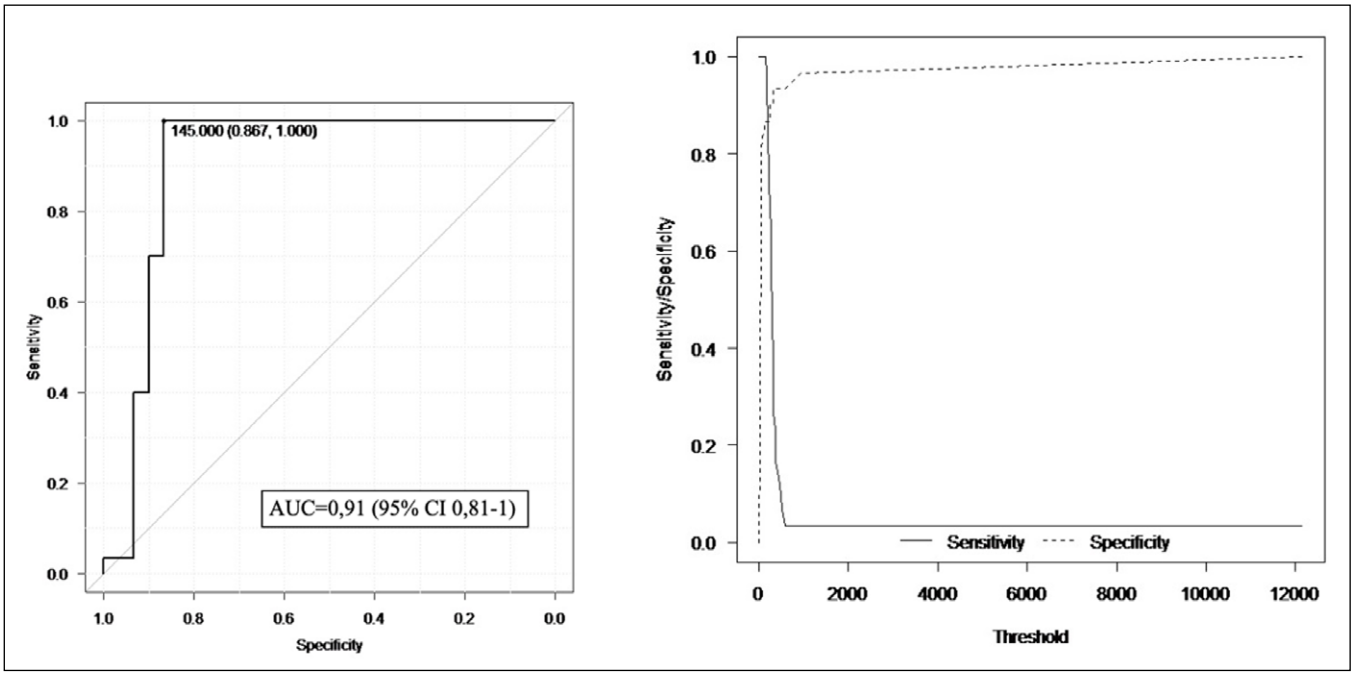

Fig. 9. ROC curve of the test for predicting the severity of the disease depending on the level of sFGL2.

DISCUSSION

Severe acute pancreatitis is a rapidly progressive disease with a high mortality rate; however, the underlying pathophysiological mechanisms have not been fully defined yet. Nowadays, the leading pathogenesis of severe acute pancreatitis is associated with microcirculatory and coagulation disorders, the development of a systemic inflammatory response syndrome and multiple organ failure. Inflammatory mediators, such as interleukin (IL)-6, IL-1β and tumour necrosis factor α (TNF-α), released during acute inflammatory reactions, are not only involved in the inflammatory process, but may also be responsible for systemic activation of haemostasis in patients with severe acute pancreatitis. It is believed that intravascular coagulation and thromboembolism play an important role in the pathogenesis of severe acute pancreatitis and are associated with its severity.

Acute inflammatory events during disease progression can lead to dysregulation of the coagulation cascade. In patients with severe acute pancreatitis,

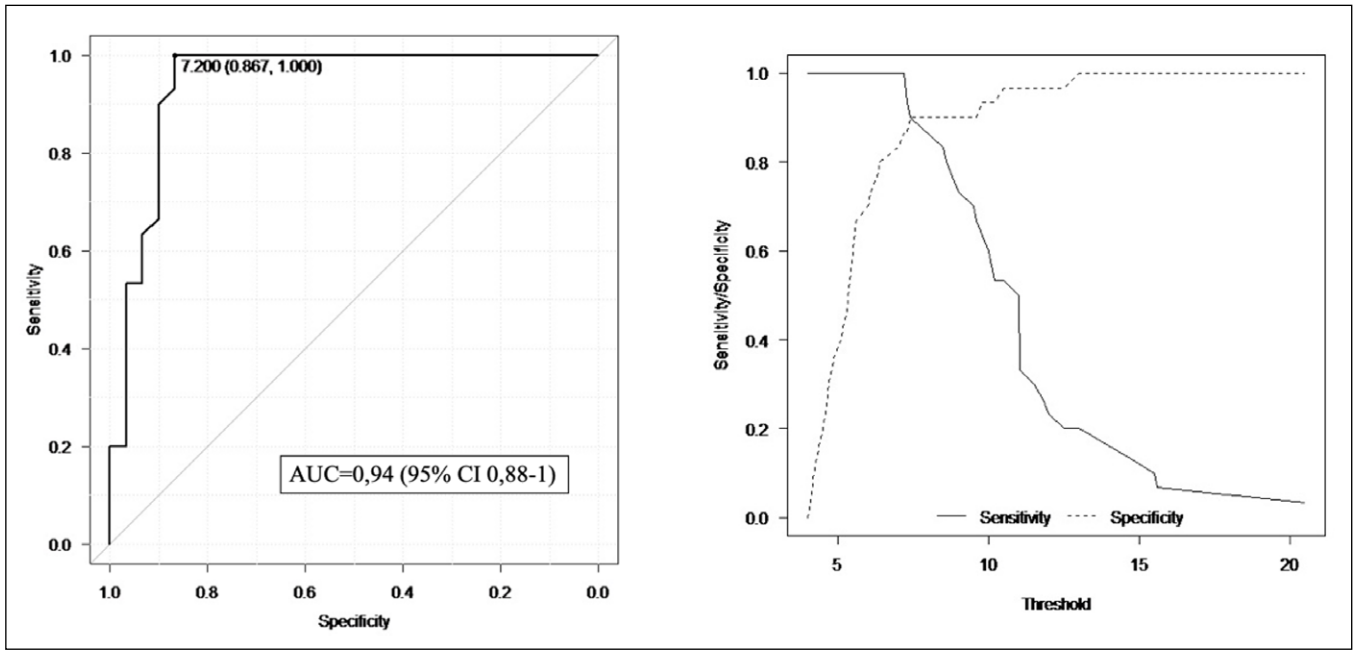

Fig. 10. ROC curve of the test for predicting the degree of severity depending on the level of intra-abdominal pressure.

thrombin and platelets are deposited not only in the local blood vessels of the pancreas, but also in the connective tissue and intercellular spaces. Studies have shown that such biochemical parameters as prothrombin time, D-dimer and coagulation time may have prognostic value, and direct anticoagulant therapy has been shown to be useful in the treatment of acute severe pancreatitis. These findings suggest that coagulation and inflammation in severe acute pancreatitis are interrelated, and thus microthrombosis plays a crucial role in the pathogenesis of the disease. Yet, the exact pathophysiological mechanism remains unknown [10].

Fibrinogen-like protein 2 (FGL2) is a multifunctional immunomodulatory protein that plays an important role in the normal physiology and pathogenesis of various diseases, including infectious, autoimmune and tumor genesis [7].

FGL2 is a new member of the fibrinogen-related protein superfamily, which includes fibrinogen, tenascin, ficolin and angiopoietin. FGL2 is a direct prothrombinase with serine protease activity. FGL2 can cleave prothrombin to thrombin in a non-canonical way, resulting in fibrin deposition. FGL2 leads to histopathological lesions and ischaemic damage through 'immune coagulation', fibrin deposition and microthrombosis. Microvascular disorders are caused by microthrombi that are activated and formed by the action of FGL2 [11].

It is believed that coagulation disorders are critical in the pathogenesis of severe acute pancreatitis. With the activation of the hemostatic system during severe acute pancreatitis, microthrombosis occurs in the microvascular bed, DIC syndrome may appear, and thrombosis of larger diameter vessels of the splanchnic basin, which was analysed in a single-centre retrospective analysis by Nawacki et al. in 2021 [12].

The given study analysed 111 patients with moderate to severe acute pancreatitis. Splanchnic vein thrombosis was detected in 30.6% of cases. Portal vein thrombosis was most common (47.1% - 16 patients)[12].

Severe complications, such as multiple organ failure syndrome, can also be associated with microcirculatory disorders, microthrombosis, endothelial damage and hypercoagulability occurring in the early phase of severe acute pancreatitis. There is indeed a link between the activation of proinflammatory cytokines and the coagulation system, but the specific pathophysiological mechanisms of this link remain unclear.

Fibrinogen-like protein 2, also known as FGL2-proteinase, has been proposed as one of the key factors, together with factor Xa, influencing microthrombosis by activating prothrombinase into thrombinase, which in turn initiates microthrombosis [13].

FGL2 exists in two structurally distinct forms: membrane-associated FFL2 (mFGL2) and soluble FGL2 (sFGL2). sFGL2 acts as a direct prothrombinase and triggers immunogenic coagulation by cleaving prothrombin in a non-classical manner [7].

In contrast, sFGL2 has an immunomodulatory effect and consequently is involved in modulating responses to tissue damage, fetal loss, malignancy, viral infection, acute allograft rejection, and autoimmune diseases[7].

In 2019, a study conducted by Wen-Bin Xu et al., aimed to investigate sFGL2 as a marker of delirium in patients with acute pancreatitis. The following study included

184 patients with acute pancreatitis. A comparison with a group of healthy patients was performed and it was determined that the elevation of sFGL2 is closely related to the severity of acute pancreatitis and has the potential to diagnose delirium after an episode of acute pancreatitis. Using statistical analysis and ROC curve construction, the threshold value of sFGL2 as a predictor of delirium in patients with acute pancreatitis was determined to be 244.6 pg/ml [14].

Our study compared patients with acute severe pancreatitis and patients with moderate acute pancreatitis to determine the possibility of using sFGL2 as a marker for the prognosis of acute pancreatitis severity and pancreatic necrosis.

CONCLUSIONS

sFGL2 can be used as a marker for assessing the severity of acute pancreatitis and predicting necrotic changes in the pancreas and surrounding tissues.

Based on the results of the study, a correlation between sFGL2 and the following parameters was found D-dimer, C-reactive protein and intra-abdominal pressure.

It was discovered that elevated levels of sFGL2, D-dimer and intra-abdominal pressure allow to predict the progression of necrotic changes in the pancreatic tissue and retroperitoneal space; and such indicators as BISAP, sFGL2, CRP, D-dimer and intra-abdominal pressure can be utilized for defining the severity of acute pancreatitis.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 08.06.2024
ACCEPTED: 22.09.2024

ORIGINAL ARTICLE

CONTENTS

From open-ended to multiple-choice: evaluating diagnostic performance and consistency of ChatGPT, Google Gemini and Claude AI

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ABSTRACT

Aim: To determine the performance and response repeatability of freely available LLMs in diagnosing diseases based on clinical case descriptions.

Materials and Methods: 100 detailed clinical case descriptions were used to evaluate the diagnostic performance of ChatGPT 3.5, ChatGPT 4o, Google Gemini, and Claude AI 3.5 Sonnet large language models (LLMs). The analysis was conducted in two phases: Phase 1 with only case descriptions, and Phase 2 with descriptions and answer variants. Each phase used specific prompts and was repeated twice to assess agreement. Response consistency was determined using agreement percentage and Cohen's Kappa (k). 95% confidence intervals for proportions were calculated using Wilson's method. Statistical significance was set at $p<0.05$ using Fisher's exact test.

Results: In Phase 1 of the study, ChatGPT 3.5, ChatGPT 4o, Google Gemini, and Claude AI 3.5 Sonnet's efficacy was 69.00%, 64.00%, 44.00%, and 72.00% respectively. All models showed high consistency as agreement percentages ranged from 93.00% to 97.00%, and k ranged from 0.86 to 0.94. In Phase 2 all models' productivity increased significantly (90.00%, 95.00%, 65.00%, and 89.00% for ChatGPT 3.5, ChatGPT 4o, Google Gemini, and Claude AI 3.5 Sonnet respectively). The agreement percentages ranged from 97.00% to 99.00%, while k values were between 0.85 and 0.93.

Conclusions: Claude AI 3.5 Sonnet and both ChatGPT models can be used effectively for the differential diagnosis process, while using these models for diagnosing from scratch should be done with caution. As Google Gemini's efficacy was low, its feasibility in real clinical practice is currently questionable.

KEY WORDS: artificial intelligence, large language model, diagnosis, performance

Wiad Lek. 2024;77(9):1852-1856. doi: 10.36740/WLek/195125 DOI

INTRODUCTION

Artificial intelligence (AI) is gaining more and more application in various spheres of modern life. The medical field, which in recent years has undergone significant changes thanks to the introduction of these technologies, was no exception. AI-based solutions are successfully used in pharmaceutical research, development of new drugs, medical documentation management, treatment strategies improvement, interpretation of medical images (including X-rays, CT and MRI), as well as in solving many other tasks [1, 2]. Recently, special attention of the medical community, including scientists, has been attracted by the possibility of using AI systems for diagnosis, forecasting and classification of diseases [3].

An important milestone was the emergence of so-called large language models (LLMs), such as ChatGPT, Google Bard, LLaMA-2 and others. They have brought AI technologies closer not only to scientists, but also to ordinary users. These complex neural network models, trained on huge amounts of data, demonstrate impressive abilities in solving a variety of tasks. The potential of the LLMs is huge and needs to be fully explored.

The use of the LLMs in routine clinical practice opens up broad perspectives from facilitating clinical decision-making to improving medical education and analyzing scientific research. Their ability to process and generate human-like text based on contextual understanding creates new opportunities for improving diagnosis, developing treatment plans and communicating with patients. However, the implementation of such powerful tools in the healthcare system requires a thorough and comprehensive analysis of their effectiveness and reliability.

Numerous studies are being conducted to assess the effectiveness and reliability of available LLMs in various medical fields. In particular, there are many publications devoted to the use of these models for the diagnosis of diseases within narrow medical specialties. A significant part of the research concerned particular diseases [4-11]. Some studies have been conducted to assess the diagnostic performance of LLMs in the context of mul-

tiple medical specialties simultaneously. These works represent a more comprehensive approach and are aimed at evaluating the effectiveness of such models for general clinical use [12-16]. In addition, specialized tests are being developed for a comprehensive assessment of the potential of LLMs in clinical practice [17].

The rapid development of this field necessitates the urgent need to conduct empirical studies that would compare various LLM-based solutions, evaluate their effectiveness according to accepted criteria, and study the possibilities of their interaction with other AI technologies in medicine. Such research is extremely important and plays a key role in shaping approaches to the responsible development and implementation of LLM in medical practice. They are designed to improve the quality and safety of patient treatment and minimize the risks associated with the implementation of AI in routine medical practice.

AIM

The aim of this study was to determine the performance of freely available LLMs in diagnosing diseases based on clinical case descriptions as well as their response repeatability.

MATERIALS AND METHODS

In our study, we evaluated the performance of ChatGPT 3.5, ChatGPT 4o (OpenAI Inc, San Francisco, CA), Google Gemini, and Claude AI 3.5 Sonnet (Anthropic, California, U.S.) in diagnosing diseases based on clinical case descriptions. For that reason 100 clinical cases were used. Each clinical case consisted of detailed information about a patient's complaints, history of present illness, past medical and family histories, results of physical, laboratory and instrumental methods of examination. An average length of clinical case description was 527±74 words. The analysis was conducted in two phases. In Phase 1 models were given only clinical case descriptions while in Phase 2 models were provided with clinical case descriptions along with variants of answer to choose from. In both phases we used an initial prompt to instruct every model. In the first phase the prompt was "In the next prompt I'll give you a description of a clinical case. Act as a professional doctor and diagnose the most suitable disease based on the description. Write the diagnosis only without any explanations". In the second phase this prompt was partially simplified to "In the next prompt I'll give you a description of a clinical case. Act as a professional doctor. Write the diagnosis only without any explanations". This change was done because the sentence "On the basis of these findings only, what is the most likely diagnosis?" was added to the end of each clinical case description before the list of answer variants. Each phase involved presenting the same set of clinical cases twice using the new chat to each LLM. The diagnostic accuracy of the models was estimated as the percentage of correct answers when given a set of clinical cases for the first time to each model. Response consistency and repeatability was determined using the agreement percentage and Cohen's Kappa coefficient (k) along with 95% confidence intervals (CI). k values were interpreted as <0.0 – poor; 0.0-0.2 – slight; 0.2-0.4 – fair; 0.4-0.6 – moderate; 0.6-0.8 – substantial; and 0.8-1.0 – almost perfect agreement [18]. 95% CI for proportions was calculated using Wilson's method. Two-tailed Fisher's exact test was used for comparative analysis of frequency tables. The difference was considered to be statistically significant if $p<0.05$.

RESULTS

Analysis of the diagnostic accuracy in the first phase of the study, which was based only on the description of clinical cases, revealed significant differences between the studied LLMs (Table 1). In particular, the result was the highest in Claude AI 3.5 Sonnet, which established the correct diagnosis in 72 cases out of 100 offered. ChatGPT 3.5 also demonstrated a strong ability to interpret clinical data. The performance of ChatGPT 4o was almost 10% lower compared to Claude AI 3.5 Sonnet. At the same time, no statistically significant difference was found between the results of these LLMs ($p>0.05$). In this phase of the study, the lowest performance was shown by Google Gemini, which correctly diagnosed less than 50% of the given cases and its performance was statistically lower compared to other models ($p<0.05$).

Providing variants of possible answers to the clinical cases description in the second phase of this study significantly increased the diagnostic accuracy of all models. In this phase, ChatGPT 4o correctly identified the largest number of diagnoses (91 cases out of 100). Both ChatGPT 3.5 and Claude AI 3.5 Sonnet showed almost the same performance (90.00 % and 89.00 % respectively, $p>0.05$). Google Gemini, despite a significant improvement compared to the results in Phase 1, showed the lowest efficiency. At the same time, its performance in this phase of the study was statistically lower compared to other LLMs ($p<0.05$).

The degree of improvement in disease diagnosis efficiency of the studied LLMs when comparing phases 2 and 1 varied depending on the model. The highest increase in productivity was demonstrated by Google Gemini (47.73%). ChatGPT 4o and ChatGPT 3.5 had

Table 1. Diagnostic Accuracy and Response Consistency of Large Language Models in Clinical Case Analysis, % (CI)

LLM	Phase 1	Agreements	Phase 2	Agreements
ChatGPT 3.5	69.00 (59.35-77.25)	96.00	90.00 (82.39 - 94.65)#	97.00
ChatGPT 4o	64.00 (54.22 - 72.74)	97.00	91.00 (83.58 - 95.38)#	99.00
Google Gemini	44.00 (34.67 - 53.77)*	93.00	65.00 (55.24 - 73.65)*#	97.00
Claude AI 3.5 Sonnet	72.00 (62.48 - 79.90)	97.00	89.00 (91.17 - 99.35)#	98.00

Note. * - the difference is statistically significant compared to ChatGPT 3.5, ChatGPT 4o and Claude AI 3.5 Sonnet ($p<0.05$), # - the difference is statistically significant compared to the results of Phase 1 ($p<0.05$)

a slightly lower degree of improvement (42.19% and 30.43%, respectively). The lowest increase in efficiency (23.61%) was demonstrated by Claude AI 3.5 Sonnet. These differences in improving disease diagnosis highlight the different ability of each model to use the diagnostic options provided to improve accuracy.

In addition to the effectiveness of LLMs in the diagnosis of various diseases, the repeatability and reproducibility of the results are also important. In Phase 1 of the study, the models showed a high consistency of responses. ChatGPT 4o and Claude AI 3.5 Sonnet showed the highest retest agreement of 97.00%. ChatGPT 3.5 and Google Gemini demonstrated slightly lower, but also high consistency. The obtained results were also confirmed when calculating k coefficients, which were 0.91 (95% CI 0.82 - 1.00), 0.94 (95% CI 0.86 - 1.00), 0.86 (95% CI 0.76 - 0.96) and 0.93 (95% CI 0.84 - 1.00) for ChatGPT 3.5, ChatGPT 4o, Google Gemini and Claude AI 3.5 Sonnet, respectively.

The consistency of LLMs' responses increased in Phase 2 of the study. The agreement percentage of ChatGPT 4o reached almost 100% (k = 0.93, 95% CI 0.862-1.000). Claude AI 3.5 Sonnet had slightly lower but still high agreement rates (98%, k = 0.91, 95% CI 0.86-1.00), as well as ChatGPT 3.5 and Google Gemini (97% each; k = 0.85, 95% CI 0.69-1.00 and 0.93, 95% CI 0.86-1.00, respectively).

DISCUSSION

The ability of LLMs to establish diagnoses based on clinical case descriptions alone is essential for their use in routine medical practice to support clinical decision making. In different studies the efficacy of different LLMs varied greatly [4-16]. Based on the results obtained in our study, three of the four models we tested showed an efficiency of more than 60%. The high results of Claude AI 3.5 Sonnet and ChatGPT 3.5 in diagnosis indicate their particular suitability for real clinical situations where there are no options for differential diagnosis. Interestingly, ChatGPT 3.5 and ChatGPT 4o had comparable performance. Despite the improvements inherent in version 4o, the lack of a significant improvement in performance in the Phase 1 study may indicate that the updates were not specifically aimed at improving medical diagnostic capabilities. At the same time, the relatively low performance of Google Gemini is an example of how important it is to carefully evaluate and validate AI models before they are put into clinical use. The identified differences in the effectiveness of the studied models are determined by many factors such as the type of training data, model architecture, fine-tuning features, understanding of the context and handling of uncertainty. A detailed study of the influence of these factors is critical for improving LLMs with the aim of their further use for medical purposes, as well as the selection of appropriate models for specific healthcare tasks. Future research should focus on identifying and studying these factors to improve the diagnostic capabilities of AI in open-ended scenarios.

The significant increase in diagnostic performance of all models in Phase 2 of this study is a key finding with important practical implications. The performance of Claude AI 3.5 Sonnet, ChatGPT 3.5 and ChatGPT 4o at 89.00-91.00 % suggests that these LLMs can be extremely useful in clinical decision support when used for differential diagnosis. Also of particular interest is the varying degree of improvement among models (from 23.61% to 47.73%) when diagnostic options are added. These differences reflect fundamental variations in how each model handles and uses additional context or constraints. The significant improvements in ChatGPT models indicate their good adaptability to multiple-choice tasks, which in turn may be related to their training methodology or architecture. The increased efficiency in the presence of choice options has several important implications for the practical application of LLMs in healthcare, such as improving human-AI collaboration, reducing diagnostic errors, training physicians and improving the efficiency of the differential diagnosis process.

When evaluating the feasibility of using LLMs in real clinical scenarios, it is important to ensure that the responses provided by these models are not random in nature. To determine the reproducibility of the result, the percentage of response repeatability and the k

coefficient are usually determined. According to the literature, these indicators for the models under study vary widely from average to significant levels [19, 20]. To a large extent, the reproducibility of the results depends on the study design, the prompt structure, and the amount of information provided for analysis.

High percentages of repeatability as well as k observed at both stages of our study indicate high reliability and reproducibility of the obtained results. Such a sequence indicates that the LLMs' responses were not random in nature, but were the result of an analysis of the provided clinical case descriptions. Furthermore, such high levels of consistency indicate that LLMs can maintain consistent performance across multiple trials, reducing the risk of random or unpredictable results. It also determines the possibility of using these models as assistants in clinical decision-making processes.

When the answer options were provided in the second phase of this study, the agreement percentage increased slightly. Although the improvement in repeatability was statistically insignificant ($p>0.05$), it demonstrates the high reliability of using LLMs in a multiple-choice format. Constraining the choice conditions helps these models produce more consistent results because they can better distinguish between the given options than generating responses from scratch, resulting in more consistent performance. The ability to generate consistent results is critical in building trust in AI systems among health care professionals. In addition, checking the repeatability of AI responses can be used in real clinical practice to determine the "confidence" of a particular model in the generated information. Although these findings are encouraging, it is important to note that consistency alone does not guarantee accuracy of results. The high levels of concordance should be considered together with the accuracy results to fully understand the potential and limitations of these models in clinical practice.

CONCLUSIONS

Our study revealed important aspects of LLMs' effectiveness when using them in diagnosis of diseases. Claude AI 3.5 Sonnet and both ChatGPT models showed moderate performance in open-ended scenarios. In the multiple-choice scenarios, their effectiveness was around 90%, which makes them particularly useful in the process of differential diagnosis. Google Gemini's efficacy was significantly lower compared to other models in both study phases, so its feasibility in real clinical practice is currently questionable.

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CONFLICT OF INTEREST

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RECEIVED: 09.06.2024
ACCEPTED: 20.09.2024

ORIGINAL ARTICLE

CONTENTS

Biomarker diagnostics of endothelial dysfunction in patients with acute coronary syndrome and non-alcoholic fatty liver disease

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ABSTRACT

Aim: To determine the severity of endothelial dysfunction (ED) in patients with acute coronary syndromes (ACS) in non-alcoholic fatty liver disease (NAFLD).

Materials and Methods: The study included 124 patients with ACS. Group 1 included 74 patients after ACS and NAFLD, and group 2 consisted of 50 patients after ACS without liver damage. The patients' ED was determined in a test with reactive hyperaemia, and the levels of endothelin-1 (ET-1), P-selectin, and von Willebrand factor (wWF) were determined.

Results: The reactive hyperaemia test revealed ED in patients with ACS regardless of liver damage. More pronounced changes in EDV and EIVD were found in patients with ACS in combination with NAFLD. Laboratory markers of ED, namely ET-1, P-selectin and WwF, were also significantly higher in patients with ACS and confirm the pronounced vasoconstrictor effect of endothelial dysregulation in these patients. A significant difference was found between the levels of ET-1, P-selectin and WwF in patients of groups 1 and 2.

Conclusions: ED is established in patients with ACS, which is more pronounced in NAFLD. Biomarkers such as ET-1, WwF, P-selectin in the blood serum are highly specific substances for determining the severity of ED in patients with ACS, especially when it is combined with NAFLD.

KEY WORDS: Acute coronary syndrome; nonalcoholic fatty liver disease; diagnostics; endothelial dysfunction, endothelin-1, P-selectin, von Willebrand factor

Wiad Lek. 2024;77(9):1857-1862. doi: 10.36740/WLek/195126 DOI

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) - the most common chronic liver disease in worldwide, affecting up to ~30% of adults in the general population, up to ~70% of patients with type 2 diabetes and almost all patients with severe obesity [1]. The pathogenesis of NAFLD includes a profound disturbance of metabolic homeostasis; reprogramming of the interaction between hepatocytes, sinusoidal endothelial cells and hepatic stellate cells; restructuring of the liver immune landscape; remodelling of the hepatic microvasculature and stromal microenvironment. The key effect of constant metabolic stress on the liver is the activation of hepatic stellate cells and the development of liver fibrosis, which usually dictates the natural course of NAFLD [2, 3].

Patients with NAFLD often suffer from obesity and/or insulin resistance and type 2 diabetes mellitus, dyslipidaemia, which are metabolic factors that complicate its course [4, 5, 6]. Namely, obesity is a key link in the chain of future metabolic disorders, in particular NAFLD, and hyperinsulinaemia leads to an increase in adipose tissue in the liver and contributes to the development of NAFLD [7].

When common diseases coexist and share common risk factors, it can be difficult to disentangle cause and effect relationships and understand the role of potential complicating factors. Metabolic syndrome (MS) is common in patients with NAFLD; however, components of MS, including obesity and hypertension, also increase the risk of developing NAFLD [8]. Non-alcoholic fatty liver disease and coronary heart disease share common pathogenetic links. Evidence of the association of NAFLD with acute coronary syndromes (ACS), complex multivessel coronary artery disease and increased mortality risk in patients with ACS is still under investigation [9]. Therefore, the study of pathogenetic links, including biomarkers of endothelial dysfunction (ED) in ACS and NAFLD, is an urgent medical issue.

AIM

The aim of the stady to determine the severity of endothelial dysfunction in patients with acute coronary syndrome in non-alcoholic fatty liver disease.

MATERIALS AND METHODS

124 patients with ACS with ST-segment elevation were examined and treated at the clinical base of Hospital therapy of the Medical Faculty of the State Higher Educational Institution «Uzhhorod National University» (Municipal Non-Profit Enterprise «Transcarpathian Regional Clinical Centre of Cardiology and Cardiac Surgery» of the Transcarpathian Regional Council) in 2019-2024. The study was conducted at the stage of outpatient observation of patients after ACS (on average up to 4.4±0.7 months). All patients who survived ST-segment elevation STEMI and were included in this study underwent stenting or coronary artery bypass grafting during inpatient treatment. After discharge, patients were prescribed individually tailored drug therapy aimed at normalising blood pressure, rhythm and conduction disorders, treating/preventing the progression of chronic heart failure, and including anticoagulants and statins.

The diagnosis of ST-segment elevation ACS was made in accordance with the unified clinical protocol for emergency, primary, secondary (specialised), tertiary (highly specialised) medical care and cardiac rehabilitation, as well as the evidence-based clinical guideline «Acute coronary syndrome with ST-segment elevation» (Order of the Ministry of Health of Ukraine No. 1936 of 14.09.2021). Patients underwent electrocardiographic examination (ECG), echocardiography and CT-coronary angiography, as well as biomarkers of myocardial infarction (troponin levels in the blood).

The average age of patients after ACS was 53.1±7.2 years. Men prevailed among the examined patients, namely 94 (75.8%), and women were 30 (24.2%). The control group consisted of 20 practically healthy individuals (16 (80.0%) men and 4 (20.0%) women). The average age was 51.1±7.4 years.

All studies were conducted in compliance with the basic provisions of the «Rules for Ethical Principles for Scientific Medical Research Involving Human Subjects» with the consent of the patients (all patients gave written consent to the relevant diagnostic and treatment measures), and the methodology was in line with the Helsinki Declaration of Human Rights (1964-2013), the Council of Europe Convention on Human Rights and Biomedicine, and Ukrainian legislation.

Patients after ACS were divided into two groups. Group 1 included 74 patients after ACS and NAFLD, and group 2 consisted of 50 patients after ACS without liver damage (no history of NAFLD). All examined patients with ACS and NAFLD were subjected to general clinical, anthropometric, instrumental and laboratory methods. All examined patients was carried out an ultrasound examination of the abdominal cavity according to the generally accepted method. The anthropometric examination included height, weight and body mass index (BMI).

Standard general and biochemical tests were performed in the blood serum to determine the functional state of the liver (alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT)), lipid metabolism (total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL)), carbohydrate metabolism (glucose, insulin, glycated haemoglobin (HbA1c, %)).

NAFLD diagnosed in according with the criteria of the unified clinical protocol (Order of the Ministry of Health of Ukraine of 06.11.2014, No. 826) and the EASL-EASD-EASO guidelines for the diagnosis and treatment of NAFLD [10]. The degree of liver damage was calculated using surrogate markers of fibrosis using online calculators, namely NAFLD fibrosis score (NFS), Fibrosis 4 calculator (FIB-4), Fibrotest. The patients also underwent liver elastometry.

The levels of von Willebrand factor (WwF), apolipoprotein, insulin, C-peptide, glycosylated haemoglobin (HbA1c) were determined by chromogenic analysis (Sysmex 500 and 560, Japan) using Siemens reagents. In the blood serum, endothelin-1 (ET-1) was measured by ELISA using test kits from Biomedica (Austria), P-selectin using a test kit from eBioscience (Austria).

The state of vascular endothelium and its dysfunction were determined by the method of D.Celermajer, assessing endothelium-dependent vasolilatation (EDV) in the brachial artery (BA). Endothelium-independent vasodilation (EIVD) was determined after 0.5 mg of nitroglycerin sublingually at rest, causing dilatation of peripheral vessels. The study was repeated 2 and 5 minutes after nitroglycerin administration. The difference between the diameter of the PA in the background of reactive hyperaemia and the initial diameter was calculated to assess the response to increased blood flow and the difference between the diameter of the PA 2 min after nitroglycerin administration and its initial diameter. An increase in the diameter of the PA against the background of reactive hyperaemia by 10.0% or more was considered normal.

The criteria for exclusion of patients from the study were: Type 1 diabetes mellitus, type 2 diabetes mellitus (severe - with severe manifestations of diabetic angioneuropathy), chronic hepatitis of alcoholic, viral (hepatitis B, C, D virus) etiologies, autoimmune hepatitis, Wilson-Conovalov disease, haemochromatosis.

Table 1. Indicators of the functional state of the liver in the blood serum of the subjects

Indicator	Control group (n=20)	Examined patients	
		Group 1 (n=74)	Group 2 (n=50)
ALT, U/l	20.4±0.8	128.7±1.8 **,++	42.3±2.6 *
AST, U/	18.6±1.1	109.2±1.6 **, ++	38.7±1.7 *
TB, mmol/l	12.5±0.7	30.1±1.0 *	20.1±0.6
ГГТ, U/l	38.9±2.4	82.4±2.1 **, +	42.7±1.2

Note: the difference of indicators between of the control group and the examined patients of groups 1 and 2 is statistically significant: * - p<0.05; ** - p<0.01; between the indicators of patients of groups 1 and 2 the difference is statistically significant: + - p<0.05; ++ - p<0.01.

Table 2. Indicators of lipid metabolism in blood serum in the subjects

Indicator	Control group (n=20)	Examined patients	
		Group 1 (n=74)	Group 2 (n=50)
TG, mmol/l	1.12±0.07	3.48±0.12 **,++	1.92±0,27 *
TC, mmol/l	4.56±0.44	7.21±0.24 **,+	5.96±0,26 *
LDL, mmol/l	1.70±.,21	3.28±0.26 **,+	2.30±0,24 *
VLDL, mmol/l	0.56±0.09	1.84±0.07 **,++	0.98±0,14 *
HDL, mmol/l	1.84±0.09	1.04±0.08 **,+	1.50±0,11 *

Note: the difference of indicators between of the control group and the examined patients of groups 1 and 2 is statistically significant: * - p<0.05; ** - p<0.01; between the indicators of patients of groups 1 and 2 the difference is statistically significant: + - p<0.05; ++ - p<0.01.

Table 3. Indicators of carbohydrate metabolism in blood serum in the subjects

Indicator	Control group (n=20)	Examined patients	
		Group 1 (n=74)	Group 2 (n=50)
Glucose, mmol/l	4.88±0.17	6.92±0.16 *	6.42±0,16 *
HbA1c, %	4.32±0.36	6.76±0.28 *	6.07±0,38 *
Insulin, U/l	8.44±0.21	22.38±0.77 **,+	14.23±0,77 *
C-peptide, ng/ml	4.12±0.18	10.92±0.51 **,+	7.12±0,21 *
HOMA-IR	1.69±0.27	8.84±0.24 ***,++	3.95±0,26 **

Note: the difference of indicators between of the control group and the examined patients of groups 1 and 2 is statistically significant: * - p<0.05; ** - p<0.01; between the indicators of patients of groups 1 and 2 the difference is statistically significant: + - p<0.05; ++ - p<0.01.

The analysis and processing of the results of examining the patients was performed using the computer program STATISTICA 10.0 (StatSoft Inc, USA) using parametric and non-parametric methods of evaluating the received results.

RESULTS

In patients after ACS, at the stage of outpatient follow-up, indicators of the functional state of the liver were determined (Table 1).

In patients of group I, a significant increase in ALT and AST activity was detected compared with patients of group 2 (3.0 and 2.8 times, respectively - p<0.01). In group 1 of patients after ACS in combination with NAFLD, a significant increase in serum levels of TB and GGT was diagnosed.

The lipid metabolism in the blood serum in both groups of patients was evaluated - Table 2.

There was a statistically significant increase in all lipid metabolism parameters in the blood serum in patients after ACS of both groups. However, it should be noted that in patients of group I (ACS in combination with NAFLD), the levels of TG, VLDL and LDL were 1.8 (p<0.01), 1.2 (p<0.05), 1.4 (p<0.05) and 1.9 (p<0.01) times higher than in patients of group 2.

Indicators of carbohydrate metabolism in patients after ACS were evaluated (Table 3). A statistically significant increase in the levels of glucose, insulin, C-peptide, and HOMA-IR index in patients of group I was found compared with those in patients of group 2.

Patients of group I (combination of ACS and NAFLD) had metabolic disorders in the body, which was manifested by a significant increase in carbohydrate and lipid metabolism in these patients.

In our patients with ACS and NAFLD, we determined the indicators of endothelial dysfunction (ED), which are presented in Table 4.

Table 4. Laboratory and instrumental parameters of endothelial dysfunction in the subjects

Indicator	Control group (n=20)	Examined patients	
		Group 1 (n=74)	Group 2 (n=50)
Diameter of BA at the beginning of the study, mm	4.38±0.06	3.52±0.07 *+	3.87±0.05
Diameter of BA for 30 sec. of reactive hyperaemia, mm	5.52±0.08	4.26±0.04 **+	4.50±0,06 *
Diameter of BA for 60 sec. of reactive hyperaemia, mm	4.78±0.05	3.81±0.05 **+	4.03±0,06 *
Blood flow rate through the BA, sm/sec.	102.26±2.14	65.12.±1.49 **+	84.12±1,71 *
EDV,%	14.59±0.65	8.06±0.21 *+	9.26±0,63
EIVD,%	25.12±0.73	14.23±0.29 *+	16.21±0,55
ET-1, fmol/ml	0.41±0.06	1.45±0.09 ***+	1.07±0,08 **,+
P-selectin, ng/ml	109.32±3.11	467.70±4.23 **+	341.12±3.91 **
wWF, %	53.7±2.5	193.7±3.2 *+	123.8±1,7**+

Note: the difference of indicators between of the control group and the examined patients of groups 1 and 2 is statistically significant: * - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$;
between the indicators of patients of groups 1 and 2 the difference is statistically significant: + - $p<0.05$.

The reactive hyperaemia test revealed endothelial dysfunction in patients with ACS regardless of liver damage. However, more pronounced changes in EDV and EIVD were found in patients with ACS in combination with NAFLD. Laboratory markers of ED, namely ET-1, P-selectin and wWF, were also significantly higher in patients with ACS and confirm the pronounced vasoconstrictor effect of endothelial dysregulation in these patients. A significant difference was found between the levels of ET-1, P-selectin and wWF in patients of groups 1 and 2.

DISCUSSION

Cardiovascular disease is the leading cause of death worldwide. Coronary artery disease (CAD) is the most common and is characterized by the accumulation of lipids and immune cells in the subendothelial space of the coronary arteries or atherosclerosis. This process involves the inflammatory response of the vascular endothelium. Endothelial cells (EC) form a semipermeable monolayer that separates the wall of the arteries from the components of intravascular flow. This barrier regulates vascular tone, prevents platelet aggregation, and maintains fluid homeostasis. The endothelium produces vasodilator and vasoconstrictor molecules such as nitric oxide and endothelin, respectively; the imbalance in production of these vasoactive substances results in the loss of its function, which is defined as endothelial dysfunction. Endothelial dysfunction plays an essential role in the development of atherosclerosis and can be triggered and exacerbated by different cardiovascular and cardiometabolic risk factors. Currently, there is a wealth of data on endothelial dysfunction and the risk of developing atherosclerosis and CAD [11].

In resistance arteries, the endothelium plays a fundamental role in the regulation of vascular tone, local blood flow and systemic blood pressure via the generation of various vasoactive stimuli. This monolayer operates to sense, integrate and transduce signals present in the blood and local tissue environment, which then initiate dynamic modulation of contractile activity of the surrounding vascular smooth muscle. In response to mechanical (e.g., shear stress due to blood flow) and chemical (e.g., acetylcholine, bradykinin, ATP) stimuli, EC release vasodilatory factors that regulate the vascular tone. The main vasoconstrictors produced by the endothelium are thromboxane A2 and ET-1, while the main endothelial vasodilator factors are NO, prostacyclin, and endothelium-derived hyperpolarization factor (EDHF) [12].

There is growing evidence of a direct link between the vascular system and NAFLD. Changes in endocannabinoids and adhesion molecules, such as P-selectin, derived from the endothelium and platelets, in children and adolescents with obesity and NAFLD were investigated. It was found that childhood obesity leads to vascular inflammation and, therefore, may contribute to the development of atherosclerosis at an early age. [13]. P-selectin is an adhesion molecule translocated to the surface of endothelial cells and platelets under inflammatory stimuli [14].

Thus, the study of changes in the levels of ED biomarkers may reveal new potentiated links between patients with cardiovascular disease and NAFLD.

CONCLUSIONS

1. Endothelial dysfunction is established in patients with ACS, which is more pronounced in NAFLD.
2. Biomarkers such as ET-1, WwF, P-selectin in the blood serum are highly specific substances for determining the severity of ED in patients with ACS, especially when it is combined with NAFLD.

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The scientific research was carried out within the scientific topic of the Department Hospital Therapy (Regional peculiarities of distribution, clinical manifestations and effectiveness of treatment of internal organ diseases in patients from different altitudinal zones of Transcarpathia (state registration number 0115U005285).

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 04.06.2024
ACCEPTED: 17.09.2024

ORIGINAL ARTICLE

CONTENTS

Anastomotic leak: genetic aspects of prediction and choice of surgical treatment tactics

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ABSTRACT

Aim: To improve the treatment results of patients with anastomotic leaks by studying genetic predisposition.

Materials and Methods: The object of this prospective study were 17 patients with anastomotic leaks. A group of 80 practically healthy people was tested as control. Real-time PCR was used to investigate polymorphisms: C-1306 →T (MMP2), rs243865 та G303→A (TIMP2), rs9900972.To assess the state of connective tissue metabolism. Free oxyproline in blood serum and the level of glycosaminoglycans (GAG) in urine were studied.

Results: Having investigated the relationship of some clinical and laboratory indicators of patients with postoperative complications with the genotypes of the studied polymorphisms, we found data indicating the pathogenetic significance of the C/C allele of the MMP-2 gene (C-1306→T) and the G/G variant of the TIMP gene 2 (G303→A) as a risk factor for the failure of anastomotic sutures, which, unlike other groups of genetic polymorphisms, are statistically reliably accompanied by hypoproteinemia, elevated indicators of markers of protein catabolism, namely free blood oxyproline and urine GAGs. Thus, in the research group with AL, the carriers of the homozygous CC genotype of the MMP2 gene had significantly lower levels of total serum protein. Indicators of urinary GAGs and free oxyproline were almost three times higher than those of carriers of the minor TT genotype.

Conclusions: Molecular genetic research is a new promising direction for the development of modern personalized diagnostic criteria and models for predicting the development and course of postoperative abdominal complications, in particular, anastomotic leaks.

KEY WORDS: postoperative complications, anastomotic leak, MMP2, TIMP2 genes, method of genetic prediction, prognostic-treatment algorithm

Wiad Lek. 2024;77(9):1863-1870. doi: 10.36740/WLek/195127 DOI

INTRODUCTION

Anastomosis formation is a complex, cellularly mediated process that restores the continuity of hollow digestive organs [1]. It involves classic inflammatory processes: alteration, exudation, proliferation, and specific reparative processes influenced by the suture technique, suture material, presence of infection, and other factors [2]. Anastomotic leak (AL) is one of the most challenging complications in abdominal surgery. According to various authors, the frequency of such complications ranges from 2% to 19% [3]. AL is accompanied by high mortality of 2 - 21,7% [4], which, with the development of generalized peritonitis, increases up to 32.2% - 82,9% [5,6]. To date, the surgical community does not have a single point of view on the causes of AL, and there is no practical way of predicting this complication. It is crucial to consider the multiple risk factors associated with the development of AL, including microcirculation disorders, tissue regeneration disorders, infection, increased intra-intestinal pressure, changes in the rheological properties of blood, and gross violations of homeostasis. Literature data suggests that these factors can contribute to the development of AL [7]. Tactical and technical errors are a separate group of risk factors in creating anastomoses [8].

AIM

To improve the treatment results of patients with intestinal suture failure by studying pathogenetic mechanisms of development, genetic determination, and the development of new informational methods of diagnosis and prognosis of this complication's course and surgical treatment.

MATERIALS AND METHODS

The object of this prospective study were 17 patients with anastomotic leaks, who were treated at the Shalimov`s National Scientific Centre of Surgery and Transplatation. To assess the polymorphism of genes in the population, group of 80 practically healthy people,

which are comparable in age and gender to the subjects were examined. Real time PCR was used to investigate polymorphisms: $C^{-1306} \rightarrow T$ (MMP2), rs243865 та $G^{303} \rightarrow A$ (TIMP2), rs9900972.

To assess the state of connective tissue metabolism. Free oxyproline in blood serum and the level of glycosaminoglycans (GAG) in urine were studied.

To assess the properties of connective tissue, immunohistochemical studies were performed with the following markers (Thermo Scientific, USA): monoclonal antibodies (MAT) to Collagen IV (clone CIV22), α-smooth muscle actin α-SMA (clone CIV22). Evaluation of the expression of markers was carried out according to the visual-analog scale. The intensity of expression was evaluated from 0 - «absent» to +++ - «expressed» [10].

Statistical calculations of research results were performed using the program «Statistica 12.6» (SPSS) and Excel 2020. When comparing quantitative characteristics in the case of a normal distribution law in two groups, the Student's criteria was used; when the distribution law differs from the normal one, non-parametric criteria were used: Mann-Whitney U Test; Wilcoxon Matched Pairs Test. Fisher's two-sided exact test was used to compare the frequency of qualitative features in two groups. The reliability of differences in average values in groups with different genotypes was determined using the method of one-factor statistical analysis (URL: http://www.dgmp.kyiv.ua/index.php/snip-ka). The appropriateness of the distribution of genotypes was checked using the Hardy-Weinberg test and the chi-square test.

RESULTS

In the studied group of patients with anastomotic leaks treated in the clinic, the vast majority (66,7%) were patients operated on in other medical institutions of Ukraine, which were transferred to the National Scientific Centre of Surgery and Transplantation for further treatment.

Notably, a great number of complications (60.2%) occurred after planned interventions, which can be explained by the predominantly elective demographic of our patients.

To identify a possible association of polymorphic variants of the MMP-2 ($C^{-1306} \rightarrow T$) and TIMP2 ($G^{303} \rightarrow A$) genes with the risk of intestinal suture failure, we performed a univariate statistical analysis of the frequency of genotypes in the studied patient groups [10].

Analysis of the multiplicative model of inheritance of the MMP-2 gene

($C^{-1306} \rightarrow T$), when comparing the control group (n=80) and the experimental group with suture failure (n=17), confirmed the conformity of the distribution of genotypes to the Hardy-Weinberg law (p>0,05). Which in the control group was tested using the $\chi 2$ test with 1 degree of freedom, without using the Yates correction. Using the $\chi 2$ test with 2 degrees of freedom, we did not find statistically significant differences in the distribution of genotypes in the group of patients and the group of practically healthy people (p>0.05).

Notably, the experimental group has half as many carriers of the homozygous TT genotype compared to the control: 5.9% versus 10% (p>0.05), respectively. However, carriers of the dominant CC genotype were present in all groups. They were most prominent in the group with suture failure: 64,7% versus 47,5% (p>0.05) in the control (Fig. 1).

When analyzing TIMP-2 ($G^{303} \rightarrow A$) inheritance patterns, we managed to find statistically significant differences in the distribution of genotypes in control group and experimental group (p<0.05).

Thus, in the group of patients with anastomotic leak, the distribution of genotype carriers was significantly different from the control. Therefore, the dominant GG variant almost doubled, reliably, the indicators of the control and experimental group (82.4% vs. 47,5%, p<0.05). The heterozygous GA genotype in the experimental group occurred more than twice as often as in the control group (17.6% versus 42,5%). Carriers of the homozygous AA genotype in the group with suture failure were not detected, while a similar variant in the control occurred in 10% of cases (Fig. 2).

As a result of genetic and statistical analysis of MMP2 ($C^{-1306} \rightarrow T$) and TIMP2 ($G^{303} \rightarrow A$) gene polymorphisms, genotype variants were determined that are associated with the risk of developing anastomotic leak. Thus, in the experimental group with AL, carriers of the homozygous SS genotype of the MMP2 gene met 1.36 times more often than the control group. At the same time, minor TT homozygotes in the group of patients with suture failure were almost twice less than in controls (5.9% vs. 10% (p>0.05)).

When analyzing carriers of TIMP-2 genotypes, we obtained statically reliable data: in the group with AL, the GG variant was 82.4%, 1,7 times higher than the indicators of the control group (82,4% vs. 47,5%, p< 0.05). Carriers of minor AA genotype homozygotes were not found in the group with suture failure, while a similar genotype in the control group occurred in 10%.

We analyzed several clinical and laboratory indicators to study the possible association of anastomotic leak occurrence to the studied genotypes during the study. The analysis of the results, according to the genotype, in patients with suture failure is presented in Table 1, where the data are given only for those indicators,

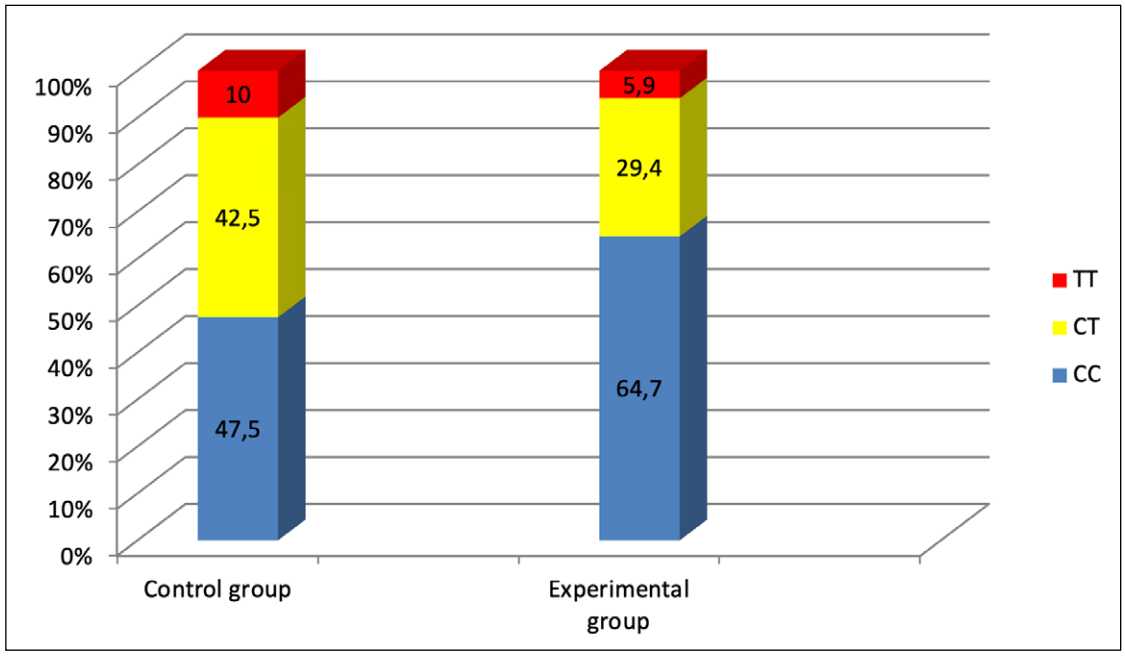

Fig. 1. Distribution of the frequency of allelic polymorphism (%) of the gene promoter MMP2 (C-1306 ⟶T).

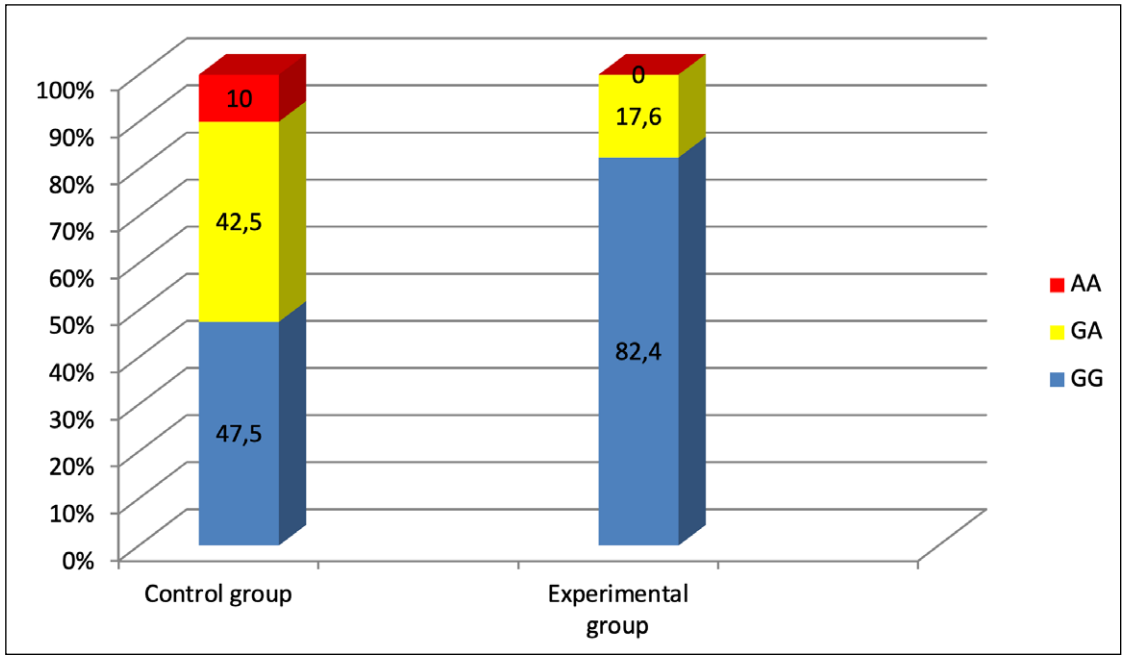

Fig. 2. Distribution of the allelic polymorphism frequency (%) of the TIMP2 gene promoter (G303⟶A).

the values of which differed statistically significantly depending on the genotype ($p<0.05$).

Having investigated the relationship of some clinical and laboratory indicators of patients with postoperative complications with the genotypes of the studied polymorphisms, we found data indicating the pathogenetic significance of the C/C allele of the MMP-2 gene ($C^{-1306}\rightarrow T$) and the G/G variant of the TIMP gene 2 ($G^{303}\rightarrow A$) as a risk factor for the failure of anastomotic sutures, which, unlike other groups of genetic polymorphisms, are statistically reliably accompanied by hypoproteinemia, elevated indicators of markers of protein catabolism, namely free blood oxyproline and urine GAGs.

Thus, in the research group with AL, the carriers of the homozygous CC genotype of the MMP2 gene had significantly lower levels of total serum protein. Indicators of urinary GAGs and free oxyproline were almost three times higher than those of carriers of the minor TT genotype.

Similar data were obtained for carriers of the GG genotype of the TIMP2 gene. In addition to significantly

Table 1. Dependence of some clinical and laboratory indicators on the genotypes of the studied polymorphisms in patients with failure of anastomoses of hollow digestive organs, n= 17

Indicator	MMP2 ($C^{-1306} \rightarrow T$)			Criteria F	P value
	C/C (n=11)	C/T (n=5)	T/T (n=1)		
Serum protein	60,12±2,74	69,44±2,20	72,50	8,193	0,04
Free Oxyproline mkmol/l	145,1±8,6	62,2±5,1	48,60	4,123	0,002
Urine GAGs Mkmol/l	140,82±7,8	68,25±4,5	50,94	5,620	0,006
	TIMP2 ($G^{303} \rightarrow A$)				
	G/G (n=14)	G/A (n=3)	A/A		
Serum protein	60,24±1,48	68,32±2,16	-	10,965	0,046
Free Oxyproline mkmol/l	187,3±9,8	55,2±3,8 *	-	5,071	0,002
Urine GAGs Mkmol/l	156,32±7,9 **	87,45±8,5 *	-	6,597	0,04
MAT Expression to α – SMA	+	++	-	-	-
MAT Expression To Collagen IV	+	++	-	-	-

Note: only statistically significant differences are given (* p1-2<0.05; ** p1-2<0.01).

lower levels of protein and high levels of free oxyproline (187.3±9.8 and 55.2±3.8, p<0.05), a reduced expression of monoclonal antibodies to α-SMA and Collagen IV was detected.

At the same time, carriers of risk alleles of TIMP-2 gene polymorphisms were distinguished by reduced expression of MAT to α-SMA, namely «+» for the G/G variant of the genotype and «++» for the G/A variant. The same trend was observed for the expression of MAT to Collagen IV, with the indicator «+» for the G/G genotype variant and «++» for the G/A variant. Thus, the indicated genetic alleles have a morphological confirmation of a genetic trigger in the pathogenesis of the development of anastomotic leak and intestinal fistulas.

The obtained data indicates the pathogenetic significance of alleles of polymorphisms of the MMP2 and TIMP2 genes, which are at risk for failure. Unlike the comparison group, presence of these alleles is accompanied by hypoproteinemia and significantly high levels of biochemical markers indicating collagen biodegradation.

Reduced expression of α-SMA cells correlates with the activation of fibrogenesis and reflects the phenotypic presence of myofibroblasts. The phenotype of myofibroblasts in the expression of α-SMA and the production of extracellular matrix coupling is regulated by β-transforming growth factor (TGF-β). The contractile properties of myofibroblasts are associated with α-SMA expression and are involved in inflammation, healing, fibrosis, and carcinogenesis [11].

All these factors have pathogenetic significance for the development of AL and are signs of connective tissue dysplasia [12].

We discovered the differences in the allelic variants of the studied genes in the groups with failure of anastomotic sutures. This became the basis for determining molecular genetic markers and developing a method of predicting the failure of anastomotic sutures.

We have proposed a method that involves a genetic study of the polymorphism of the MMP-2 ($C^{-1306} \rightarrow T$) and TIMP-2 ($G^{303} \rightarrow A$) genes and is distinguished by the fact that when the GG variant of the TIMP-2 gene ($G^{303} \rightarrow A$) is detected, we predict the development of AL if the AA variant of the genotype is detected, anastomotic leak is unlikely.

Based on the role of connective tissue pathology in anastomotic leaks, we have identified morphological signs of reparative regeneration disorders and proteolysis activation. We have also established a connection between these signs and genetic polymorphisms of MMP-2 ($C^{-1306} \rightarrow T$) and TIMP-2 ($G^{303} \rightarrow A$). Using this knowledge, we have developed a predictive treatment algorithm to prevent complications and increase treatment reliability at all stages. Our approach involves preventive measures and pathogenetically justified treatment of complications, as shown in Fig.3.

The developed prognostic-treatment algorithm involves screening patients for the presence of undifferentiated connective tissue dysplasia using the developed method (patent No. 120158) followed by genetic research of polymorphisms of the MMP-2 ($C^{-1306} \rightarrow T$) and TIMP-2 ($G^{303} \rightarrow A$). The determination of genetic polymorphism allows for the prediction of the development of postoperative complications. Patients who are carriers of alleles of the GG-variant of the TIMP-2 genotype and the CC-variant of the MMP-2 genotype are a risk group for the development of suture failure and require

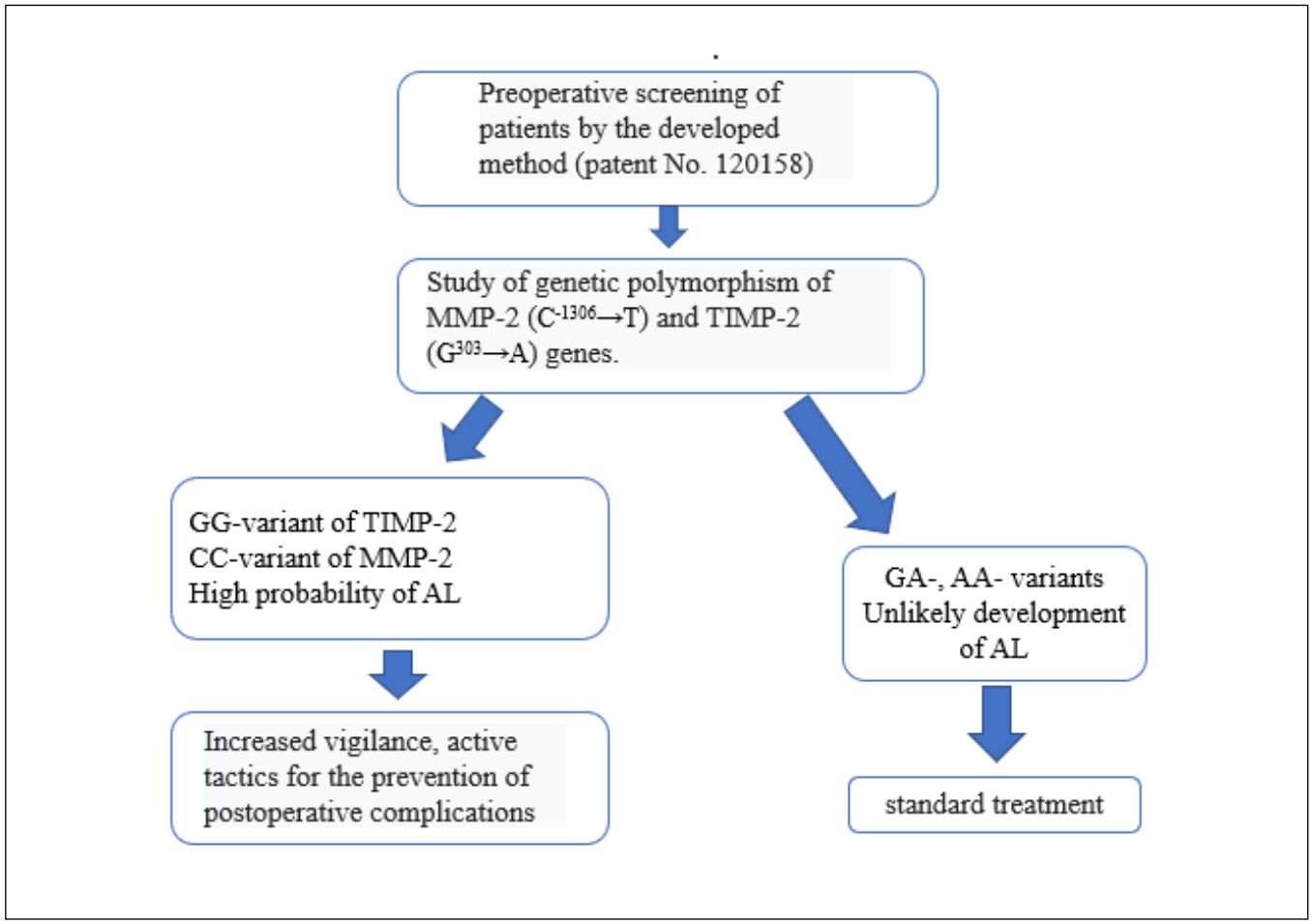

Fig. 3. A prognostic and therapeutic algorithm for the prevention of anastomotic leak in patients with undifferentiated connective tissue dysplasia.

comprehensive preventive and therapeutic measures starting from the pre-, intra-, and postoperative-stages.
Preoperative stage:
- Sparing food intake.
- Mechanical bowel preparation.
- Bowel decontamination.
- Epidural anesthesia.

Intraoperative stage:
- Precise technique of anastomosis and minimization of the tension in the anastomosed area;
- giving preference to the "side to side" small-intestinal, small-large-intestinal anastomosis
- Use of monofilament long-absorbable suture material.
- Use of "reinforcing" sutures in staple lines of colo-rectal and esophago-jejunal anastomosis
- Feeding Microjejunostomy formation in operations on proximal sections of GI tract
- Expansion of indications for protective stomas during operations on the distal parts of the hollow digestive organs (colonic and colo-anal anastomoses);
- Colonic lavage with use of antiseptic solutions.

Postoperative stage:
- Conservative treatment aimed at enhancing of regenerative processes in tissues;
- Rational antibiotic prophylaxis and antibacterial therapy;
- Prolonged use of epidural anesthesia;
- Early enteral nutrition.

Based on the analysis of the obtained research results, we believe that the surgical treatment of such postoperative complications as anastomotic leak in patients with identified and genetically determined pathology of connective tissue should be included in the rule «less is better,» i.e., reducing trauma and the volume of surgical intervention, with a preference for minimally invasive methods.

DISCUSSION

Anastomotic leak is a major complication that is associated with major postoperative morbidity, mortality, and prolonged hospital stay [13]. Depending on the site of anastomosis incidence ranges from 2 to 19 %, and may be up to 25 % in the case of pancreatic anastomoses [14]. Short-term consequences of AL

such as septic or hemorrhagic complications drastically increase postoperative morbidity and mortality, and long-term outcomes may require additional interventions due to a stricture formation, functional limitations to the patient`s life, and also have a negative impact on recurrence-free survival [15]. So prevention of such complications, and identification of patients, susceptible to AL with further tailored management in such cases is of great importance for reducing morbidity and mortality.

The absence of tension, adequate blood supply, and the correct opposition of tissues are fundamentals in the technical aspects of anastomotic formation [16]. Despite this, there is no common point of view on the reasons for anastomotic leak. In the conducted studies on anastomotic leaks, multiple factors that may add to the chance of AL have been described, some are non-modifiable such as male gender due to a more narrow pelvis. Some are based on preoperative therapy – radiation and chemotherapy, blood products, and patient-dependent factors: smoking, alcohol consumption, obesity, and malnutrition [17,18]. Lin et al. in their study of 999 patients that were operated on for rectal cancer link a patient`s old age to an anastomotic leak formation (P = 0.009) [19]. But there are several studies, that show that a patient`s age is not correlated with AL [20-22]. The way anastomosis should be constructed, hand-sewn, or stapled is also a point of dispute, while there are studies advocating for lower AL incidence in stapled anastomoses [23]. They are opposed by studies, some of which demonstrate a two-fold increase in anastomotic dehiscence after the use of stapling devices in comparison to hand-sewn [24,25].

In conclusion, most authors identify four main risk factors: state and morpho-functional processes occurring in anastomosed tissues, unfavorable factors for which sutures were applied, technical features of stitching, and adverse factors occurring in the postoperative period. The first group of factors is decisive and reflects the viability of tissues and the extent of reparative processes. Despite its undisputed role, research on regenerative processes in anastomosed tissues is lacking [1].

Several research papers have developed a hypothetical relationship between genetic polymorphisms in the mechanisms of inflammation development and the occurrence of anastomotic leaks. The impact of genetic factors on anastomotic healing requires further research. [9, 10]. The lack of clear prognostic criteria for the possibility of AL, the practically unexplored role of genetic predisposition for the development of anastomotic complications, and the possibility of correcting surgical tactics in such patients require new scientific research in this direction. Based on the genetic research findings, our proposed prognostic treatment algorithm promises to significantly improve the treatment outcomes for patients with intestinal suture failure.

CONCLUSIONS

1. The pathogenetic significance of alleles of polymorphisms of the MMP2 and TIMP2 genes, which are a risk factor for AL, accompanied by hypoproteinemia, high levels of biochemical markers of collagen biodegradation, and reduced expression of monoclonal antibodies to α-SMA and Collagen IV was revealed.
2. The proposed method of prognosis, which involves genetic research of MMP-2 ($C^{-1306}\rightarrow T$) and TIMP-2 ($G^{303}\rightarrow A$) gene polymorphisms, makes it possible to determine the probability of the development of anastomotic leak, which affects the choice of treatment tactics for such patients.
3. Molecular genetic research is a new promising direction for the development of modern personalized diagnostic criteria and models for prediction of postoperative abdominal complications, in particular, anastomotic leaks.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 13.06.2024
ACCEPTED: 09.09.2024

ORIGINAL ARTICLE

CONTENTS

Intra-abdominal hypertension and pancreatic destruction in patients with acute necrotizing pancreatitis

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ABSTRACT

Aim: To determine the relationship between intra-abdominal hypertension and the volume of pancreatogenic necrosis in patients with acute necrotizing pancreatitis.

Materials and Methods: A prospective single-center study of 32 adults with acute necrotizing pancreatitis (ANP). A correlation was made between the maximum intra-abdominal pressure (IAP) in the early phase of the disease and the area of pancreatic necrosis and extrapancreatic necrosis (EPN) according to CT data. A one-factor linear regression model was built, based on the linear dependence of the volume of EPN on the maximum value of IAP.

Results: A positive correlation between the IAP level and the volume of EPN was revealed, $\rho = 0.547$ ($p=0.0012$). No linear correlation between the level of IAP and the degree of necrosis of the pancreas ($p=0.368$). The volume of EPN was related to the indicator of IAP($p<0.001$). When the IAP level increases for each mm Hg., the volume of EPN increased, on average by 146.29 ± 37.74 ml ($p<0.001$).

Conclusions: The increase in IAP in the early phase of ANP was accompanied by an increase in the volume of EPN, $\rho = 0.547$ ($p=0.0012$). In the study it was possible to predict EPN volume by measuring the level of IAP in the early phase of the disease with an 15.02 ml error. An increased IAP can be considered one of the markers of an increase in the volume of EPN in patients with ANP.

KEY WORDS: acute pancreatitis, intraabdominal pressure, pancreatic necrosis, extrapancreatic necrosis

Wiad Lek. 2024;77(9):1871-1876. doi: 10.36740/WLek/195128 DOI

INTRODUCTION

Necrotic pancreatitis occurs with a frequency of 20% among diagnosed cases of acute pancreatitis with mortality rate of 20-40% depending on the volume of the necrotic lesion [1-2]. The duration of early organ failure caused by the systemic inflammatory response determines the severity of acute pancreatitis and predicts mortality [3-7]. Pancreatogenic necrosis with associated infection, in turn, determines organ failure in the second phase of the disease and increases mortality in these patients to 30-65% [8-11].

A persistent increase in intra-abdominal pressure (IAP) is a proven predictor of the severity of acute pancreatitis: microcirculation disorders associated with cytokine storm and organ failure and intra-abdominal hypertension (IAH) create a vicious circle, which is another challenge in the complex of intensive care of acute severe pancreatitis [12-13]. Systemic inflammatory response syndrome, increased capillary permeability, swelling of retroperitoneal tissue, functional intestinal obstruction, decreased compliance of abdominal wall lead to mechanical compression of vessels, cellular hypoxia, organ ischemia, increased interstitial edema, and increased IAP [14-16]. The effect of increased abdominal pressure on the organs of the abdominal cavity and retroperitoneal space with subsequent laboratory-clinical manifestation of multiple organ failure is described in experimental works related to induction of abdominal compartment syndrome in laboratory animals [17-19]. Hepatic, venous, arterial and microcirculatory blood flow significantly decreases with even a slight increase in IAP [20-21]. Necrosis of the pancreas and extrapancreatic tissue in the manifestation of acute pancreatitis, which is pathophysiologically caused by premature activation of pancreatic enzymes, may also depend on circulatory changes in IAH syndrome.

AIM

The aim of the study was to determine the relationship between intra-abdominal hypertension and the volume of pancreatic and extrapancreatic necrosis in patients with acute necrotizing pancreatitis.

Table 1. Data presentation

Indicator	Median	QI-QIII
Intra-abdominal pressure, max, mm Hg	15	13-16
Volume of extrapancreatic necrosis, ml	1790	1590-2025
Pancreatic necrosis area, ranks	**Absolute value**	**Percentage, CI% (confidence interval)**
I (<30%)	1	3.1 (1 - 16.2)
II (30-50%)	2	6.2 (0.8-20.8)
III (>50%)	29	90 (75-98)

Table 2. Coefficients of a one-factor model for predicting the volume of extrapancreatic necrosis in patients with acute necrotizing pancreatitis

Indicator	Value of model coefficient b±m(b)	Significance level of the coefficient difference from 0, p
Const	-257.79±551.32	p=0.64
X1	146.29±37.74	p=0.0005

MATERIALS AND METHODS

The study was a prospective cross-sectional non-randomized single-center study of 32 adult patients with acute necrotizing pancreatitis (ANP) treated in 2023 at the Department of General Surgery No. 1 of the Bogomolets National Medical University. Of the patients, 56% (18/32) were male, 44% (14/32) were female, with an average age of 49±3 years. The study included patients with acute pancreatitis, confirmed in compliance with the 2012 revised criteria of Atlanta [5], having signs of transient or persistent organ failure. The patients underwent computed tomography of abdominal cavity and retroperitoneal space with intravenous contrast in the second week after the onset of the disease with pancreatic necrosis confirmed by calculating the pancreatic necrosis index (PNI) and measuring the volume of extrapancreatic necrosis (EPN) in millilitres. Exclusion criteria: acute mild pancreatitis, absolute contraindications to performing computed tomography, death in the first week of the disease, patients with neurogenic bladder disease, which made it impossible to measure IAP.

IAP monitoring was carried out in the studied patients in the early phase of the disease. For further calculations, the maximum indicator among those measured ones was selected. An indirect method was chosen to measure IAP: 100 ml of sterile physiological solution was slowly injected into the lumen of an empty bladder using a Foley catheter and a drip system. After that, the intravesical pressure was measured using a ruler in mm H2O, whereas the level of the pubic symphysis was taken as zero. The obtained results were translated into mm Hg: 1 mm Hg. = 13.5951 mm H2O [22].

The first step was to determine the relationship between the maximum value of IAP, mm Hg, the volume of EPN in millilitres, and the degree of pancreas necrosis, which was ranked depending on the percentage of lesions according to PNI (I-<30%, II- 30 - 50%, III- > 50%) Spearman's rank correlation index ρ was calculated after checking the distribution of values for normality.

The second step included the construction and analysis of a univariate linear regression model. The analysis was based on the linear dependence of the resulting feature Y (volume of extrapancreatic lesion, EPN) on the factor feature X1 (maximum value of IAP in the early phase of the disease, IAP). The value of the model coefficient, b±standard error m(b), adjusted coefficient of determination R^2adj, F value, root mean square error RSE were calculated. A value of $p<0.05$ was considered statistically significant. The EZR (R-statistics) package was used for data calculation and analysis [23].

RESULTS

The median (QI-QIII) of the maximum IAP among the patients was 15 (13-16) mmHg, the median volume of EPN was 1790 (1590-2025) ml, respectively. Pancreatic necrosis of more than 50% was recorded in 90% (CI% 75-98) of patients. The distribution of ICP values and EPN volume differed from normal, $p<0.05$. (Table 1)

The first thing to be determined was a relationship between the level of IAP in the early phase of the disease and the volume of EPN. For this purpose, Spearman's rank correlation index was calculated. The value of the correlation coefficient $\rho = 0.547$ (statistically significantly different from 0, p=0.0012). Consequently, the existence of a positive correlation between the level of IAP and the volume of EPN was revealed. An increase in IAP in the early phase of ANP disease was on average accompanied by an increase in the volume of EPN (Fig. 1).

Another thing to find out was the relationship be-

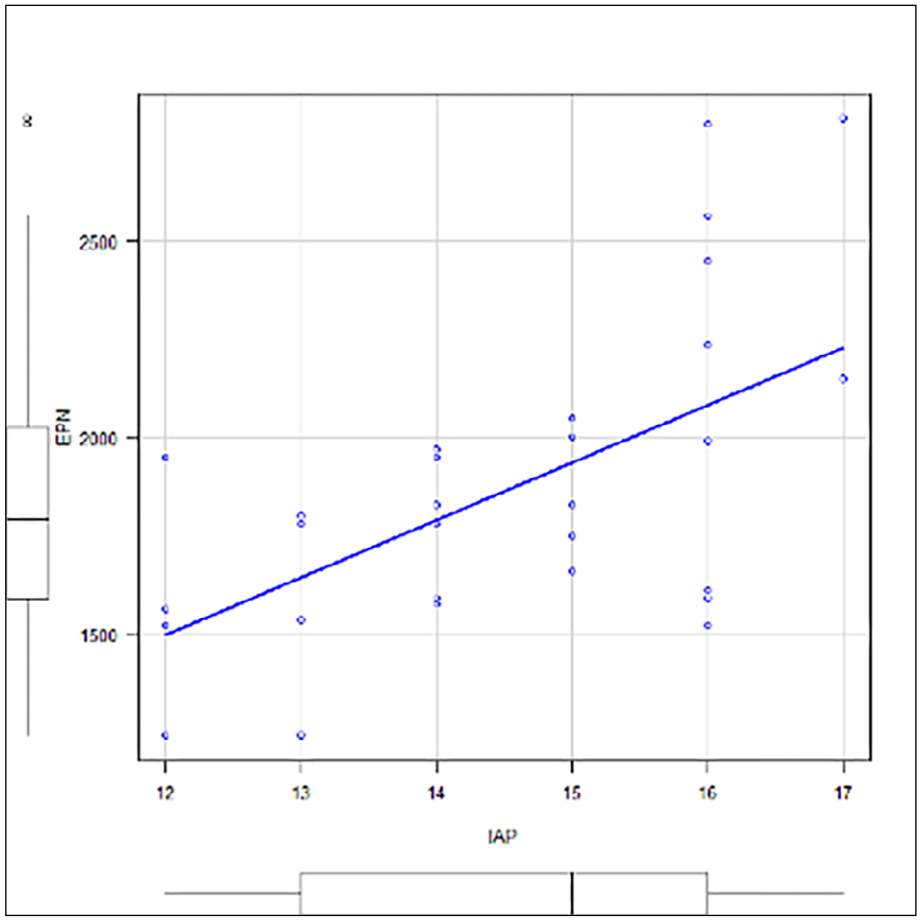

Fig. 1. Correlation field of intraabdominal pressure (IAP) mm Hg, and the volume of extrapancreatic necrosis (EPN) ml, $p<0.001$.

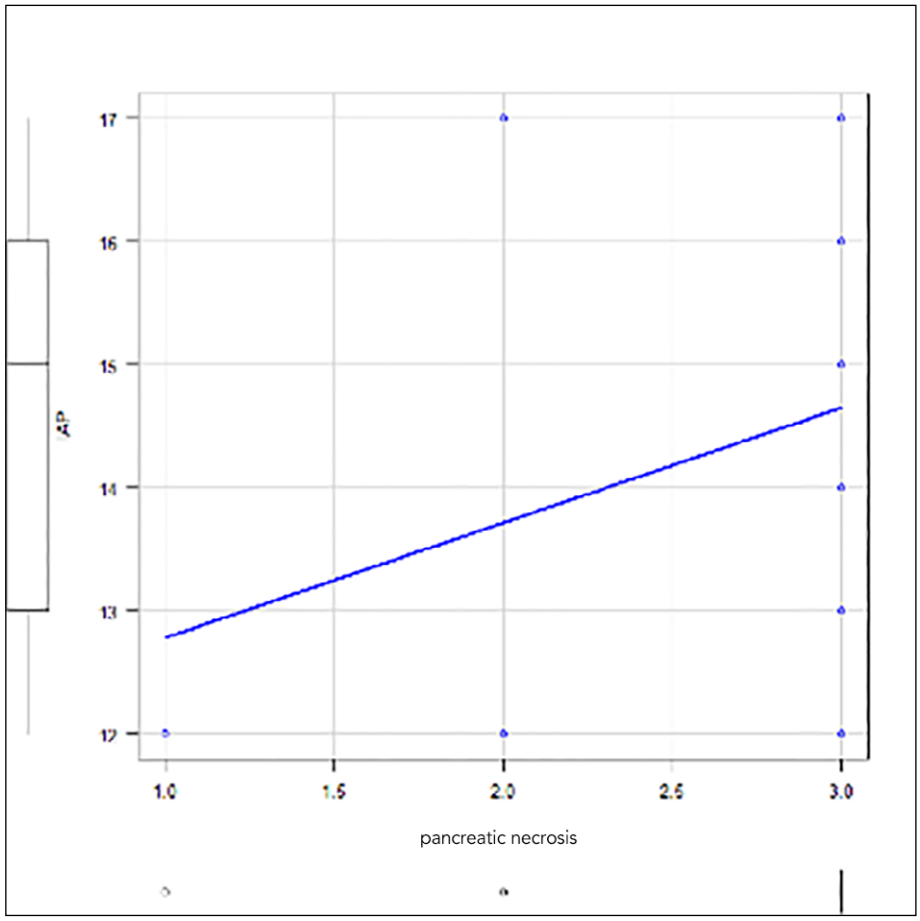

Fig. 2. Correlation field of intraabdominal pressure (IAP), mm Hg, and pancreatic necrosis area, ml, $p<0.001$.

tween the level of IAP in the early phase of the disease and the area of pancreas necrosis in accordance to three ranks. For this purpose, Spearman's rank correlation index was calculated. The value of the correlation coefficient ρ = 0.165 was not statistically significantly different from 0 (p=0.368). Thus, a linear correlation between the level of IAP and the degree of necrosis of the pancreas was not detected (p>0.05) (Fig. 2)

The method of building and analyzing a one-factor linear regression model was used for further analysis of the EPN volume dependence on the IAP indicator in the early phase of the disease, estimated in mmHg, Dependence of the initial variable (the volume of extrapancreatic necrosis) on the IAP value was revealed. Adjusted coefficient of determination R^2adj = 0.3115, F value 15.02, p<0.001, root mean square error RSE = 320.2 (with 30 degrees of freedom). Table 2 shows the results of estimating model coefficients.

Thus, it was established that the volume of EPN in patients with ANP is related (p<0.001) to the indicator of IAP in the early phase of the disease. When the IAP level increases for each mm Hg., the volume of EPN increased on average by 146.29±37.74 ml (p<0.001). The obtained mathematical model for forecasting the volume of the EPN can be expressed by the formula:

Y=(-257.79) + 146.29×X1

Formula X1 - IAP in the early phase of the disease, Y - volume of extrapancreatic necrosis

DISCUSSION

The area of pancreatic destruction is determined in order to choose treatment tactics, just as well as for the purpose of the expected disease results, i.e. patient's stay in hospital, frequency of complications and risk of organ failure development [24-26].

The classic CT index of acute pancreatitis severity, which among other things evaluates in percentage the area of pancreas parenchyma damage [27], is now increasingly frequently supplemented by the calculation of extrapancreatic damage volume [28-30], which also applies to the early phase of the disease [31].

Pancreatogenic necrosis infection significantly worsens the disease prognosis [32-33], the volume of the lesion correlates with the severity of septic complications. High IAP increases the risk of hypoperfusion and translocation of intestinal microflora to lesions. Earlier, we also considered the derivative of intra-abdominal pressure - abdominal perfusion pressure as a predictor of infection duration in patients with ANP [34].

A 2021 study scrutinized a difference in CT findings in acute pancreatitis in a group with and without intra-abdominal hypertension. A significant difference was disclosed in the presence, volume, and maximum size of clusters, volume of pleural effusion, and bile duct dilatation [35]. Compared with the results of our study, Pankaj Gupta et al found no difference in the presence of extrapancreatic necrosis, presence and area of pancreatic necrosis between patients with and without IAH. In order to compare the data, it is necessary to compare retrospectively the CT findings of our studied patients with ANP and selected patients without IAH.

In the presented study, the increase in IAP at the beginning of the disease affected only the volume of the extrapancreatic necrotic lesion. In the future, it is necessary to compare the values of IAP and EPN in one time interval and determine the critical level of IAP increasing the volume of EPN.

CONCLUSIONS

1. The increase in intra-abdominal pressure in the early phase of acute necrotizing pancreatitis was, on average, accompanied by an increase in the volume of extrapancreatic necrosis, ρ = 0.547 (p=0.0012).
2. No linear correlation was found between the level of intra-abdominal pressure and the degree of pancreatic necrosis (p>0.05).
3. In the studied sample, it was possible to predict the volume of extrapancreatic necrosis by measuring the level of intra-abdominal pressure in the early phase of the disease with an error of 15.02 ml. When the IAP level increases for each mm Hg., the volume of EPN increases, on average by 146.29±37.74 ml (p<0.001).
4. An increase in intra-abdominal pressure can be considered one of the markers of an increase in the volume of extrapancreatic lesions in patients with acute necrotizing pancreatitis.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 10.06.2024
ACCEPTED: 22.09.2024

ORIGINAL ARTICLE

CONTENTS

Minimally invasive methods of surgical treatment of patients with varicose disease of the lower extremities

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ABSTRACT

Aim: to improve the results of surgical treatment of patients with varicose veins of the lower extremities.

Materials and Methods: A retrospective evaluation of the treatment of 97 patients with a diagnosis of «varicose veins of the lower extremities».

Patients were divided into three groups: Group I (study), patients underwent EVLC, sclerotherapy foam-foam (STFF) and mini-phlebectomy - 61 cases; Group II, patients underwent EVLC and STFF - 43 cases and Group III, patients underwent EVLC and mini-phlebectomy - 45 cases.

The results of the performed intervention were evaluated clinically and with the help of duplex scanning (DS) the next day, after a week, 1 month, 6 and 12 months.

Results: In group I, the total frequency of postoperative complications reached 6.4% (4 cases). The overall frequency of complications in the group II reached 27.9% (13 cases). The overall frequency of complications in the group III reached 33.3% (15 cases).

Conclusions: 1) EVLK in combination with STFF and mini-phlebectomy gives the best results of surgical treatment of varicose veins.

2) In the studied group, where mini-phlebectomy and STFF were used, there is a mutual exclusion of postoperative complications.

KEY WORDS: varicose disease of the lower extremities, endovenous laser coagulation, radiofrequency ablation, trunk foam sclerotherapy, mechanochemical obliteration

Wiad Lek. 2024;77(9):1877-1882. doi: 10.36740/WLek/195129 DOI

INTRODUCTION

Varicose veins of the lower extremities is one of the most important medical and social problems associated with the high prevalence of the disease and dissatisfaction with the results of its treatment. The frequency of postoperative recurrences of the disease reaches up to 50%. In industrialized countries, the total cost of treating patients with varicose veins of the lower extremities is from 1.5 to 3% of the health care budget, which determines an important medical and social component of medical and surgical treatment of patients with varicose veins of the lower extremities [1-4].

Over the past decades, significant progress has been made in the treatment of varicose veins. In the treatment of patients, various surgical techniques are used, the list of which is growing over time. Open methods of treatment were replaced by such minimally invasive methods as endovenous laser coagulation (EVLC), radiofrequency obliteration, trunk foam sclerotherapy, cyanoacrylate obliteration, mechanochemical obliteration, cryostripping and others [4-8]. These techniques are used to eliminate trunk reflux in the great saphenous vein (GSV) and small saphenous vein (SSV), their main tributaries, as well as in perforating veins. After elimination of trunk or perforating insufficiency, as a rule, it is necessary to perform an intervention on varicose tributaries. According to the literature, the choice of the method and scope of surgical treatment of varicose affected tributaries is quite variable [7-12].

Endovasal treatment methods make it possible to implement an individual approach to each patient and can be used both independently and in the combined treatment of varicose veins.

In modern conditions, in the presence of a wide range of operative methods for the elimination of pathological veno-venous refluxes and varicose and dilated subcutaneous veins, the doctor is faced with the question of choosing the optimal intervention option, which would combine minimal invasiveness and radicality.

AIM

To improve the results of surgical treatment of patients with varicose veins of the lower extremities.

MATERIALS AND METHODS

A retrospective evaluation of the treatment of 97 patients (149 lower extremities; 26 with bilateral lesions) with a diagnosis of «varicose veins of the lower extremities» who were treated in the surgical department of the National Medical Center «UMBKL» of the UMR was carried out.

Patients were examined using general clinical methods. To assess the degree and dynamics of edema, we used the anthropometric method of measuring the circumference of the limb at four standard levels (the middle of the foot, the level of the ankle joint, the level of the middle third of the leg, the level of the upper third of the leg).

The main instrumental method of examination was ultrasound angioscanning with Alpinion color Doppler mapping with a set of linear (5-10 MHz) and convex (2-5 MHz) sensors.

Patients were divided into three groups: Group I (study), patients underwent EVLC, sclerotherapy foam-foam (STFF) and mini-phlebectomy - 61 cases; Group II, patients underwent EVLC and STFF - 43 cases and Group III, patients underwent EVLC and mini-phlebectomy - 45 cases. In patients of all groups, varicose disease of the lower extremities was diagnosed for the first time.

All patients of the study group underwent EVLC of the main vein in combination with STFF and mini-phlebectomy of varicose tributaries in «key points». As a sclerosant, a foam form of a 0.5-2% solution of polidocanol, prepared according to the Tessari technique [13], was used. Mini-phlebectomy was applied according to the Varadi technique. The results of the performed intervention were evaluated clinically and with the help of duplex scanning (DS) the next day, after a week, 1 month, 6 and 12 months. Demographic and clinical data of patients who underwent endovasal laser coagulation of the main vein with one-moment microfoam sclerotherapy in combination with mini-phlebectomy are presented in Table 1.

All patients had reflux through the IVC and varicose tributaries. In order to eliminate trunk insufficiency, the EVLC technique was chosen. The procedure was performed under ultrasound control under local tumescent anesthesia. In the case of bilateral damage, operations were performed consecutively with an interval of 7 days. At the preoperative stage, all patients were marked with a permanent marker for varicose veins in a standing position. For the EVLC of the GSV trunk, the generally accepted technique was used (diode laser, wavelength 1470 nm and optical fibers with radial emission). After the treatment of the trunk vein was completed, STFF of varicose tributaries was first performed. The sclerosant was injected in small volumes (1-2 ml) at several points, and not in a large volume for one injection. When the simultaneous ultrasound examination determined foam filling of all target veins, the injections were stopped. The total volume of the injected drug did not exceed 10 ml per operation. After completion of STFF, tumescent anesthesia and mini-phlebectomy according to Varada were performed in the marked veins. Skin punctures were performed with an 18G puncture needle along the marked tributaries. The «key points» were defined in advance: the first is the closest point to the point of confluence of the varicose inflow into the main vein, the second is the closest point to the point of reflux drainage into the deep venous system, the others are separated by about 10 cm from each other. Then, through skin punctures, the wall of the vein was grasped with a Varadi hook and its extraction was carried out. When the vein was brought out, it was picked up with clips of the «mosquito» type and with smooth pulling movements, it was removed by the cranial and distal ends at the maximum extent. The number of punctures varied in different patients and depended on the extent of the varicose syndrome. Most often it was 5–7. After this stage, the operated leg was treated with a solution of hydrogen peroxide and an antiseptic. Suturing of puncture holes was not required due to their small diameter. Sterile napkins were applied to the puncture sites and a class 2 compression stocking was put on. Patients were advised to walk calmly for 30-60 minutes immediately after the operation. Ultrasound control were performed the next day, after 1, 6 and 12 months. after intervention or as needed in case of complaints.

RESULTS

EVLC GSV was successfully performed in all cases. In the observation period up to 12 months. There was no case of IVC recanalization, corresponding to 100% obliteration of the target segment. The success of sclerotherapy and mini-phlebectomy was evaluated by the frequency of postoperative complications in the form of infectious complications and matting, postoperative hyperpigmentation, formation of coagulum and ecchymoses, skin necrosis, recanalization of segments, and inflammation in the postoperative period (Table 2).

In group I, the total frequency of postoperative complications reached 6.4% (4 cases). All patients with CVI C5 with residual hyperpigmentation after 12 months.

The overall frequency of complications in the group where patients with EVLC combined with STFF reached 27.9% (13 cases). In addition, most often

Table 1. Demographic and clinical data of patients

Characteristic	Value
Number of patients	97
Number of lower limbs/ on both sides	149 / 26
Gender, men and women	27 / 70
Average age	51,4 (±14,1)
Clinical picture by CEAP classification	
C2	47 (32%)
C3	76 (51%)
C4a	20 (13%)
C5	6 (4%)

Table 2. Postoperative complications in patients of different groups

Complication	Number (%)		
	I	II	III
Hyperpigmentation	4	7	4 (6,4%)
Formation of coagulum	0	6	0
Matting and ecchymoses	0	2	10
Thrombophlebitis of residual veins	0	0	6
Skin necrosis	0	2	0
Suppuration of p/o wounds	0	2	2
Recanalization	0	2	7
Total patients:	4(6,4%)	13(27,9%)	15(33,3%)

patients experienced hyperpigmentation (7 cases), formation of clots that required surgical intervention (6 cases), recanalization (2 cases); skin necrosis due to perivasal injection (2 cases). A combination of several complications (hyperpigmentation and clot formation) was observed in 2 patients.

The overall frequency of complications in the group where patients with EVLC combined with mini-phlebectomy reached 33.3% (15 cases). In addition, the patients most often had ecchymoses and bruises (10 cases); varicothrombophlebitis of residual veins (6 cases), paresthesias of various areas of the lower extremities (7 cases); suppuration of p/o wounds (2 cases); A combination of two complications was observed in 2 patients (bruises, varicothrombophlebitis of residual veins, paresthesias). Other 2 have a combination of 3 or more complications.

Deep vein thrombosis was not detected in any group.

To eliminate the tympanic reflux in all studied groups, we chose EVLC, because it is not inferior to traditional surgical intervention in terms of effectiveness and is characterized by minimal trauma, quick recovery of working capacity, good cosmetic result and minimal negative impact on the quality of life [14]. Our occlusion indicators during 1 year of observation (100%) confirm the data of world literature and other studies [2, 3, 5]. Currently, there are two main ways to eliminate varicose veins of the STFF and mini-phlebectomy. Each of these treatment options has its advantages and disadvantages.

The best treatment results were presented in group I, for which we used EVLC in combination with STFF and mini-phlebectomy. The frequency of complications in this group reached 6.4%.

The highest frequency of complications (33.3%) was noted in the group where mini-phlebectomy was used in parallel with EVLK. The most frequent complications were bruises and ecchymoses, thrombophlebitis of residual veins and paresthesias, which is explained by the peculiarity of the mini-phlebectomy technique.

When using a combination of both methods, we observe the mutual exclusion of the disadvantages of each individual procedure.

DISCUSSION

The ultimate goal of any method of treating varicose veins of the lower extremities is to restore the somatic level of health and ensure a high quality of life for patients after surgery. The main goal of surgical treatment of varicose veins of the lower extremities today is the elimination of the pathogenetic mechanism

of the disease, namely, pathological veno-venous refluxes, which is achieved by crossing and ligation of insufficient perforating veins, sapheno-femoral and sapheno-popliteal joints and removing varicose dilated subcutaneous veins.

The traumatic nature of saphenectomy and related complications led to the search for ways to change treatment tactics - from mechanical removal to intravascular manipulations accompanied by delayed fibrous transformation caused by chemical or high-energy damage to the venous wall.

Endovasal laser coagulation (EVLC) is a method of «caking» veins, which is based on the mechanism of selective photocoagulation - selective absorption of laser energy of a certain wavelength by various components of biological tissues, which leads to their destruction. The main advantage of EVLC, in addition to minimal invasiveness and cosmeticity, is a low risk of complications, the use of local anesthesia, a reduction in the duration of treatment and incapacity for work.

Currently, better tolerability of EVLC with the use of W-laser and reduction of the rehabilitation period compared to traditional operative treatment have been proven, in the absence of significant differences in the frequency of disease recurrence. Due to the transition from the use of hemoglobin-absorbing H-lasers (0.81-1.06 μm), which cause excessive carbonization and tissue necrosis due to low water absorption, to W-lasers (1.47-1.56 μm), for which at short-term exposure is characterized by the predominance of denaturation over carbonization, the efficiency and safety of laser obliteration has increased significantly. There are no such complications as skin burns and thrombophlebitis, and less pronounced pain syndrome when EVLC is performed on laser devices with a long-wave spectrum. EVLC is devoid of the risk of side effects associated with regional anesthesia and the risk of complications typical for endoscopic dissection of perforating veins. In addition, after conducting EVLC, patients usually do not need bed rest and long-term anesthesia, since they have practically no pain syndrome [1, 11, 12, 14, 15].

Sclerotherapy is a method of treating varicose veins by introducing a chemically active agent into the lumen. Traditionally, it was performed with the help of the introduction of a liquid form of sclerosant. With the development of phlebology and the widespread introduction of ultrasound diagnostic methods for vein diseases, microfoam sclerotherapy (STFF) was introduced and proved itself as a more effective technique. Its advantages are the visual controllability of the spread of the sclerosant, short duration, low cost, the possibility of eliminating pelvic-gonadal and perforating venous insufficiency, recurrent variant of varicose syndrome or progression of varicose disease. Disadvantages can be recanalization of sclerosed tributaries at low concentrations, thrombophlebitis, hyperpigmentation, matting, skin necrosis, deep vein thrombosis, allergic reactions, pulmonary embolism [13].

Mini-phlebectomy (MFE) is a method of treating varicose veins, which is most often performed under local anesthesia in an outpatient setting using special tools (18G needles, hooks for mini-phlebectomy, «mosquito» type clamps). Varicose veins are removed through point punctures and/or microincisions of the skin. Mini-accesses give a good cosmetic result compared to traditional surgical interventions, but this surgical method also has its drawbacks. In the case of incomplete removal of varicose veins, their segments may remain in the subcutaneous tissue, in which local inflammation often develops, causing significant discomfort in the immediate postoperative period. In some cases, incomplete removal may require repeated interventions. It should also be remembered that most often the marking of varicose tributaries is carried out in a vertical position and when the patient changes the position to a horizontal one, the anatomical course of the «target veins» may change. This nuance makes it difficult to find veins and, as a result, leads to a large number of accesses and traumatization of tissues, as well as to the occurrence of a greater number of hematomas and ecchymoses in the postoperative period. Other complications can be infections and matting, extremely rarely - postoperative bleeding and deep vein thrombosis. Such a complication as postoperative neuralgia is the result of unintentional damage to skin nerves with the tip of a needle or a hook, which leads to the emergence of a pronounced pain syndrome with temporary sensory disturbances.

Removal of subcutaneous veins by cryostriping is a relatively new method of treating varicose veins, which is now being used more and more often. Removal of a vein with the help of a cryoprobe allows to shorten the operation time, shorten the patient's stay in the hospital, reduce the severity of the pain syndrome, and improve the cosmetic results. The cold effect during cryoextraction leads to a decrease in the volume of hematomas, a decrease in the intensity of the inflammatory process and an increase in the patient's comfort level, which in turn reduces the rehabilitation period. The use of cryoprobes allows you to minimize the number of accesses and achieve a better cosmetic effect. The possibility of paravasal use of a cryoprobe without an anechoic lumen or pronounced tortuosity of the venous trunk of the saphenous vein, in addition to reducing the number of accesses, also leads to a

reduction in the duration of surgical intervention. The analysis of immediate results allows us to state that cryostripping is the most promising method aimed at removing the trunks of the subcutaneous veins of the lower extremities. It combines efficiency and safety, which allows its wide application in the surgical treatment of varicose veins of the lower extremities [16].

An alternative to EVLC was the method of radiofrequency ablation of main subcutaneous veins. The RFA technique entered clinical practice in 1998. Intravasal thermolysis is carried out using the Closure Vein Treatment System (VNUS), due to which a dosed thermal effect causes irreversible damage to the proteins of the vascular wall, while not violating the integrity of the wall itself [11, 14].

Mini-invasive surgical interventions can be performed on an outpatient basis, which has a positive effect on the psychological state of patients, reduces the risk of infectious and thromboembolic complications, and expands the range of patients who are shown modern specialized care for varicose veins of the lower extremities.

In connection with the introduction of new surgical technologies in the treatment of varicose veins of the lower extremities, there was a need for a more thorough study of endovasal methods, the results of their use, improvement of the technical aspects of the operation, which contribute to increasing the effectiveness of these treatment methods.

CONCLUSIONS

1) EVLK in combination with STFF and mini-phlebectomy gives the best results of surgical treatment of varicose veins. The frequency of complications reached 6.4%, more than 5 times less than in the group where isolated mini-phlebectomy was used, and more than 4 times less than when using isolated STFF.

2) In the studied group, where mini-phlebectomy and STFF were used, there is a mutual exclusion of postoperative complications.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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RECEIVED: 09.06.2024
ACCEPTED: 17.09.2024

ORIGINAL ARTICLE

CONTENTS

Analysis of the prevalence of allergic rhinitis among children in Uzhhorod

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ABSTRACT

Aim: To analyze data on the prevalence of allergic rhinitis (AR) among children in Uzhhorod**.**

Materials and Methods: 373 patients who consulted a pediatric allergist at the Uzhgorod City Children's Polyclinic were under observation.

Results: After examination of 373 patients with AR, it was found that girls predominated by gender, namely 54.7%, against 45.3% boys. In the age structure, younger preschool age prevailed -32.5%. Aggravated heredity, namely the presence of allergies, bronchial asthma and atopic dermatitis (AD) in parents was found in a total of 78.1%. Of them, the presence of burdened heredity in both parents was found in 29.3%, in one of the parents - 48.7%.

Analyzing the features of the clinical course of AR, it was established that the vast majority of patients were diagnosed with persistent AR, namely 53.4%, against intermittent AR in 46.6%. According to the data of VAS, the clinical course of AR was characterized by a moderate or severe course, with the greatest severity in the group of primary school age, namely 7.6±2.1 points.

Conclusions: The highest prevalence of AR is noted in the age group of primary school age in 32.5% of patients. According to the clinical course of AR, persistent AR prevails in 53.4% of patients.

KEY WORDS: allergic rhinitis, prevalence, children, comorbidity

Wiad Lek. 2024;77(9):1883-1887. doi: 10.36740/WLek/195136 DOI

INTRODUCTION

According to the European Academy of Allergology and Clinical Immunology (EAACI), allergy is the most common chronic disease in Europe[1]. According to the prognosis of the World Health Organization (WHO), during the 21st century, allergic diseases will take second place, after to mental diseases in terms of prevalence in the world[2]. Today, this pathology represents a global medical and social problem, because up to 40% of the Earth's population has allergic diseases, among the children's population up to 15% [3]. In Ukraine, according to statistics, 10-15% of the population suffers from allergic diseases, which is significantly less than the world indicators[4].

One of the most widespread are respiratory allergic diseases, among which bronchial asthma and allergic rhinitis (AR) are the most common [5].

According to the WHO, the prevalence of seasonal AR in different countries of the world varies from 1 to 40%, year-round - from 1 to 18% [6].

AR does not threaten the life of the patient, but affects its aspects, in particular, social, mental, physical (reduced work capacity, reduced productivity, impaired cognitive function, impaired learning, increased fatigue, disturbed sleep), and is also a risk factor for the development of bronchial asthma and in 43-64% of cases it precedes its development [7].

According to ARIA, the prevalence of spontaneous AR allergic rhinitis is 2% to 25% in children and 1% to 40% in adults. The prevalence of confirmed allergic rhinitis in adults in European countries ranges from 17% to 28.5% [8].

According to official statistics in Ukraine, the highest prevalence rates of allergic rhinitis were registered in Dnipro - 596.0 and Kharkiv - 398.6 regions. The lowest prevalence rates of the studied pathology were registered in the Volyn region - 78.5 per 100,000 adult population. The overall prevalence rate of AR in Ukraine is 267.3 per 100,000 adult population, which is only 0.3% of the total population (the prevalence of this pathology among children over the past 10 years in Ukraine is 0.5%)[9] . The given data of official statistics on the prevalence of AR indicate the underdiagnosis of this pathology [10].

Therefore, the study of the true prevalence of AR and its clinical features is relevant.

Table 1. Distribution of AR patients by age and gender

Age groups	The total number of patients n=373		Boys n=169		Girls n=204	
	Abs.	%	Abs.	%	Abs.	%
1-2 years	52	13,9	28	7,5	24	6,5
3-6 years	90	24,1	42	11,2	48	12,9
7-11 years	121	32,5	48	12,9	73	19,5
12-18 years	110	29,5	51	13,7	59	15,8
The total number of patients	373	100	169	45,3	204	54,7

AIM

The aim was to analyze data on the prevalence of allergic rhinitis (AR) among children in Uzhhorod**.**

MATERIALS AND METHODS

Under observation were 373 patients who consulted a pediatric allergist at the Uzhhorod Children's Polyclinic with a diagnosis of AR for 2023 year. The studies were conducted with the informed consent of the patients, and their methodology was in accordance with the Helsinki Declaration of 1975 and it was revised in 1983 and approved by the UzhNU local bioethics commission (Protocol No. 7/10 dated May 18, 2024).

The diagnosis of AR was established according to the recommendations of the Unified Clinical Protocol of Primary, Secondary and Tertiary Care «Allergic Rhinitis» (2019) and EAACI recommendations (2022) [11].

The research was conducted by means of a questionnaire using a special questionnaire to identify AR symptoms[12]. To assess the severity of symptoms, a visual analog scale (VAS) was used, where 0 is the worst condition and 10 is the best condition [13].

Only verified diagnoses and consultation data of related specialists were taken into account. Data analysis was performed using Janovi version 2.3.28. Means, standard errors, and significance of differences were calculated and considered statistically significant at $p<0.05$.

RESULTS

After examination of 373 patients with AR, it was found that girls predominated by gender, namely 54.7% (204 out of 373), against 45.3% boys (169 out of 373), which indicates a greater prevalence of this pathology among the female.

In the age structure, younger preschool age prevailed -32.5% (121 out of 373), the next most common was high school age - 29.5% (110 out of 373), the third - preschool age - 24.1% (90 out of 373) , and the smallest was before preschool age – 13.9% (52 out of 373) (table 1).

Aggravated heredity, namely, the presence of allergies (food and inhalation), bronchial asthma and atopic dermatitis (AD) in parents, in general, was found in 78.1% (291 out of 373). Of them, the presence of burdened heredity in both parents was found in 29.3% (109 out of 373), in one parent -48.7% (182 out of 373). The absence of aggravating heredity was noted in 21.9% (82 out of 373).

At the next stage of the study, the peculiarities of the course of AR in age groups and its influence on the quality of life according to VAS were studied.

Analyzing the features of the clinical course of AR, it was established that the vast majority of patients were diagnosed with persistent AR, namely 53.4% (199 out of 373), against intermittent AR in 46.6% (174 out of 373) (table 2).

According to VAS data, the clinical course of AR was characterized by a moderate or severe course, the highest severity in the group of primary school age, namely 7.6±2.1 points (table 3).

Also, among this sample of patients, an analysis of the frequency of concomitant pathology was conducted (Table 4).

Therefore, the results of the study indicate that the most frequent concomitant diseases were AD and bronchial asthma, the third most frequent concomitant condition was allergic conjunctivitis (AK).

DISCUSSION

Therefore, the revealed data regarding the higher prevalence of AR among women coincide with the data of a study by Hungarian scientists (2020) regarding the prevalence of AR among children [14].

The actual data on the distribution of AR in the age structure, which were observed in persons aged 1 to 18 years inclusive, had an obvious tendency to increase until primary school age, with a subsequent decrease, which can be explained by the age-related features of immunity, the influence of sex hormones on the immunological mechanisms of occurrence and course AR, as well as the nature of the treatment given to these patients. The obtained data complement the work of

Table 2. Distribution of the clinical course of AR in different age groups

Age groups	Clinical course			
	Intermittent		Persisting	
	abs.	%	abs.	%
1-2 years	12	3,2	40	10,8
3-6 years	30	8,0	60	16,0
7-11 years	62	16,7	59	15,8
12-18 years	70	18,7	40	10,8
The total number of patients	174	46,6	199	53,4

Table 3. Indicators of VAS in different age groups

Age groups	Indicators of VAS
1-2 years	6,7±1,8*
3-6 years	6,3±2,4*
7-11 years	7,6±2,1*
12-18 years	5,1±1,7*

Note. Difference validity: * – p>0,05.

foreign scientists SN Hong et al. on the distribution of AR among children [15].

The obtained results regarding the presence of burdened heredity only confirm many years of research by scientists regarding the significant influence of heredity on the development of allergic diseases in children[16].

The obtained data on the clinical course of AR are probably related to the prevalence of the causative allergens that cause AR, because according to the data of many population studies, the most frequent allergens that cause allergic diseases among children are house dust mites, which in turn will cause complaints in patients throughout the year[4,5].

The obtained results for VAS are probably related to the peculiarities of the immune system in children, which can be excessively activated in response to allergens, which leads to more severe manifestations of allergies. As well as frequent infections of the upper respiratory tract, which can complicate the course of allergic rhinitis and lead to chronic inflammation with a high level of morbidity in this category of patients with various ARVs, which aggravates the symptoms of allergic diseases. The obtained data coincide with the data of scientists, who also associate the more severe course of AR with the peculiarities of the immune response in children at different age periods[17].

The data obtained regarding the presence of concomitant pathology in patients with AR only confirm the long-term studies of scientists from around the world[18,19], according to which the most common concomitant conditions in AR are bronchial asthma and AD. Allergic rhinitis, bronchial asthma and atopic dermatitis often occur together due to common development mechanisms that are associated with hyperactivity of the immune system. The main cause is atopy — the body's genetic predisposition to an excessive immune response to common irritants (allergens). These conditions often develop in a complex due to the so-called «atopic march» - a gradual transition of one disease to another, which begins with atopic dermatitis in childhood, continues with allergic rhinitis and can lead to asthma**.**

CONCLUSIONS

1. The highest prevalence of AR is noted in the age group of primary school age in 32.5% of patients.

Table 4. Prevalence of concomitant pathology in patients with AR in different age groups

Nosology	Age groups								Total number	
	1-2 years		3-6 years		7-11 years		12-18 years			
	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%
Bronchial asthma	0	0	35	9,4	54	14,4	58	15,5	112	30,0
Atopic dermatitis	42	8,6	48	10,1	49	10,5	40	10,7	179	48,0
Allergic conjunctivitis	0	0	10	2,7	15	4,0	14	3,7	30	8,0
Vasomotor rhinitis	0	0	5	1,3	11	2,9	7	1,8	23	6,2
Narrowing of the nasal passages	5	1,3	4	1	2	0,5	0	0	11	3,0
Chronic adenoiditis	6	1,6	10	2,6	4	1	0	0	17	9,0

2. According to the clinical course of AR, persistent AR prevails in 53.4% of patients.
3. The most frequent comorbid conditions in RA are atopic dermatitis and bronchial asthma.

PROSPECTS FOR FURTHER RESEARCH

Study of the prevalence of AR, features of its clinical course among different age groups of the population.

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The research was performed within the departmental topic of the Department of Internal Medicine of the Uzhhorod National University; "Features of the clinical course, pathogenetic mechanisms and treatment approaches for diseases of internal organs in the presence of comorbidity" № stateregistration 0123U1011508.

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

RECEIVED: 08.06.2024
ACCEPTED: 21.09.2024

ORIGINAL ARTICLE

CONTENTS

Endometrial hyperplasia as a consequence of mixed urogenital infections

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ABSTRACT

Aim: Investigation of hyperproliferative diseases of the female genital organs as a consequence of mixed urogenital infections.

Materials and Methods: The study included 56 women of reproductive age who experienced discomfort in the external genital area in the form of excessive vaginal discharge and/or unpleasant odour of the discharge, itching in the external genital area (main group). The control group consisted of 30 somatically and gynaecologically healthy patients.

Results: The following complaints were noted in mixed infections: increased amount of vaginal discharge (28 women / 50% of the main group), unpleasant odour of discharge (19 women / 34%), less frequently - itching (10 women / 18%), burning (11 women / 20%), pain during intercourse (8 women / 14%), digestive tract disorders - bloating and irregular bowel movements (13 women / 23%). Two or more complaints were noted in 29 patients (52% of the main group). The microscopy revealed a small number or complete absence of lactobacilli, abundant polymorphic gram-negative and gram-positive bacillus and coccusmicroflora, and the presence of 'key cells'.

Conclusions: Taking into account the information described above, it can be noted that mixed urogenital infections affect the pathogenesis of endometrial hyperplastic processes.

KEY WORDS: urogenital infections, microbiota, inflammatory diseases of pelvic organs, hyperplastic processes

Wiad Lek. 2024;77(9):1888-1891. doi: 10.36740/WLek/195137 DOI

INTRODUCTION

The inflammatory processes of female genital organs make up 55-70% of cases in the structure of all gynaecological diseases, 40-50% of which are vaginal infections. In spite of a considerable amount of scientific research and the latest antibacterial drugs, there is no tendency for the inflammatory diseases to decrease.

The vaginal microbiome is a dynamic system that can change periodically under the influence of endogenous physiological factors (age, phase of the menstrual cycle, etc.).These changes are usually temporary and do not affect a woman's health [1].Many exogenous factors also have a significant impact on the state of the vaginal microbiome. These include poor hygiene and frequent douching, smoking and other unhealthy habits, psycho-emotional stress, the use of antibiotics, hormones, contraceptives, immunosuppressants, cytostatics and radiotherapy.

Over the recent years social and behavioral factors have negatively impacted the women`s genital organs. This has resulted in a decrease in immune defense against infections and has enabled the diseases to spread [2, 3].Chronic alcoholism, drug addiction, low living standards (insufficient and irrational nutrition), and ongoing stressful situations are examples of social factors [4, 5]. The behavioral factors include the early onset of sexual life, a great number of sexual contacts and sexual partners, unconventional forms of sexual contact(orogenital, anal), sexual relations during menstruation etc.

Thepathogenetic mechanisms of proliferative diseases of the reproductive system are unclear. Since the 1990s, the leading role in the development of hyperplastic processes has been attributed to increased oestrogen concentration - absolute or relative hyperestrogenism and imbalance of oestrogenhydroxymetabolites [6-8].

In the last few decades in Ukraine, as in most countries of the world, there has been an increase in the incidence of hormone-dependent tumours, especially endometrial hyperplastic processes (EHP).According to both domestic and foreign authors, they represent

a multifaceted problem of theoretical and clinical medicine and hold one of the leading places in the structure of gynecological pathology.Their frequency ranges from 14% to 83% [6, 7].The relevance of the problem is determined by the possibility of HPE degeneration into endometrial cancer.Numerous studies confirm the likelihood of developing oncological processes in the setting of HPE, which is possible in 4-67.2% of patients. This pathology is also of interest due to the tendency to a long and recurrent course, the absence of specific, pathognomonic symptoms, the complexity of differential diagnosis, and the difficulty of individualising treatment [7].

AIM

Investigation of hyperproliferative diseases of the female genital organs as a consequence of mixed urogenital infections.

MATERIALS AND METHODS

The study included 56 women of reproductive age who experienced discomfort in the external genital area in the form of excessive vaginal discharge and/or unpleasant odour of the discharge, itching in the external genital area (main group). The control group consisted of 30 somatically and gynaecologically healthy patients.

The contingent of the examined women did not differ greatly in terms of age, general and obstetric and gynaecological history. Comparisons were made based on the principles of selecting patients with the same nosologies and somatic background.

The microbiocenosis of the urogenital tract was assessed by real-time PCR using the Femoflor-16 test system. As for the additional examination of hormonal status, the day of the menstrual cycle (MC) and preparation for the tests were taken into account. On the 3-5 day of the menstrual cycle, estradiol, NOMA index, and prolactin were additionally examined. On the 21-22 day of the MC - prolactin, progesterone, estradiol. Material: venous blood, testing method: immunochemical with electrochemiluminescent detection (ECLIA), analyser and test system: Cobas 6000/ Cobas 8000, Roche Diagnostics (Switzerland). Ultrasound examination of the pelvic organs was performed on the 7-8 and the 21-22days of MC.

RESULTS

The following complaints were noted in mixed infections: increased amount of vaginal discharge (28 women / 50% of the main group), unpleasant odour of discharge (19 women / 34%), less frequently - itching (10 women / 18%), burning (11 women / 20%), pain during intercourse (8 women / 14%), digestive tract disorders - bloating and irregular bowel movements (13 women / 23%). Two or more complaints were noted in 29 patients (52% of the main group). The microscopy revealed a small number or complete absence of lactobacilli, abundant polymorphic gram-negative and gram-positive bacillus and coccusmicroflora, and the presence of'key cells'.The number of leukocytes was variable and often did not correspond to the severity of vaginal dysbiosis.

In the majority of patients (84%), the PCR examination revealed severe vaginal dysbiosis. The disruption of the vaginal microbiome was characterised by a significant predominance of aerobic and anaerobic opportunistic microorganisms with a significant decrease (80%) and in some cases (4%) a complete absence of lactobacilli. Moderate dysbiosis was diagnosed in 9 (16%) of the women in the main group. Among the facultative anaerobic microorganisms, the most commonly detected were Enterobacterium spp. Obligate anaerobic microorganisms were mostly represented by the groups Gardnerellavaginalis / Prevotellabivia / Porphyromonas spp.,Lactobacteriumspp. / Clostridiumspp. andAtopobiumvaginae.

Before coming to the clinic, 20 (36%) patients were receiving antibiotic therapy for extragenital pathology; 12 (21%) were being monitored for infertility; 6 (11%) women were preparing for assisted reproductive technologies. The average age of patients was 27.8±6.4 years. Treatment for mixed urogenital infections in the history was noted in 26 (46%) women, and recurrent disease (2-4 relapses per year) was diagnosed in 11 (20%) patients.

Detection was based on complaints and the nature of vaginal discharge, as well as the use of laboratory methods, according to current understanding of the clinical diagnosis of mixed urogenital infections.Normal human flora is a set of microbiocenoses that occupy numerous ecological niches on the skin and mucous membranes in places of contact of the human body with the environment. The microbiocenosis is a highly sensitive indicator that responds with quantitative and qualitative changes to any disturbances in the external and internal environment. A change in the number of a particular species of microorganisms in a habitat or the appearance of bacteria that are not typical for a given habitat is a signal of adaptive or irreversible changes in the relevant link of the microecological system [1].

Lactobacilli of various types are an important component of the vaginal microflora, with a normal content of $1x10^7$-10^9 CFU/ml. Lactobacilli, in turn, produce lactic acid, which acidifies the vaginal environment (normal pH is 3.8-4.5), as well as a number of microbicidal factors and hydrogen peroxide (H2O2), which inhibit the growth of many microorganisms [2, 5].

The microorganisms that can cause inflammatory diseases of the genital organs are mainly sexually transmitted mi-

crobial-protozoan-viral associations, characterized by new properties, specific clinical features and are not pathological components of individual infectious components. They can persist in a human body lifelong, leading to periodic exacerbation of adnexitis, cervicitis, vaginitis, bartholinitis, cystitis, etc., which result in infertility [8].

The study included women with minimal or no lactobacilli with polymorphic gram-negative and gram-positive bacillus and coccus flora. A microbiological examination of vaginal discharge was performed before treatment.

The following pattern was observed when examining the hormonal status of the above mentioned groups: in patients of the main group, the level of estradiol was elevated - 256.0 pg/ml; the NOMA index was also observed in the range of 3.2-3.5 (increased) in 50% of patients of the main group; prolactin level - 10.2-18.0 ng/ml (normal). All these examinations were performed in the first phase of MC (on day 3-5). As for the patients in the control group, these indicators were within the normal range. During the studies in phase II of MC (on day 21-22): prolactin levels in patients of the main group were slightly elevated - in the range of 25.4-27.9 ng/ml, in patients of the control group they remained within the normal range (8.8-16.7 ng/ml); progesterone levels - 0.8-1.1 ng/ml (reduced) in patients of the main group and 4.6-19.2 ng/ml (normal) in patients of the control group; estradiol levels were similarly elevated as in phase I of MC - 360.2 pg/ml in patients of the main group, compared to the control group, where they were normal (within 130.4-183.5 pg/ml).

The composition of the urogenital microbiota of a healthy woman may have characteristics depending on the phase of the menstrual cycle and age [5, 6]. The optimal number and species composition of lactobacilli ensures the acidity of the vaginal secretion within 4-4.5, the formation of a barrier to the fixation of opportunistic and pathogenic flora to vaginal epithelial cells and participates in a number of immune mechanisms [5, 7]. The composition of the microbiome is strongly correlated with the state of the vaginal mucosa which depends primarily on the levels of sex hormones, in particular estrogens. The latter stimulate the proliferation of stratified squamous epithelium and the production of glycogen in surface cells, which is a substrate for the vital activity of lactobacilli [5, 8]. Due to the influence of estrogen, the mucosal plug of the cervical canal is saturated with bactericidal enzymes and is able to act as a barrier, a kind of filter that prevents the spread of pathogenic pathogens (specific or nonspecific infection) to the upper parts of the urogenital tract and the generalisation of the inflammatory process. Progesterone slows down the maturation of the stratified squamous epithelium. It causes cytolysis and desquamation of stratified squamous epithelium with the release of glycogen into the vaginal lumen. Under the influence of cellular enzymes sugars, maltose and glucose are formed from glycogen, which is a nutrient medium for lactic acid bacteria (lactobacilli).

During the pelvic ultrasound examination on days 7-8 and 21-22 of the MC, the following pattern was observed: the endometrial level in phase I was 10-11 mm in patients of the main group, and in phase II, its thickening was also observed (up to 16-17 mm). We interpreted this as a discrepancy with the phase of the menstrual cycle - endometrial hyperplasia.

DISCUSSION

According to the data available in the literature, 20-55% of women suffering from vaginal dysbiosis are also diagnosed with intestinal dysbiosis, which indicates a single dysbiotic process in the body with a dominant display in the genital or digestive system [1, 2]. The main representatives of the obligate vaginal microflora found in women of reproductive age are lactobacilli, which play a leading role in maintaining normal vaginal biocenosis due to high competition and antagonism to most pathogenic and opportunistic bacteria. Lactobacilli metabolise glycogen into glucose and eventually into lactic acid, which maintains the acidic reaction of the vaginal contents (pH 3.8-4.4), which is necessary for the growth of lactobacilli.

The restoration of an adequate state of the genital tract microenvironment in women with mixed infections is based on the creation of an optimal pH level for the dominant influence of normal representatives of this biotope. The predominance of natural bacterial protective agents will not only restore the colonization resistance of the vaginal biotope, but will also reduce the frequency of recurrence of vaginal microcenosis disorders.

Lactobacilli of various types are an important component of the vaginal microflora, with a normal content of $1x10^7$-10^9 CFU/ml. Lactobacilli, in turn, produce lactic acid, which acidifies the vaginal environment (normal pH is 3.8-4.5), as well as a number of microbicidal factors and hydrogen peroxide (H2O2), which inhibit the growth of many microorganisms [2, 5].

The microorganisms that can cause inflammatory diseases of the genital organs are mainly sexually transmitted microbial-protozoan-viral associations, characterized by new properties, specific clinical features and are not pathological components of individual infectious components. They can persist in a human body lifelong, leading to periodic exacerbation of adnexitis, cervicitis, vaginitis, bartholinitis, cystitis, etc., which result in infertility [8].

CONCLUSIONS

Taking into account the information described above, it can be noted that mixed urogenital infections affect the pathogenesis of endometrial hyperplas-

tic processes. The concept of vaginal biocenosis is currently in the focus of attention of many scientists and doctors. The functioning and coordinated interaction of all parts of the microecosystem is ensured by the immune and endocrine systems. It reflects their functional state and depends on factors of both the internal and external environment. A disruption in one of these links invariably leads to disruption of the vaginal microenvironment which can further lead to the development of inflammatory processes in the genital tract, which in turn can lead to hyperplastic processes in the endometrium.

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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

RECEIVED: 11.06.2024
ACCEPTED: 24.09.2024

ORIGINAL ARTICLE

CONTENTS

Influence of elemental composition on the stability of restorative structures in permanent teeth in children from different geographical areas of residence

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ABSTRACT

Aim: To evaluate the correlation between the mineral composition of the hard tissues of the teeth and the percentage of defects and loss of various restorative materials in permanent teeth in children living in the lowland and mountainous geographical zones of Transcarpathian region.

Materials and Methods: 1050 permanent teeth of different groups filled with different materials were studied. An ultra-microscopic examination by the method of raster electron microscopy was carried out and the mineral composition of the teeth was evaluated. On the AZtecOne microanalytical accelerator complex with the X-MaxN20 detector.

Results: Improbable differences in indicators were established in children living in lowland and mountainous areas of Zakarpattia region, when using glass ionomer cements both after 6 months (6.7%; 7.2%; p>0.05) and after 12 months. months (20.0%; 16.3%; p>0.05); with direct composite restorations after 6 months (4.4%; 6.0%; p>0.05) and after 12 months (9.6%; 11.1%; p>0.05); with indirect composite restorations after 6 months (2.1%; 4.0%; p>0.05) and after 12 months (10.5%; 10.0%; p>0.05).

Conclusions: The reason for the higher percentage of fillings made of glass ionomer cements in the permanent teeth of children from mountainous areas is the significantly lower calcium content and their mineral composition (p>0.05)

KEY WORDS: permanent teeth, restorative structures, glass ionomer cements, light-curing composites, indirect restorations

Wiad Lek. 2024;77(9):1892-1901. doi: 10.36740/WLek/195138 DOI

INTRODUCTION

The restoration of the lost hard tissues of the teeth is carried out by direct and indirect fixed structures made of various types of restorative materials according to the indications [1]. Direct restorations mean fillings using glass ionomer cements (GC) and composites, mostly light-cured, and indirect restorations include inlays, veneers, and when more than 2/3 of the tooth tissue volume is lost, crowns [2]. With strict adherence to manufacturing technologies, all types of modern structures are durable, which will satisfy aesthetic and functional requirements. However, even in the absence of manufacturing errors, defects may occur in restorative structures, even their complete loss [3, 4]. An important etiological role in this is played by the condition of the hard tissues of the teeth, in particular, the mineral composition [5-7]. The mineral state of permanent teeth largely depends on the intake of minerals into the human body with drinking water and food [8]. Drinking water has physical and chemical characteristics specific to different geographical zones and regions of residence [9]. The Transcarpathian region, which is a natural zone of biogeochemical deficiency of fluorine and iodine, is an unfavorable background for the formation of a dental prosthesis system in children who live permanently in these territories and consume drinking water available for these territories [10].

The question of the influence of the mineral composition of the hard tissues of permanent teeth and the stability of restorative structures has not been given sufficient importance in the scientific literature, which served the purpose of our study.

AIM

The aim of the study was to assess the correlation between the mineral composition of the hard tissue of the teeth and the percentage of defects and the loss of various restorative structures in the permanent teeth of children living in the lowland and mountainous geographical zones of the Transcarpathian region.

MATERIALS AND METHODS

525 medical records of dental patients (form 043) of patients of lowland (Uzhgorod) and mountain (Rakhiv) geographic areas of residence in the Transcarpathian region were subjected to paraclinical content analysis to evaluate the performed restorative structures in permanent teeth and their dispensary observation after 6 and 12 months after treatment. Statistical analysis was carried out on the basis of 1050 filled permanent teeth (630 in children from the lowland area of residence and 420 from the mountain area), their distribution was carried out by group affiliation and by the material from which the filling was made. An ultra-microscopic study was carried out by the method of raster electron microscopy (electron microscope SEO-SEM Inspect S50-B) and the mineral composition of the teeth was assessed (characteristic X-ray radiation induced by a proton beam (PIXE, μ-PIXE methods) was carried out on the AZtecOne microanalytical accelerator complex with the X-MaxN20 detector (manufacturer Oxford Instruments plc) B, (Sumy State University, Ukraine). Statistical research was carried out using the standard program Microsoft Excel, 2010 and the package of statistical programs «Statistica 13.0» [11-13].

RESULTS

Restorative structures in permanent teeth are represented by direct restorations, that is, fillings made of light-curing composite materials and glass ionomer cements, and indirect restorations in the form of inlays and veneers.

The percentage distribution in relation to the material of restorative structures was as follows: fillings made of light-curing composite materials accounted for 88.1%, of which 38.1% were in molars, 30.2% in incisors, 12.7% in premolars, 7.1% in canines ; fillings made of glass ionomer cements accounted for 2.4%, of which in molars - 1.6%, in premolars - 0.8%; indirect restorations accounted for 9.5%, of which 3.9% in molars, 3.2% in incisors, and 2.4% in canines.

Figure 1 shows the percentage ratio of the number of filled permanent teeth with different types of materials in lowland and mountainous geographical zones (Fig. 1).

Therefore, the number of permanent teeth treated with glass ionomer cements in the lowland geographical area (the city of Uzhhorod) is different compared to the mountainous geographical area (the city of Rakhiv). Yes, incisors and canines were not filled with this material; premolars filled (5-4.7=) by 0.3 percent more in Rakhiv; molars - by (10-8.3=) 1.7 percent more in the lowland zone. Regarding the use of direct composite restorations: incisors filled more in Uzhhorod by 13.5 percent; canines - by 1.2 percent, molars - by 1.2 percent; premolars filled 2.8 percent less. Regarding indirect restorations, inlays or veneers: mainly molars were treated in the city of Rakhiv - a mountainous geographical area with an index of 7.2 - the maximum compared to other teeth and a lowland geographical area.

In general, the percentage of teeth treated with the materials analyzed above is higher compared to such treatment carried out in Uzhhorod for incisors and canines and in Rakhiv for premolars and molars (Fig. 2).

Figure 3 shows the total percentage of filled teeth in the two geographical areas studied for permanent teeth. From the figure, there is an obvious advantage of using direct composite restorations in Uzhhorod and a slightly lower percentage of the use of this treatment in Rakhiv (Fig. 3).

Thus, the share of permanent teeth filled with different types of restorative materials relative to the two studied geographical areas and types of teeth is presented as follows: in general, the share of use of these materials for the treatment of permanent teeth is different in different geographical areas. In all cases, the difference is small, from 0.5 to 1.8, and in total up to 5.0 for indirect restorations - inlays, veneers.

Figure 4 presents the results of the obtained data regarding the placed fillings and their defects in permanent teeth for the lowland and mountainous geographical zones of the Transcarpathian region, which were treated with glass ionomer cements (Fig. 4).

It is noticeable that in both geographical zones the defects and loss of teeth are similar quantitatively and after a year they prevail mainly in the lowland zone. It is also evident that the rate of filling defects or loss increases by 12 months in both geographic areas for all types of teeth.

Figure 5 shows the percentage distribution of fillings placed for direct composite restorations in two geographic areas (Fig. 5).

The number of defects and loss of fillings increases reliably up to 12 months in molars.

Figure 6 shows the results of teeth treatment through indirect restorations - inlays, veneers in percentage. Also shown are the indicators of fillings and defects after 6 and 12 months with this material. It is obvious that premolars and molars were treated the least with this material (Fig. 6).

The number of defects is the highest after 12 months in both geographical areas.

According to the results of the correlation analysis of the relationship between the materials used for the treatment of permanent teeth in terms of the prevalence of tooth defects after 6 and 12 months, the following pairs were established that showed a reliable

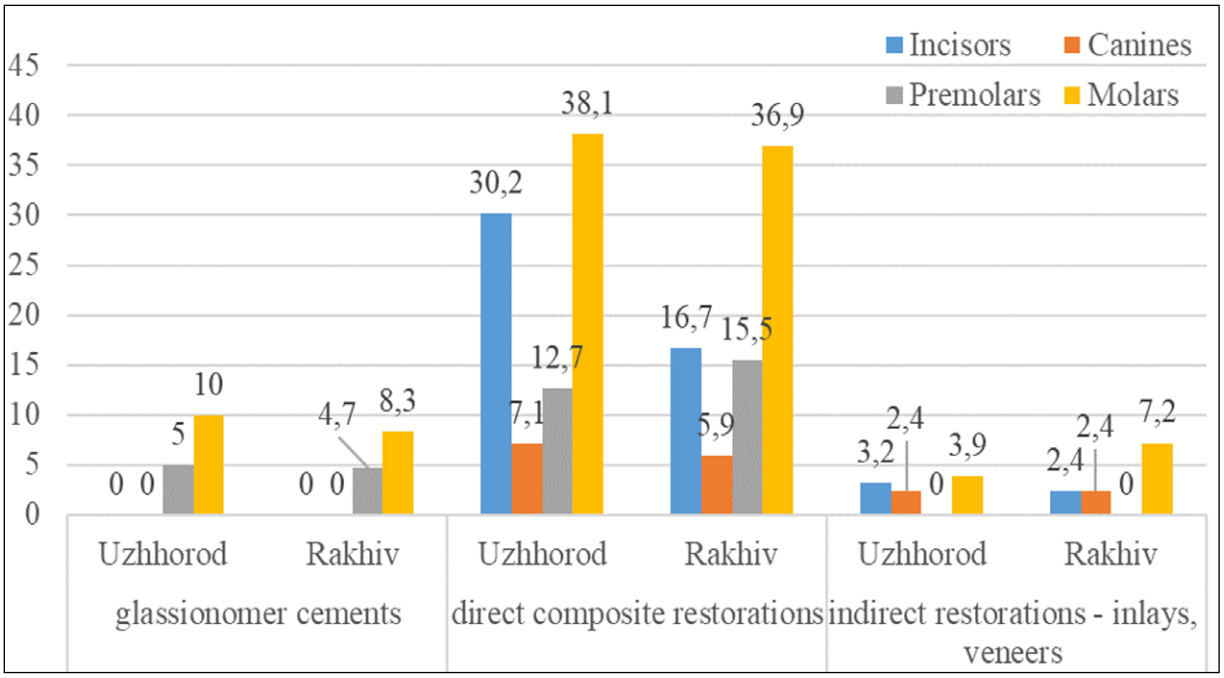

Fig. 1. The percentage ratio of the number of filled permanent teeth with different types of materials in lowland and mountainous geographical zones.

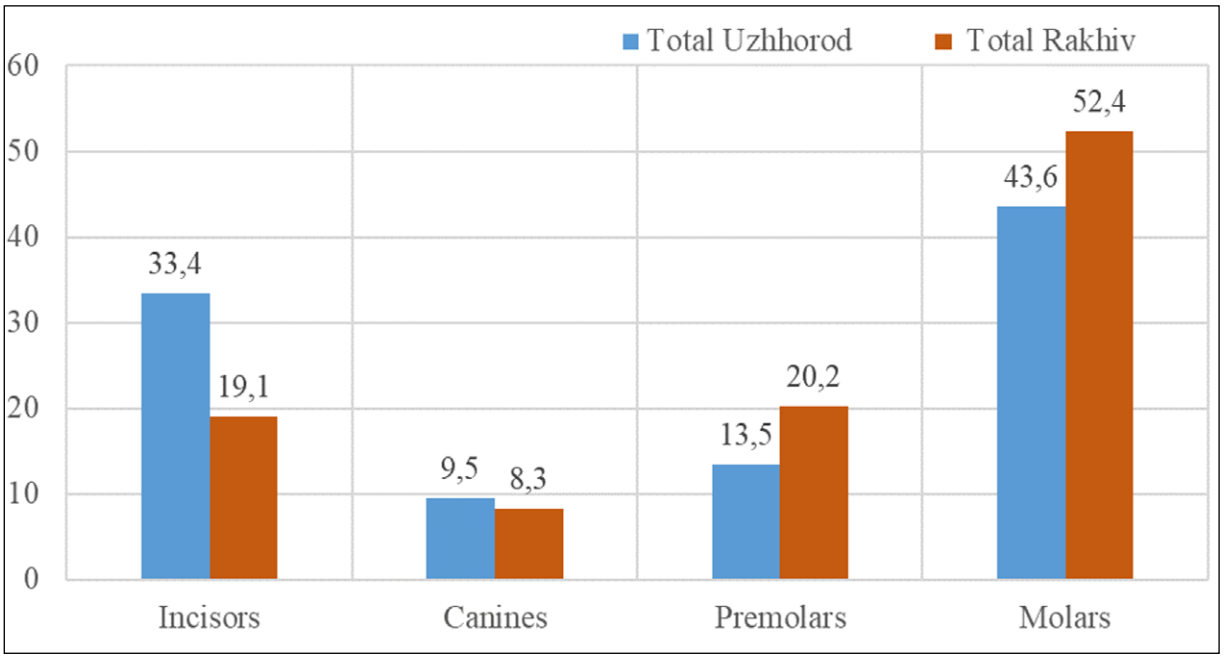

Fig. 2. Total percentage of filled permanent teeth with different restorative materials.

relationship, where GC – Glass ionomer cements; DCR - Direct composite restorations; IR – Indirect restorations, LZ - lowland zone, MZ - mountain zone.

GC, placed fillings (LZ) and GC, defects 6 months (LZ), IR, defects 6 months (LZ), IR, defects 6 months (MZ) with R=0.88; P<0.05 and DCR, defects 6 months (MZ), DCR, defects 12 months (MZ) with R=0.92; P<0.05.

GC, placed fillings (MZ) and GC, defects 6 months (LZ), IR, defects 6 months (MZ), IR, defects 6 months (MZ) with R=0.88; P<0.05 and DCR, defects 6 months (MZ), DCR, defects 12 months (MZ) with R=0.92; P<0.05.

DCR, placed fillings (LZ) and DCR, defects 6 months (MZ), DCR, defects 12 months (MZ) with R=0.97; P<0.05; and IR, placed fillings (IR) with R=0.90; P<0.05.

DCR, placed fillings (MZ) and DCR, defects 6 months (MZ), DCR, defects 12 months (MZ) with R=0.97; P<0.05; and IR, placed fillings (IR) with R=0.90; P<0.05.

IR, placed fillings (LZ) and DCR, placed fillings (LZ), DCR, placed fillings (MZ), DCR, defects 6 months (LZ), DCR, defects 12 months (LZ), IR, defects 12 months

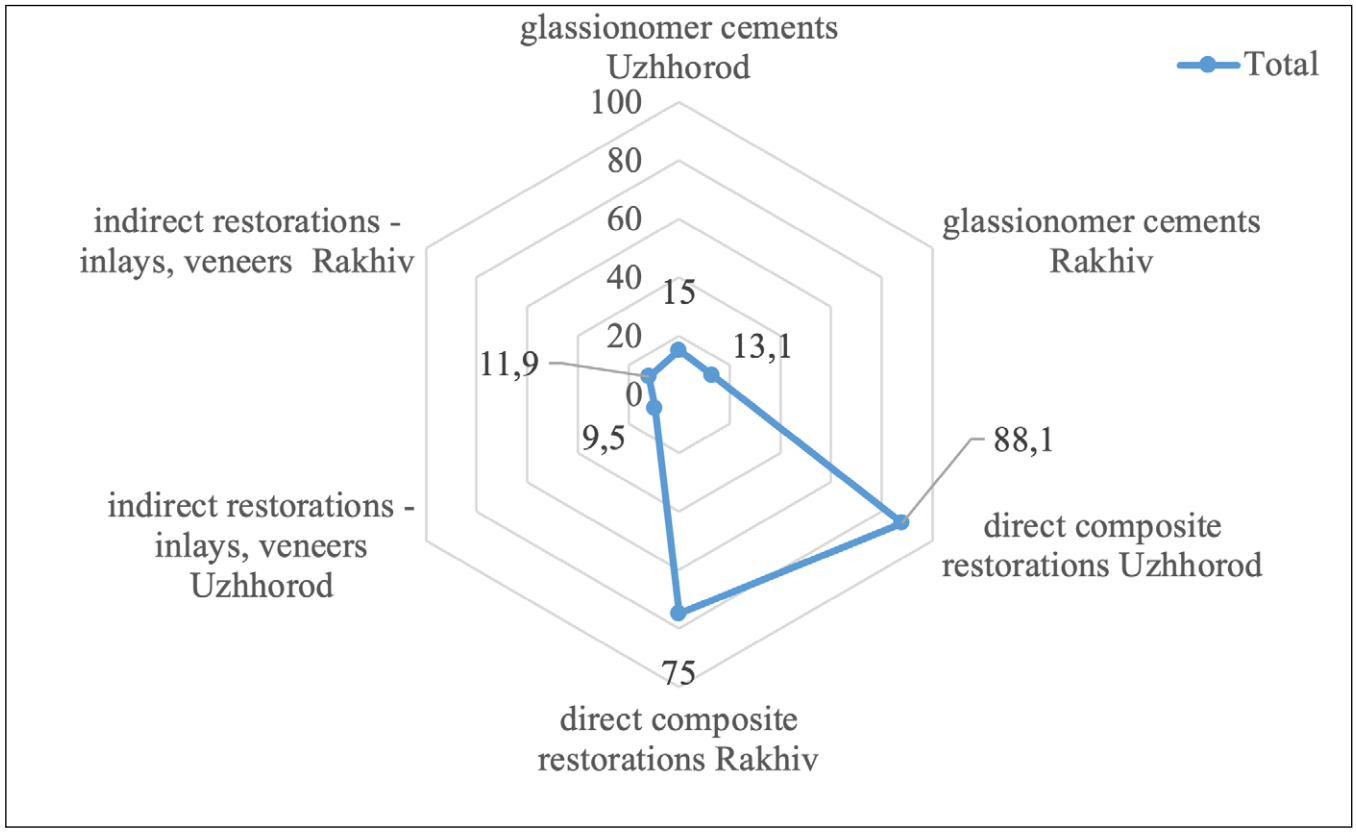

Fig. 3. Distribution of the prevalence of various restorative structures and fillings in permanent teeth by group affiliation of teeth in children who live in two geographical zones together.

(LZ) with R=0.90; P<0.05; and IR, placed fillings (MZ), IR, defects 12 months (MZ) with R=0.97; P<0.05.

IR, placed fillings (MZ) and GC, defects 6 months (LZ), IR, defects 6 months (LZ), IR, defects 6 months (MZ) with R=0.89; P<0.05; and IR, fillings placed (LZ), IR, defects 12 months (LZ) with R=0.97; P<0.05.

Thus, it can be seen that the correlation indicators are very high and amount to R=0.90 with a statistical significance level of p<0.05. Figure 7 presents the results of the cluster analysis, which shows the degree of difference or similarity between the studied indicators. It is noticeable that there is a certain similarity between the group of materials - glass ionomer cements and compomers and their difference from light curing composite materials. The figure shows only the presence of two separate groups - placed seals and defects (Fig. 7).

Somewhat separately, forming a subgroup, defects were placed after 12 months in the lowland and mountain zone for teeth treated with glass ionomer cements, which may indicate some difference relative to all other materials and defects.

Table 1 shows the results of spectral measurement of the weight of elements present in the enamel and dentin of permanent teeth in children from lowland and mountainous geographical areas (Table 1).

Only 9 elements are observed in different quantities. All elements are present in the teeth of both geographical areas. It can be seen from the table that in the enamel and dentin of the patients' teeth, carbon C (51.34%; 45.79%; p<0.05) prevails in the lowland zone and oxygen O (52.13%; 52.42%; p< 0.05) in the mountain zone. Figure 8 shows the average weight of microelements of enamel and dentin in % (Fig. 8).

Al is also present everywhere, the indicators of which prevail in the mountainous zone. Regarding Mg, which is present everywhere in a relatively small amount relative to all other trace elements, and S is not observed in permanent teeth. Thus, sulfur in the patients' temporary teeth can be considered an indicator of the mountainous area of their residence, and a high calcium index can be a characteristic of the patients' permanent teeth. When comparing the percentages of the appearance of defects of various restorative structures and their loss in permanent teeth of children living in the lowland and mountain areas of the Transcarpathian region, differences were found when using glass ionomer cements after 6 months (6.7%; 7.2%; p>0.05) and after 12 months (20.0%; 16.3%; p>0.05); with direct composite restorations after 6 months (4.4%; 6.0%; p>0.05) and after 12 months (9.6%; 11.1%; p>0.05); with indirect composite restorations after 6 months (2.1%; 4.0%; p>0.05) and after 12 months (10.5%; 10.0%; p>0.05).

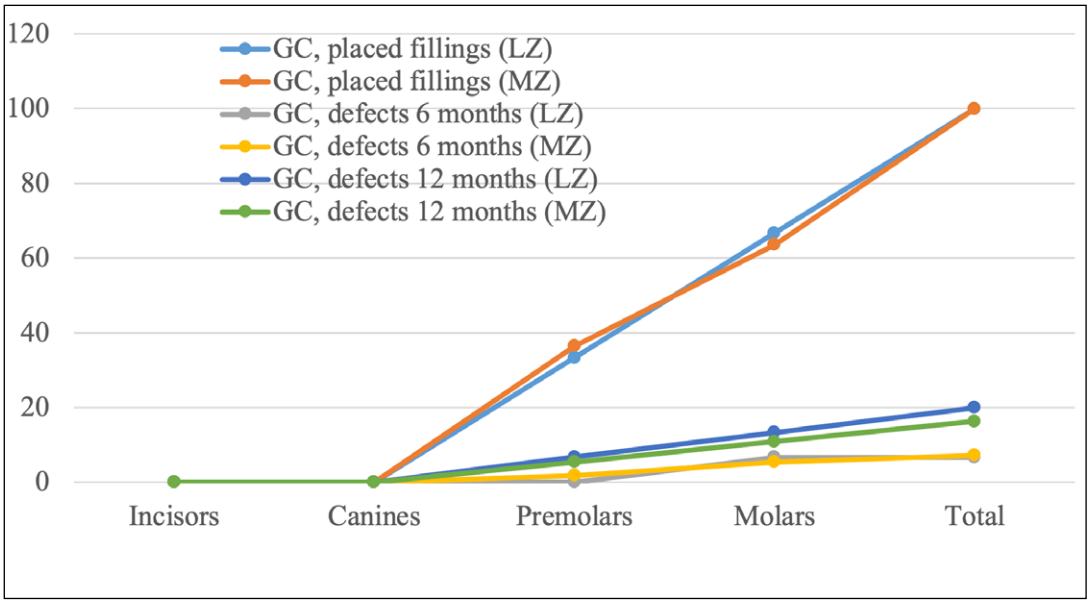

Fig. 4. Percentage of placed fillings in permanent teeth that were treated with glass ionomer cements in two geographical areas.

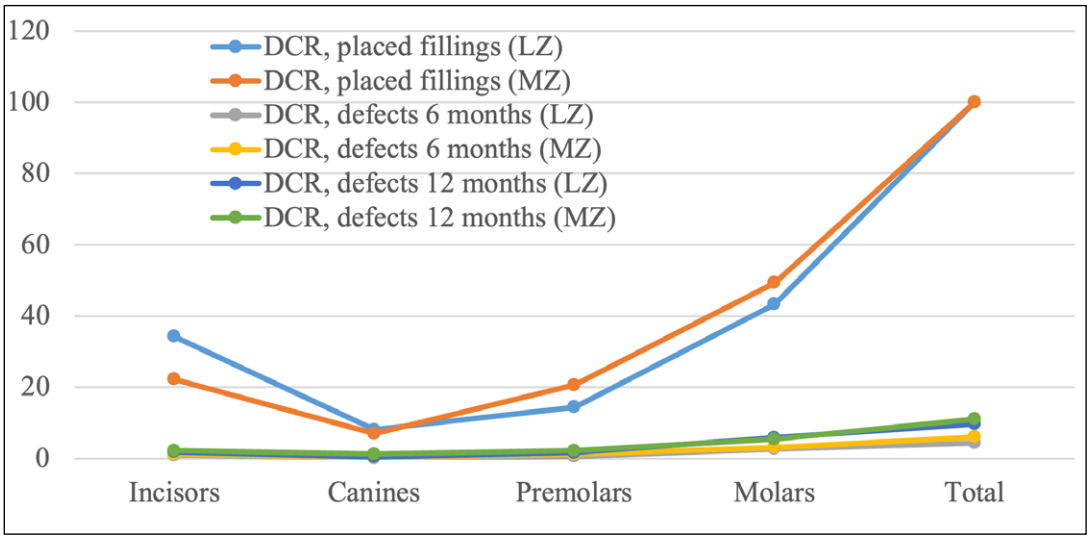

Fig. 5. Percentage of fillings placed in permanent teeth where direct composite restorations were performed in two geographic areas.

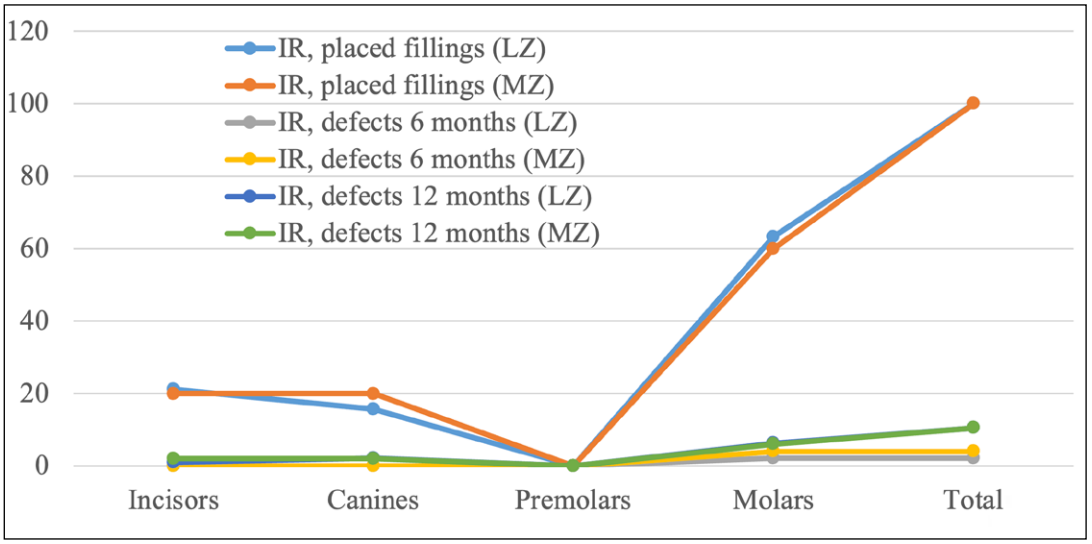

Fig. 6. Percentage of placed fillings and defects after 6 and 12 months in permanent teeth that were treated through indirect restorations - inlays, veneers in percentages in two geographical areas.

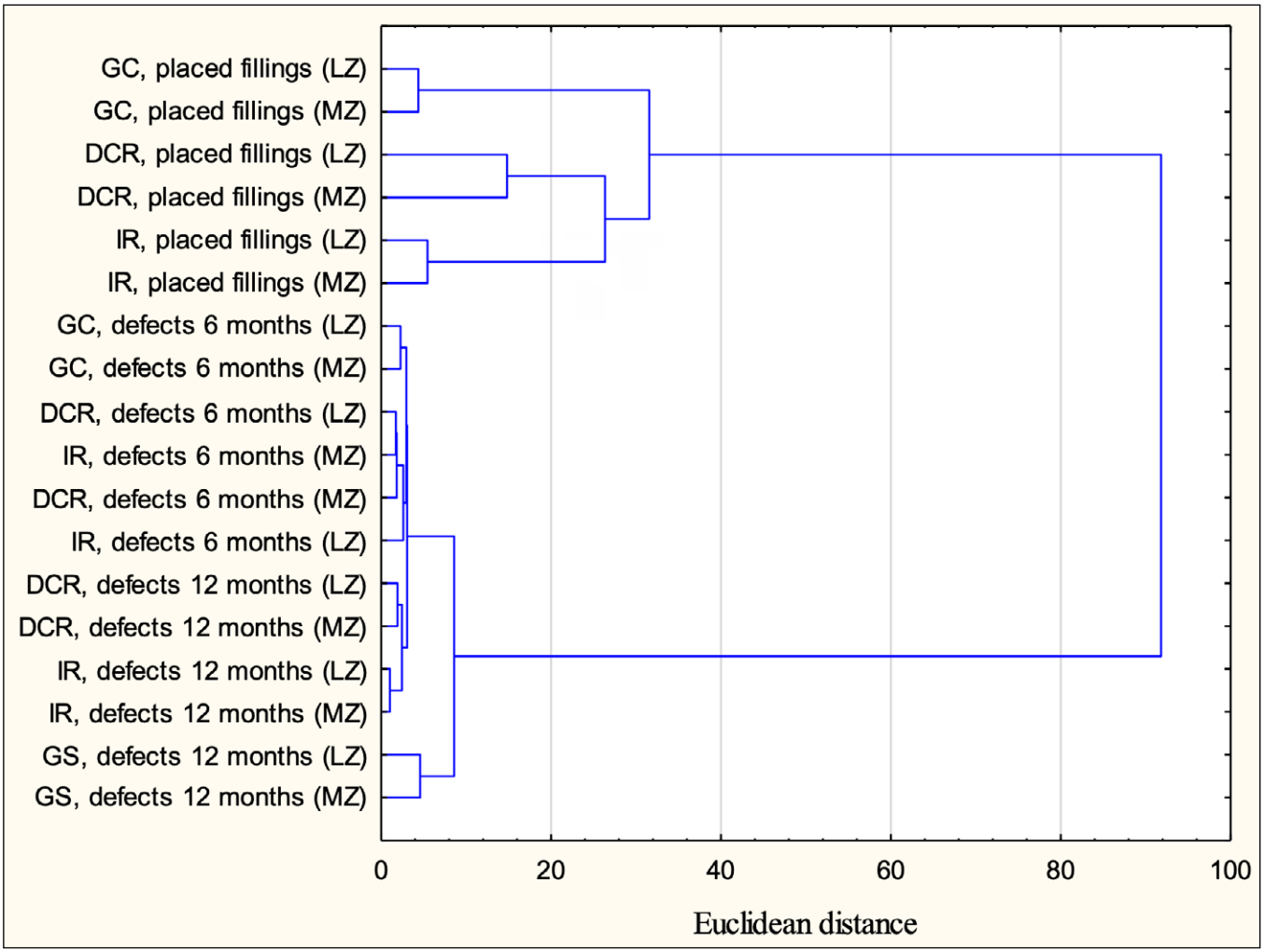

Fig. 7. Results of cluster analysis showing the degree of difference or similarity between the studied indicators.

According to Figure 9, we can hypothesize that, probably, in the permanent teeth of children in the mountain zone, in a higher percentage of cases, fillings also made of glass ionomer cements fall out, which manifests itself mainly after 12 months, because the mineral composition of their teeth is recorded statistically reliably (p>0, 05) lower calcium content (Fig. 9).

DISCUSSION

Despite the rather complete and perfect study of the macroelement composition of the hard tissues of the teeth, the importance of microelements in maintaining the health of the oral cavity is still a subject of research and debate. Some microelements are etiological factors of the occurrence and progression of caries, while others, on the contrary, contribute to the remineralization of hard tooth tissues. The influence of metals on the condition and functioning of the hard tissues of the teeth has been studied by a number of authors.

Researches related to the layer-by-layer, zonal distribution of essential microelements in the dentin and enamel of intact teeth and the distribution of such cariostatic elements as calcium, fluorine, and phosphorus on the surface of the enamel and in the parapulpous dentin attracted the attention of scientists. Thus, Mamaladze M. et al. (2022) conducted an X-ray spectral analysis of 6 intact extracted teeth using scanning electron microscopy (SEM) in order to identify trace elements in the structures of human teeth and determine their localization and concentration in 6 areas of these teeth: surface enamel, enamel thickness, enamel-dentin border, parapulpal dentin, root dentin and cementum. The researchers found that the distribution of essential trace elements in the hard tissues of the teeth is uneven, and fluoride in the hard tissues is contained only in minimal concentrations [14].

To improve the optical properties of restorative materials, Pink K. et al. 2022 applied collimated transmission spectroscopy, in particular, determined the extinction coefficient of human tooth enamel to

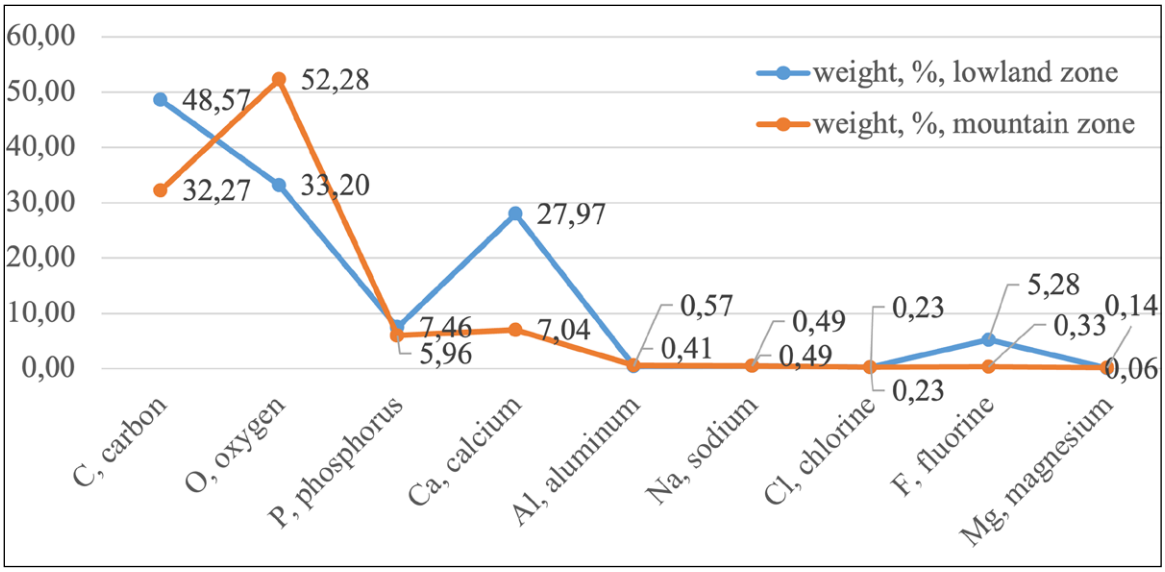

Fig. 8. Average weight values of microelements of enamel and dentin, %.

Table 1. Interrelationship of the elemental composition of permanent teeth of patients from the lowland and mountain areas of residence of the patients

Element, permanent teeth	Weight, %, enamel, lowland	Weight,%, dentin, lowland	Weight,%, enamel, mountain zone	Weight,%, dentin, mountain zone
C, carbon	51,34±6,32	45,79±0,25	27,53±2,35	37,00±4,34
O, oxygen	34,14±3,35	32,26±0,20	52,13±5,27	52,42±5,78
P, phosphorus	8,34±2,01	6,58±0,12	7,79±1,09	4,13±0,12
Ca, calcium	31,62±4,03	24,31±0,12	9,96±1,26	4,11±0,24
Al, aluminum	0,29±0,01	0,52±0,01	0,49±0,01	0,64±0,03
Na, sodium	0,53±0,02	0,45±0,04	0,53±0,02	0,45±0,02
Cl, chlorine	0,28±0,01	0,17±0,01	0,28±0,01	0,17±0,01
F, fluorine	5,48±0,93	5,07±0,21	0,48±0,01	0,17±0,01
Mg, magnesium	0,06±0,01	0,05±0,01	0,16±0,01	0,12±0,01
P<0,05				

provide additional optical properties of enamel and improve the propagation of light in teeth and improve the optical properties of restorative materials. The authors determined the uniqueness of the optical behavior of each sample, which is of great importance in the development of restorative dental materials [15].

Fischer A. et al. 2009 studied the concentration of metals (Cd, Pb, Mn, Cu, Cr, Fe, Zn, Na, K, Mg, Ca) in temporary and permanent teeth, taking into account their location in the oral cavity, that is, on the upper or lower jaw and found that the concentration of metals in temporary teeth is probably higher than in permanent teeth, and the relationship between the concentration of metals in permanent and temporary teeth and their location on the jaw was revealed, higher metal concentrations were found in the teeth of the upper jaw than in the lower jaw [16].

Shaik I. et al. 2021, reviewed the presence of trace elements in teeth and their role in dental health and development. The role of elements such as calcium and phosphate has been carefully studied, unlike elements that are present in small amounts in the enamel and dentin of the teeth, although their absence can disrupt the healthy development of enamel and dentin and lead to defects in the development of teeth, as well as caries. In addition, excessive consumption of some trace elements can adversely affect the development and health of teeth [17].

The exact impact of micronutrients on dental and oral health is still unknown. Researchers have found that lead ions replace calcium, as well as calcium and phosphorus in bone mineral crystals, causing hypercalcemia and hyperphosphatemia, meaning lead

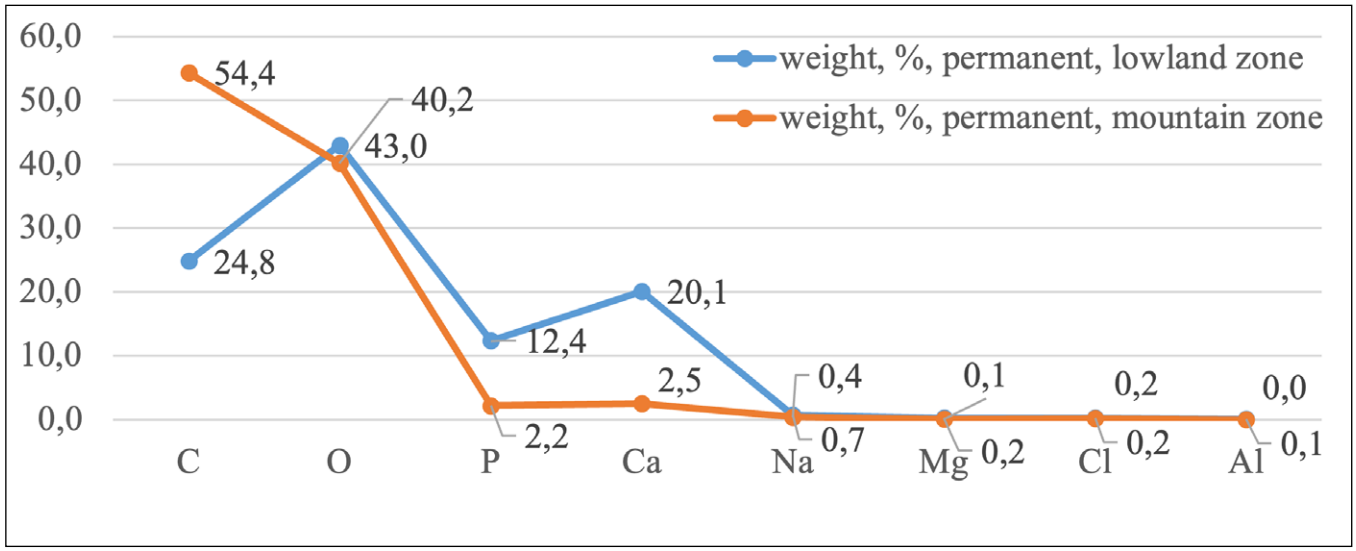

Fig. 9. Average values of the weight of trace elements and the percentage of defects in permanent teeth in two geographical areas, %.

is considered a caries-promoting element. A direct link between enamel hypoplasia and lead exposure in children was found [18].

For the first time, we conducted studies that determined the relationship between the mineral composition of enamel and dentin of permanent teeth and the stability of restorative structures made of different restorative materials. For this, a paraclinical content analysis and statistical analysis was carried out on the basis of 1050 filled permanent teeth (630 in children from the lowland area of residence and 420 from the mountain area), their distribution was carried out by group affiliation and by the material from which the filling was made. It was determined that permanent teeth were dominated by direct restorations in permanent molars made of light-cured composite materials in 38.1% of cases (240 units) in children from the lowland zone and in 36.9% of cases (155 units) in children from the mountain zone.

The percentage of loss or defects of fillings in permanent teeth made of glass ionomer cements and light-curing composite materials in children living in the mountainous area does not significantly differ from similar indicators in children living in the lowland area, namely after 6 months ($p>0.05$), and after 12 months ($p>0.05$); indicators regarding indirect restorations differ significantly after 6 months ($p<0.05$) and not significantly after 12 months ($p>0.05$). The appearance of defects or loss of fillings made of glass ionomer cements prevails after both 6 and 12 months.

Improbable differences in the appearance of instability of restorative structures in the permanent teeth of children from the mountain zone made of glass ionomer cements, light-curing composites on indirect restorations were determined after 6 months (7.2%; 6.0%; 4.0%; $p>0.05$), and after 12 months (16.3%; 11.1%; 10.0%; $p>0.05$); in children of the lowland zone, the differences are probable, and are after 6 months (6.7%; 4.4%; 2.1%; $p<0.05$) and after 12 months (20.0%; 9.6%; 10 .5%; $p<0.05$).

Estimation of the mineral composition of tooth enamel using spectrometry is an interesting and modern type of research that will allow with a high degree of probability to establish the micro- and macroelemental composition of enamel and determine the optimal ways of normalizing its composition. In addition, during restorative manipulations with the hard tissues of the teeth, the determination of the mineral composition of the teeth and the study of the relationship between the mineral composition of the hard tissues and the adhesive properties of the restorative filling materials with a high degree of efficiency will improve the quality of caries treatment.

The results of a spectral study of the hard tissues of the teeth will help to choose a filling material taking into account its adhesive properties, which will improve the long-term results of caries treatment and the quality of the performed restoration work.

CONCLUSIONS

Improbable differences in the stability of restorative structures in the permanent teeth of children living in the lowland and mountain areas of the Transcarpathian region were established when using glass ionomer cements both after 6 months (6.7%; 7.2%; $p>0.05$) and after 12 months (20.0%; 16.3%; $p>0.05$); with direct composite restorations after 6 months (4.4%; 6.0%; $p>0.05$) and after 12 months (9.6%; 11.1%; $p>0.05$); with indirect composite restorations after 6 months (2.1%; 4.0%; $p>0.05$) and after 12 months (10.5%; 10.0%; $p>0.05$).

According to the analysis of the mineral composition of the enamel and dentin of the teeth and its relationship with the prevalence of defects, the loss of fillings in different geographical areas, it is possible to hypothesize that, probably, fillings made of glass ionomer cements fall out in a higher percentage of cases in the permanent teeth of children in the mountainous area, because a statistically significantly ($p>0.05$) lower calcium content was recorded in the mineral composition of their teeth. Also, the loss of fillings will correlate with these indicators at an average or high level ($R=0.6$-0.8; $p<0.05$) and with the content of magnesium and sodium at a low or average level ($R=0.3$-0.6 ; $p<0.05$).

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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

RECEIVED: 14.06.2024
ACCEPTED: 25.09.2024

ORIGINAL ARTICLE

CONTENTS

Epidemiology of complications associated with gynecological laparoscopy procedures in Ukraine: results a multicenter study

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ABSTRACT

Aim: To estimate the frequency of complications during laparoscopic gynecologic surgery for benign diseases in women and identify associated risk factors in Ukraine.

Materials and Methods: A multicenter, prospective cohort study was performed in gynecological departments from 10 regional hospitals of Ukraine between January 1, 2020, to December 31, 2022. The study included gynecologic laparoscopies for benign diseases in women performed at these hospitals. To identify risk factors and variables associated with complications, crude and adjusted odds ratios were calculated with unconditional logistic regression. Surveillance was performed during 30 days after gynecological laparoscopy procedures.

Results: A total of 14,440 laparoscopic surgeries were performed, 2,340 (16.2%) complications cases were observed. Of all complication's cases, 74.9% were detected after hospital discharge. The overall frequency of major complications was 5.04%, and that of minor complications was 11.2%. The most frequently reported complications types were serious bleeding complications (20.6%), intestinal perforation (17.9%), mild anemia (16.7%), severe anemia (transfusion) (16.1%), failed laparoscopy (9.3%), minor bleeding complications (8.3%), postoperative hematoma (6.7%), urinary tract infection (5.6%), and fever (5.0%). The level of technical difficulty and existence of prior abdominal surgery, and obesity were associated with a higher risk of complications associated with gynecological laparoscopy procedures.

Conclusions: Results this study suggest a high frequency of complications associated laparoscopic gynecologic surgery in Ukraine. Greater technical difficulty and prior surgery were factors associated with a higher frequency of complications.

KEY WORDS: laparoscopic gynecologic surgery, complications, risk factors, outcomes, Ukraine

Wiad Lek. 2024;77(9):1902-1909. doi: 10.36740/WLek/195172 DOI

INTRODUCTION

Laparoscopic surgeries have evolved over decades to become a safe and the standard of care for abdominal surgeries. Laparoscopic surgery has become widely accepted by surgeons and patients as an effective technique to treat gynecologic pathologies [1]. Although laparoscopic surgeries have evolved and have become the standard of care over the years, the rate of major complications, namely vascular, bowel, and urological injuries, have remained rare but constant over the last 3 decades. Complications associated laparoscopic gynecologic surgery are comparatively rare, especially injuries of major vessels like the aorta, which is a retroperitoneal structure [2, 3].

Currently, as the technology has improved and surgical skills have increased, the nature and characteristics of laparoscopic procedures have also become more complex. At gynecological departments equipped for advanced laparoscopic surgery, procedures such as surgery for complex adnexal lesions, hysterectomies, pelvic floor repair, and resection for severe endometriosis are now performed by this approach [4].

It is an evidence-based fact that minimal access surgery is superior to conventional open surgery since this is beneficial to the women, community and the healthcare system. Over the past 50 years, many techniques, technologies and guidelines have been introduced to eliminate the risks associated with laparoscopic entry. No single technique or instrument has been proved to eliminate laparoscopic entry associated injury [2]. Proper evaluation of the women, supported by surgical skills and good knowledge of the technology and instrumentation is the keystone to safe access and prevention of complications during laparoscopic surgery.

Laparoscopic gynecologic surgery is associated with a low frequency of complications but is a procedure that is not without risk. Greater technical difficulty and prior surgery were factors associated with a higher frequency of complications [1].

According to the literature, the incidence of complications decreases as surgeons gain experience with laparoscopy [5], the growing difficulty of some procedures in gynecologic surgery may increase the frequency of severe complications (visceral and great vessel injuries) [6].

It is important for major complications to be diagnosed promptly during laparoscopy so that corrective measures can be taken intraoperatively [7, 8]. However, shortened hospital stays and minimally invasive or outpatient surgery have led to the delayed diagnosis of complications in the gynecology department rather than during the postoperatve hospitalization period.

The epidemiology of complications during laparoscopic gynecologic surgery and identify associated risk factors is not well understood and remains underestimated. The true incidence of complications during laparoscopic gynecologic surgery is not fully understood as outpatient surveillance data are lacking.

AIM

The aim this study to estimate the frequency of complications during laparoscopic gynecologic surgery for benign diseases in women and identify associated risk factors in Ukraine.

MATERIALS AND METHODS

DESIGN, SETTING AND PATIENTS

A multicenter, prospective cohort study was performed in gynecological departments from 10 regional hospitals of Ukraine between January 1, 2020, to December 31, 2022. The study including women who have undergone gynecologic laparoscopic surgery for benign diseases. All patients were local residents. Inclusion criteria: any women who underwent gynecological laparoscopic procedures, and had relevant medical data. Exclusion criteria: subjects who malignant tumor, severe dysfunction in the heart, lung, liver, kidney, mental diseases, fever due to medical causes, infection after cesarean section, urinary tract infection or thrombophlebitis.

DEFINITIONS

In this study the surgical indications for laparoscopy were classified into 3 groups according to the level of technical difficulty. These 3 groups were chosen based on the classifications of Chapron et al [7], Leonard et al [9], and Härkki-Sirén and Kurki [10], although in this study, groups 1 and 2 together were considered technically simple procedures, group 3 was considered moderate difficulty, and group 4 was considered complex surgery. In this study, the simple surgery group included tubal electrocoagulation, coagulation of bleeding areas without other procedures, and diagnostic laparoscopy with or without biopsy. The moderate difficulty group comprised ovarian surgery, including endometriosis (management of ectopic pregnancy, adnexectomy, cystectomy, salpingectomy, tubal plasty, and ovarian drilling), and removal of an intrauterine device that had migrated to the abdominal cavity. The complex surgery group included total and subtotal hysterectomy with or without pelvic lymphadenectomy and myomectomy.

DATA COLLECTION

In our study surveillance was performed during 30 days after gynecological laparoscopy procedures for to detect complications cases. All clinical records of patients undergoing laparoscopy procedures were included in the study. Data were collected from hospital records.

Data were collected by a questionnaire given to the women in ambulatory setting and combined with data from general practitioner and hospital records. Clinically relevant data of subjects with complications during laparoscopic gynecologic surgery were analyzed, and all clinical data were collected by obstetricians and related investigators receiving unified training. Information about patient characteristics, surgical procedure, laparoscopy-related complications, and length of hospital stay was entered into a database for later analysis. The following variables for patients' characteristics were recorded: age, morbid obesity (body mass index >35 kg/m^2), prior abdominal surgery, year of surgery, and length of hospital stay (in days). Age was classified into 3 categories: <30 years, 30 to 60 years, and >60 years.

Table 1. Characteristics of 14,440 gynecological laparoscopy procedures in perinatal centers of Ukraine, 2020-2022

Variable	No (%)
Age group (yrs.)	
<30	3,915 (27.1)
30–60	10,050 (69.6)
>60	475 (3.3)
Morbid obesity	255 (1.8)
Prior surgery	2,275 (15.8)
Level of technical difficulty	
Simple	5,370 (37.2)
Tubal ligation	4,435 (30.7)
Diagnostic laparoscopy	785 (5.4)
Laparoscopy and biopsy	80 (0.6)
Coagulation	70 (0.5)
Moderate	7,820 (54.2)
Unilateral adnexectomy	1,490 (10.3)
Bilateral adnexectomy	755 (5.2)
Salpingectomy	1,470 (10.2)
Cystectomy	3,770 (26.1)
Tubal plasty	30 (0.2)
Adhesiolysis	205 (1.4)
Ovarian drilling	25 (0.2)
Complex	1245 (8.6)
Subtotal hysterectomy	190 (1.3)
Total hysterectomy	520 (3.6)
LAVHa	420 (2.9)
Myomectomy	190 (1.3)
Length of hospital stay	
0–3 d	12215 (84.6)
4–5 d	1950 (13.5)
≥6 d	275 (1.9)

LAVH, laparoscopy-assisted vaginal hysterectomy.

ETHICS

Approval was granted by the Ethics Committee of Shupyk National Healthcare University of Ukraine. Consent was obtained by all participants in this study.

STATISTICAL ANALYSIS

All data were collected with Microsoft Excel. The statistical analysis was performed using IBM SPSS (Version 21.0, IBM SPSS Inc., Chicago, IL, USA). A descriptive analysis was produced for each variable. Differences between groups were identified with the $\chi 2$ test for qualitative variables. In all analyses based on bilateral comparisons, $P < 0.05$ was considered statistically significant. To identify the factors associated with major and minor complications, conversion to laparotomy, or failed laparoscopy, a specific logistic regression model was constructed for each dependent variable, and the crude and adjusted odds ratios were calculated together with their 95% confidence interval (CI).

RESULTS

INCIDENCE OF COMPLICATIONS

During the study period (2020-2022), a total of 14,440 laparoscopic surgeries were performed, 2,340 complications cases were observed. Of all complication's cases, 74.9% were detected after hospital discharge. The mean age was ±35.8 years, and 70% of the patients were aged between 30 and 60 years. Prior abdominal surgery was recorded in 15.8% of the patients in this group. Most of the laparoscopies during the study period were of moderate technical difficulty (54.2%). The mean length of hospital stay was ≤3 days, and 84.6% of the patients were discharged during the first 3 days after the procedure. Patient characteristics and the indications for the laparoscopic approach are presented in Table 1.

Incidence of complications associated with gynecological laparoscopy procedures was 16.2% (95% CI, 15.9-16.5). Of these cases, 5.04% (95% CI, 4.8-5.6) were major complications and 11.16% (95% CI, 10.9-11.5) were minor complications. Bleeding was the most frequent major complication, with 13 due to a major vessel injury. The distribution of 2,340 complications associated with gynecological laparoscopy procedures in regional (tertiary care) hospitals is shown in Table 2.

RISK FACTORS

In our study, patients with prior abdominal surgery had significantly more ($P < 0.001$) serious complications (21.1% vs 10.9%) and more failed attempts at laparoscopy. A greater level of difficulty of the procedure was associated with both complications and failed laparoscopy ($P < 0.001$). Obesity and age were also significantly associated with failed laparoscopy ($P < 0.001$) and serious complications ($P = 0.02$), respectively. The factors associated with major and minor complications, conversion, and failed laparoscopy are shown in Table 3.

Multiple logistic regression analyses (Table 4 and Table 5) showed that serious complications were significantly more frequent in patients with prior abdominal surgery (adjusted odds ratio, 2.78; 95% CI, 1.54–4.95), and the adjusted odds ratio tended to increase with increasing level of technical difficulty of the procedure. Level of difficulty was also directly associated with conversion to laparotomy and failed laparoscopy.

Table 2. Distribution of 2,340 complications associated with gynecological laparoscopy procedures in perinatal centers of Ukraine, 2020-2022

Type of complications	No. (%)	95% CI
Major complications	728 (31.1)	30.1-32.1
Intestinal perforation	130 (17.9)	17.1-18.7
Bladder perforation	52 (2.2)	1.9-2.5
Serious bleeding complications	481 (20.6)	19.8-21.4
Serious complications from infection	52 (2.2)	1.9-2.5
Acute pulmonary edema	39 (1.7)	1.4-2.0
Minor complications	1,612 (68.9)	67.9-69.9
Mild anemia	390 (16.7)	15.9-17.5
Severe anemia (transfusion)	377 (16.1)	15.3-16.9
Minor bleeding complications	195 (8.3)	7.7-8.9
Minor complications from infection	52 (2.2)	1.9-2.5
Wall abscess	13 (0.6)	0.4-0.8
Vaginal vault abscess	26 (1.1)	0.9-1.3
Pelvic abscess	13 (0.6)	0.4-0.8
Nerve lesion	13 (0.6)	0.4-0.8
Fever	117 (5.0)	4.5-5.5
Pain of undetermined cause	52 (2.2)	1.9-2.5
Subcutaneous emphysema	13 (0.6)	0.4-0.8
External genitalia edema	26 (1.1)	0.9-1.3
Paralytic ileum	39 (1.7)	1.4-2.0
Hernia at laparoscopy trocar	13 (0.6)	0.4-0.8
Urinary tract infection	130 (5.6)	5.1-6.1
Urinary retention	26 (1.1)	0.9-1.3
Hematoma (postoperative)	156 (6.7)	6.2-7.2
Postoperative wall hematoma	143 (6.1)	5.6-6.6
Postoperative vaginal vault hematoma	13 (0.6)	0.4-0.8
Uterine perforation	13 (0.6)	0.4-0.8
Failed laparoscopy	1,053 (9.3)	8.7-9,9
Conversion to laparotomy because of complications	468 (4.2)	3.8-4.6

CI, confidence interval.

In this study we found 130 cases of intestinal perforation, 39 of which were diagnosed intraoperatively and 91 postoperatively. In all 130 cases conversion to laparotomy was necessary to manage the perforation. There were 52 cases of injury to the bladder; 13 was managed during laparoscopy. Among the severe bleeding complications, 13 great vessel injury occurred during insertion of the Veress needle into the abdominal cavity, and urgent laparotomy was required. There were 39 cases of serious infection that required further surgery.

We found that obesity played an important role as a risk factor for failed laparoscopy. Women with obesity were likely than women without obesity to require open surgery because laparoscopy could not be initiated ($P < 0.001$).

DISCUSSION

This multicentre, prospective cohort study is the first Ukrainian study to estimate the frequency of complications during laparoscopic gynecologic surgery for benign diseases in women and identify associated risk factors in different regions of the country. This study also provides information about the relative proportion of gynecological laparoscopy procedures for the different types of benign diseases in Ukrainian women. The association between socio-demographic characteristics, different type of laparoscopic gynecologic procedures, and clinical profile for women with complications was analyzed in this study.

Results our study suggest a high (16.2%) frequency of complications associated laparoscopic gynecologic surgery in Ukraine. Of all complication's cases, 5.04%

Table 3. Factors associated with complications in gynecological laparoscopy procedures in perinatal centers of Ukraine, 2020-2022

Variable	Serious complications (n = 728)	Minor complications (n = 1,612)	Conversion (n=468)	Failed laparoscopy (n=1,053)
	No. (%)	No. (%)	No. (%)	No. (%)
Age (yrs.)				
<30 (3,915)	78 (2.0)	578 (14.8)	40 (1.0)	286 (7.3)
30–60 (10,050)	598 (5.9)	955 (9.5)	408 (4.1)	702 (7.0)
>60 (475)	52 (10.9)	79 (16.6)	20 (4.2)	65 (13.7)
P value	0.02	0.12	0.14	0.22
Prior abdominal surgery				
Yes	481 (21.1)	1183 (52.0)	312 (13.7)	741 (32.6)
No	247 (10.9)	429 (18.9)	156 (6.9)	312 (13.7)
P value	< 0.001	0.01	0.013	0.004
Obesity				
Yes	602 (29.5)	1290 (63.2)	381 (18.7)	823 (40.3)
No	126 (6.2)	322 (15.9)	87 (4.3)	230 (11.3)
P value	0.011	0.004	0.013	< 0.001
Level of difficulty				
Simple	130 (2.4)	208 (3.9)	26 (0.5)	130 (2.4)
Moderate	403 (5.2)	585 (7.5)	273 (3.5)	572 (7.3)
Complex	195 (15.7)	299 (24.0)	169 (13.6)	351 (28.2)
P value	< 0.001	< 0.001	< 0.001	< 0.001

Table 4. Multiple logistic regression analyses factors associated with serious and mild complications in gynecological laparoscopy procedures in perinatal centers of Ukraine, 2020-2022

Variable	Serious complications		Mild complications	
	cOR (95% CI)	aOR (95% CI)	cOR (95% CI)	aOR (95% CI)
Age group (yrs.)				
<30	Ref	Ref	Ref	Ref
30–60	2.41 (0.98–5.80)	2.43 (1.03–5.89)	0.69 (0.44–1.09)	0.56 (0.34–0.93)
>60	1.28 (0.13–1.14)	1.51 (0.68–3.11)	1.59 (0.47–5.42)	0.95 (0.26–3.45)
Prior abdominal surgery (Yes vs No)	2.68 (1.48–4.78)	2.78 (1.54–4.95)	0.57 (0.28–1.60)	0.59 (0.29–1.26)
Obesity (Yes vs No)	2.11 (0.51–8.88)			
Level of difficulty				
Simple	Ref	Ref	Ref	Ref
Moderate	2.7 (1.24–5.96)	2.85 (1.27–6.41)	1.97 (1.12–3.49)	1.89 (1.02–3.44)
Complex	7.67 (3.17–8.45)	8.58 (3.39–1.82)	6.74 (3.51–12.95)	7.65 (3.71–15.75)

aOR, adjusted odds ratio; cOR, crude odds ratio; CI, confidence interval.

(95% CI, 4.8-5.6) were major complications and 11.16% (95% CI, 10.9-11.5) were minor complications. Estimating the incidence of complications associated with gynecological laparoscopy procedures is a challenge since many women with minor complications are asymptomatic, while others may report non-specific symptoms. In addition, shortened hospital stays have led to the delayed diagnosis of complications in the gynecological department. The most cases (74.9%) of complications were detected after hospital discharge. The most frequently reported complications types were serious bleeding complications (20.6%), intestinal perforation (17.9%), mild anemia (16.7%), severe anemia (transfusion) (16.1%), failed laparoscopy (9.3%), minor bleeding complications (8.3%), postoperative hematoma (6.7%), urinary tract infection (5.6%), and fever (5.0%). The level of technical difficulty and existence of prior abdominal surgery, and obesity were associated

Table 5. Multiple logistic regression analyses of factors associated with conversion to laparotomy to laparotomy and failed laparoscopies in perinatal centers of Ukraine, 2020-2022

Variable	Conversion to laparotomy		Failed laparoscopy	
	cOR (95% CI)	aOR (95% CI)	cOR (95% CI)	aOR (95% CI)
Age group (yrs.)				
<30	Ref	Ref	Ref	Ref
30–60	2.75 (0.96–7.84)	2.07 (0.69–6.22)	0.92 (0.55–0.52)	0.62 (0.36–1.09)
>60	2.95 (0.32–26.91)	1.66 (0.17–15.78)	2.21 (0.73–0.69)	0.75 (0.21–2.63)
Prior abdominal surgery	2.37 (1.17–4.78)	2.35 (1.13–4.91)	2.02 (1.24–3.30)	2.17 (1.29–3.65)
Obesity	2.86 (0.67–2.29)		8.20 (3.93–17.14)	7.04 (3.09–16.03)
Level of difficulty				
Simple	1	1	1	Ref
Moderate	5.91 (1.38–25.26)	12.25 (1.61–93.25)	2.49 (1.25–4.98)	2.52 (1.23–5.17)
Complex	22.57 (5.05–98.79)	47.14 (5.86–98.99)	9.94 (4.73–20.89)	10.81 (4.76–24.59)

aOR, adjusted odds ratio; cOR, crude odds ratio; CI, confidence interval.

with a higher risk of complications associated with gynecological laparoscopy procedures. We believe that this study constitutes a unique addition to the currently available literature on surgical complications since it has included and analysed even minor complications.

Laparoscopic surgery has evolved over the past two decades to now be accepted as the method of first choice for tackling most gynecological problems. Better recovery, a shorter hospital stay, less postoperative pain, and lower blood loss are the main arguments in favor of this approach [11]. Gynecologic laparoscopic surgical procedures are increasing throughout the world, and with this increase in its utilization, a renewed interest in its possible complications. Diagnostic and sterilization laparoscopies appear to be safe, but more complex laparoscopies are associated with an unacceptably high number of serious complications requiring continuous follow-up and expertise. Despite advanced technology and experience, complications during the installation phase of laparoscopy remain a major cause of significant morbidity and most operative complications occurred in advanced operative procedures. Complications associated with all types of laparoscopic procedures should not be underestimated.

Complications during gynecologic laparoscopic surgical procedures result from the proximity of the uterus and ovaries to other critical pelvic structures. These structures include the urinary tract, bowel, nerves, and vasculature. Knowledge of pelvic anatomy is important when performing these procedures and is critical in cases of altered anatomy from adhesive disease and during intraoperative hemorrhage. Recognition and repair of an unintended injury gives the best chance for minimizing sequelae from these complications.

Complications of gynecologic laparoscopic procedures are relatively uncommon but may be devastating. As the number of endoscopic procedures has increased tremendously over the years, several different complications may arise affecting different systems. According to the literature, the overall rate of complications ranges from 0.4% to 5.57% [4, 7, 8, 12, 13]. Although the incidence of complications decreases as surgeons gain experience with laparoscopy [5, 6, 14, 15], the growing difficulty of some procedures in gynecologic surgery may increase the frequency of severe complications. Vascular structures, gastro-intestinal organs and urinary tract are the most common affected sites [14, 16]. As well as being the rarest, major vascular complications occurring after closed or direct trocar entry are observed in approximately 0.04-0.1% of laparoscopic procedures [17]. During laparoscopic entry, gastrointestinal system injuries may also be observed (0.06%) [18].

Surgical complications can arise either intraoperatively or postoperatively. The factors that lead to complications in gynecological surgeries could be both patient-related or surgeon-related [19]. There are several patients and surgeon related factors that might influence complications, however in the presence of adequate expertise, most of these complications can be avoided. In the researchers' reports majority of the cases where major vascular complications were encountered was overweight or obese patients [20, 21]. Furthermore, inadequate surgical experience and inappropriate patient positioning may also contribute to increased complication rates. It is crucial to determine the site of the aortic bifurcation conjecturally before starting the procedure. This region may not always be palpated or detected according to the umbilicus. Sub-specialization fellowship training and high surgical volume have previously been linked to improved gynecological surgical

outcomes. [22-24]. Conversely, this additional training narrows a physician's scope of practice and, on a larger scale, could limit access to routine or preventive health care from general Obstetricians Gynecologists. [25]. Many surgeons in our study are sub-specialty-trained in urogynecology and/or minimally invasive surgery. However, surgeon training and/or experience was not a factor assessed in this study.

STRENGTH AND LIMITATIONS

This is one of the largest series reported of laparoscopic gynecologic surgery for benign diseases in women and the first focused on complications in Ukraine. These results add valuable information to the literature regarding the occurrence of postoperative complications after gynecologic procedures.

A limitation our study based on a 10 perinatal centers data is that the results this study may not be generalizable to other hospitals of Ukraine. However, knowledge of centre-specific surgical outcome data can help in providing patients with better preoperative counselling.

CONCLUSIONS

Results this study suggest a high frequency of complications associated laparoscopic gynecologic surgery in Ukraine. Shortened hospital stays have led to the delayed diagnosis of complications in the gynecological department. The most cases of complications were detected after hospital discharge. The level of technical difficulty and existence of prior abdominal surgery, and obesity were associated with a higher risk of complications associated with gynecological laparoscopy procedures. Major operative complications are more likely to occur in complex procedures. Through the comprehensive understanding of the relevant anatomy, surgical instruments, complex maneuvers, and optimal surgical technique, gynecologic laparoscopists can avoid most of the complications described. Surgeons should be diligent in recognizing and managing these events. The accumulation of surgical experience with the aid of preventive maneuvers is helpful to reduce the complication rate significantly. Further research needs to be conducted on this topic to design necessary strategies to decrease the burden on patients facing these complications.

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We thank the participating hospitals, doctors and nurses for their diligent efforts in performing the frequency surveys for complications during laparoscopic gynecologic surgery for benign diseases and identify associated risk factors in Ukraine. The findings and conclusions in this study are those of the authors.

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

RECEIVED: 10.06.2024
ACCEPTED: 21.09.2024

ORIGINAL ARTICLE

CONTENTS

The second week corneal changes in rodents model of streptozotocin-induced diabetes

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ABSTRACT

Aim: Installation the changes of the microstructural rearrangement of the layers of the rat cornea at the end of the second week of experimental streptozotocin-induced diabetes.

Materials and Methods: The research was conducted on 15 sexually mature, outbred white male rats, weighing 120-130 g. Two groups of animals were used in the work: the first group with developing diabetes (2 weeks after administration of streptozotocin); the second group served as control and received injections of 0.9% physiological solution for 2 weeks.

Results: Basal cells were located loosely, lost their columnar shape, basal cells of a rounded shape were visualized. The cytoplasm of individual basal cells was swollen and contained small acidophilic granules. At the same time, the cells of the basal layer with lighted vacuolated cytoplasm were visualized. Epitheliocytes of the middle layer of the outer epithelium of the cornea were chaotically located in individual areas, lumps of intensively condensed chromatin were often visualized in their nuclei. Focal destruction of the cells of the surface layer of the cornea and focal layering of fine-grained acidophilic masses in such areas were observed in places. The endothelium of the anterior chamber of the eye was preserved in most areas of the cornea.

Conclusions: At the end of the second week of experimental streptozotocin induced diabetes mellitus, we established pathomorphological manifestations indicating the initial phenomena of diabetic keratitis.

KEY WORDS: eye, membranes, cornea, experiment, diabetes, rat

Wiad Lek. 2024;77(9):1910-1915. doi: 10.36740/WLek/195141 DOI

INTRODUCTION

Diabetes mellitus is one of the most widespread endocrine diseases, which currently occupies one of the leading places in terms of medical and social importance, next to cardiovascular and oncological pathologies. Nowadays, the number of people with diabetes in Ukraine reaches 1.5-3% of the entire population [1]. Due to wide distribution of this pathology, the variety of complications, the severity of manifestations and the difficulty in selecting treatment, a thorough and comprehensive study of changes in the microstructural organization of tissues of the affected organs is indicated. Generalized vascular damage, specifically at the microcirculatory level is characteristic of both insulin-dependent and non-insulin-dependent types of diabetes, and largely determines the course and prognosis of the disease [2].

The incidence of ophthalmic diseases ranges globally approximately from 65% to 98%. Numerous clinical and experimental studies have established the relationship between eye tissue diseases and the state of somatic health, in particular, the state of the endocrine system and, as a result, the development of keratitis [3-8]. Diabetes mellitus significantly involves changes in tissues of the eyeball. The most numerous and at the same time contradictory information concerns the vascular concept of chronic damage to the membranes of the eyeball, which was formulated in the last century [9,10]. To date, it has not been definitively established whether the nerve fiber is primarily affected, as a result of a trophic disturbance, or a vessel, as the object of neurotrophic influence [11].

With a chronic, ongoing state of hyperglycemia, there is a higher probability of bacterial damage to the structures of the eyeball, in particular the cornea [12, 13]. On the one hand, this may be related to a change in the conjunctival flora in this group of patients due to an increase in the content of gram-positive cultures (mainly coagulase-negative

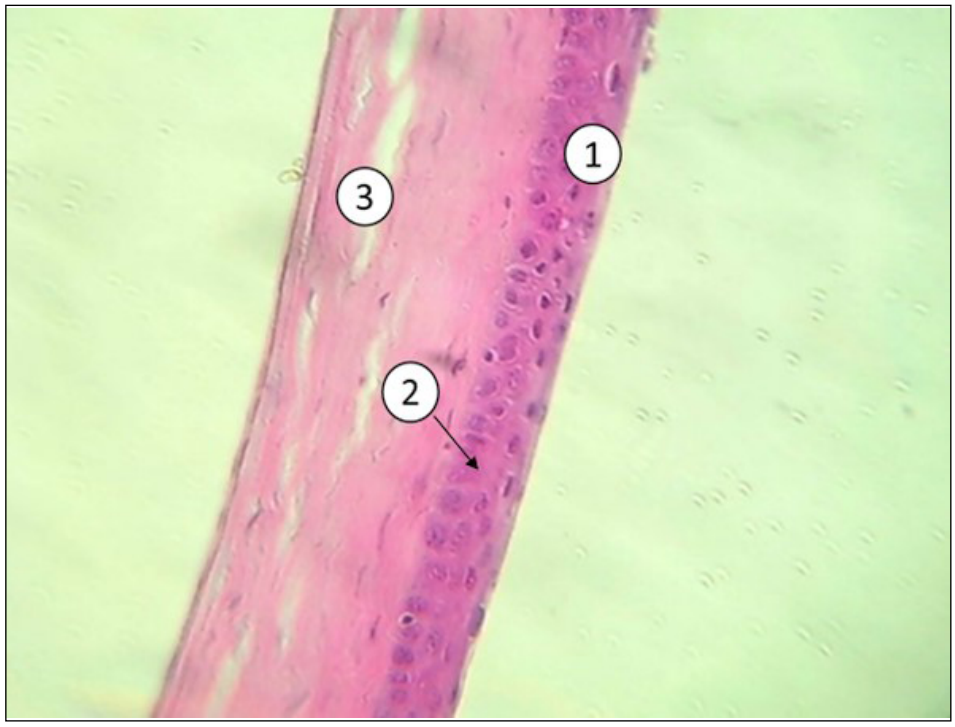

Fig. 1. Cornea at the end of the second week of experimental diabetes. Hematoxylin and eosin staining. Coll. x 400.
1-loose arrangement of cells of the basal layer of the outer epithelium of the cornea; 2-enlightenment of the cytoplasm of individual epitheliocytes of the basal layer; 3-delamination of the connective tissue plates of the main substance of the cornea.

staphylococcus) due to an increase in the concentration of glucose in tears and the frequent use of antibiotics [14].

On the other hand, specific structural and functional changes of the corneal tissues due to diabetes, is characterised by the development of diabetic keratopathy, which occurs as the background of diabetic corneal neuropathy. According to various authors, diabetic keratopathy occurs in 50–70% of patients with diabetes and is characterized by an asymptomatic course [12, 15].

However, eye trauma or any other surgical injury of a cornea being in such condition can lead to incomplete and delayed regeneration and is often the cause of reduced visual acuity due to clouding of the stroma and uneven surface [12, 15].

Bacterial keratitis occurs more often in patients with diabetes than in the general population, and has a more severe course [16–18]. A key role in the development and regulation of the inflammatory reaction is controled by cytokines — a group of mediators of intercellular interaction of a protein nature, which is the main non-specific humoral factor of immunity. According to their biological effect, cytokines are conditionally divided into pro-inflammatory and anti-inflammatory [19]. In bacterial keratitis, there is an increase in the expression of pro-inflammatory cytokines (interleukin 1α (IL-1 α) and interleukin 6 (IL-6)) in the tear fluid of the diseased eye [20, 21] and anti-inflammatory (interleukin 10 (IL-10)) — in tear fluid of the contralateral eye [20]. In patients with diabetes mellitus, the presence of chronic systemic inflammation of low intensity has been proven, which leads to an increase in the concentration of pro-inflammatory factors in blood plasma and biological fluids in the absence of an immunostimulator [22] and a change in the body's response to inflammatory diseases [23–26].

However, immunological aspects of bacterial keratitis in patients with diabetes have not been identified to date. Taking into account the above, diabetic damage to the structures of the eyeball will continue to be an actual problem of the possible development of diabetic damage to the cornea.

AIM

The aim was to find out the peculiarities of the microstructural rearrangement of the layers of the rat cornea at the end of the second week of experimental streptozotocin-induced diabetes.

MATERIALS AND METHODS

The research was conducted on 15 sexually mature, outbred white male rats, weighing 120-130 g. Experimental diabetes was induced by a single intraperitoneal injection of streptozotocin (Sigma, ltd) at the rate of 7 mg per 100 g of body weight (prepared in 0.1 mol citrate buffer, pH=4.5). The diabetes development was monitored during 2 weeks of blood glucose level increase, which was measured by the glucose oxidase method. The research was conducted from the second week of the experiment on animals with a glucose level of more than 13.48 mmol per 1 liter. Two groups of animals were used in the work: the first group (10 animals) with

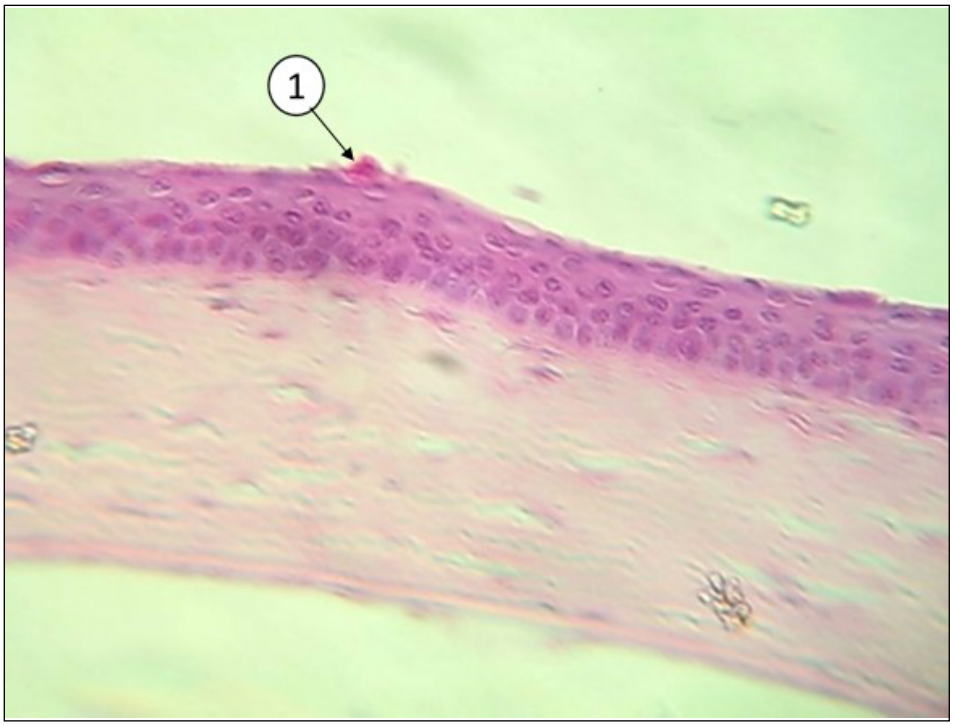

Fig. 2. Cornea at the end of the second week of experimental streptozotocin diabetes. Hematoxylin and eosin staining. Coll. x 400.
1-acidophilic layers on the surface of the outer epithelium of the cornea.

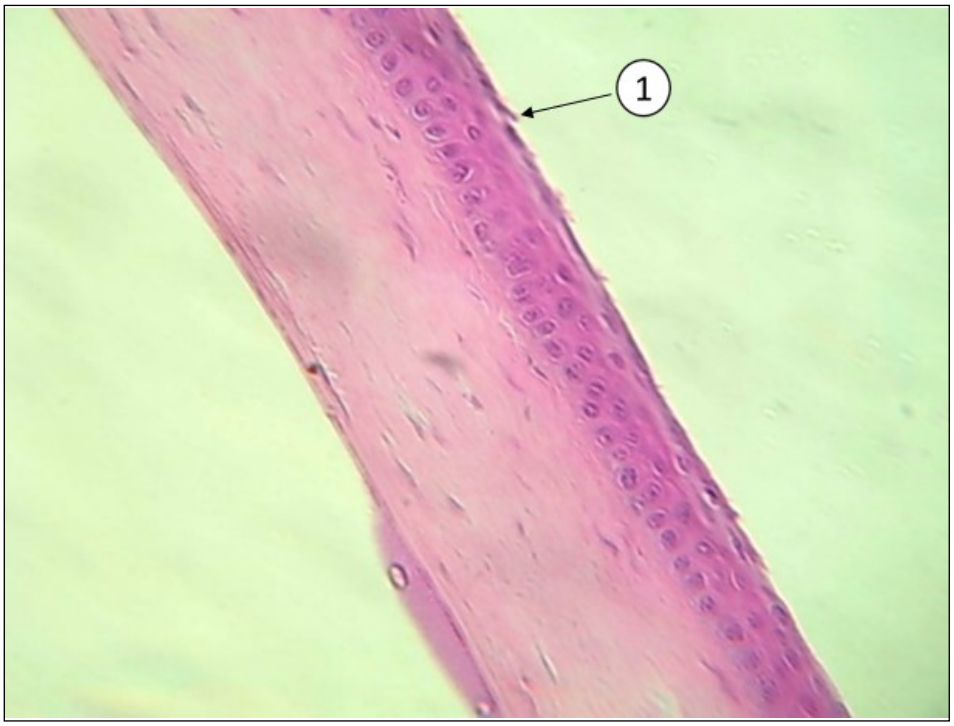

Fig. 3. Cornea at the end of the second week of experimental streptozotocin diabetes. Hematoxylin and eosin staining. Coll. x 400.
1- focal destruction of the surface layer of the outer epithelium of the cornea.

developing diabetes (2 weeks after administration of streptozotocin); the second group served as control (5 animals) and received injections of 0.9% physiological solution for 2 weeks.

All animals were kept in vivarium conditions and the procedures related to housing, care, labeling and all other manipulations were carried out in compliance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [Strasbourg, 1985], "General ethical principles of experiments on animals", adopted by the First National Congress on Bioethics [Kyiv, 2001], Law of Ukraine No. 3447 - IV "On the Protection of Animals from Cruelty". The Bioethics Commission of the Lviv National Medical University named after Danylo Halytsky found that the conducted scientific research meets the ethical requirements in accordance with the order of the Ministry of Health of Ukraine No.

231 of November 1, 2000 (protocol No. 10 of December 26, 2011), (protocol No. 2 of February 20, 2012 year). Before taking the material of the experimental site, the animal was removed from the experiment with the help of diethyl ether. The eyeballs of rats were used for microscopic examination (intersecting in the area of the limbus). Preparations for histological examination were prepared according to the generally accepted method [28]. Microscopic studies and photography of the preparations were carried out using an MBI-1 microscope and a Nicon D 3100 digital camera.

RESULTS

Microstructural examination of the cornea at the end of the second week of experimental streptozotocin-induced diabetes of the cornea of laboratory rats revealed that the outer epithelium of the cornea retained its integrity. In the epithelium, the basal, intermediate and surface layers were clearly visualized. In some areas of the cornea, the basal cells were located loosely, lost their columnar shape, and basal cells of a rounded shape were visualized (Fig. 1).

The cytoplasm of individual basal cells was swollen and contained small acidophilic granules. At the same time, the cells of the basal layer with lighted vacuolated cytoplasm were visualized.

Epitheliocytes of the middle layer of the outer epithelium of the cornea were chaotically located in individual areas, lumps of intensively condensed chromatin were often visualized in their nuclei.

Focal destruction of the cells of the surface layer of the cornea and focal layering in such areas of fine-grained acidophilic masses of rice were noted in places. (Fig. 2, Fig. 3).

In most areas of the corneal stroma, the connective tissue plates were arranged in an orderly manner, and their collagen fibers were preserved. In some areas of the corneal stroma, connective tissue plates were swollen, their main substance was slightly swollen.

The loose arrangement of connective tissue plates, their delamination, and the accumulation of optically light masses in such areas were also noted (Fig. 1). The endothelium of the anterior chamber of the eye was preserved in most areas of the cornea.

However, in some areas of the cornea, swelling of the cytoplasm of cells of the posterior corneal epithelium was noted.

DISCUSSIONS

The most frequently diagnosed eye diseases is a group of corneal lesions of various origins, which have different etiology, pathogenesis, and clinical manifestations [3-8]. Among the main causes of their occurrence, various authors name injuries, bacterial lesions, as well as general metabolic changes in the body, which develop under the influence of both exo- and endogenous factors, as well as background of diseases that lead to the development of polyneuro- and polyangiopathies, in particular - the background of diabetes.

Chang Y.S. et al. (2020) and Badawi A.E. et al. (2017) in their works emphasize the significantly more frequent damage to the cornea by pathological processes in patients with diabetes than in the general population [6, 8].

The same opinion is held by Vieira-Potter V.J. et al. (2016), Wang B et al. (2018) [12, 13]. In order to understand the causes of diabetic keratopathies and to develop effective methods of their prevention and treatment, it is necessary to study in-depth the changes that occur in the structures of the cornea at the background of diabetes, starting from the early stages of its development. Understanding the mechanisms of their development will also allow to optimize and accelerate the processes of corneal regeneration after trauma or surgery in patients with diabetes.

The results of our study indicate that condition of the rat cornea influenced by experimental diabetes at the end of the second week of experiment showed the development of focal destruction of the corneal surface layer cells, which can be interpreted as the initial phenomena of diabetic keratitis. The detected changes explain why even at the initial stages of the development of this pathology, the examined tissues become more vulnerable to external stimuli, and in the absence of treatment (both general and local), these changes can progress and, as a result, cause the development of acute or chronic keratitis and significant decrease in visual acuity.

In order to preserve patients' vision and prevent the development of more serious complications, in our opinion, it is advisable to develop measures for early correction of detected changes already at the initial stages of the of diabetes development.

CONCLUSIONS

1. The result of our study of the cornea, at the end of the second week of experimental streptozotocin-induced diabetes found that in some areas of the cornea, the basal cells were located loosely, lost their columnar shape, and the cells of the basal layer with lightened vacuolated cytoplasm were visualized.
2. There were local zones where focal destruction of the cells of the surface layer of the cornea and focal layering of fine-grained acidophilic masses were noted. In some areas of the corneal stroma, connective tissue plates were swollen, their main substance was slightly swollen.

3. There were foci of swelling of the cytoplasm of cells of the posterior corneal epithelium. These pathomorphological manifestations at the end of the second week of experimental streptozotocin-induced diabetes indicate the initial phenomena of diabetic keratitis.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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RECEIVED: 20.06.2024
ACCEPTED: 29.09.2024

ORIGINAL ARTICLE

CONTENTS

Molecular-genetic characteristics of hmgb1 mrna expression in blood of women with endometriosis associated with infertility

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ABSTRACT

Aim: To examine HMGB1 expression in the blood of women with endometriosis-associated infertility and to establish its role in disease progression.

Materials and Methods: We analyzed HMGB1 gene expression levels in two groups: 20 women with endometriosis-associated infertility (main group) and 10 healthy women (control group). The study was conducted at Bukovinian State Medical University and the Centre of Reproductive Medicine. Primary infertility was significantly higher in the main group. We used real-time reverse transcription polymerase chain reaction (RT-PCR) to determine HMGB1 mRNA levels in mononuclear cells isolated from whole blood. Statistical significance was determined using the Student's t-test, with $p < 0.05$ considered significant.

Results: Results indicated a significant increase in HMGB1 mRNA expression in the main group compared to the control group ($p < 0.001$). The relative normalized expression ranged from 1.8924 to 33.426 (median = 8.01). Expression levels were categorized into three groups: borderline with the control, moderate increase, and significant increase. Only one sample (5%) had values equal to the control. Fifteen percent of samples had values slightly above control (1.89–3.45), 30% had moderate increases (3.45–8.01), and 50% had significant increases (>8.01). In 95% of the main group, HMGB1 mRNA expression was elevated, predominantly at high values ($p < 0.001$).

Conclusions: Women with endometriosis-associated infertility show significantly increased HMGB1 mRNA expression, particularly in moderate and severe cases compared to mild cases, indicating HMGB1's role in disease progression ($p < 0.001$).

KEY WORDS: endometriosis, infertility, assisted reproductive technologies, HMGB1

Wiad Lek. 2024;77(9):1916-1921. doi: 10.36740/WLek/195142 DOI

INTRODUCTION

HMGB1 is a nuclear protein that is present in almost all cell types. It can also be released extracellularly, where it mediates the activation of innate immune responses, including chemotaxis and cytokine release. HMGB1 was originally described as a protein that binds nuclear DNA [1,2]. It is evolutionarily highly conserved and functions as a nuclear cofactor in transcriptional regulation. HMGB1, like many other cofactors, was later found to play another role as an intercellular molecule that is released from various cells into the extracellular environment to act on specific cell surface receptors. In this latter role, HMGB1 is a proinflammatory cytokine that can contribute to many inflammatory diseases, including sepsis [3-5]. Through interaction with immune cell cell surface receptors, HMGB1 activates intracellular cascades that regulate immune cell functions, including chemotaxis and immune modulation. HMGB1 is a critical mediator of lethality in sterile and infectious inflammation. Similar inflammatory responses are initiated by strokes caused by sterile trauma or infection. During infection, innate immunity is activated by foreign molecular products called PAMPs, which include, for example, lipopolysaccharides [6]. During sterile injury or ischemia, the cells themselves are activated under the influence of endogenous DAMPs, which include molecules such as heat shock proteins, uric acid, annexin, and IL1β. HMGB1, released by activated immune cells and damaged or necrotic cells, plays an important role in host responses to both types of threats; thus, it is a critical mediator in the final common pathway to morbidity and mortality during infection and sterile trauma [7-10].

In a recent study (Expression of high-mobility group box-1 in eutopic/ectopic endometrium and correlations with inflammation-related factors in adenomyosis Xiu-Ni Liu and Zhong-Ping Cheng) it was described.

Possible pathogenesis of adenomyosis: the strong HMGB1/TLR4 system may be involved in the inflammatory pathological process of adenomyosis development. It generates a local inflammatory reaction and activates the body's specific immune system, builds

and maintains a stable inflammatory inflammatory microenvironment in the local lesion, and forms a local inflammatory pathology of adenomyopathy, which may involve the pathogenesis of angiomyopathy.

Therefore, HMGB1 may also play an important role in the pathophysiology of endometriosis, a gynecological disease associated with a chronic immunoinflammatory process.

AIM

The aim of our study was to examine the expression HMGB1 in the blood of women with endometriosis-associated and to establish the role of HMGB1 in the progression of endometriosis by analyzing the level of gene expression in the blood of women with infertility-associated infertility, depending on the severity of endometriosis.

MATERIALS AND METHODS

We determined the level of HMGB1 mRNA gene expression in two groups. The first group (main) consisted of 20 women with endometriosis associated with infertility. The control group consists of 10 practically healthy women. The absence of signs of acute inflammatory processes in the reproductive sphere and the negative results of microbiological and virological studies were evidence of a long-overdue inflammatory process that caused tubal obstruction.

This study was conducted at the Bukovyna State Medical University and the clinic "Yuzko medical center".

To analyze the expression of the HMGB1 gene and to determine the relative normalized expression of HMGB1 mRNA, the polymerase chain reaction with reverse transcription in real time (RT-PCR) was used. The object for molecular genetic studies by the RT-PCR method was the fraction of mononuclear cells isolated from the whole blood of patients with endometriosis.

Specific pairs of primers for the analysis of the studied and reference genes were selected using the Primer-BLAST software (www.ncβi.nlm.nih.gov/tools/primer-βlast) and manufactured by Thermo Scientific (USA). The actin, βeta (Actβ) gene was used as a reference gene to determine the relative value of the change in the expression level of the studied genes. The comparative Ct method (ΔΔCt method) was used to express the relative level of gene expression. Calculations were made according to the formulas: ΔCt (target gene) = Ct (target gene) – Ct (calibrator gene / ACT1); ΔΔCt = ΔCt (target gene) – ΔCt (base gene); The relative expression level was expressed as 2–ΔΔCt. Statistical analysis of PCR data was performed using CFX Manager ™ software (Bio-Rad, USA). Optimal RT-PCR conditions were selected to achieve a linear relationship between the number of cycles and the number of PCR products. Negative controls were included in the experiment: without addition of cDNA matrix in the PCR reaction, without addition of mRNA matrix in cDNA synthesis, without addition of enzyme in cDNA synthesis. All amplification reactions were performed on individual samples in triplicate. Quantitative variables were evaluated using the Shapiro-Wilk test (if the number of subjects was less than 50) or the Kolmogorov-Smirnov test (when the number of subjects was more than 50).

Comparison of two groups for a quantitative variable according to a normal distribution, under the condition of equality of variances, was performed using the Student's t-test.

Statistical processing of the results of this section was carried out using non-parametric methods, namely the Wilcoxon-Mann-Whitney test. Differences between groups were considered probable at the significance level of $p<0.05$.

RESULTS

In the analysis, depending on the degree of severity of endometriosis, stage I and II were combined into so-called «small» forms of endometriosis, or mild stage, stage III corresponded to the average severity of endometriosis, and stage IV corresponded to the severe stage of the disease.

The results of the study of the relative normalized expression of HMGB1 mRNA in the whole blood of women with endometriosis associated with infertility are shown in Fig. 1.

Based on our research, it was established that the expression of HMGB1 mRNA in the blood of women with endometriosis associated with infertility is significantly increased compared to the indicators of the control group ($p< 0.001$).

The range of all obtained values of the relative normalized amount of mRNA of the HMGB1 gene was 1,8924 – 33,426 (median – 8,01). For a broader characterization of the features of the level of HMGB1 mRNA expression in the blood of women with endometriosis, associated with infertility, and not only the limitation of the data of the average value, conditionally, the ranges of fluctuations of the indicator were separated into the following groups: borderline with the control group, lower than the median (moderate increase HMGB1 expression level) and higher than the median (significant increase in HMGB1 expression level). As can be seen, the lower range of values of the HMGB1 gene is equal to the

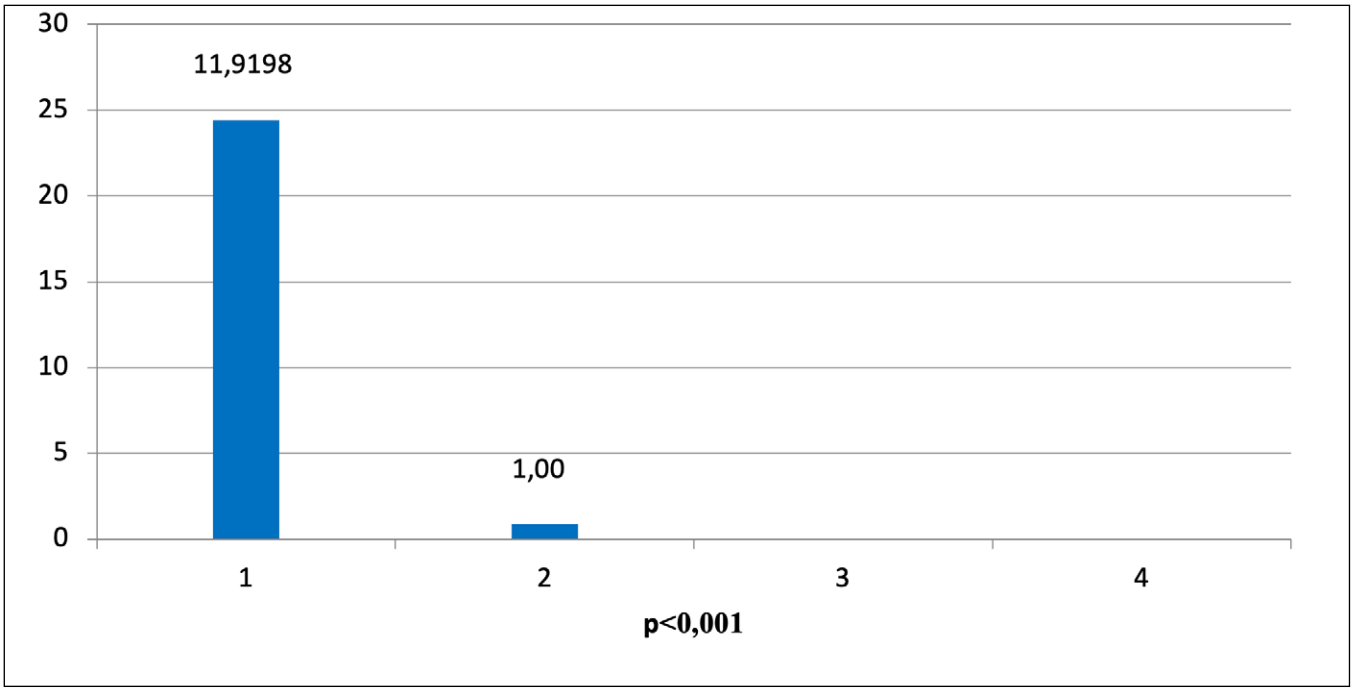

Fig. 1. Relative normalized HMGB1 mRNA gene expression
Normalization by the ΔΔCt method with a reference gene Actin, βeta

control, however, this indicator was measured only in 1 out of 20 studied blood samples, which was 5% of cases (p<0,001). There were only three results that slightly differed from the control (>1,89-<3,45), which was 15% (p<0,001). The number of blood samples in which low values (>3,45-<8,01) of relative normalized expression were found was 6 cases (30%). Relative normalized expression above the median (>8,01) was found in 10 blood samples, which accounted for half of the cases - 50%. Thus, in the blood samples of 95% of the women in the study group, increased expression of the HMGB1 mRNA gene was detected relative to the control with a preference for high values (p<0,001) (Table 1).

Thus, the obtained results indicate an increase in the relative normalized amount of mRNA of the HMGB1 gene in the blood of women with endometriosis associated with infertility. The results of the work indicated in the previous subsection demonstrate a pronounced probable increase in the expression of the HMGB1 gene in the blood of women with endometriosis associated with infertility, which coincides with the trends of scientific works of recent years. Free HMGB1IL participates in all phases of inflammation, from damage to repair. It is able to activate endothelial cells and their precursors, which determines a certain contribution of HMGB1 to angiogenesis in pathological processes.

To implement the tasks of this work unit, patients are divided into three groups depending on the severity of endometriosis. Stages of the disease were determined according to the recommendations of AFS-85, however, in order to simplify the analysis, stages I and II were combined into the so-called "small" forms of endometriosis, or mild stage, stage III corresponded to the average severity of endometriosis, and stage IV corresponded to the severe stage of the disease. The first group included 10 women with mild endometriosis, the second group included 3 patients with moderate endometriosis, and the third group included 7 women with severe endometriosis.

Table 2 shows the results of the HMGB1 mRNA gene expression study in the blood of women depending on the severity of endometriosis.

As can be seen from the table 2, the levels of relative normalized expression of the HMGB1 gene increased in direct proportion to the increasing stage of endometriosis. This was reflected in the statistically significant difference found between the indicators of the first and third, and second and third studied groups, i.e., compared between the groups of women with mild and severe endometriosis. It is worth noting that the range of relative normalized values of HMGB1 gene mRNA in mild cases was 1,89 – 7,32 (median – 4,76). No specific features of the distribution, concentration of low values or predominance of lower values of HMGB1 expression in the group of patients with "small" forms of endometriosis were found - all values of the relative normalized mRNA expression of the studied genes are "scattered" among the entire range of the sample as a whole. In the group of patients with an average degree of endometriosis, the range of values of the relative

Table 1. Comparative characteristics of relative normalized expression HMGB1 gene mRNA in the blood of women with endometriosis is associated with infertility

Groups	HMGB1 mRNA	p
Researched Group	11,9198 ±0,01	p<0.05
Control Group	1,00±0,01	*

Note: Relative normalized amount of HMGB1 gene mRNA. Normalization by the ΔΔCt method with the reference gene Actin βeta p<0,05 reflects a probable difference.* - normalization by relative amount.

Table 2. Expression of the HMGB1 mRNA gene level in the blood of women, of patients with endometriosis associated with infertility, depending on the severity of endometriosis (M±m)

No /p	Groups of patients	Indexes
		HMGB1
1	1 group (mild endometriosis ("small" forms) (n=10))	4,76±0,22
2	2nd group (moderate endometriosis (n=3))	9,94±0,73
3	3 group (severe endometriosis (n=7))	22,97 ± 1,23
	p	p1 > 0,05 p2 <0,001 p3 < 0,001

Notes:p reflects the statistical probability of the difference between the indicators of the studied and control groups; p < 0.05, p < 0.01 - probable difference, p > 0.05 - no probable difference. p1 - the probability of the difference between the indicators of the 1st and 2nd groups, p2 - the probability of the difference between the indicators of the 2nd and 3rd groups, p3 - the probability of the difference between the indicators of the 1st and 3rd groups.

normalized expression of the HMGB1 gene ranged from 8,28 to 12,87 (median – 9,94), without certain trends.

In the group of women with severe endometriosis, the range of relative normalized expression of the HMGB1 gene was 13,94 – 33,42 (median – 22,97). It should also be noted that (100,00%) of the observations in this group belonged to significant deviations from the control and the median (p<0,001). Accordingly, the expressed values of HMGB1 gene expression were concentrated even in groups of women suffering from mild endometriosis (I stage).

Thus, statistical differences in the relative normalized expression of HMGB1 were found between groups of women with mild, moderate, and severe endometriosis, trends indicate an increase in this indicator directly proportional to the increase in the degree of endometrioid lesions. This is consistent with the assumption of some authors that endometrioid growth is supported by the activation of a number of innate immune molecules with subsequent production of factors such as cytokines and vascular growth factors [1, 16, 20, 21].

DISCUSSION

The resulting increase in IL1β expression can be explained from the standpoint that HMGB1 is part of a system that binds to immunogenic nucleotides to activate the innate immune response during tissue damage or microbial infection. The biological activity of extracellular HMGB1 is largely related to the interaction with target cells involved in inflammatory and immune responses, which emphasizes the importance of HMGB1 blood levels as a valuable biomarker of inflammation or inflammatory diseases [11-15]. HMGB1 provokes cell proliferation, invasion and cellular inflammation in the ectopic endometrial environment through Toll-like receptors [16-20]. By itself, HMGB1 has little or no pro-inflammatory activity, but forms complexes with pro-inflammatory factors such as IL 1β or lipopolysaccharide and thus enhances their biological activity [21]. Thus, HMGB1 complexes stimulate the synthesis of pro-inflammatory cytokines. During infection, innate immunity is activated by foreign molecular products called PAMPs (pathogen-associated molecular patterns), which include, lipopolysaccharides. During damage or ischemia, the cells themselves are activated under the influence of endogenous DAMPs (damage associated molecular patterns), which include such molecules as heat shock proteins, annexin, IL1β. HMGB1, released by activated immune cells and damaged or necrotic cells, plays an important role in host responses to both

types of threats; thus, it is a critical mediator in the final common pathway to morbidity during infection and trauma. A possible pathogenesis of adenomyosis a strong HMGB1/TLR4 system may be involved in the inflammatory pathological process of adenomyosis development. It generates a local inflammatory reaction and activates the body's specific immune system, builds and maintains a persistant inflammatory microenvironment in the local lesion, and forms a local inflammatory pathology of adenomyopathy, which may involve the pathogenesis of angiomyopathy, include IL1β. Etracellular matrix acts an endogenous ligand for molecules such as TLR-4 (tumor like receptor) to stimulate the activation of TLR-4-mediated cellular inflammatory signaling and participate in the inflammatory response [17]. It has been proven that HMGB1, TLR-4-mediated inflammatory signaling system is involved in the formation of multiple tumor inflammatory pathological microenvironments and participates in inflammatory tumor proliferation, invasion and metastasis and other pathological mechanisms [18].

CONCLUSIONS

In the blood of women with endometriosis associated with infertility, there is a significant increase in the level of mRNA expression of the HMGB1 gene. In the blood of women with endometriosis associated with infertility, a probable increase in the expression of HMGB1 ($p<0,001$) in women with moderate and severe endometriosis compared to the mild course of the disease and HMGB1 ($p<0.05$) in the groups of women with mild and severe endometriosis, which indicates the role of these factors in the progression of the disease.

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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

RECEIVED: 04.06.2024
ACCEPTED: 26.09.2024

ORIGINAL ARTICLE

CONTENTS

Local anesthesia in adolescents with high anxiety levels

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ABSTRACT

Aim: To assess the effectiveness of local anesthesia in the treatment of acute forms of caries, pulpitis, and periodontitis in adolescents with a high level of baseline anxiety.

Materials and Methods: 1.7 ml of local amide anesthetic from the articaine series was used. The assessment of anxiety was carried out according to the method of Ch.D. Spielberger - Y.L. Khanina). Pain intensity was measured on the VAS scale. Statistical data processing was carried out in the program "MS Excel 7" Windows and "STATISTICA" v. 6.0.

Results: Complete pain relief was observed during anesthesia in 5.0% (4 cases) after 10 minutes and in 56.9% (45 cases) after 15 minutes for acute medium caries; in 3.9% (5 cases) after 5 minutes, in 19.6% (25 cases) after 10 minutes, and in 43.8% (56 cases) after 15 minutes for acute deep caries; in 29.0% (18 cases) after 10 minutes and in 45.2% (28 cases) after 15 minutes for acute pulpitis; and in 34.8% (8 cases) after 10 minutes and in 65.1% (15 cases) after 15 minutes for acute periodontitis.

Conclusions: The reduction in pain syndrome during the treatment of acute forms of medium and deep caries, pulpitis, and periodontitis was significant compared to premanipulation values ($p<0.05$). Complete pain relief was observed during anesthesia in 5.0% (4 cases) after 10 minutes and in 56.9% (45 cases) after 15 minutes for acute medium caries; in 3.9% (5 cases) after 5 minutes, in 19.6% (25 cases) after 10 minutes, and in 43.8% (56 cases) after 15 minutes for acute deep caries; in 29.0% (18 cases) after 10 minutes and in 45.2% (28 cases) after 15 minutes for acute pulpitis; and in 34.8% (8 cases) after 10 minutes and in 65.1% (15 cases) after 15 minutes for acute periodontitis.

KEY WORDS: adolescents, dental visit, effectiveness of local anesthesia, acute periodontitis, acute pulpitis, acute caries, psychoemotional state, high anxiety

Wiad Lek. 2024;77(9):1922-1927. doi: 10.36740/WLek/195143 DOI

INTRODUCTION

Dental care is the most widespread type of medical care for the population. However, recently the issue of patients' demand for quality and safety of dental services has become particularly relevant [1, 2]. An important factor affecting the quality is effective pain relief, as almost all medical procedures are accompanied by pain of varying severity. At the same time, about 84% of patients suffer from various forms of dentophobia [3, 4]. That is why painless dental interventions are of great importance in the structure of the quality of treatment in general and eliminate the feeling of fear in patients [5-7].

The level of patients' anxiety, both personal and reactive, caused by a visit to the dentist plays a significant role during the visit, affects trust during communication, and affects the quality of dental services in general [8, 9].

According to some authors, the effectiveness of anesthesia in adolescents largely depends on the level of the patient's baseline anxiety, and these factors are directly related [10-12].

AIM

To determine the effectiveness of local anesthesia in the treatment of acute forms of caries, pulpitis and periodontitis in adolescents with a high level of basic anxiety.

MATERIALS AND METHODS

The analysis was carried out by examining 244 patients (175 girls and 69 boys) aged 12-17 years, who had a high level of baseline anxiety according to the methodology of C.D. Spielberger (adapted by Y.L. Khanin).

Acute medium caries was diagnosed in 79 adolescents, acute deep caries in 80 adolescents, acute pulpitis in 62 adolescents, and acute periodontitis in 23 adolescents. All pathologies required treatment under local

anesthesia. As a local anesthetic, we used a local amide anesthetic of the articaine series, containing articaine hydrochloride 40 mg and epinephrine hydrochloride 0.012 mg (equivalent to 0.01 mg of epinephrine) in a volume of 1.7 ml.

In all patients, the level of pain was determined using a modified VAS scale, taking into account the level of baseline anxiety. Measurements were performed before medical procedures, 5, 10, and 15 minutes after anesthesia [13].

The study was performed in compliance with the basic provisions of the «Rules for Ethical Principles for Scientific Medical Research Involving Human Subjects» approved by the Declaration of Helsinki (1964-2013), ICH GCP (1996), EEC Directive 609 (24.11.1986), orders of the Ministry of Health of Ukraine No. 690 of 23.09.2009 No. 944 of 14.12.2009, No. 616 of 03.08.2012. All participants were informed about the objectives, organization, and methods of the study and signed an informed consent to participate in it, and all measures were taken to ensure patient anonymity.

The statistical analysis of the data obtained was carried out using the methods of mathematical statistics with the determination of the mean value, standard deviation, error of the mean value, reliability of the compared values with the determination of parametric indicators, as well as paired and partial Pearson correlations (r) with the reliability interval (p) based on absolute data were analyzed. All calculations were performed on a personal computer using licensed programs ″MS Excel 7″ for the Windows operating system and the standard software package «STATISTICA» v. 6.0. [14-16].

RESULTS

The intensity of pain syndrome in patients diagnosed with acute medium caries at the initial stage of testing according to the VAS scale was as follows: severely expressed pain was noted by 18 (22.8%) adolescents, moderate - 49 (62.0%) adolescents, and mild - 12 adolescents.

After anesthesia, there was a decrease in the intensity of PS and the frequency of its fixation. Unbearable pain was never noted. Severe pain syndrome was diagnosed in 5 minutes in 12 (15.2%) adolescents; in 10 minutes in 4 (5.0%) people; in 15 minutes severe pain syndrome was not noted (Fig. 1).

A decrease in moderate intensity PS was detected in 5 minutes in 49 (62.0%) adolescents; in 10 minutes in 35 (44.4%); in 15 minutes in 12 adolescents (15.3%). An increase in the percentage of adolescents with low-intensity PS was diagnosed in 10 and 15 minutes after the manipulation compared to the pre-treatment indicators (15.2%, 22.8%, 45.6%, 27.8%; $p<0.05$); complete disappearance of BS was observed in 10 minutes in 4 (5.0%) adolescents; in 15 minutes in 45 (56.9%) adolescents.

In adolescents with a high level of anxiety, the decrease in the intensity of BS was significant compared to the pre-manipulation values, with complete disappearance of BS in 5.0% (4 patients) in 10 minutes and in 56.9% (45 patients) in 15 minutes after anesthesia.

Investigating the intensity of the pain symptom in dental patients with a verified diagnosis of acute deep caries, it was found that in 100% of cases, before the start of manipulations, they indicated the presence of BS. Severe pain was noted by 16 (20.0%) adolescents, moderate intensity was noted by 38 (47.5%) patients; 26 patients (32.5%) characterized the intensity of the pain syndrome as «weak» and recorded as the violet-blue part of the scale ($0 <VAS\leq 4$) (Fig. 2).

Severe pain was observed in 5 adolescents (6.3%) after 5 minutes, and in 5 adolescents (6.3%) after 10 minutes. After 15 minutes, only 2 adolescents (2.5%) reported severe pain. The reduction in severe pain intensity was statistically significant ($p < 0.05$).

The reduction in moderate pain intensity also showed significant improvement over time. At 5 minutes, 33 adolescents (41.2%) experienced moderate pain, which decreased to 17 adolescents (21.3%) at 10 minutes, and 8 adolescents (10.0%) at 15 minutes.

Furthermore, the percentage of adolescents with low-intensity pain increased significantly 10 and 15 minutes after treatment compared to the initial levels (32.5%, 52.5%, 65.0%, 56.3%; $p < 0.05$).

Complete disappearance of PS was observed in 5 minutes in 1 (2.8%) adolescent; in 10 minutes in 6 (7.4%) adolescents; in 15 minutes in 25 (31.2%) adolescents. The decrease in the intensity of BS was significant compared with the pre-manipulation values; complete disappearance of PS was observed in 3.9%-5 patients in 5 minutes, in 19.6% (25 patients) in 10 minutes, and in 43.8% (56 patients) in 15 minutes after anesthesia.

In the treatment of acute pulpitis, the intensity of pain at the initial stage of testing according to the VAS scale was as follows: 8 (12.8%) patients indicated the presence of severe BS (unbearable degree of PS): $7 < VAS \geq 10$ (red-orange shades of the VAS scale); severely pronounced PS was noted by 28 (45.2%) adolescents; moderately pronounced intensity was noted by 20 (32.3%) patients (35.7%), which corresponded to $4 < VAS < 7$ (green and yellow shades of the scale). 6 patients (9.7%) characterized the intensity of the pain syndrome as «weak» and recorded it as the violet-blue part of the scale ($0 <VAS\leq 4$).

After 5 minutes, unbearable pain was recorded in 6 (9.7%) adolescents; after 10 minutes in 1 (1.5%)

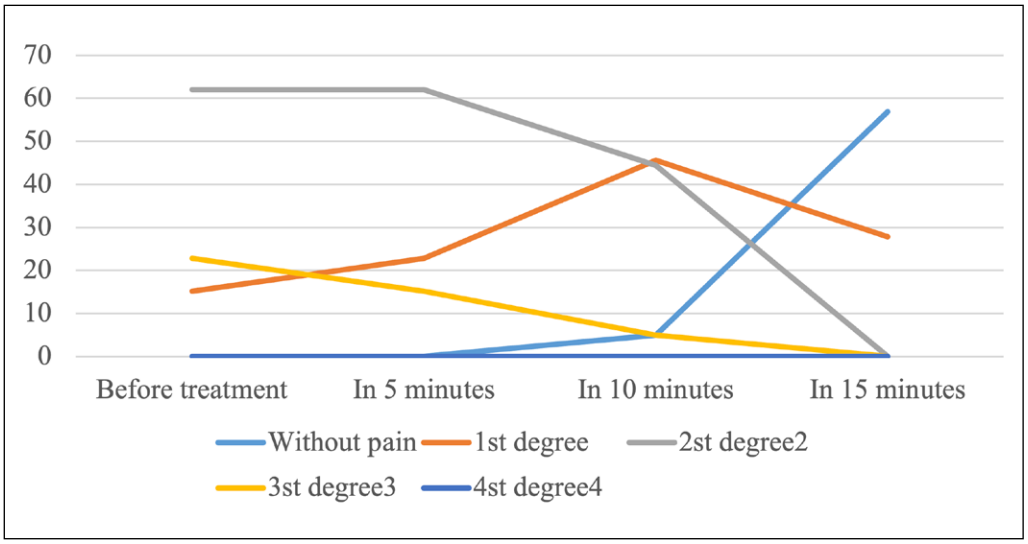

Fig. 1. Diagram of changes in the intensity of pain syndrome in acute medium caries in clinical groups in the dynamics of treatment in adolescents with high anxiety.

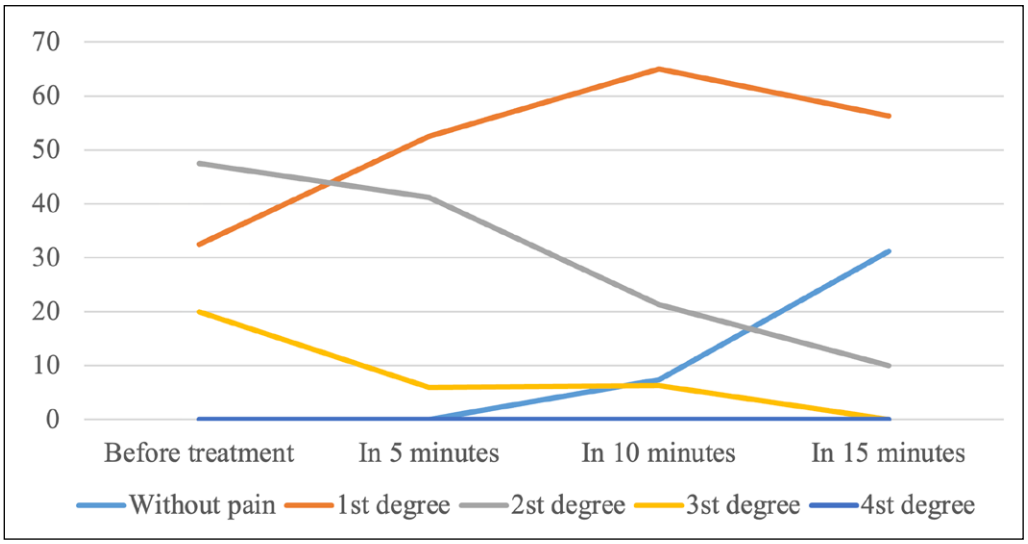

Fig. 2. Diagram of changes in the intensity of pain syndrome in acute deep caries in clinical groups in the dynamics of treatment in adolescents with high anxiety.

adolescent; after 15 minutes, no pain of this intensity was noted (Fig. 3).

The decrease in the intensity of unbearable pain was significant ($p<0.05$). Severe pain syndrome was diagnosed in 6 (21.3%) adolescents in 5 minutes; in 10 minutes in 6 (9.8%) adolescents; in 15 minutes - in 1 patient (1.5%). Reduction of moderate intensity BS, in particular, in 5 minutes in 26 (41.9%) adolescents; in 10 minutes in 16 (25.8%) adolescents; in 15 minutes in 5 adolescents (8.1%). An increase in the percentage of adolescents with low-intensity BS was observed in all groups 10 and 15 minutes after the manipulation compared to the pre-treatment indicators (9.7%, 33.9%, 45.2%; $p<0.05$); complete disappearance of BS was observed in 10 minutes in 18 (29.0%) adolescents; in 15 minutes in 28 (45.2%) adolescents.

In adolescents with a high level of anxiety, the decrease in the intensity of BS was significant compared to the pre-manipulation values, with complete disappearance of BS observed in 29.0% (18 patients) in 10 minutes and 45.2% (28 patients) in 15 minutes after anesthesia.

In the treatment of acute forms of periodontitis, before treatment, only 11 (47.8%) patients indicated the presence of severe BS (intolerable BS) (37.5%): 7 < VAS ≥10 (red-orange shades of the VAS scale). Severe BS was reported by 9 (39.1%) patients with VAS <7 (37.5%) (Fig. 4).

Moderate intensity was noted by 3 patients (13.1%), which corresponded to 4 <VAS <7 (green and yellow shades of the scale);. one patient (3.1%) characterized the intensity of the pain syndrome as «weak» and was recorded as the violet-blue part of the scale (0 <VAS≤ 4).

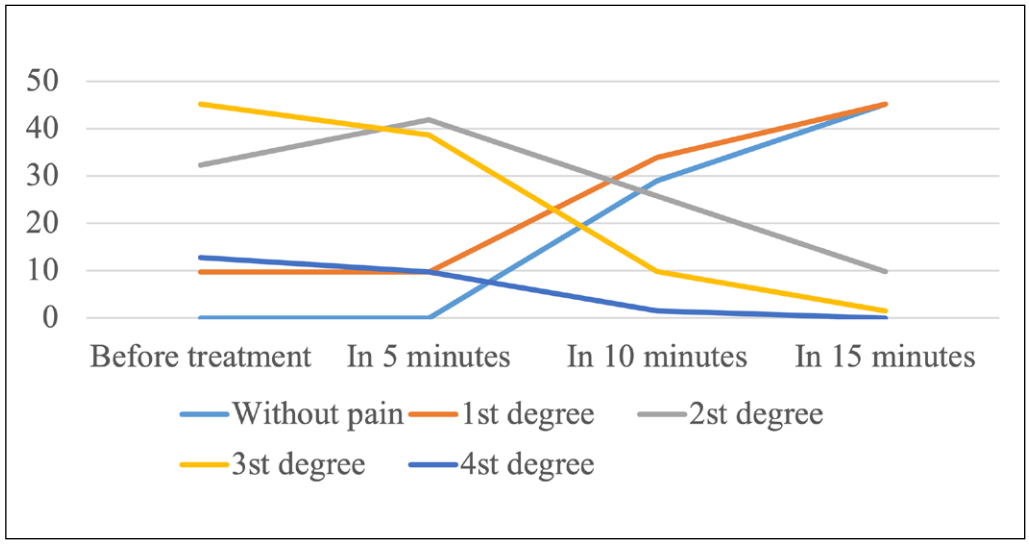

Fig. 3. Diagram of changes in the intensity of pain syndrome in acute pulpitis in clinical groups in the dynamics of treatment in adolescents with high anxiety.

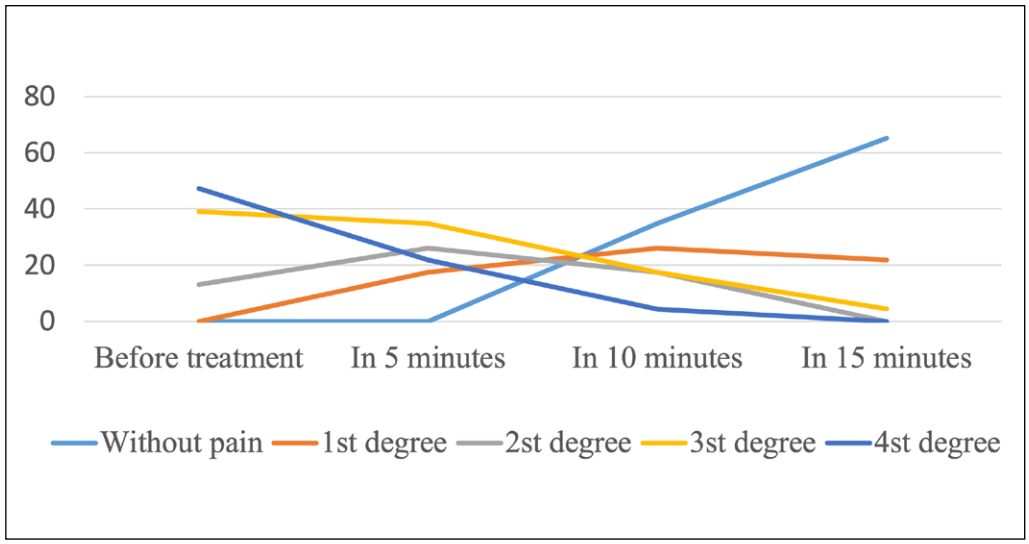

Fig. 4. Diagram of changes in the intensity of pain syndrome in acute periodontitis in clinical groups during the course of treatment in adolescents with high anxiety levels.

In all patients, a significant decrease in the intensity of PS and the frequency of its fixation in the dymanic zone was noted ($p<0.05$). After 5 minutes, unbearable pain was recorded in 1 (4.4%) adolescent; after 15 minutes, no pain of this intensity was noted. Severe pain syndrome was diagnosed in 8 (34.8%) adolescents in 5 minutes; in 10 minutes in 4 (17.4%) people; in 15 minutes - in 1 (4.4%) patient. The decrease in moderate intensity PS was significant, in particular, in 5 minutes in 6 (26.0%) adolescents; in 10 minutes in 4 (17.4%) adolescents; in 15 minutes in 2 adolescents (8.7%). An increase in the percentage of adolescents with low-intensity PS was observed in 10 and 15 minutes after the manipulation compared to the pre-treatment indicators (0.0%, 17.4%, 26.0%, 21.8%; $p<0.05$); Complete disappearance of PS was observed in 10 minutes in 8 (34.8%) adolescents; in 15 minutes in 15 (65.1%) adolescents.

The decrease in the intensity of BS was significant compared with the pre-manipulation values; complete disappearance of PS was noted in 34.8% - 8 patients in 10 minutes and in 65.1% - 15 patients in 15 minutes after anesthesia.

DISCUSSION

The implementation of modern anesthesia technologies is essential for safe and high-quality dental interventions, which was confirmed by the research of Boychenko O.M. at al. (2021), and Avetikov D.S. at al. (2022). Contemporary local anesthesia techniques require knowledge and practical skills in anesthesiology, anatomy, age-specific topography, psychophysiology, pediatrics, local anesthesia methods, anesthetic drugs (both anesthetics and vasoconstrictors), and the use of instruments (syringes, needles, and accessories) [1, 12].

Based on the Guideline on Use of Local Anesthesia for Pediatric Dental Patients. Council on Clinical Affairs, American Academy of Pediatric Dentistry (2015) and results from Janssen DF. (2021), modern anesthesia should ensure the patient's comfort and create optimal working conditions for the dentist. The authors confirm that, the primary requirements for anesthesia are its adequacy and safety [2, 4].

Pain management is particularly important in pediatric and adolescent dentistry, as there is a growing prevalence and severity of caries and its complications in children of all ages. Painful sensations limit the scope of treatment and reduce its quality, which is confirmed by the studies of Avetikov DS and co-authors (2022) [1].

Additionally, the pain a child may experience during a dental visit can lead to a refusal of treatment and foster a fear of visiting doctors of any specialty.

According to Bista P, Imlach W. (2019) a child's behavior is primarily driven by emotions, which can exacerbate their physiological intolerance to pain. The dentist should help the patient overcome fear and create an environment of safety and painlessness during examination and treatment. The unique physiological characteristics of children at different ages should guide the provision of differentiated dental care [10, 11].

Key conditions for providing quality treatment and preventive care for children include ensuring effective and safe anesthetic support for each child, preventing the development of fear, and fostering a positive attitude toward dental visits before addressing fears rooted in previous negative experiences. To assess the level of fear, we used the VAS scale proposed by Huang Z, Kohler IV, Kämpfen FA, 2019 [13].

Even with an appropriate choice of anesthesia method, correct dosage of the local anesthetic, and proper technique, anesthesia does not always achieve maximum effectiveness and may require additional measures to ensure comfortable, painless treatment. This is especially relevant for children and adolescents, where the psycho-emotional factor plays a significant role, as evidenced by the studies of Klitynska OV, Layosh NV., 2022 [8].

The psycho-emotional state of patients can be negatively affected, leading to psychological disorders and social adaptation issues. Dental pathology not only impacts a patient's health but also their quality of life, potentially leading to stigmatization among peers, limiting future career choices, and affecting overall professional development [1, 9].

The available literature contains limited studies on the relationship between the effectiveness of local anesthesia and the patient's psycho-emotional state. There is also a lack of data on the criteria for addressing the emotional component, particularly in adolescents, highlighting the relevance of research conducted.

Effective anesthesia during dental procedures is one of the key factors in providing high-quality dental care, and its success is directly proportional to the patient's level of anxiety, particularly in children and adolescents. Managing anxiety in this population will enhance the effectiveness of local anesthesia and, consequently, improve the overall quality of dental treatment.

CONCLUSIONS

The reduction in pain syndrome during the treatment of acute forms of moderate and deep caries, pulpitis, and periodontitis was significant compared to pre-treatment levels ($p<0.05$). Complete pain relief was observed during anesthesia in 5.0% of cases (4 cases) after 10 minutes and in 56.9% (45 cases) after 15 minutes in the treatment of acute forms of moderate caries; in 3.9% (5 cases) after 5 minutes, in 19.6% (25 patients) after 10 minutes, and in 43.8% (56 cases) after 15 minutes in the treatment of acute forms of deep caries; in 29.0% (18 cases) after 10 minutes and in 45.2% (28 cases) after 15 minutes in the treatment of acute pulpitis; and in 34.8% (8 patients) after 10 minutes and in 65.1% (15 patients) after 15 minutes in the treatment of acute periodontitis.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 19.06.2024
ACCEPTED: 25.09.2024

ORIGINAL ARTICLE

CONTENTS

Dynamic indicators of the antioxidant system in children with acute respiratory pathology depending on the therapy scheme

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ABSTRACT

Aim: To improve the early diagnosis, course, prediction of the development of Acute Respiratory pathology in children, taking into account the state of antioxidant system (AOS).

Materials and Methods: The research group included school-age children (10-14 years old) with Acute Respiratory pathologe (n=111) and a control group (n=25).diseases in comparation.

Results: The highest positive correlation between ferritin and TNF- α (r=0,41, p=0,001); Cortisol with Glutathione peroxidase (r=0,35, p=0,006) were observed. Ascorbic acid presented positive interactions on the values of Il-6 and Il-2 (r=0,27, 0,26, respectively). The value of vitamin D is represented in positive interactions with γ-IFN (r=0,30), leptin (r=0,38) and Cu (r=0,32). The negative relationship of Zn with Il-6 was transformed for supporting immune barriers and protein metabolism.

Conclusions: The level of Glutathione peroxidase increased in 1,6 times, on the other hand, when the basic therapy was prescribed - in 1,1 times (p_3<0,01; p_4<0,01; p_5<0,01), cortisol level had a reliable tendency to decrease in 1,6. The values of Ascorbic acid increased by almost in 2 times in first group andin 1,4 times in the second group (p_3<0,01; p_4<0,01; p_5<0,01). After the treatment, the level of vitamin D was identified within the physiological range in the first group. There are also positive effects of optimized therapy on the state of Copper (p_3<0,01), Zinc (p_3<0,01), Iron (p_3=0,04) trace elements in comparison with the data of the second group.

KEY WORDS: acute respiratory disease (acute pharyngitis, acute bronchitis, acute tonsillitis), antioxidant system, reactive changes of the hepatobiliary system, treatment, children

Wiad Lek. 2024;77(9):1928-1937. doi: 10.36740/WLek/195151 DOI

INTRODUCTION

Oxidative stress (OS) is widely studied in the pathology of various diseases and their effects on the development of an imbalance in the links of homeostasis. The mechanisms of formation of free radicals and regulation of their levels with the participation of oxidant and antioxidant systems have been studied, but there is a need for scientific research on the identification of early and highly sensitive biomarkers of OS in patients and their role [1]. The main target of oxidative stress is phospholipid membranes. Violation of the integrity of the protective membrane leads to an increase in its permeability to viruses, changes in the receptor apparatus and, as a result, the penetration of free radicals into the cell with subsequent damage to DNA and mitochondria, which is accompanied by a decrease in the transmembrane potential and an acceleration of the release of apoptosis factors [2,3].

In the cells of a living organism, there is a balance between the synthesis of reactive molecules and their leveling by protective mechanisms. As a rule, this balance is aimed at maintaining pro-oxidant conditions, which ensures permanent moderate oxidative eustress [4].

Disturbance of the balance can cause development of oxidative stress, that is, a state of the body characterized by an increase in the oxidative potential, which can lead to damage to the structure of DNA, proteins, carbohydrates and lipids and, thus, physiological homeostasis is disturbed. As a result, there is a shift in cell functioning and redox signal transmission, accumulation of cytotoxic compounds. Stress is defined as an imbalance between the formation of oxidants and antioxidant protection in favor of oxidants, which leads to cellular dysfunction and tissue damage, apoptosis. An imbalance of pro-oxidants and antioxidant system components is at the root of many diseases, as well as in acute respiratory pathology [5].

Among all the systems of the body, the respiratory tract, especially the lungs, are the most sensitive to oxidative stress compared to other organs and systems, due to the effect on them of a higher concentration of oxygen, exogenous oxidants that increase the synthesis

of oxidants and activate the formation of free radicals as markers of inflammation. Thus, free radicals are constantly formed in the human body during normal metabolism. They have both positive and negative properties [6].

AIM

To improve the early diagnosis, course, prediction of the development of Acute Respiratory pathology in children, taking into account the state of Antioxidant system for the development of optimized therapy.

MATERIALS AND METHODS

The research group included school-age children (10-14 years old). The general group of inflammatory diseases of the respiratory tract (J06, 106.8, 106.9) with a diagnosis of Acute Respiratory infection (ARI) of bacterial origin was considered (n=111) and included local inflammatory lesions of the upper respiratory tract with presentation of acute Pharyngitis, J02 (68,0%), acute Bronchitis, J20, J20.9 (22,0%) acute Tonsillitis J03, J03.9 (10,0%) and a control group (n=25), identical in age and gender. Two research groups were created, depending on the method of treatment: 1st group - 60 patients (optimized therapy), 2nd group - 51 patients (basic therapy) and general clinical, biochemical, immunological studies were performed in the dynamics under the influence of the addition of mineral -vitamin complex and lysozyme.

RESULTS

In our research, we used the classification of natural antioxidants. The vast majority are exogenous components obtained from natural sources [7]. In the group of exogenous antioxidants, there are several subgroups that a person receives mainly with food, in particular, vitamins D3, C, minerals (Zinc, Iron and Copper).

The scientific study included determination of the levels of the following antioxidants (AO) in the blood of the examined patients:

a) representative of the enzyme link - glutathione-peroxidase (GPO);

b) non-enzymatic link:

1) low molecular weight AO – Bilirubin, Urea; Thyroxine (T4), steroid hormones (cortisol), vitamins D3, C);

2) macromolecular antioxidants of protein nature - total Protein, Ferritin;

3) trace elements: Zn, Fe, Cu.

Compounds that slightly inhibit or stimulate the action of antioxidants, but have no effect on the intensity of BRO processes, are also considered.

The obtained data on the studied indicators of AOS in children is presented in the following table (Table 1).

Based on the obtained date, the level of GPO increased by 1,6 times, when receiving basic therapy – by 1,1 times. Dynamic data varied within reference values, but with significant differences between groups and control group data ($p3<0,01$; $p4<0,01$; $p5<0,01$). It is known that the general cohort of biological antioxidants creates a buffer antioxidant system that has a certain capacity and spheres of influence, and the interrelationships of prooxidants/antioxidants determine the AO status of a child's organism. Antioxidant enzymes form a single metabolic chain, in which the product of the first reaction is the substrate of the next one, therefore, for the normal functioning of the entire system, it is important to maintain certain ratios in the activity of individual enzymes of the chain. The enzymatic link of the AOS of the body has significant associative links with the non-enzymatic link to protect cells from free radical oxidation products, taking into account their hydrophobic and hydrophilic characteristics and as a result, the formation of a complex antioxidant effect [4].

Dynamic changes in indicators under the influence of various treatment methods are observed. The predominance of positive probable differences in the group of children with an optimized therapy scheme in the levels of total bilirubin ($p5=0,002$), total protein ($p5<0,01$), and urea ($p5=0,03$) are especially worth noting.

The level of ferritin varied within the reference range, but with a tendency to increase ($p1=0,003$; $p2=0,46$; $p3=0,36$; $p4=0,2$; $p5=0,004$). The values of vitamin D3 at the initial stage were equal, below the reference range ($18,77 \pm 4,14$ and $18,41 \pm 3,26$ ng/ml, respectively, by group). After the treatment, the level of vitamin D3 was identified within the physiological range in the first group ($32,12 \pm 3,48$ ng/ml; $p1<0,01$, $p2<0,01$), while the upper limit was not reached in the second group ($28, 43 \pm 4,43$; $p3<0,01$, $p4<0,01$), but with a significant upward trend. The values of ascorbic acid increased almost 2 times in the first group and 1,4 times in the second group with reliable indicators ($p3<0,01$; $p4<0,01$; $p5<0,01$) and within the reference range. There are also positive effects of optimized therapy on the state of copper ($p5<0,01$), zinc ($p5<0,01$), iron ($p5=0,04$) trace elements. Increasing the level of zinc, which is part of approximately 300 different proteins and plays an enormous role in the functioning of the body, contributes to the reaction of antioxidant protection, stabilization of biological membranes and functioning of the endocrine glands. The trace element copper participates in the immune reactions of the child's organism, tissue respiration, mechanisms of enzymatic catalysis, contributes to the processes of cell proliferation, which

Table 1. Indicators of AOS links in children with Acute Respiratory diseases

Indicator	Group 1 (n=60)		Group 2 (n=51)		Control Group (n=25)
	Before treatment	After treatment	Before treatment	After treatment	
1	2	3	4	5	6
GTP (4171-10881, U/1)	3774,08±124,85	5974,29±344,22 (p1<0,01; p2=0,13)	3807,88±111,89	4192,81±219,25 (p3 <0,01; p4 <0,01; p5 <0,01)	6108,72±425,93
Ascorbic acid (5,0 – 15,0 mg/l)	4,30±0,25	8,09±0,57 (p1<0,01; p2=0,66)	4,34±0,21	6,25±0,45 (p3 <0,01; p4 <0,01; p5 <0,01)	8,21±1,96
Vitamin D3 (30-70 ng/ml)	18,77±4,14	32,12±3,48 (p1<0,01; p2 < 0,01)	18,41±3,26	28,43±4,43 (p3 <0,01; p4 <0,01; p5 <0,01)	39,57±5,86
Total protein g/l	66,49±7,04	74,42±3,98 (p1<0,01; p2= 0,20)	67,72±7,30	71,09±3,18 (p3 =0,003; p4=0,06; p5 <0,01)	73,03±5,68
Total bilirubin, µmol/l	15,72±9,3	12,68±2,91 (p1=0,02; p2= 0,19)	15,98±9,01	14,75±3,95 (p3 =0,37; p4=0,31; p5=0,002)	13,74±4,26
Ureal, µmol/l	4,72±1,26	3,87±0,82 (p1 < 0,01; p2= 0,77)	4,92±1,37	4,27±1,05 (p3 =0,008; p4=0,07)	3,81±0,99

Table 2. Correlation relationships of AOS indicators in children at the initial stage

Laboratory parameters		Correlation coefficient (r)	Statistical significance (p)
Free thyroxine	Total protein	-0,29	0,002
GPO	Thyroid hormone	-0,43	<0,01
Ascorbic acid	Vitamin B_{12}	0,25	0,007
Vitamin D3	CRP	-0,21	0,03
	ATPO	0,21	0,03
	IL-1	0,22	0,02
	TNF-α	0,26	0,005
Ferritin	Urea	-0,27	0,004
	Na	0,19	0,04
	TNF-α	0,23	0.01
Total protein	IL-4	0,19	0,05
Urea	CRP	-0,26	0,005
Cu	Bilirubin	0,20	0,03
	Creatinine	0,30	0,001
Zn	IL-6	-0,19	0,04
Fe	Urea	0,19	0,05
	Na	0,22	0,02
	Neoptein	-0,26	0,005
Cortisol	Creatinine	0,22	0,02
	Adiponectin	0,27	0,005
	IL-1	-0,26	0,005

determines its importance in the regeneration of the mucous membrane. Its biological significance is due to the fact that Cu+ and Cu2+ ions are components of numerous enzymes and proteins. Currently, about 20 are known, in particular, cytochrome C oxidase is a terminal protein complex that plays a decisive role in the regulation of the entire respiratory chain [8]. Also, Cu/Zn-dependent Superoxide dismutase is the starting chain of the antioxidant defense system of the child's body. Interdependence with iron exchange is demonstrated by presence of copper in Ceruloplasmin. Scientists have proven the existence of physiological

Table 3. Correlation relationships of AOS components under the optimized therapy influence

Laboratory parameters		Correlation coefficient (r)	Statistical significance (p)
Glutathione peroxidase	Cortisol	0,35	0,006
Ascorbic acid	IL-6	0,27	0,04
	IL-2	0,26	0,05
Vitamin D3	Y-IFN	0,30	0,02
	Leptin	0,28	0,003
	Cu	0,32	0,01
ferritin	Total protein	0,26	0,04
	TNF-α	0,41	0,001
	Antibodies to thyroperoxidase	0,39	0,02
Urea	IL-2	0,30	0,02
	IL-4	0,26	0,005
	IgG	0,29	0,02
Cortisol	IgE	-0,28	0,03
Bilirubin	Vitamin B_{12}	-0,28	0,03
Cu	Glucose	-0,34	0,007
	Alkaline phosphatase	0,28	0,03
	Vitamin D3	0,32	0,01
Zn	Creatinin	0,27	0,04
Fe	-	-	-

antagonism between copper, on the one hand, and zinc, molybdenum, and magnesium, on the other. Iron participates in the synthesis of hemoglobin and myoglobin, catalase and peroxidase; in direct and indirect oxidative processes (includes 72 enzymes), ensuring normal functioning of the Immune system [9].

The level of thyroid hormones also varied within the reference range with unreliable differences between groups (free Triiodothyronine, p5=0,37; free Thyroxine, (p5=0,16) in contrast to the level of Antibodies to Thyroperoxidase (p5=0,009), with a predominance of decrease of the level in the first group, but also within the reference values.

Cortisol values during our study varied within the physiological range, but with a reliable tendency to decrease by 1,6 times in the group of children with an optimized treatment regimen (p4<0,01; p5<0,01).

An important stage of scientific research is the analysis of correlation relationships of the obtained values at the initial stage and at the end of the treatment.

The components of the antioxidant protection of the child's organism at the initial stage are presented in Table 2.

The most numerous (n=5) correlations of vitamin D3 were with CRP (r =-0,21, p=0,03) with a positive direction from antibodies to thyroperoxidase, Il-1, and TNF-a (r=0,21-0,26 with p=0,02-0,005). The correlations of ferritin with the levels of urea (r=-0,27, p=0,004), the positive direction - with the levels of Na (r=0,19, p=0.04), TNF-α (r=0,03, p=0,01) and the indicator of Fe with urea (r=0,19, p=0,05), Na (r=0,22, p=0,02, neopterin (r =-0,26 , p=0,005) should be noted. The cortisol level was positively correlated with the values of creatinine (r=0,22, p=0,02) and adiponectin (r=0,27, p=0,005) and negative interactions were found with IL-1 (r=-0,26, p=0,005).

According to our data, AOS indicators have a suppressive effect on the inflammatory process in the organism, in particular, Fe on the level of neopterin (r=-0,26), Zn on the level of IL-6 (r=-0,19), cortisol on IL-10 (r=-0,26), vitamin D3 on CRP (r=-0,21), free thyroxine on the level of total protein (r=-0,29).

Glutathione peroxidase correlates with the level of Creatinine (r=-0,27), which is involved in the energy exchange of tissues. Ascorbic acid has a positive interaction with the level of vitamin B12 (r=0,25), which regulates carbohydrate and fat metabolism. This way, the results of these indicators testify the positive effects on the main metabolic processes in order to preserve energy exchange. The level of copper correlates with the levels of bilirubin and creatinine, as the final product of the exchange of protein compounds, which indicates the positive effects of this trace element on the biliary function of the hepatobiliary system and energy metabolism [10, 11]. In clinical pediatric Gastroenterology, the vast majority of diseases of the hepatobiliary system are of a functional nature, accompanying and aggravating the course of the underlying disease. An

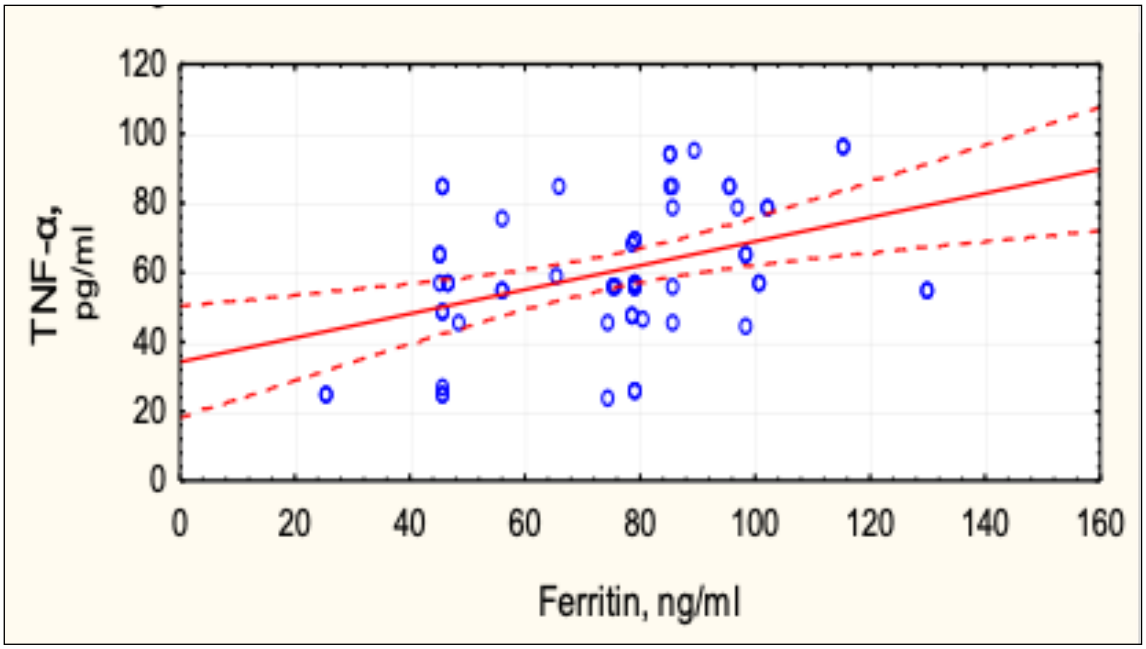

Fig.1. Correlation between the levels of TNF-a and ferritin levels (r= 0,41; p=0,001).

Table 4. Correlation relationships of data in children with Acute Respiratory diseases when using basic therapy

Laboratory parameters		Correlation coefficient (r)	Statistical significance (p)
Ascorbic acid	Urea	0,31	0,03
	K	0,27	0,04
Cu	Adiponectin	-0,31	0,03
	C-peptid	0,32	0,02
Zn	Glucose	0,37	0,008
	Ferritin	0,28	0,04
	TNF-α	0,35	0,01
Fe	CRP	-0,30	0,03
	Alkaline phosphatase	0,36	0,01
Free Thyroxine	Leucocyres	0,35	0,01
	Ca	-0,33	0,02
	Adiponectin	-0,33	0,02
	Leptin	-0,31	0,03
Vitamin D3	CRP	-0,39	0,004
	IgM	-0,30	0,03
	Vitamin B12	-0,39	0,005
	GPO	-0,36	0,009
Total Protein	Vitamin B12	-0,28	0,04
	IL-1	-0,36	0,009

interesting fact is that the development of hepatopathies with acute respiratory pathology, which have a short-term course and clinical presentation, are often undiagnosed and ignored. However, there are cases of functional disorders that dynamically transform into organic pathology. In particular, metabolic disorders and violations of components of AOS in acute Respiratory pathology can lead to the development of nonalcoholic fatty liver disease [12].

To optimize the treatment of ARD, we have developed a complex of vitamin-mineral composition and Lysozyme. The main regularities and relationships of the investigated components of AOS are considered in the following table (Table 3).

It should be noted that the number of relationships decreased from 22 to 18, of which 15 were positive (r=0,26-0,41, p=0,05-0,006). The highest positive correlation was observed in ferritin from TNF-α (r=0,41, p=0,001). The correlogram is presented on the following figure (Fig. 1).

The relationships between the levels of Fe, free Thyroxine, and Adiponectin were reduced, which indicates the positive effects of therapy in this study group. Negative relationships were observed between the levels of Cortisol and IgE (r=-0,28, p=0,03), which is evidence of suppression of allergic complications in respiratory pathology. Bilirubin values have a negative correlation with vitamin B12 (r=0,28, p=0,03), proving the effect on the biliary system and the decrease of Bilirubin depending on the increase of vitamin B12. Correlations of the level of Cu with glucose values (r=- 0,34, p=0,007) characterize the influence on the regulation of carbohydrate metabolism.

As for positive correlations, there is a synergism of the action of GPO with cortisol (r=0,35, p=0,006), which means the balancing of the antioxidant action in the organism, when negative relationships with Creatinine were observed at the beginning of the research. Cortisol at the initial stage was also positively correlated with Creatinine and Adiponectin with negative influence with pro-inflammatory IL-1. The final studies presented the synergism of the relationship between cortisol and GPO and the suppressive effects on the level of IgE.

Ascorbic acid presented positive interactions on the values of pro-inflammatory IL-6 and IL-2 (r=0,27 and 0,26, respectively at p=0,04, 0,05) in contrast to the initial interactions on vitamin B12 (r=0,25), which regulates carbohydrate and fat metabolism. It indicates there is still a need for the child's organism to regulate pro-inflammatory cytokines (IL-2,6) as means of preventing the development of the inflammatory process and promoting the secretion of immunoglobulins.

The value of vitamin D is represented by positive interactions with γ-IFN (r=0,30), Leptin (r=0,38) and Cu (r=0,32). The data can be interpreted as suppression of the activity of affected cells, stimulation of immunogenesis and its maintenance due to γ-IFN and the irreplaceable trace element Cu, taking into account the metabolic component represented by the leptin indicator. Correlations of the ferritin level have interactions with the values of total Protein (r=0,26), TNF-**α** (g=0,41) and Antibodies to Thyroperoxidase (r=0,39).

The level of Urea is positively correlated with Il-2 (r=0,30), Il-4 (r=0,26) and IgG (r=0,29), which indicates stabilization of the cytokine profile and prevention of chronic respiratory pathology.

Correlation between Zinc and Creatinine levels were also considered (r=0,7, p=0,04). Zinc is an important trace element, a representative of AOS, which modulates the functions of approximately 2,000 enzymes and 750 transcription factors to participate in various biological and physiological processes. There are scientific studies on its maintenance of the integrity of immune barriers as a Cofactor in Metalloenzymes, enhancement of cytotoxic activity of natural killers, proliferation and differentiation of innate immune cells, production of interferon. It has anti-inflammatory properties due to the modulation of the release of cytokines and the production of antibodies, mainly IgG [13]. According to our data, negative connections of Zn with IL-6 as a pro-inflammatory marker were transformed at the final stage into modulating characteristics of the trace element. both in terms of supporting immune barriers and protein metabolism. Although at the initial stage, there was a negative correlation with CRP and a positive correlation with the levels of pro-inflammatory Cytokines - IL-1, TNF-a. We will analyze the state of the links of AOS homeostasis in the treatment of children with basic therapy for Acute Respiratory diseases (Table 4).

Positive correlations are presented in the values of Ascorbic acid with the levels of Urea (r=0,31) and Potassium (r=0,27). Ascorbic acid is a non-enzymatic component of AOS. Ascorbic acid is an unusual antioxidant, because it reacts mainly with radicals, and not with non-radical compounds. The protective function of this vitamin includes the prevention of damage to protein, lipid and carbohydrate compounds and the acceptance of active forms of oxygen, nitrogen, and carbon. Vitamin C can also act as a pro-oxidant, especially in the presence of transition metals such as iron and copper, triggering various dangerous radical reactions and behaving as a radical promoter [14].

The formation of urea is a necessary link in protein synthesis and amino acid metabolism, it also participates in osmoregulation, affects the distribution of fluid between body cells and the extracellular space. The urea level indicator also correlates in a positive range with IgG values (r=0,29, p=0,02), as components of the body's protein metabolism.

The next element of the studied triad is the value of potassium. Which, as the main intracellular cation, regulates intracellular osmotic pressure and participates in protein metabolism.

The next investigated component is vitamin D level. The value of the indicator is presented by negative correlations with CRP (r=-0,39, p=0,004), IgM (r=-0,30, p=0,03), vitamin B12 (r=-0,39, p=0,005), GPO (r=-0.36, p=0.009). According to our data, the value of vitamin D3 has a suppressive effect on the synthesis of inflammatory markers IgM, CRP. The negative interactions on GPO level, on the background of

the metabolic factor vitamin B12, suggest a suppressive direction, which can be explained by vitamin D3 levels below reference, even after treatment. However, at the initial stage, there was a negative correlation with CRP and a positive correlation with the levels of pro-inflammatory cytokines - IL-1, TNF-a.

Thyroxine correlations are also informative. Thyroxine is one of the hormones that is synthesized by the thyroid gland and regulates energy metabolism in the human body. The study of the features of the antioxidant reaction shows that thyroxine stops oxidation by reacting with common types of chain oxidation. Studies confirms the hypothesis that thyroxine participates in the system of free radical reactions and as a result of these reactions it decomposes [15].

According to our data, there is a predominance of negative correlations of Thyroxine with Ca levels (r=-0,33, p=0,02), Adiponectin (r=- 0,33, P=0,02), Leptin (r=-0,31, p=0,03), except for the level of the Leukocyte pool (r=0,35, p=0,01), which is presented by positive relationships. Regarding the initial correlations, negative correlations with the levels of Total Protein (r=-0,29) and TSH (r=-0,43) were observed. Thyroxine functions as a highly specific antioxidant. According to the final results, it can be suggested that the level of thyroxine stabilizes the metabolic component and mineral exchange in the organism after treatment [16].

As for the correlation relationships of the total protein indicator, negative effects on the level of vitamin B12 (r=-0,28, p=0,04), IL-1 (r=-0,36, p=0,009) and positive correlations with the level of Neopterin (r=0,31, p=0,03) were observed. The anti-inflammatory effects of the Total Protein level with IL-1 are particularly pronounced, with a high degree of reliability, along with positive effects on the Neopterin level , which characterizes the state of the Immune system.

Correlative interrelationships of microelements are indicative as well. In particular, Cu presents multidirectional effects on adipose tissue hormones - with Adiponectin (r=-0,31, p=0,03), C-peptide (r=0.32, p=0.02), which indicates a metabolic component in copper exchange.

The level of Zn is characterized by positive correlation effects with the values of Glucose (r=0,37, p=0,008), Ferritin (r=0,28, p=0,04) and TNF- α (r=0,35, p=0,01). The level of Fe correlates in a negative range with suppression of the synthesis of the inflammatory marker CRP (r=-0,30, p=0,03) and with a positive vector with the value of Alkaline Phosphatase (r=0,36, p=0,01). Oxidative stress is a common denominator in the pathogenesis of various chronic diseases. Therefore, Antioxidants are often used to protect cells and tissues and eliminate oxidative damage. It is well known that iron metabolism is the basis of the dynamic interaction between Oxidative stress and antioxidants in many pathophysiological processes. Both Iron deficiency and Iron overload can affect the redox state.

DISCUSSION

Oxidative processes and the formation of free radicals are an integral part of human metabolism. Redox biology encompasses events involving a shift in the balance between reactive oxygen species (ROS) and their removal [17]. Infection with respiratory viruses is generally associated with cytokine production, inflammation, cell death, and other pathological processes that can be triggered by increased ROS production. [18].

The Respiratory tract occupies the second place after the Gastrointestinal tract in terms of the area of the mucous membranes. Therefore, understanding the unique nature of the Immune system of the respiratory mucosa is extremely important [19]. The consequence of vitamin A hypovitaminosis is, in particular, poses damage to the mucous membranes of the Respiratory tract: the glandular epithelium is replaced by a keratinized one due to keratinization. The loss of the mucociliary epithelium of the Respiratory tract reduces the barrier role of the mucous membranes. Also, vitamin A deficiency reduces the production of Lysozyme and Interferons, further weakening the body's non-specific resistance. One of the consequences of vitamin A deficiency is also the weakening of specific resistance during reproduction, growth and differentiation of Immunocompetent cells [20]. Therefore, Hypovitaminosis A results in increased susceptibility to the causative agents of Acute Respiratory nfections and their more severe course [21]. At the same time, the presence of Hypovitaminosis A, taking into account the involvement of vitamin A in the synthesis of Iron (insufficiency of which leads to a decrease in the Oxygen capacity of the blood) and the synergistic effect of vitamins A and D3 (activation of receptors for Calcitriol), is being actively investigated [22].

Nowadays, scientific literature contains more and more data on the role of vitamin D3 and its metabolites in the regulation of body homeostasis, the effect on cell differentiation and proliferation, the expression of antimicrobial peptides, as well as its involvement in the formation of innate and adaptive immunity. It is recommended to prevent vitamin D3 deficiency and maintain a serum 25(OH)D level >75 nmol/L. [23, 24]. It is vitamin D3 that is receiving special attention regarding the risk of acute respiratory tract infections in the pediatric

population. Children with respiratory tract infections were found to have significantly lower mean vitamin D3 levels compared to control groups. A relationship between the level of vitamin D3 and the frequency and severity of infections was also observed [23].

A disease caused by vitamin C deficiency can affect susceptibility to respiratory infections [24]. The level of vitamin C in human blood plasma rapidly decreases under conditions of physiological stress, including infection. The antioxidant, anti-inflammatory and immunomodulatory effect of vitamin C makes it a potential therapeutic agent both for the prevention and relief of the course of respiratory infections, and as an adjunctive therapy as well [25].

Therefore, oxidative processes, including a shift in the balance between active forms of oxygen and their removal, are an integral part of human metabolism. Infection with respiratory viruses, which is associated with the production of cytokines, inflammation, cell death and other pathological processes, can be provoked by oxidative stress, changes in the content of certain vitamins and trace elements, which creates conditions for the development of a number of pathological processes, including acute and recurrent respiratory diseases.

CONCLUSIONS

1. Based on the analysis of dynamic indicators under the influence of optimized treatment, the level of GPO increased in 1,6 times, on the other hand, when the basic therapy was prescribed - in 1,1 times (p3<0,01; p4<0,01; p5<0,01), the level of Cortisol had a reliable tendency to decrease by 1,6 but within the physiological range (p4<0,01; p5<0,01), the level of Ascorbic acid increased in almost 2 times in the first group and in 1,4 times in the second group (p3<0,01; p4<0,01; p5<0,01). Vitamin D3 levels at the initial stage were equal, below the reference range (18,77 ± 4,14 and 18,41 ± 3,26 ng/ml, respectively, by group). After the treatment, the level of vitamin D3 was identified within the physiological range in the first group (32,12 ± 3,48 ng/ml; p1<0,01, p2<0,01), in contrast, the upper limit was not reached in the second group (28,43 ± 4,43; p3<0,01, p4<0,01, p5<0,01), but with a significant upward trend. There are also positive effects of optimized therapy on the state of Copper (p3<0,01), Zinc (p3<0,01), Iron (p3=0,04) trace elements in comparison with the data of the second group.
2. At the initial stage, the most numerous (n=5) negative correlations of vitamin D3 with CRP (r=-0,21, p=0,03) and a positive correlation with antibodies to TPO, IL-1, and TNF-α were found (r=0,21-0,26, p=0,02-0,005). AOS indicators had a suppressive effect on the inflammatory process in the body, in particular, Fe on the level of Neopterin (r=-0,26), Zn on the level of IL-6 (r=-0,19), Cortisol on IL-10 (r=- 0,26), vitamin D3 on CRP (r=-0,21), free Thyroxine on the level of Total Protein (r=-0,29). GPO was correlated with the level of Creatinine (r=-0,27), which is involved in the energy exchange of tissues. Ascorbic acid had a positive interaction with the level of vitamin B12 (r=0,25), the level of copper was correlated with the levels of Bilirubin and Creatinine, as the end product of the exchange of Protein compounds. In other words, the results of these indicators prove the positive effects on the main metabolic processes in order to preserve energy exchange.
3. The relationships between the levels of Fe, free Thyroxine and Adiponectin were reduced, which indicates the positive effects of therapy in this study group. The highest positive correlation was found between Ferritin and TNF-α (r=0,41, p=0,001); along with the the synergism of the action of cortisol with GPO (r=0,35, p=0,006) and the suppressive effect on IgE (r=-0,28, p=0,03), which indicates the balancing of the antioxidant action in the organism, ascorbic acid presented positive interactions on the level of pro-inflammatory IL-6 and IL-2 (r=0,27, 0,26, respectively at p=0,04, 0,05), which is considered as prevention of the development of the inflammatory process. Vitamin D3 is represented by positive interactions with γ-IFN (r=0,30), Leptin (r=0,38) and Cu (r=0,32), which can be interpreted as inhibition of the activity of affected cells, stimulation of immunogenesis and its maintenance due to γ-IFN and Cu taking into account the metabolic component - Leptin. The level of Urea was positively correlated with IL-2 (r=0,30), IL-4 (r=0,26) and IgG (r=0,29), which indicates stabilization of the Cytokine profile and prevention of Chronic Respiratory pathology.
4. Positive correlations are presented in the values of Ascorbic acid with the levels of Urea (r=0,31) and Potassium (r=0,27). The indicator of vitamin D3 presented negative correlations with CRP (r=-0,39, p=0,00 IgM (r=-0,30, p=0,03), vitamin B12 (r=-0,39, p=0,005), GPO (r=-0,36 and p=0,009), which can be regarded as a suppressive effect on the synthesis of inflammatory markers IgM, CRP. Negative interactions on the level of GPO against the background of the metabolic factor of vitamin B12 indicate a suppressive direction, which can be explained by the levels of vitamin D3 being below the reference range even after treatment. The level

of Zn is characterized by positive correlations with the levels of Glucose (r=0,37, p=0,008), Ferritin (r=0,28, p=0,04) and TNF-α (r=0,35, p=0,01). The level of Fe correlates in a negative way with the suppression of the synthesis of the inflammatory marker CRP (r=-0,30, p=0,03) and with a positive vector with the value of Alkaline Phosphatase (r=0.36, p=0.01).

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 10.06.2024
ACCEPTED: 11.09.2024

ORIGINAL ARTICLE

CONTENTS

Violation of vitamin and mineral homeostasis in children with recurrent respiratory diseases

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ABSTRACT

Aim: To examine and analyze the state of vitamin and mineral homeostasis in children with recurrent respiratory diseases.

Materials and Methods: To achieve the goal, 62 children of primary school age with a diagnosis of Recurrent Respiratory diseases were examined in comparison with data of healthy children of the control group (n=26). The study included a clinical examination of children, determination of serum vitamin and mineral levels.

Results: A dinamic decreasing in the level of vitamins beyond the reference range was found: vitamin D (in 1.4 times) vitamin C (in 1.4 times lower than the data of the control group, and Cyanocobalamin (in 1.9 times) in relation to the data of the control group. Calcium levels were below the limit of reference values, both total (2.14 ± 0.04 mmol/l vs. 2.53 ± 0.07 mmol/l, $p<0.01$) and ionized (1.15 ± 0.01 mmol/l compared 1.26 ± 0.05 mmol/l, $p<0.01$). At the lower limit of the reference, there were significant differences in the levels of Zinc ($p<0.01$, 1.6 times), Copper ($p<0.01$, 1.4 times) and unreliable - Magnesium ($p<0.12$), sodium ($p<0.62$).

Conclusions: Positive correlations were observed with vitamins - Cyanocobalamin ($r=0.40$, $p<0.01$), vitamin C ($r=0.43$, $p<0.01$), Ferritin ($r=0.35$, $p<0.01$), Magnesium ($r=0.27$, $p<0.003$). Negative correlations of vitamins, in particular, Folic acid ($r=-0.50$, $p<0.01$), vitamin A ($r=-0.40$, $p<0.01$) and Phosphorus minerals ($r=-0.75$, $p<0.01$), Sodium ($r=-0.51$, $p<0.01$), Potassium ($r=-0.24$, $p=0.008$), Chlorine ($r=-0.38$, $p<0$,01).

KEY WORDS: acute respiratory infections, Recurrent Respiratory infections, vitamins, minerals, homeostasis disorders, children

Wiad Lek. 2024;77(9):1938-1946. doi: 10.36740/WLek/195155 DOI

INTRODUCTION

Recurrent respiratory infections (RRIs) are a very common clinical condition in childhood with an important social and economic impact. According to estimates, about 25% of children under the age of 1 year and 6% of children in the first 6 years of life have PRIs, which makes them one of the most common reasons for visiting a pediatrician in the first years of life [1–3]. Although it is a benign condition that is likely to gradually improve by age 12, it significantly interferes with the child's well-being and causes significant medical and social costs. Within RRIs, a specific definition of relapse has yet to find consensus in the literature, on the contrary, the relapse of some specific respiratory diseases is well expressed. These include infectious rhinitis [4], which is defined as recurrent if it occurs more than 5 times per year, or acute otitis media, which is classified as recurrent with 3 episodes in 6 months or 4 episodes in 12 months [5].

In the past, a clinical score for the assessment of PRIs was proposed for children, and it was based on the type of infectious episode, its duration, visits to the pediatrician, therapy, and absence from the community [1]. Cases that scored more than 30 points in 6 months were classified as PRIs. Alternatively, recently, a definition has been introduced that takes into account different trends in the development of respiratory infections depending on age: to define them as PRIs, 8 or more infections per year are required in subjects under 3 years of age, 6 or more infections in children older than 3 years [6].

To guide physicians in the treatment and prevention of children with PRIs, based on the analysis, an intersocietal consensus document was developed, which includes an updated definition of PRIs, a practical diagnostic algorithm, and recommendations for the use of possible measures to prevent PRIs in children. Available international scientific literature, developed according to the GRADE (Grading of Recommendations Assess-

ment, Development and Evaluation) method. Regarding the treatment of individual infectious episodes, the commission recommends that each individual infection be controlled according to national and international guidelines published for each respiratory disease (eg, tonsillitis, rhinitis, otitis, etc.) [7].

AIM

The aim was to examine and analyze the state of vitamin and mineral homeostasis in children with recurrent respiratory diseases.

MATERIALS AND METHODS

To achieve the goal, 62 children of primary school age with a diagnosis of recurrent respiratory diseases were examined in comparison with the data of healthy children of the control group (n=26) identical in age and anthropometric data. The study included a clinical examination of children, determination of serum vitamin and mineral levels.

RESULTS

Recurrent respiratory infections (RRIs) are characterized by impaired immune function and deficiency of micronutrient reserves, particularly vitamins, including vitamins A, B9, B12, C, D3, and E [8]. Some nutritional components are reported to be actively involved in the proper functioning and strengthening of the immune system, including proteins, omega-3 fatty acids, vitamins A, D3, E, B1, B6, B12, and C [8].

Based on studies that show that vitamins are effective in the prevention of respiratory pathology, we will consider the role of vitamins and their effect on PRIs (Table 1).

According to Table 1, there is a decrease in the level of vitamins beyond the reference limits: vitamin D3 (26.82 ± 2.90 ng/ml and in comparison with the data of the control group 36.05 ± 2.58 ng/ml, $p<0.01$), vitamin C (4.78 ± 2.41 mg/l vs. 9.17 ± 2.63 mg/l, $p<0.01$) and cyanocobalamin (206.39 ± 24.91 pg/ml vs. 385.57 ± 54 .26 pg/ml, $p<0.01$).

In infectious diseases, vitamin D3 plays a role in maintaining the physical barrier of the immune system by maintaining the integrity of the mucous membrane, which is usually disrupted by viruses and other microorganisms, enhances innate and cellular immunity, partly through the induction of antimicrobial peptides, directly exhibits antimicrobial, antiviral, antifungal effects, reduces the replication of the influenza A virus. In the adaptive immune response, vitamin D3 suppresses pro-inflammatory cytokines mediated by T-helper cells type 1 (Th1), and enhances the production of anti-inflammatory cytokines by T-helper cells 2 (Th2), promotes the induction of T-regulatory cells, thereby suppressing inflammatory processes [9].

Despite strong evidence for the important role of vitamin D3 in the immune system, clinical trials have shown conflicting findings regarding the effects of vitamin D3 supplementation on respiratory infections. A systematic review that included 39 studies showed a significant association between low vitamin D3 and an increased risk of upper and lower respiratory tract infections [10,11].

According to our research, the values of vitamin D3 were lower than the reference and 1.4 times lower than the data of the control group. In the context of respiratory infections, there is increasing scientific evidence that vitamin D3 can have a protective effect against various infections and reduce the severity of the disease. These findings have increased scientific interest in the relationship between vitamin D3 and infections, particularly the molecular mechanisms involved in the effect of vitamin D3 status on respiratory tract infections. Although between-study heterogeneity is high for most outcomes, and publication bias may have led to an overestimation of the effect size of the incidence data, vitamin D3 supplementation may be beneficial in increasing resistance to common respiratory infections, especially when used daily [12].

From a functional point of view, vitamin C is involved in many biological processes: it prevents oxidative damage to biomolecules by directly absorbing free radicals, by donating electrons to these radicals, and indirectly absorbing free radicals by reactivating other free radical scavengers, such as alpha-tocopherol (vitamin E) and glutathione (GSH), also acts as a cofactor for more than sixty enzymes that catalyze important reactions, including the synthesis of collagen, carnitine, serotonin, and norepinephrine (Ang et al. 2018) [13].

In addition, suppressive effects on the level of inflammatory mediators are observed by inhibiting the activity of nuclear factor kappa B (NF-κB) and the penetration of immune cells into the microcirculation by inhibiting the expression of intracellular adhesion molecules (Du et al. 2022) [14]. In line with this extensive biological activity, beneficial effects of vitamin C have also been reported in the context of various diseases. In addition, numerous findings have been published on the effects of vitamin C on respiratory, allergic, and immunological diseases. In this regard, treatment with vitamin C has been shown to improve

Table 1. Level of vitamins and proliferative activity of lymphocytes in children with PRIs

Laboratory parameters	Control group (n=26)	1st group – OT (n=62)	Statistical significance of differences
Vitamin D3 (30-50, ng/ml)	36,05±2,58	26,82±2,90	p<0,01
Vitamin A (0.26-0.49 ng/ml)	0,39±0,07	0,38±0,05	p<0,045
Vitamin E (3.0-9.0, ng/ml)	7,28±1,47	10,56±2,67	p<0,01
Vitamin C (5.00-15.00, ng/ml)	9,17±2,63	4,78±2,41	p<0,01
Folic acid (B9)(3.00-17.00, ng/ml)	8,26±2,61	3,75±0,54	p<0,01
Cyanocobalamin (211.0-911.0, pg/ml)	385,57±54,26	206,39±24,91	p<0,01
Proliferative activity of lymphocytes with mitogen (1.2-1.68, opt.unit)	1,45±0,07	1,54±0,08	p<0,01

Notes: p – the statistical significance of the differences between the indicators of the 1st and control group

Table 2. Levels of mineral metabolism in children with RRI

Laboratory parameters	Control group	1st group (n=62)	Statistical significance of differences
Za (46-150, µ/dL)	75,35±18,05	47,27±3,46	p<0,01
Cu (10.4-21.4, µmol/l)	15,89±1,32	11,05±1,53	p<0,01
Se (0.05-0.28, µg/l)	0,17±0.05	0,11±0,02	p<0,01
Mg (0.7-0.86, mmol/l)	0,80±0,04	0,79±0,02	p<0,12
Total Ca (2.2-2.7, µmol/l)	2,53±0,07	2,14±0,04	p<0,01
Ionazed Ca (1.16-1.32, mmol/l)	1,26±0,05	1,15±0,01	p<0,01
P (phosphorus)(0.95-1.75, mmo;/l)	1,64±0,18	1,69±0,19	p<0,26
Na (136.0-145.0, mmol/l)	139,66±2,07	139,89±1,94	p<0,62
K (3.5-5.1, mmol/l)	4,43±0,29	4,42±0,34	P=0,89
Cl (98.0-107.0, mmol/l)	103,07±2,64	102,35±2,15	P=0,18

Notes. P2 – statistical significance of differences between indicators of the 1st and control groups

the course of influenza and upper respiratory tract infections (Colunga Biancatelli et al. 2020; van Driel et al. 2019) [15].

In studies on vitamin C and Colds, 3 out of 5 studies showed an effect of reducing the frequency of cold diagnoses and the duration of cold symptoms with the consumption of 500–6000 mg of vitamin C [16-19].

Considering that vitamin B12 plays a crucial role in metabolic processes, cardiovascular and circulatory systems, as well as controls the immune system and antiviral activity, participates in the recovery of damaged tissues and compensates for the reduction of reserves in the liver during viral hepatitis [20], it is possible to think about the reduction of positive effects in our studied contingent on the above biological characteristics. The values of the level of vitamin B12 show a decrease in the level relative to the reference values and a significant decrease of 1.9 times in relation to the data of the control group (206.39 ± 24.91 pg/ml vs. 385.57 ± 54.26 pg/ml, p<0.01).

According to our research, there was a slight increase in vitamin E beyond the reference values (10.56 ± 2.67 mg/l vs. 7.28 ± 1.47 mg/l, p<0.01). According to the researchers, compared with the pneumonia group, the vitamin E level increased in the recurrent respiratory infection group, and the difference was statistically significant (P<0.05), which is consistent with our research. Vitamin E levels in the asthma with cough group were decreased compared to the recurrent respiratory infection group, asthma and pneumonia group (P<0.05) [21].

According to our data, the level of folic acid is within the reference range (3.75 ± 0.54 ng/ml vs. 8.26 ± 2.61 ng/ml data of the control group), that is, it is 2 times lower than in the children of the control group. Given the properties of the functioning of the body system, folic acid is necessary for the creation and maintenance of new cells in a healthy state, therefore its presence is especially necessary for the development of the body, especially in childhood. One can think about the pre-pathological level of folic acid in our patients, which needs correction. [22]. Two studies related to folic acid have shown that folic acid has a positive effect on the prevention of viral infections.

Table 3. Correlation relationships of lymphocytes proliferative activity with mitogen and vitamin-mineral components

Parameter	Parameter	r	p
Lymphocytes proliferative activity with mitogen	Focil acid	-0,50	<0,01
	Cyanocobalamin	0,40	<0,01
	Ferritin	0,35	<0,01
	Vitamin A	-0,40	<0,01
	Vitamib C	0,43	<0,01
	Mg	0,27	<0,003
	Phoshorus	-0,75	<0,01
	Na	-0,51	<0,01
	K	-0,24	<0,008
	CL	-0,38	<0,01

A study related to folic acid and respiratory infections indicated that deficiency of micronutrients such as folic acid was strongly associated with the occurrence of pneumonia or colds ($p < 0.001$) [23].

Considering the level of vitamin A in our patients, no increase was found, and no significant differences were identified between the studied parameters ($p=0.45$). From numerous scientific studies, it has been proven that children with low serum vitamin A levels were significantly associated with respiratory diseases in children and adolescents, contrary to our results. [24]

The study of indicators of the proliferative activity of lymphocytes with mitogen in patients with recurrent respiratory diseases (RRD) showed that its level is significantly different from the values of the control group of children and varies within the reference values of anti-infective protection of patients (1.54 ± 0.08 opt. units vs. 1.45 ± 0.07 opt. units, $p<0.01$).

Saturation of the children's body was considerated with micro and macroelements in the RRI childs (table 2).

According to the data of table II, Calcium levels were observed below the limit of reference values, both total (2.14 ± 0.04 μmol/l vs. 2.53 ± 0.07 μmol/l, $p<0.01$) and ionized (1.15 ± 0.01 mmol/l vs. 1.26 ± 0.05 mmol/l, $p<0.01$). At the lower reference limit, there were significant differences in the levels of Zinc (47.27 ± 3.46 μg/dL vs. 75.35 ± 18.05 μg/dL, $p<0.01$, 1.6 times), Copper (11.05 ± 1.53 μmol/l versus 15.89 ± 1.32 μmol/l, $p<0.01$ in 1.4 times) and unreliable Magnesium ($p<0.12$), Sodium ($p<0.62$). The value of the Selenium level varied within the reference range, but with a significant decrease of 1.5 times compared to the data of the control group (0.11 ± 0.02 μg/l vs. 0.17 ± 0.05 μg/l, $p<0.01$). The values of Potassium ($p=0.89$), Chlorine ($p=0.18$), Phosphorus ($p=0.26$) were also presented as unreliable differences.

Calcium has many important functions, including intracellular signaling, muscle function, nerve transmission, and mediating vasoconstriction and dilation. Total serum calcium concentration varies significantly with serum albumin concentration and hydration status, without any change in ionized calcium concentration. The gold standard for assessing calcium status is measurement of ionized calcium. Although Hypocalcemia can lead to acute respiratory failure due to muscle weakness, tetany, laryngospasm, and bronchospasm, patients with severe Hypercalcemia may present with lethargy, confusion, and coma. Low serum ionized calcium can also cause cardiac dysfunction, including QTc prolongation and reduced left ventricular systolic function, leading to acute pulmonary edema. Low serum ionized Calcium may also reflect the severity of respiratory Alkalosis, which eventually leads to fatigue and diaphragmatic weakness due to hypocalcemia, as well as heavy diaphragmatic stress due to the underlying disease itself [25]. Zinc, Copper and Iron are trace elements that are involved in the development of the immune response. Regarding the effectiveness of micronutrients in the prevention of RRIs, there are few low-quality studies available, burdened by insufficient reproducibility, methodological imprecision, small population size, and heterogeneity of the study population and the results obtained, so it is not possible to recommend the use of micronutrients for the prevention of RRIs [26].

Zinc is involved in the body's cell-mediated and humoral immune responses, each of which is generated for a specific pathogen. Its low level can affect the ability of the immune system to fight infections [27]. Copper is an important trace element for various physiological processes in almost all types of human cells. Dysfunctional Cu metabolism or regulatory pathways can lead to an imbalance

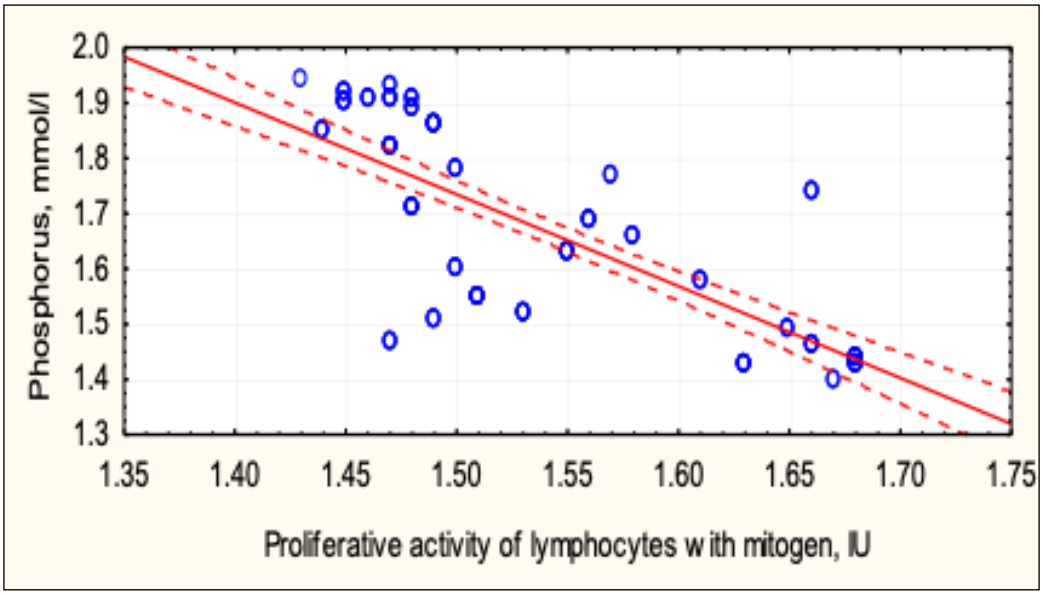

Fig.1. Correlation between Phosphorus concentration and Lymphocytes Proliferative activity with mitogen in the blood (r=-0.75; p<0.01).

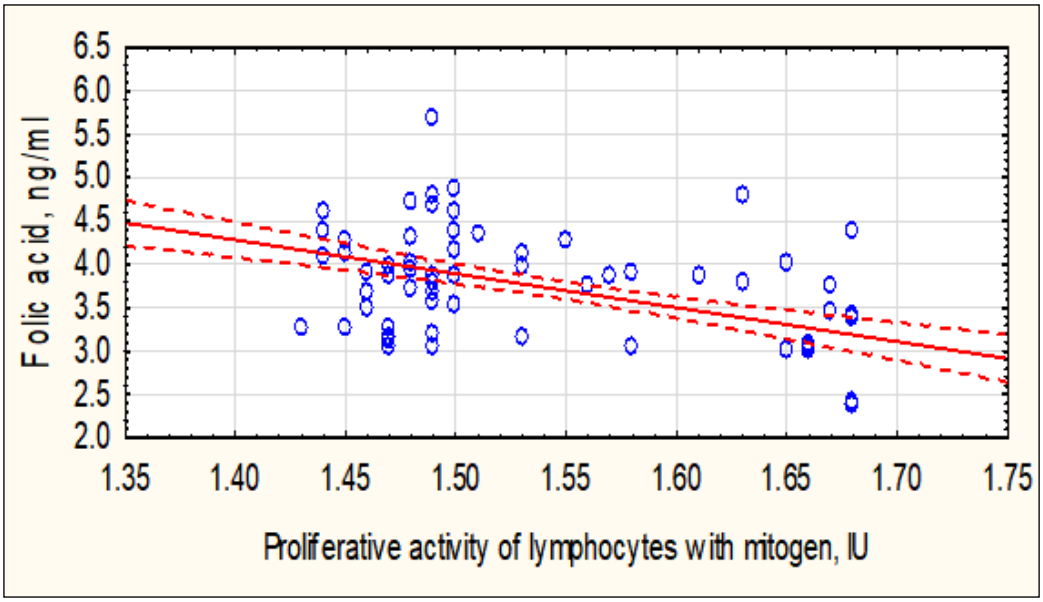

Fig. 2 Correlation between the Lymphocytes Proliferative activity with mitogen and the level of Folic acid in the blood (r=-0.50; p<0.01).

of Cu homeostasis in the lung, affecting both acute and chronic pathological processes. Recent studies have identified a new form of Cu-dependent cell death called cuproptosis, which has sparked renewed interest in the role of Cu homeostasis in disease. Cuproptosis differs from other known pathways of cell death. This occurs through direct binding of Cu ions to lipoylated components of the tricarboxylic acid cycle during mitochondrial respiration, which causes toxic stress to proteins and ultimately leads to cell death. When considering the homeostasis of Cu on the pathogenesis of various respiratory diseases, it is necessary to take into account the influence of the presence of unregulated metabolism. The therapeutic potential of copper interaction mechanisms with infections and immune inflammation as a component of cellular processes involved in airway homeostasis in the context of respiratory diseases and studying the potential of therapeutic strategies is also possible [28].

In the group with a low content of Selenium in blood serum, serum levels of CRP were increased, which served as an indicator of systemic inflammatory reactions. Consistent with this, multivariate analysis also showed that low serum Selenium levels were also significantly correlated with higher serum CRP levels. Se deficiency affects immune function, Selenoprotein expression, alters the antioxidant response, contributing to greater susceptibility to severe viral and bacterial infections [29].

Lymphocyte proliferation tests are based on the ability of Lymphocytes to proliferate when cultured. Proliferation can be enhanced by mitogens that are polyclonal activators of T-lymphocytes (eg, concanavalin A) or B-lymphocytes (eg, LPS). Activation-induced proliferation and clonal expansion of antigen-specific Lymphocytes is a hallmark of

the adaptive Immune response to pathogens. This proliferative burst is autonomously synchronized, ensuring an appropriate magnitude of response while preventing uncontrolled expansion. Steady clonal expansion of a small number of antigen-specific T cells, as well as the simultaneous emergence of a large cellular diversity creates immunity to a large number of different pathogens. Thus, a precise description of the regulatory principles governing Lymphocyte proliferation, differentiation, and survival is essential for a unified understanding of the Immune system [30].

We also analyzed the proliferative capacity of Lymphocytes with mitogen in the studied children. The correlations of vitamin-mineral components and Lymphocytes Proliferative activity with mitogen are considerated (Table 3).

According to the obtained data, positive correlations are observed with vitamins Cyanocobalamin (r=0.40, p<0.01), vitamin C (r=0.43, p<0.01) and Ferritin, as an intracellular depot of Iron (r=0.35, p<0.01), the mineral Magnesium (r=0.27, p<0.003), which testifies to the protective role of the components of the metabolic direction at the stage of development of the cellular immune response. Negative correlations of vitamins, in particular, Folic acid (r=-0.50, p<0.01), vitamin A (r=-0.40, p<0.01) and minerals Phosphorus (r=-0.75,p<0.01) and Potassium (r=-0.24, p=0.008),Sodium (r=-0.51, p<0.01), Chlorine (r=-0.38, p<0 ,01). Negative correlations may indicate the exclusion of components from possible preventive factors to optimize prevention [7].

We presented some correlograms of the relationship between the Lymphocytes Proliferative activity with mitogen in the blood and the level of Phosphorus and the values of Folic acid (Fig. 1-2).

The negative direction of relationships (Fig.1) shows that the body has a balanced saturation of the body with phosphorus and does not need to add this mineral to the adaptive nutritional balance. Phosphorus, like Phosphates, are negatively charged ions that are highly reactive with oxygen to form phosphate ions that form complexes with several ions such as calcium and form ester bonds with other molecules such as peptides and proteins. Phosphorus is needed for bone growth, cell metabolism, energy and signaling through phosphorylation reactions, structures (Phospholipid membranes and skeletal tissue), protein and nucleic acid synthesis (DNA and RNA), and oxygen delivery. In children, serum Phosphorus levels are higher, vary throughout the day, and depend on age, genetics, sex, chemical factors in environmental pollution, food intake, use of certain medications, serum pH, disease, and levels of other nutrients and hormones involved in the Phosphorus balance. As for excessive Phosphorus intake at a higher level than the norm, it is associated with an increased risk of chronic diseases [22].

The relationship between Folic acid and the Lymphocytes proliferative activity with mitogen also has a negative direction of relationship (Fig. 2), which indicates the actual balancing of the vitamin with an adequate adaptive immune response. Folic acid plays a role in mitigating inflammatory reactions in the human body. High serum Folate was positively associated with measures of Lung function in patients with COPD. Further longitudinal studies are needed to elucidate the underlying mechanisms [22].

DISCUSSION

Vitamins are multifunctional compounds that carry out biological activity necessary for the completion of enzymatic processes in the child's organism and the possibility of modulating the functions of the Immune system. Trace elements, which are present in the body in very small quantities, play a basic role in metabolic processes and adequate functioning of the Immune system. The increased risk of infection in deficient states has led to the hypothesis that optimization by micronutrient supplementation may improve the efficiency of the Immune response [23].

The results of numerous scientific studies are contradictory and present significantly lower serum levels of vitamin D3 in children with RRIs, while other studies do not indicate a significant difference in serum levels of vitamin D, which are low in both children with RRIs and controls [12].

The effects of vitamin D3 administration in the prevention of RRIs have also been the subject of systematic reviews and meta-analyses. When conducting a study in children under 5 years of age, the effect of vitamin D3 on the prevention of infections in general, including gastrointestinal ones, was considered [7].

The authors concluded that vitamin D3 was effective in preventing infections (adjusted Odds ratio), and that the effect was stronger in subjects with vitamin D3 < 25 nmol/L. A modest statistically significant protective effect of vitamin D3 supplementation compared with placebo was found (OR 0.91, 95% CI 0.84–0.99), in contrast to the results of a previous meta-analysis [11]. Vitamin D has attracted the most attention during the COVID-19 pandemic due to its purported protective effects, among of all the metabolic components. Several systematic reviews and meta-analyses have been published about vitamin

D3 in the prevention of ARI, of which the most recent and large-scale study was by Joliffe et al. in 2020 [12,22]. Recent evidence has shown no positive effects of vitamin D3 and vitamin C together with zinc in the treatment of moderate and mild cases of COVID-19, respectively [9].

Currently, there are no studies in the literature which indicate that low levels of vitamins A and E is leaded to the respiratory infections predispose in children, so their use cannot be recommended for the prevention of RRIs [21,24].

Zinc is a micronutrient whose role in supporting the immune response against viral infections has been extensively studied, especially in children. A systematic review and meta-analysis of the preventive effect of Zinc supplements in children was conducted. Despite many published studies on Zinc supplementation for the prevention of viral respiratory diseases, some studies have reported only modest effects in children, which warrants further research [26,27].

Prevention of RRD in healthy children has recently become the subject of several clinical studies. There is no consensus as to which children should be considered susceptible to RRD (i.e., those who suffer from recurrent ARIs), as different countries use different criteria. The specific sites of respiratory tract infection, the total number of ARIs, and the period of life during which infection should occur vary between countries [27]. On the basis of the conducted research, numerous factors influencing the implementation of RRD in children were identified, which have a significant impact on the condition of sick children, their families, society and the health care system. It is possible to draw conclusions about the importance of metabolic and pro-inflammatory disorders in the prevention of this pathology. It is possible to draw conclusions about the importance of metabolic and pro-inflammatory disorders in the prevention of this pathology.

CONCLUSIONS

1. A decrease in the level of vitamins beyond the reference range: vitamin D3 (26.82 ± 2.90 ng/ml and in comparison with the data of the control group 36.05 ± 2.58 ng/ml, p<0.01) and in 1, 4 times lower than the data of the control group; vitamin C (4.78 ± 2.41 mg/l vs. 9.17 ± 2.63 mg/l, p<0.01) and 1.4 times lower than the data of the control group and Cyanocobalamin (206.39 ± 24.91 pg/ml versus 385.57 ± 54.26 pg/ml, p<0.01), the level of which had a significant decrease of 1.9 times in relation to the data of the control group.
2. The values of Folic acid were within the reference range (3.75 ± 0.54 ng/ml vs. 8.26 ± 2.61 ng/ml, p<0.01), i.e. in 2 times lower than in children of the control group. Considering the properties of the functioning of the body system, one can think about the pre-pathological level of folic acid in our patients.
3. According to our research, there was a slight increase in vitamin E beyond the reference values (10.56 ± 2.67 mg/l vs. 7.28 ± 1.47 mg/l, p<0.01).
4. Considering the level of vitamin A in our patients, no increase was found, and no significant differences were identified between the studied parameters (p=0.45).
5. The study of indicators of the proliferative activity of lymphocytes with mitogen in patients with RRD showed that its level is significantly different from the values of the control group of children and varies within the reference values of anti-infective protection of patients (1.54 ± 0.08 opt. units vs. 1.45 ± 0. 07 opt. units, p<0.01).
6. Calcium levels were observed below the limit of reference values, both total (2.14 ± 0.04 μmol/l vs. 2.53 ± 0.07 μmol/l, p<0.01), and ionized (1.15 ± 0.01 mmol/l versus 1.26 ± 0.05 mmol/l, p<0.01). At the lower limit of the reference, there were significant differences in the levels of Zinc (47.27 ± 3.46 μg/dL vs. 75.35 ± 18.05 μg/dL, p<0.01, 1.6 times), Copper (11.05 ± 1.53 μmol/l versus 15.89 ± 1.32 μmol/l, p<0.01, 1.4 times) and unreliable - Magnesium (p<0.12), Sodium (p<0.62). The value of the Selenium level varied within the reference range, but with a significant decrease of 1.5 times compared to the data of the control group (0.11 ± 0.02 μg/l vs. 0.17 ± 0.05 μg/l, p<0, 01). The values of Potassium (p=0.89), Chlorine (p=0.18), Phosphorus (p=0.26) were also presented as unreliable differences.
7. Positive correlations of Lymphocytes Proliferative activity with mitogen are observed with vitamins – Cyanocobalamin (r=0.40, p<0.01), vitamin C (r=0.43, p<0.01) and Ferritin, as an intracellular Iron depot (r= 0.35 p<0.01). Corelationship with mineral Magnesium (r=0.27, p<0.003) testifies about the protective role of the metabolic direction components at the stage of the Cellular Immune response development. Negative correlations of vitamins, in particular, Folic acid (r=-0.50, p<0.01), vitamin A (r=-0.40, p<0.01) and Phosphorus minerals (r=-0.75 , p<0.01), Sodium (r=-0.51, p<0.01), Potassium (r=-0.24, p=0.008), Chlorine (r=-0.38, p<0 ,01) were identified. Negative correlations may indicate the exclusion of components from possible preventive factors to optimize prophylactic measure.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 18.06.2024
ACCEPTED: 22.09.2024

ORIGINAL ARTICLE

CONTENTS

The burdened medical history of the mothers-in-partum – risks for the newborn

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ABSTRACT

Aim: To improve early diagnosis in the mothers-in-partum, with a burdened of medical case history, taking into account the pathological pattern «mother-newborn» analysis

Materials and Methods: Two groups of mothers-in-partum (n=109) with burdened anamnesis were analyzed, in comparison with mothers of children with control group date(n=31).

Results: As for the course of pregnancy, there was a predominance of values of chronic Fetoplacental insuffieciency data (33,91±2,16 vs. 12,92±2,36%, p<0,001), Preeclampsia (38,54±1,67 vs. 22,67±2,48 %, p<0,001) in comparation with control group date. Regarding the course of labour, deliveries by Cesarean section were the most frequent among mothers in the first group in comparison with the data of the control group (55,03±3,28 vs. 45,21±2,48%, p<0,01) along with early discharge of amniotic fluid (44,95±1,39 vs. 38,70±1,46%, p<0,01). Suppression of IL-10 level, is observed at high levels of Anti-Toxoplasma-IgG, as evidenced by the negative correlation between them (r=-0,27, p=0,004). However, Anti-HSV 1/2-IgG does not have a negative effect on the level of Neopterin in infants. The relationship between Anti-CMV-IgG and IL-1 (r=0,75, p<0,001) indicates the stimulation of the inflammatory process in premature infants and inhibition of IL-10 synthesis (r=-0,29, p=0,002).

Conclusions: A negative correlation between IL-10 level in infants and levels of Anti-Toxoplasma-IgG in mothers, similar relationship was detected regarding Anti-HSV 1/2-IgG on level of TNF-α. Anti-CMV-IgG and IL-1 correlation analysis detected the stimulation of inflammatory process in premature infants and inhibition of IL-10 synthesis.

KEY WORDS: mothers-in-partum, burdened medical history, risk factors, Caesarean section, intrauterine infections, newborns

Wiad Lek. 2024;77(9):1947-1955. doi: 10.36740/WLek/195157 DOI

INTRODUCTION

Diagnosis of intrauterine infections (IUI), given the non-specificity of clinical manifestations, is possible taking into account risk factors in a pregnant woman, data from functional studies, as well as a general blood test, which allows us to assume the presence of an inflammatory process, even in the absence of clinical manifestations. Examination of 65 pregnant women at 32-36 weeks of gestation and 62 newborns with IUI (32 full-term and 30 premature) was carried out [1]. The diagnosis of IUI was verified by peripheral blood tests for the presence of antigens and antibodies to the causative agents of streptococcal, CMV, HSV, Candidal, Chlamydial, Mycoplasmal, and Ureaplasmal infections.

A CBC test in pregnant women showed a decrease in the concentration of hemoglobin by 21%, erythrocytes by 38%. Leukocytosis was observed with a shift of the leukocyte formula to the left by 18%. In severe infections, the leukocyte formula changed due to an increase in the number of segmented neutrophils and the appearance of more young forms. ESR levels were elevated in 17% of cases. However, in 6% of cases, no changes were detected.

During a comprehensive clinical examination, 75% of newborns were diagnosed with intrauterine pneumonia, 14% with conjunctivitis, 6% with enterocolitis, and 3% with pyelonephritis. In other children in the early neonatal period, clinical manifestations of IUI were not detected.

In the general blood test, specific changes characteristic for infection were found in all children. An increase in the number of lymphocytes and segmented neutrophils was found in 56% of cases. At the same time, the number of leukocytes did not exceed the normal level in 25% of cases. The most pronounced changes in the general blood analysis were noted in streptococcal infection, as well as in its combination with CMV. No significant differences were found in ureaplasma infection.

A significant increase in the concentration of Na ions (151.1±2.7 mmol/l) in blood serum and a tendency to increased concentration of Ca ions (2.9±0.01 mmol/l) were found in newborns with IUI. In contrast, there was a decrease in the concentration of K ions (2.4±0.05 mg-eq/l), which indicates the development of an imbalance in the serum electrolyte composition in children with IUI. In addition, with a very severe course of the disease with pronounced respiratory insufficiency, with colitic and hyperthermic syndrome, more pronounced hypernatremia with increasing hypokalemia appears. The ionic composition of blood plasma differed depending on the nosological form of BUI. Infectious pathology intensifies changes in the electrolyte balance, as it disrupts the transport of microelements at the cellular level [1].

The transfer of maternal IgG begins at approximately 13 weeks of pregnancy, at the beginning of the second trimester, and has long been recognized as a central component of the formation of fetal immunity against pathogens. Infectious diseases of the mother change the system of signals that form a complex network of interactions and, thus, can change the immunity of the fetus [2].

In case of viral infection, trophoblasts constitutively secrete antiviral IFNs, which limit the infection both autocrinely and paracrinely. The release of type III IFNs is a unique feature of trophoblasts, as IFNs are usually induced only in response to viral infection. As antiviral effectors of the interferon pathway, IFN-stimulated genes (ISGs) can exhibit potent cytotoxic and proinflammatory properties [3, 4]. In addition to IFNs, trophoblasts also secrete antiviral micro-RNAs in the placental exosome, which provide antiviral protection in non-placental cells and can be isolated from the blood serum of pregnant women [5, 6].

Thus, the problem of diagnostic possibilities of intrauterine infections of newborns remains relevant due to the lack of a specific clinical presentation in the early stages of the pathological process and the low diagnostic capacity of the available examination methods. At the same time, the use of only clinical signs causes numerous diagnostic errors, which requires the mandatory use of laboratory, microbiological, morphometric, and molecular genetic studies.

AIM

To improve early diagnosis in the mothers-in-partum, with a burdened of medical case history, predicting the consequences of intrauterine infections in neonates, taking into account the analysis of the pathological pattern «mother-newborn» for the development of optimized therapy.

MATERIALS AND METHODS

In order to find out the predictors of the development of the pathological pattern «mother-newborn», two groups of mothers-in-partum (n=109) who had laboratory-confirmed TORCH-infection (IgG above the level of reference values) were analyzed, in comparison with mothers of children with control group (n=31).

INCLUSION CRITERIA WERE

women in labor, premature birth, burdened medical history, identified TORCH infection (IgG above the level of reference values).

EXCLUSIONS

physiological childbirth, oncological and autoimmune diseases, laboratory detection of TORCH infection (IgM), human immunodeficiency virus (HIV), viral diseases (rubella, hepatitis, chicken pox), tetanus, syphilis, gonococcal infection, tuberculosis, malaria, intestinal infectious diseases , candidiasis, severe developmental disabilities, alcohol and drug use.

RESULTS

In order to identify and analyze patterns of pathology development in children, a detailed analysis of the somatic and gynecological status of mothers was carried out in comparison with the data of the control group. The accompanying pathology in the mothers of the studied contingent of infants is considered in the table (Table 1).

According to Table 1, only 13 (41,9%) mothers of the control group were observed without pathology. Attention is drawn to the highest prevalence of anemia during pregnancy (52(48, 4±5,2%) versus 3(9,6±2,1%) in pregnant women of the control group. A high level of extragenital pathology in women in childbirth was observed due to diseases of the urinary system (21(19,2±2,4%) and diseases of the digestive system (18 (16,5±2,9%)). During the study of the morbidity of the digestive system in pregnant women with identified TORCH infection, pathology of the hepatobiliary system, in particular non-alcoholic fatty liver dystrophy was found, along with upper digestive tract involvement. According to scientific studies, disruption of biochemical processes of the liver and pronounced reflux esophagitis indicate negative effects of TORCH infection on mothers and, potentially, on newborns [7, 8].

The highest reliable indicator (<0.001) was observed in the category of cases of inflammatory diseases of the genital organs of women in childbirth in the main group (60(55,2±3,1%) versus 2(6,4±1.3%) of the control group, p<0.001). There is also a high reliable indicator for the diagnosis of anemia (52(48, 4±5,2%)

Table 1. Somatic and gynecological status of mothers of the studied contingent of infants

Parameters	The studied group (n=109)	Control group (n=31)	P
Extragenital pathology			
Urinary system diseases	21(19,2±2,4%)	7(22,6±%)	>0,05
Digestive system diseases	18(16,5±2,9%)	4(12,9±%)	<0,05
Cardiovascular system diseases	5(4,6±1,7%)	1(3,2±%)	<0,05
Anemias	52(48,4±5,2%)	3(9,6±2,1%)	<0,002
Associated gynecological diaseases			
Significant perinatal infections	9(8,3±%)	0	
Polycystic ovaries	2(1,8±0,8%)	1(3,2±1,1%)	<0,001
Inflammatory diseases of the genital organs	60(55,2±3,1%)	2(6,4±1,3%)	<0,001
Without pathology	0	13(41,9±3,2%)	

Table 2. Reproductive anamnesis of the mothers of the studied contingent of infants

Parameters	N=109	N=31	P
Artificial termination of pregnancy	21(19,3±1,7%)	6(19,4±2,1%)	>0,05
Miscarriages	26(23,9±2,1%)	3(9,7±2,2%)	<0,001
Work under toxic conditions	4(3,7±%)	1(3,2±%)	>0.05
Total	51(46,9%)	10(32,3%)	

against the data of the control group (3(9,6±2,1%), p<0,002). Comparative characteristics of other extragenital nosologies were uninformative.

Reproductive history of mothers is analyzed in the following table (Table 2).

According to women's anamnesis, reproductive dysfunction was detected in 51 (46,9%) mothers of children from the first and 10 mothers (32,3%) from the control group. Significant differences were observed as for the number of miscarriages (26 (23,9±2,1%) vs. 3(9,7±2,2%), (p<0,001), which is confirmation of fetopathy, caused by TORCH infections in most cases.

The information about the course of pregnancy in mothers is important as well (Table 3, Fig. 1).

According to table 3 and fig. 1 there is a predominance of the values of CFPI data (33,91±2,16 vs. 12,92±2,36% in the control group, p<0,001), cases of preeclampsia (38,54±1,67 vs. 22,67±2,48 %, p<0,001) and threatenes miscarriage (16,54±2,91 vs. 9,21±1,27%, p<0,001). Determination of CFPI, threatened miscarriage and premature birth as the main manifestations of fetopathy due to IUI are observed in the studied newborns.

Data of the course of childbirth among the mothers of the studied groups of infants is reflected in the following table (Table 4).

Among the individual indicators (Table 4), deliveries by Cesarean section were the most frequent among mothers in the first group in comparison with the data of the control group (55,03±3,28 vs. 45,21±2,48%, p<0,01) along with early discharge of amniotic fluid (44,95±1,39 vs. 38,70±1,46%, p<0,01). Rapid childbirth, on the contrary, was more often observed in the mothers of the control group of infants (32,26±1,18 vs. 18,34±2,17, p<0,001).

The analysis of the data of mothers with identified TORCH infection, by number of pregnancies, is presented on Fig. 2.

According to the data of Fig. 2, among the contingent of mothers, the most frequently odbserved cases were of second (44%) and the less frequent - of third pregnancies (6%).

Information on the distribution of women by the number of births is presented on Fig. 3.

Levels of childbirth cases by their number: the highest level was recorded at the first - 48% and the second - 40% birth, the lowest - at the fourth birth (4%). Mothers underwent screening for TORCH infection, when serum was collected for the study in the first trimester of pregnancy (10,32±1,18 weeks of gestation).

For research and sampling, a group of pregnant mothers formed a contingent with Anti-TORCH IgG levels above the reference values (Table 5).

The value of the Anti-Toxoplasma-IgG level (100,67 ± 94,67 IU/ml), which exceeds the upper limit of reference values by more than 12 times, is particularly indicative. High levels (>200,0 IU/ml) were observed in 32 pregnant women. Anti-HSV 1/2-IgG levels also exceeded physiological ranges up to 8 times, Anti-CMV-IgG - up to 8 times.

Table 3. Characteristics of pregnancy in mothers of infants

Parameters	First group (n=109)		Control group (n=31)		The index of P
	Abs.	%	Abs.	%	
Chronic fetoplacental insufficiency (CFPI)	37	33,91±2,16	4	12,92±2,36	<0.001
Toxicosis	18	16,54±2,91	4	12,92±2,36	>0,05
Threatened miscarriage	18	33,91±2,91	3	9,21±1,27	<0.001
Preeclampsy	42	38,54±1,67	7	22,67±2,48	<0.001

Note. P – reliability between groups.

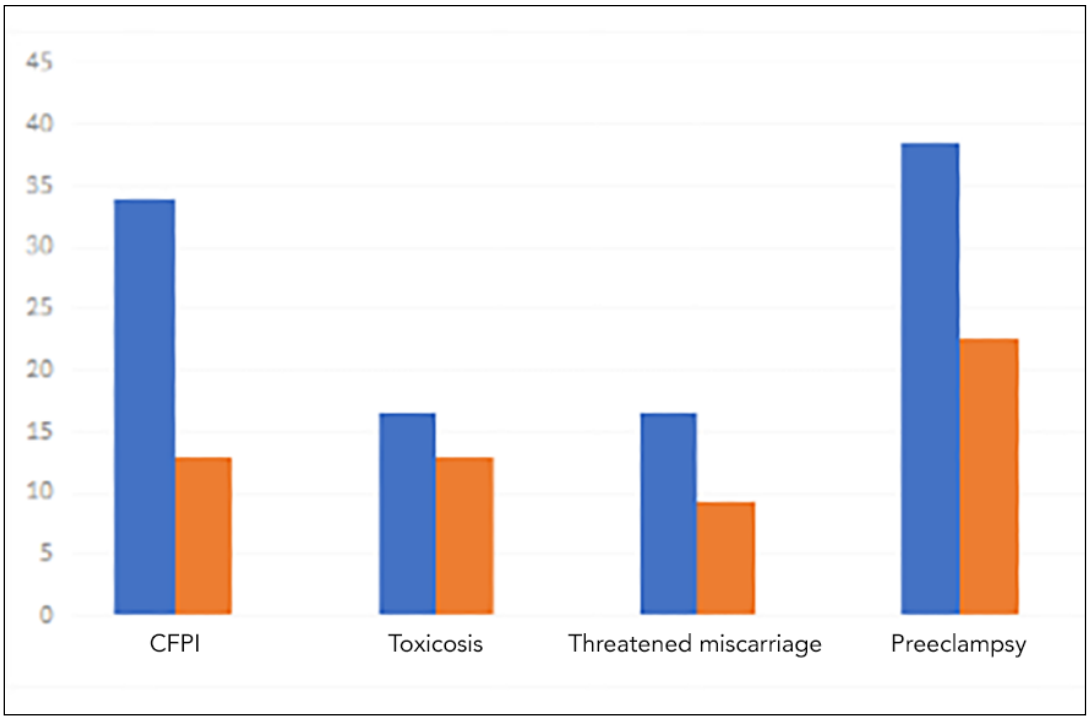

Fig. 1. The course of pregnancy in the mothers of the research groups.

To analyze and assess the risks of having children from mothers with TORCH infection, a correlation analysis of the relationships between the levels of TORCH antibodies in mothers and parameters of the inflammatory response in infants was conducted (Table 6).

Suppression of IL-10 level, as an anti-inflammatory factor in infants, is observed at high levels of Anti-Toxoplasma-IgG, as evidenced by the negative correlation between them (r=-0,27, p=0,004). The effect of Anti-HSV 1/2-IgG on the level of TNF-α has similar negative effects (r=-0,24, p=0,01). However, Anti-HSV 1/2-IgG does not have a negative effect on the level of neopterin in infants, on the contrary, it has a positive correlation (r=0,92, p<0,001), which indicates the absence of an effect on the inhibition of neopterin synthesis. The relationship between Anti-CMV-IgG and IL-1 (r=0,75, p<0,001) indicates the stimulation of the inflammatory process in premature infants and inhibition of IL-10 synthesis (r=-0,29, p=0,002).

The most indicative correlograms of the relationships between antibodies to HSV 1/2 IgG and neopterin and antibodies to CMV IgG and IL-1 are presented on Fig. 4, Fig. 5., respectively.

According to our data, chronic fetoplacental insufficiency (CPFPI) of mothers with a history of TORCH infections was observed in 37 (33,91±2,16%) women with an identified TORCH infection. The obtained data should be taken into account by neonatologists for more careful monitoring of newborns at risk of perinatal infections.

DISCUSSION

The fact that most of the diseases of pregnant women leading to IUI occur in a subclinical latent form with activation of the process in case of any homeostasis violation, can complicate the clinical diagnosis. At the same time, diagnosis based on clinical manifestations, without the involvement of specific microbiological

Table 4. Characteristics of childbirth in mothers of infants

Course of childbirth	First group (n=109)		Second group (n=31)		The index of P
	Abs.	%	Abs.	%	
Cesarean section	60	55,03±3,28	14	45,21±2,48	<0,01
Prolonged childbirth	29	26,60±2,17	7	22,58±1,37	>0,05
Rapid childbirth	20	18,34±2,17	10	32,26±1,18	<0.001
Stimulation of labor activity	49	44,95±2,31	14	45,16±2,48	>0.05
Early discharge of amniotic fluid	49	44,95±1,39	12	38,70±1,46	<0.01

Notes. P-reliability between groups

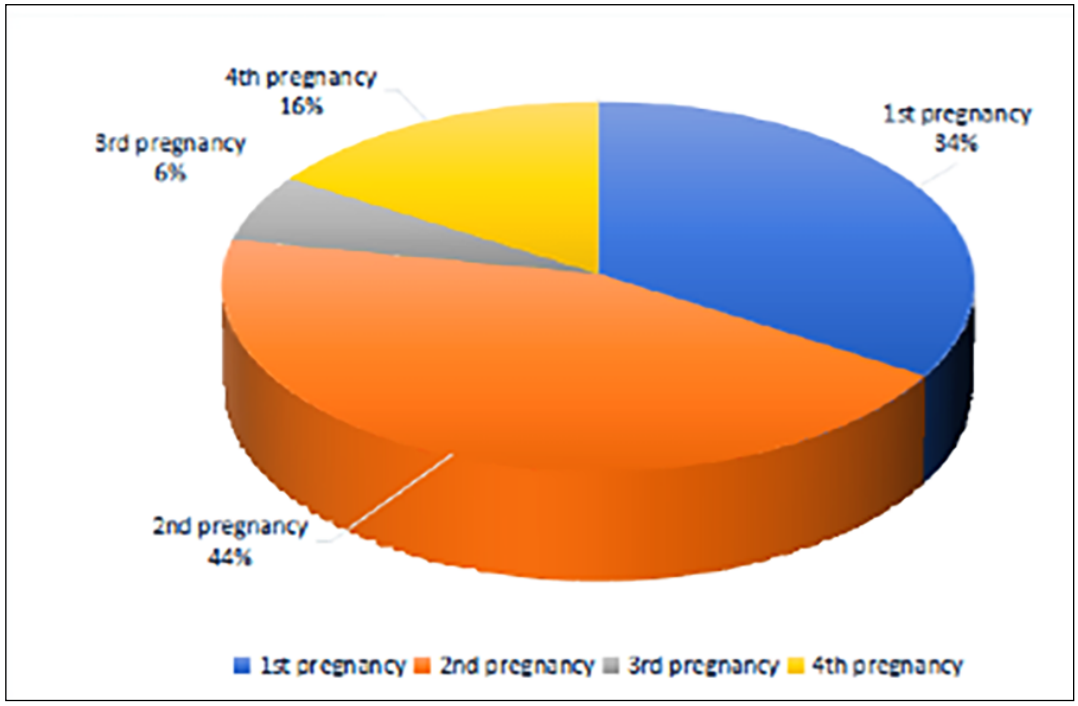

Fig. 2. Distribution of women by number of pregnancies.

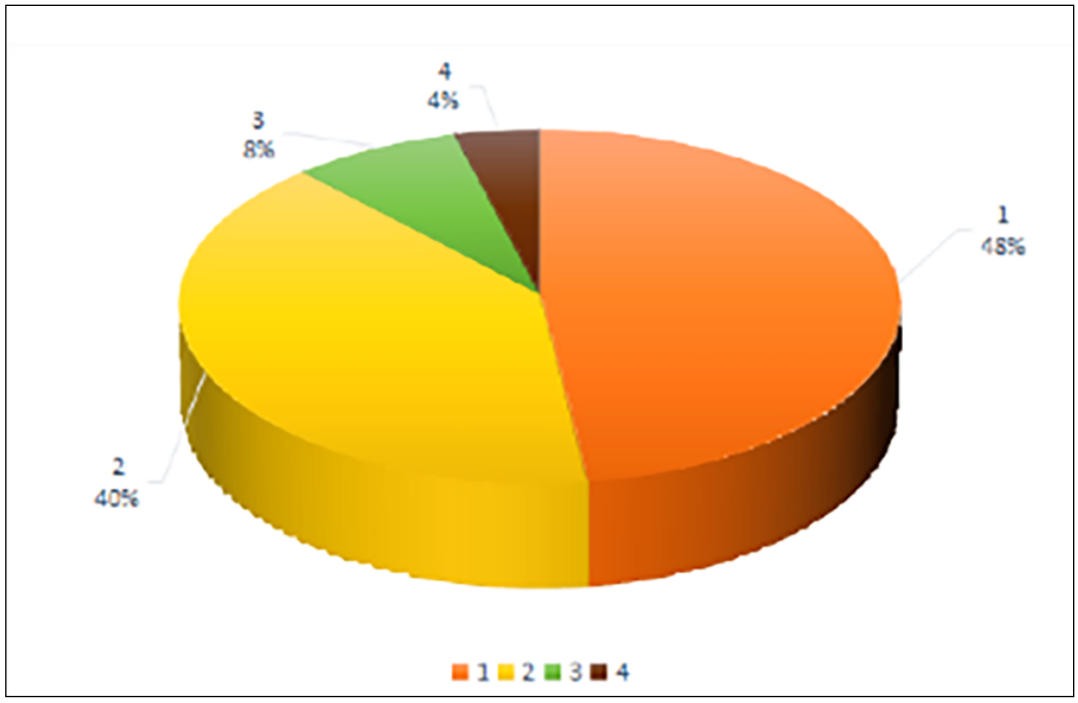

Fig.3. Distribution of women by number of deliveries (no.; %).

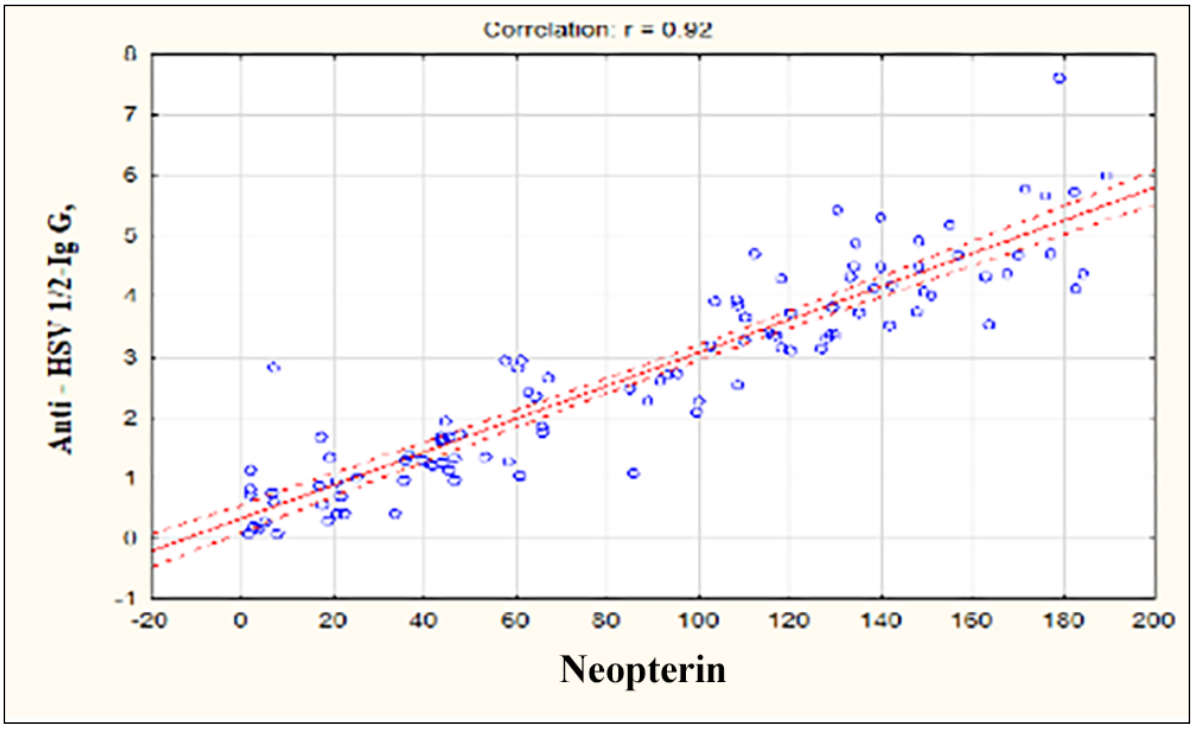

Fig. 4. Correlation between the level of Anti-HSV 1/2-IgG in the blood of mothers and the concentration of neopterin in the blood of newborns.

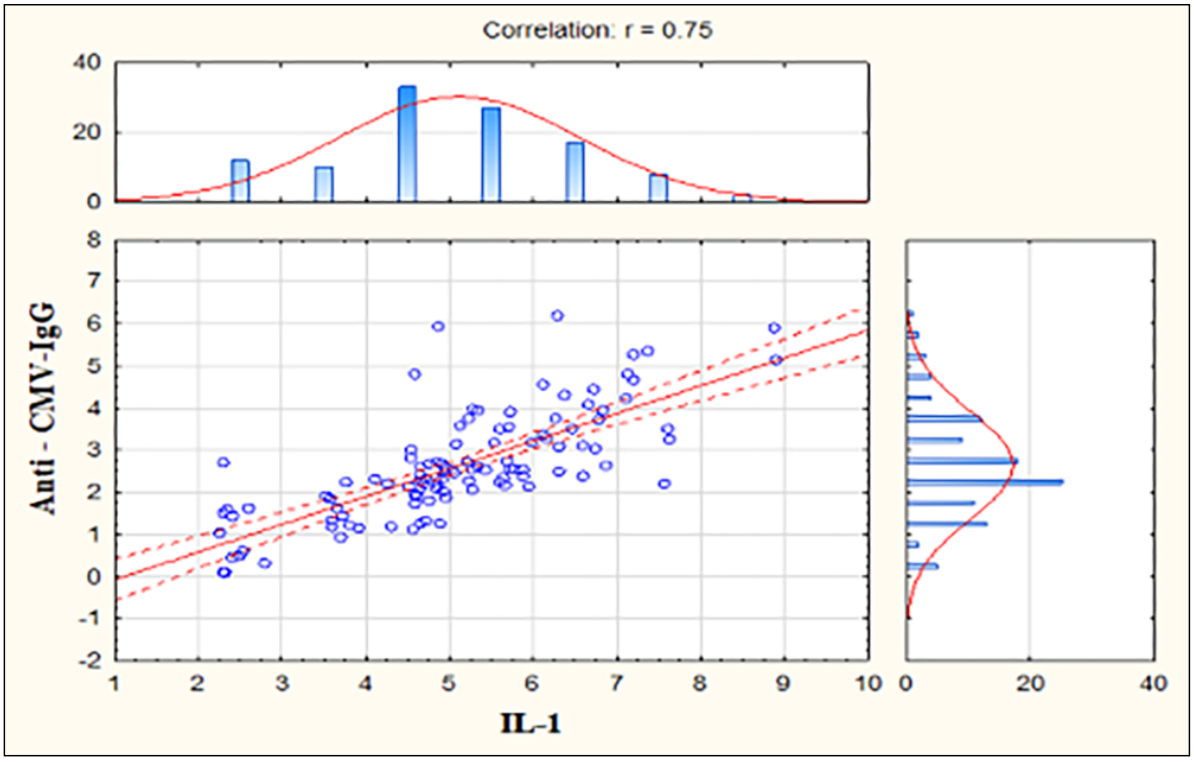

Fig. 5. Correlation between the level of Anti-CMV-IgG in the blood of mothers and the concentration of IL-1 in the blood of newborns.

studies, leads to diagnostic errors in 90-95% of cases [9, 10]. The identification of the causative agent itself is not enough to diagnose active IUI. Determination of cellular or humoral immunity does not provide an opportunity to adequately predict the risk of IUI at all stages of gestation. After the release of cytokines, the inflammatory reaction is realized in typical pathological processes, which mainly depend not so much on the type of the pathogen as on the gestation period, when the infection was realized. The study of cytokine

Table 5. Parameters of IgG levels to TORCH-infections in pregnant mothers

Parameters (n=109)	M±m	Min	Max
Anti-Toxopatoplasma-IgG, IUO/ml (>8,0)	100,67±	1,36	423,00
Anti-CMV-IgG, IU/ml (>0,9)	2,62±1,27	0,11	6,18
Anti-HSV ½-IgG, IU/ml (>0,9)	2,73±1,66	0,08	7,63

Table 6. Correlations between levels of Anti-Toxoplasma-IgG, Anti-CMV-IgG, Anti-HSV 1/2-IgG in mothers and laboratory parameters in infants

Parameters	Anti-Toxoplasma-IgG	Anti-HSV 1/2-IgG			Anti-CMV-IgG
IL-10	R= -0,27; p=0,004	Neopterin	r=0,92; p<0,001	IL-1	r=0,75; p<0,001
		TNF-α	r=-0,24; p=0,001	IL-10	r=-0,29; p=0,002

and interferon (IFN) status makes it possible to predict the implementation of infection in the early stages of pregnancy [11].

Cesarean section is the most common surgical procedure which performed in women of worldwide. The frequency of cesarean section should range from 10 to 15%? according to the recommendations of the World Health Organization (WHO) [12]. However, more and more often, women choose to give birth by cesarean section [13,14]. Perinatal mortality is up to 19 children per 1000, according to the world scientific literature [15]. In developed countries, is considered that this surgical intervention can prevent severe perinatal complications.

Respiratory and neurological disorders are more often observed in children borned by Caesarean section: autism spectrum disorders [16], schizophrenia [17]) and immune-dependent diseases [18,19], atopic dermatitis [20] and other childhood pathology [21]. The most common complications after cesarean section include the following: respiratory disorders, transient tachypnea, postpartum hypoglycemia [22,23]. Hansen et al. [24] announced data that the percentage of complications depends on the Caesarean section procedure and the length of pregnancy.

CONCLUSIONS

1. During a detailed analysis of the somatic and gynecological status of mothers high level of extragenital pathology in women in childbirth was observed due to diseases of the urinary system (21(19,2±2,4%) and diseases of the digestive system (18 (16,5±2,9%), thus indicating negative effects of TORCH infection on mothers and, potentially, on newborns. Reproductive dysfunction was detected in 51 (46,9%) mothers of children from the first and 10 mothers (32,3%) from the control group, significant differences were observed as for the number of miscarriages (26 (23,9±2,1%) vs. 3(9,7±2,2%), (p<0,001), confirming fetopathies, caused by TORCH infections in most cases.
2. Anti-Toxoplasma-IgG level (100,67 ± 94,67 IU/ml), which exceeded the upper limit of reference values by more than 12 times, Anti-HSV 1/2-IgG and Anti-CMV-IgG levels also exceeded physiological ranges up to 8 times.
3. Suppression of IL-10 level, as an anti-inflammatory factor in infants, is observed at high levels of Anti-Toxoplasma-IgG, as evidenced by the negative correlation between them (r=-0,27, p=0,004). The effect of Anti-HSV 1/2-IgG on the level of TNF-α has similar negative effects (r=-0,24, p=0,01). However, Anti-HSV 1/2-IgG does not have a negative effect on the level of neopterin in infants, on the contrary, it has a positive correlation (r=0,92, p<0,001), which indicates the absence of an effect on the inhibition of neopterin synthesis. The relationship between Anti-CMV-IgG and IL-1 (r=0,75, p<0,001) indicates the stimulation of the inflammatory process in premature infants and inhibition of IL-10 synthesis (r=-0,29, p=0,002).

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 14.06.2024
ACCEPTED: 27.09.2024

ORIGINAL ARTICLE

CONTENTS

Serotonin levels in children with cognitive impairment in delayed speech development in non-alcoholic fatty liver disease with obesity and Covid-19

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ABSTRACT

Aim: To identify features of changes in serum serotonin and tryptophan levels in children with cognitive impairment (CI) and delayed speech development (DSD) at non-alcoholic fatty liver disease (NAFLD) and COVID-19.

Materials and Methods: The study included 108 children with CI and DSD at NAFLD. The children were divided into 2 groups: group 1 included children with COVID-19 (n=58); group 2 consisted of 50 children without COVID-19.

Results: All the examined children were diagnosed the weight gain and NAFLD (non-alcoholic steatohepatitis), mainly of minimal to moderate activity. The results indicate a decrease of serotonin and tryptophan levels in the examined children with CI and DSD and NAFLD. However, in children of group 1 (after COVID-19), the level of ST was 2.4 times (p-value <0.01) higher than in children of group 2. Tryptophan levels were also 1.4 times higher in children from group 1 (p-value <0.05).

Conclusions: In children with CI and DSD at NAFLD, a decrease in serum levels of ST and Trp was diagnosed, which is more pronounced after COVID-19. A direct relationship between the decrease in serum Trp and serotonin levels has been established, which directly depends on the functional state of the liver and negatively correlates with the severity of insulin resistance.

KEY WORDS: children, cognitive impairment, delayed speech development, non-alcoholic fatty liver disease, obesity, COVID-19, insulin resistance, diagnostics, serotonin, tryptophan

Wiad Lek. 2024;77(9):1956-1961. doi: 10.36740/WLek/195158 DOI

INTRODUCTION

Metabolic dysfunction-associated fatty liver disease (MAFLD) is a term proposed in 2020 to refer to fatty liver disease associated with systemic metabolic disorders instead of non-alcoholic fatty liver disease (NAFLD) [1].

The World Health Organization (WHO) estimates that non-alcoholic fatty liver disease (NAFLD) affects 25% of the world's population, and approximately 80 million people, both children and adults, in the United States have NAFLD. The prevalence of NAFLD in the paediatric population is estimated to be 13% (9.8% adjusted) with an age-related increase in prevalence from less than 1% in children aged 2 to 4 years to 17% in adolescents. The incidence of NAFLD in children has increased dramatically from 36/100,000 in 2009 to 58.2/100,000 in 2018, in parallel with the worsening childhood obesity epidemic. However, given the low level of adherence to NAFLD screening guidelines, the true prevalence and incidence of NAFLD in children is likely underestimated. In a study using histological assessment of children with chronic hepatitis, more than one-third of obese children had NAFLD. Male children are at higher risk of developing NAFLD than female children, with males predominating among obese adolescents [2].

The metabolic risk factors for MAFLD/NAFLD are type 2 diabetes mellitus and overweight/obesity according to ethnic body mass index (BMI) classifications. Risk factors include increased waist circumference, high blood pressure, plasma triglycerides, plasma high-density lipoprotein cholesterol, prediabetes, insulin resistance model score, and high-sensitivity C-reactive protein in the blood. The combination of liver steatosis with one of these three metabolic risk stratifications leads to the diagnosis of NAFLD [3, 4].

The COVID-19 pandemic has also had a negative impact on the lives of children and young people around the world. Public health measures aimed at reducing the transmission of SARS-CoV-2 in the community have included school closures and stay-at-home orders. The role of social inequality in exacerbating the negative impacts of quarantine on children's health and well-being was already evident after the first wave of

COVID-19. The new findings highlight the impact of the pandemic and socioeconomic impact on childhood obesity and, as a result, NAFLD [5].

COVID-19 has caused numerous stressful situations for many families due to job losses and has negatively impacted already vulnerable communities. The closure of schools has had a particularly negative impact on children living in poverty, for whom school provides access to healthy food, physical activity, medical and social care, and social networks. Stay-at-home orders and restrictions on outdoor recreation have led to an increase in sedentary lifestyles and screen time, particularly affecting children living in densely populated urban areas without access to green spaces. Maintaining healthy behaviours requires a high level of personal commitment, time, and cognitive, psychological and material resources, which vulnerable families struggled to manage before the COVID-19 pandemic. Stress and parental control, mental illness, and disrupted social environments in childhood are associated with weight gain and obesity in children, and the combination of COVID-19-related stressors has led to an increase in obesity prevalence among children as well [5-7].

Thus, the study of cognitive impairment (CI) and delayed speech development (DSD) in children with NAFLD during COVID-19 is still an urgent issue for the medical community.

AIM

The aim to identify features of changes in serum serotonin and tryptophan levels in children with CI and DSD in NAFLD and COVID-19.

MATERIALS AND METHODS

A comprehensive examination and treatment of children conducted on the clinical basis of the Department of Neurology, Neurosurgery and Psychiatry at the Medical Faculty of state higher educational establishment «Uzhhorod National University». The study included 108 childrens with NAFLD and cognitive dysfunction.

Among the examined children there were 60 boys (55.6 %) and 48 girls (44.4 %). The control group consisted of 20 children (12 boys (60.0%) and 8 girls (40.0%)). The average age at children of group 1 was 11.4±3.6 years, and 12.7±4.3 years – at children of group 2.

Children with CI and DSD at NAFLD were divided into two clinical groups: group 1 (n=58) included children with COVID-19, and group 2 included 50 children without COVID-19. All children's in group 1 had a previous confirmed diagnosis of COVID-19 (positive polymerase chain reaction (PCR) result for SARS-CoV-2 RNA (SARS-CoV-2 RdRP gene, SARS-CoV-2 gene E) in anamnesis.

Criteria for exclusion from the study were the presence of autoimmune, viral (hepatitis B, C, D viruses) liver damage, Wilson-Konovalov disease, haemochromatosis, acute traumatic brain injury, type 1 diabetes mellitus, type 2 diabetes mellitus (decompensation stage).

All studies were carried out with the consent of the parents of the children examined (they gave written consent to the relevant diagnostic and treatment measures), and the research methodology was in line with the Helsinki Declaration of 1975 and its revision of 1983, the Convention on Human Rights and Biomedicine developed by the Council of Europe, as well as Ukrainian legislation.

A general clinical and neurological examination was carried out, which included the collection of anamnesis, complaints, standard clinical, laboratory and instrumental examination, examination of the neurological status according to the generally accepted scheme, as well as neuropsychological testing of cognitive functions. The following methods were used for the latter: Montreal Cognitive Assessment (MCA); Mini-Mental State Examination (MMSE); 10-Word Memory Test by Luria A.R. (for the study of auditory-verbal memory). The Spielberger-Hanin method was used to determine the level of anxiety, and autonomic dysfunction was determined by the Wayne Questionnaire. Children underwent electroencephalography (EEG).

All examined children were subjects to research carried out in accordance with the general clinical, anthropometric, instrumental and laboratory methods. In order to verify the diagnosis attention was paid to the nature of complaints, medical history. In anthropometric research, height, weight, waist circumference were measured, and the body mass index (BMI) was calculated [8].

The ultrasound examination of all the children abdominal organs was carried out according to the generally accepted procedure. There were done standard general and biochemical tests based on the blood serum to determine the functional state of the liver (alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBL), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT)), indicators of lipid metabolism (total cholesterol (TT), triglycerides (TG), high-density lipoproteins (HDL), low-density lipoproteins (LDL), very-low-density lipoproteins (VLDL)), carbohydrate metabolism (glucose, insulin, glycated hemoglobin (HbA1c,%)).

Table 1. Indicators of the liver functional state in the examined children

Indicator	Control group (n=20)	Examined children	
		group 1 (n=58)	group 2 (n=50)
ALT, U/L	22.1±1.4	97.4±3.9**,+	63.1±3.1**
AST, U/L	19.7±1.1	78.0±2.7**,+	53.4±3.4*
TBL, mmol/l	15.2±0.7	34.4±2.1**	30.6±0.9*
ALP, UI/L	163.9±5.4	339.6±6.3**,+	277.0±5.5**
GGT, U/L	17.1±2.6	56.3±2.5**	41.2±2.9*

Note: the difference is statistically reliable between the parameters of the control group and the examined children of groups 1 and 2:* – p<0.05; ** – p<0.01; the difference between the indicators in group 1 and 2 children is statistically reliable: + – p<0.05.

Table 2. Indicators of carbohydrate ьetabolism in the examined children in blood serum

Indicator	Control group (n=20)	Examined children	
		group 1 (n=58)	group 2 (n=50)
Fasting blood glucose, mmol/l	4.5±0.3	5.2±0.2	4.9±0.5
HbA1c, %	4.2±0.4	5.0±0.3*	4.7±0.4
Insulin, U/L	8.8±0.7	33.2±1.7**,+	26.1 ±1.5**
C-peptide, ng/ml	4.0±0.5	17.8±0.8**	13.4±1.1**
HOMA-IR	1.5±0.4	8.6±0.6**,+	6.9±0.4**

Note: the difference is statistically reliable between the parameters of the control group and the examined children of groups 1 and 2:* – p<0.05; ** – p<0.01; the difference between the indicators in group 1 and 2 children is statistically reliable: + – p<0.05.

Table 3. Levels of Serotonin and Tryptophan in the in the examined children in blood serum

Indicator	Control group (n=20)	Examined patients	
		group 1 (n=58)	group 2 (n=50)
ST, mcg/L	344.7±15.6	98.1±5.4**,++	235.4±7.7*
Trp, nmol/ml	53.4±1.1	28.2±0.8**,+	40.7±1.0*

Note: the difference is statistically reliable between the parameters of the control group and the examined children of groups 1 and 2:* – p<0.05; ** – p<0.01; the difference between the indicators in group 1 and 2 children is statistically reliable: + – p<0.05.

Table 4. Correlation of ST and Trp indicators and functional state of the liver, carbohydrate metabolism in the examined children

Indicator	Indicator			
	ST, mcg/L		Trp, nmol/ml	
	group 1 (n=58)	group 2 (n=50)	group 1 (n=58)	group 2 (n=50)
ALT, U/L	r= 0.84; p<0.01	r= 0.72; p<0.05	r= 0.76; p<0.01	r= 0.66; p<0.05
AST, U/L	r= 0.68; p<0.05	_	_	_
Insulin, U/L	r= -0.80; p<0.01	r= -0.62; p<0.05	r= -0.72; p<0.05	r= -0.56; p<0.05
HOMA-IR	r= -0.86; p<0.01	_	r= -0.76; p<0.01	_

NAFLD was diagnosed in accordance with the criteria of the unified clinical protocol and the EASL-EASD-EASO clinical recommendations on the diagnosis and treatment of NAFLD [9]. The degree of liver damage was calculated using surrogate markers of fibrosis by means of online calculators NAFLD fibrosis score (NFS), the Fibrosis 4 calculator (FIB-4) as well as FibroTest and the liver elastograpthy results.

Quantitative determination of tryptophan (Try) levels in blood serum was performed by reversed-phase high-performance liquid chromatography in isocratic elution mode with electrochemical detection. Serum serotonin (ST) levels were determined by high-performance liquid chromatography on an Agoilent 1100 chromatograph using the Agilent Technologies (USA) test system.

The analysis and processing of the results of examining the patients was performed using the computer program STATISTICA 10.0 (StatSoft Inc, USA) using parametric and non-parametric methods of evaluating the received results.

RESULTS

All the examined children with CI and DSD at NAFLD were diagnosed the weight gain, namely overweight and obesity. All the children included in the study were, diagnosed the NAFLD, namely, non-alcoholic steatohepatitis, mainly of minimal to moderate activity. At the same time, more pronounced changes in transaminase levels were found in children of group 1. In children with CI and DSD at NAFLD during COVID-19 infection, a significant increase in the activity of transaminases, as well as TBL and GGT levels, was diagnosted (Table 1).

The determination of carbohydrate metabolism indicates insulin resistance in children with CI and DSD at NAFLD, which is more pronounced in group 1 at COVID-19 (Table 2).

Indicators of the neurohormone serotonin and the amino acid tryptophan in the blood serum of children included in the study were determined (Table 3).

The results indicate a decrease of serotonin and tryptophan levels in the examined children with CI and DSD and NAFLD. However, in children of group 1 (after COVID-19), the level of ST was 2.4 times (p-value <0.01) higher than in children of group 2. Tryptophan levels were also 1.4 times higher in children from group 1 (p-value <0.05).

We analysed the relationship between the level of neurohormone ST and tryptophan in the blood serum and indicators of the functional state of the liver and carbohydrate metabolism in the examined children. It should be noted that there was a strong correlation between ST and tryptophan in both groups, namely, r= 0.88; p<0.01 in children of group 1 and r= 0.76; p<0.01 in children of group 2 with CI and DSD at NAFLD.

Statistical analysis revealed a correlation between the progression of liver damage and a decrease in the levels of ST and Trp. The decrease in serum levels of ST and Trp correlated significantly with insulin and HOMA-IR levels at children with Ci and DSD at NAFLD (table 4).

Thus, the studies indicate changes in neurohormone ST and amino acid Trp levels in the blood of children with CI and DSD at NAFLD, which are more pronounced after COVID-19.

DISCUSSION

Clinical studies have defined COVID-19 as a multisystemic infection that can affect various systems, such as cardiovascular, hematologic, renal, gastrointestinal, neurologic and hepatobiliary, and endocrinologic [10]. Children and/or adolescents tend to generally have a symptomatic but mild COVID-19 course with few requiring intensive care treatment and a very low rate of mortality [11-13]. However, cognitive impairment is often reported after SARS-CoV-2 infection [14].

New evidence suggests that SARS-CoV-2 can infect the brain, leading to CI. It can spread to other parts of the brain via transsynaptic neurons, including the olfactory, optic and vagus nerves. It can also enter the central nervous system via the bloodstream or lymphatic system. However, the mechanisms underlying COVID-19-associated CI are currently under active investigation. They include non-immune effects, such as viral proteins, tissue hypoxia, hypercoagulation and pathological changes in neuronal cells, and immune effects, such as microglial and astrocyte activation, peripheral infiltration by immune cells, blood-brain barrier disruption, dysregulation of the cytokine network and gut microbiota. Inflammation is a central feature, and both central and systemic inflammation can cause acute and persistent neurological changes, including CI [15].

Thus, the study of mechanisms that influence the formation and progression of existing cognitive impairment in children, especially in metabolic diseases such as NAFLD, obesity, type 2 diabetes, is still an important and controversial issue in medicine.

The relationship between NAFLD and mood disorders may be bi-directional in nature as metabolic liver disease has been reported to be an independent risk factor for emerging anxiety and depression [16, 17].

The relationship between mental illness in children and metabolic liver disease is an under-researched issue. NAFLD in children and adolescents is the most common cause of liver disease and has a high comorbidity rate with childhood obesity. NAFLD in children depends on many factors, including genetics, low birth weight, and male gender [18-20].

Serotonin is a multifunctional bioamine that plays an important role in the regulation of numerous physiological and pathological pathways. Approximately 90-95% of the total 5-HT content in the body is synthesised and secreted from enterochromaffin cells found in the epithelium of the gastrointestinal tract (GIT). 5-HT exerts its biological effects primarily by binding to 5-HT receptors (HTRs), from where it is then taken up by 5-HT transporters (SERT, slc6a4) and metabolised by monoamine oxidase. As a gastrointestinal hormone, 5-HT is able to directly regulate liver function, thus mediating liver regeneration [21].

The results of our examinations indicate a decrease of serum serotonin levels at children with CI and DSD at NAFLD, which is more pronounced in group 1 (with COVID-19) and directly depends on the level of tryptophan in the blood.

The mechanism of interaction between the GIT and NAFLD remains poorly understood. A deeper understanding of the role of 5-HT in the pathogenesis of NAFLD could lead to significant progress in pharmaceutical development, especially in children with cognitive impairment, which is an extremely relevant issue during the COVID-19 pandemic.

CONCLUSIONS

In children with CI and DSD at NAFLD, a decrease in serum levels of ST and Trp was diagnosed, which is more pronounced after COVID-19. A direct relationship between the decrease in serum Trp and serotonin levels has been established, which directly depends on the functional state of the liver and negatively correlates with the severity of insulin resistance.

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The study was performed within the framework of the scientific topics "The latest methods of studying the central and peripheral nervous system" (state registration number 0121U112168) researched by the Department of Neurology, Neurosurgery and Psychiatry of State University "Uzhhorod National University".

CONFLICT OF INTEREST
The Authors declare no conflict of interest

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RECEIVED: 12.06.2024
ACCEPTED: 27.09.2024

ORIGINAL ARTICLE

CONTENTS

Effectiveness of the quercetin use in patients with COVID-19 with concomitant type 2 diabetes mellitus

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ABSTRACT

Aim: To conduct a comparative analysis of the effectiveness of basic therapy and basic therapy with the inclusion of quercetin in patients with COVID-19 with concomitant type 2 diabetes.

Materials and Methods: There were examined 60 patients with COVID-19 with concomitant T2DM. Upon admission into the hospital and again after 10 days, serum levels of interleukin-6, C-reactive protein, procalcitonin, ferritin, endothelin-1 were determined, and capillaroscopy of the nail plate was performed. Patients of the group I (30) against the background of protocol therapy received 0.5 g of quercetin intravenously once a day for 10 days. Patients of the group II (30) received to basic therapy.

Results: After the treatment in patients of the group I general weakness decreased, body temperature normalized, improved saturation indicators, the level of acute-phase parameters (interleukin-6, CRP and ferritin) significantly decreased, a positive effect of quercetin on the level of D-dimer in blood serum was noted, indices of pericapillary edema and hemosiderin deposition significantly decreased, indices diameter of the arterial part of the capillary and capillary network density significantly increased.

Conclusions: The use of quercetin against the background of basic therapy in patients with COVID-19 and concomitant T2DM reliably reduces the level of acute-phase indices, has an important clinical significance for reducing endothelial dysfunction and for preventing thrombotic complications.

KEY WORDS: capillaroscopy, D-dimer, endothelin, respiratory insufficiency

Wiad Lek. 2024;77(9):1962-1968. doi: 10.36740/WLek/191875 DOI

INTRODUCTION

The problem of coronavirus disease in patients with diabetes mellitus (DM) has become extremely relevant. Predictors of a severe course of the COVID-19 are considered to be the age of 65 years and over, pregnancy, the presence of DM, chronic pathology of the respiratory and cardiovascular systems [1-5]. The course of COVID-19 in patients with DM worsens the results of patients treatment due to the presence of background angiopathy and endothelial dysfunction [6, 7]. The data of many studies indicate the development of endothelial dysfunction as a determining pathological factor of the COVID-19. During capillaroscopy of the nail plate in the acute period of the disease there were noted: the deposition of hemosiderin and microthrombosis, sludge and pericapillary edema [8, 9].

The increased degree of severity of COVID-19 may be related to the chronic inflammation and impaired immune system present in patients with T2DM. Therefore, in our opinion, a combination of antiviral, anti-inflammatory and anti-diabetic therapy will be an effective strategy to combat SARS-CoV-2 infection in patients with type 2 diabetes mellitus [10, 11].

Numerous evidences of the effectiveness of quercetin in reducing the activation of pro-inflammatory cytokines are described in the literature. Quercetin contributes to the restoration of impaired functions of the endothelium through the activation of endothelial NO-synthase, an increase in the contents of NO and prostaglandin F2, and a decrease in endothelin-1 in the blood [11, 12].

AIM

To conduct a comparative analysis of the effectiveness of basic therapy and basic therapy with the inclusion of quercetin in patients with COVID-19 with concomitant type 2 diabetes.

MATERIALS AND METHODS

There were examined 60 patients with the COVID-19 with concomitant T2DM. There were used 60 of

"Medical records of inpatients" (form 003/o) of the Ivano-Frankivsk Regional Clinical Infectious Diseases Hospital, Ukraine, for 2020-2022 years.

Examinations of patients were performed during the first-second days after admission of patients to the hospital and again after 10 days Indicators of treatment effectiveness were body temperature, presence or absence of cough, general weakness, saturation level, length of stay in the hospital, level of ferritin, CRP, procalcitonin, D-dimer and endothelin-1 blood serum, quantitative and qualitative parameters of capillaroscopy.

Capillaroscopy of the nail plate was performed using a digital Capillaroscope 200 Rgt (MEDLA4N Pro), Dino-lite. Capillary network morphometry was performed in the Dino Direct-release_V1/10 program (1). Quantitative morphological parameters were determined: the length of the visible part of the capillary, the diameter of the arterial, venous, transition part of the capillary. Qualitative morphological indices were also studied: dilated capillaries (increased capillary diameter between 20 and 50 μm), giant capillaries (uniformly dilated capillaries with a diameter ≥ 50 μm), microhemorrhages, capillary ramifications, the density of capillaries per linear millimeter (normal density ≥7 capillaries) [9, 13, 14].

All examinations were performed with the consent of the patients, according to the Declaration of Helsinki, 1975 (and its revision of 1983). The research was approved by the commission on bioethics of IFNMU (Expert Decision № 121/21 dated May 13, 2021).

Criteria for the inclusion of patients with coronavirus disease (COVID-19) in study are the presence of confirmation of coronavirus disease (COVID-19) (RNA SARS-COV-2 smear from the naso- and oropharynx) and concomitant type 2 diabetes mellitus, moderately severe and severe course of the disease, age – older than 60 years, and the presence of signed informed consent. Exclusion criteria from the study: the presence of other severe chronic diseases in patients: COPD, bronchial asthma, oncological diseases, lymphoproliferative and onco hematological diseases, HIV-infection, immunodeficiency conditions – congenital, acquired, medically induced, severe chronic heart failure.

Patients were randomly divided into two groups. Patients of the main group I (30) received 0.5 g of quercetin intravenously once a day during 10 days against the background of basic therapy. Patients of the control group II (30) were prescribed basic therapy medicines: antiviral drugs (remdesivir), infusion (detoxification) agents, nonsteroidal anti-inflammatory drugs, anticoagulants, antibacterial agents for appropriate indications, oxygen therapy.

Statistical analysis of data was performed using the Microsoft Excel Statistical Package for Microsoft 365 MSO (setup 2311 of version 16.0.17029.20068) (32-bit version). License ID: EWW_58cc64b2-cc32-48b6-bd4b-cce379e20247_574357c00167ce3139.

The results obtained during the research are represented in the form of absolute numbers or proportions (for the analysis of categorical data) and average values and their errors (for the analysis of quantitative data). The values were calculated according to standard formulas. To analyze the difference between the compared results of various groups or the results before and after the treatment, the parametric Student's criterion and the non-parametric Pearson's test were used (calculation and analysis of the results were performed according to standard methods; the results were considered reliable in the p-value less than 0.05).

RESULTS

As a result of the performed research, it was determined that there were 33 male patients – 55%, 27 female patients – 45%; the age of the patients ranged from 60 to 82 years (on average, 66.02±1.29 years). Patients were admitted for the inpatient treatment, on average, during the 5.50±0.20 day of the disease. All patients (100%) had concomitant type 2 diabetes mellitus. 11 people (18.3%) were diagnosed with the COVID-19 of moderate severity, 49 people (81.7%) were diagnosed with severe disease.

Under the influence of treatment, positive dynamics of all clinical symptoms of the disease was observed in both study groups. Specifically, in patients of the main group receiving the medicine quercetin in combination with basic therapy, normalization of body temperature was noted in 96.7% of patients, while in patients of the control group, a decrease in temperature to normal indices was observed in only 76.7% of patients, and another 23.3% of patients had elevated body temperature (non-parametric Pearson's test χ^2= 5.1923, p = 0.023, p<0.05). Complaints about general weakness in the main group bothered another 23.3% of patients, while in the control group – 53.3% (non-parametric Pearson's test χ^2= 5.7109, p = 0.017, p<0.05). Upon admission to the hospital, cough was noted to the same extent in patients of both groups (main group – in 83.3%, control group – in 80% of patients, non-parametric Pearson's test χ^2= 0.111, p = 0.739, p>0.05). After the treatment, cough decreased in 30% of patients in the main group and in 50% of patients in the control group (non-parametric Pearson's test χ^2= 2.5 p = 0.114, p>0.05).

When assessing the length of patients' stay in the inpatient treatment, it was determined that patients in the main group were treated as inpatients 2 days

Table 1. Dynamics of clinical symptoms of the coronavirus disease (COVID-19) in patients with concomitant T2DM under the influence of treatment with the use of quercetin

Symptoms	Main group, n=30		Control group, n=30	
	Before treatment	After treatment	Before treatment	After treatment
Temperature increase	29 (96,7%)	1 (3,3%)	28 (93,3%)	7 (23,3%)
General weakness	26 (86,7%)	7 (23,3%)	27 (90%)	16 (53,3%)
Cough	25 (83,3%)	9 (30%)	24 (80%)	15 (50%)
Duration of inpatient treatment, days	13,77±0,75		16,13±0,79	

Table 2. Qualitative morphological parameters of capillaroscopy in patients with COVID-19 with concomitant T2DM under the influence of treatment with quercetin

Indicators	Main group (n=30)		Control group (n=30)	
	Before treatment	After treatment	Before treatment	After treatment
Dilated capillaries, n (%)	7 (23,3)	5 (16,7)	8 (26,6)	7 (23,3)
Giant capillaries, n (%)	4 (13,3)	3 (10,0)	4 (13,3)	3 (10,0)
Tortuous capillaries, n (%)	19 (63,3)	16 (53,3)	18 (60,0)	16 (53,3)
Dilatation, n (%)	18 (60,0)	17 (56,7)	16 (53,3)	16 (53,3)
Avascular area, n (%)	16 (53,3)	16 (53,3)	15 (50,0)	15 (50,0)
Pericapillary edema, n (%)	29 (96,7)	19 (63,3) *	30 (100)	28 (93,33)
Capillary ramifications, n (%)	8 (25,8)	8 (25,8)	10 (33,3)	10 (33,3)
Bushy capillaries, n (%)	8 (25,8)	8 (25,8)	10 (33,3)	10 (33,3)
Deposition of hemosiderin, n (%)	21 (70,0)	13 (43,3) *	23 (76,7)	20 (66,7)
Microthromboses, n (%)	8 (26,7)	6 (20,0)	7 (23,3)	6 (20)
Microhemorrhages, n (%)	13 (43,3)	11 (36,7)	15 (50,0)	9 (30)

Notes: * - significant difference between the indices of patients before and after the treatment ($p<0.05$).

less compared to patients in the control group, 13.77±0.75 bed days versus 16.13±0.79 bed days (parametric Student's criterion t=2.17, p=0.017, p<0.05) (Table 1).

Analyzing the development of respiratory insufficiency (RI) at the time of hospitalization of patients of the main group in the hospital, RI of the II degree was determined in 10 (33.33%) patients, SpO_2 85% - 89%, RI of the I degree was found in 10 (33.33%) patients, SpO_2 90% - 94%, no signs of respiratory insufficiency of the 0 degree, in 10 (33.33%) people, $SpO_2 \geq 95\%$. Among the persons of the control group when admitted for the treatment of RI of the I degree was observed in 10 (33.33%) patients, RI of the I degree – in 8 (26.67%) patients, 12 (40.0%) people – without signs of RI. Therefore, the distribution of patients according to the degree of development of respiratory failure at the time of hospitalization did not differ in the main and control groups (non-parametric Pearson's test $\chi^2= 0.404$ p = 0.817, p>0.05).

After the treatment the patients of the main group noted a positive dynamics in reducing the manifestations of respiratory insufficiency. In patients with RI of the I degree saturation significantly increased from 92.5±0.37% before the treatment to 96.5±0.27% after the treatment, such patients no longer needed oxygen donation (Student's parametric test t=8.73, p<0.001). In patients who were admitted to the hospital with RI of the II degree, blood oxygen saturation indices have also improved, the changes were reliable: 88.0±0.26% before the treatment and 90.7±0.33% after treatment (p<0.001). In this group, after the treatment with the inclusion of quercetin, RI of the II degree was observed in 3 (10.0%) patients, RI of the I degree – was observed in 7 (23.33%) patients, such patients continued to receive medical support with the help of an oxygen concentrator.

In patients of the control group with RI of the I degree against the background of the received basic treatment, the saturation indices have also improved, the changes were reliable – 92.0±0.42% and 93.10±0.28%, respectively (p<0.05), but 4 (13.33%) patients required supply of oxygen-enriched air through an oxygen concentrator. In patients with RI of the II degree, blood oxygen saturation indices were 87.9±0.43% and 88.80±0.39%, respectively, (p>0.05). In this group RI of

Table 3. Quantitative morphological parameters of capillaroscopy in patients with COVID-19 with concomitant T2DM under the influence of treatment with quercetin, M±m

Indicators	Main group (n=30)		Control group (n=30)	
	Before treatment	After treatment	Before treatment	After treatment
Capillary length (l cap.), μm	289,17±3,20	302,13±3,65*	289,84±3,43	300,51±1,19*
Intercapillary distance, μm	148,62±14,26	112,58±2,59*	149,12±1,89	130,14± 1,93*
Density of capillaries, n /mm	5,63±0,48	6,12±0,13*	5,79±0,18	5,91±0,18
Diameter of the arterial part of the capillary (d art.), μm	8,31±1,93	9,87±0,18*	8,68±0,20	9,0±0,21
Diameter of the venous part of the capillary (d ven.), μm	15,61±1,82	13,93±0,30*	15,44±1,57	14,90± 0,22*
Diameter of the transition part of the capillary (d trans.) μm	18,79±1,68	16,56±0,23*	18,56±0,32	17,78± 0,49*

Notes: * - significant difference between the indices of patients before and after the treatment ($p<0.05$).

the II degree was observed in 5 (16.67%) patients, they continued to receive a donation of high-flow oxygen, RI of the I degree – in 3 (10.0%) patients; such patients continued to receive oxygen support with the help of a concentrator.

As a result of the initial examination of patients with COVID-19 with concomitant T2DM upon admission to the hospital, the increase of the level of acute-phase indices of the inflammatory response (interleukin-6, CRP, procalcitonin, ferritin) compared to normal values.

The level of interleukin-6, CRP, procalcitonin and ferritin in blood serum in patients of the main group was significantly decreased after the treatment, $p<0.05$. In patients of the control group, after the treatment, a significant decrease in the level of procalcitonin was noted ($p<0.05$), other acute inflammatory indices remained elevated.

As a result of the study, the increase of the level of D-dimer in blood serum was found in patients with COVID-19 with concomitant T2DM during hospitalization compared to similar normal values. After the treatment with the use of quercetin, a slight increase in the average level of D-dimer was noted in the main group of patients at 219 ngFEU/ml, while in the control group it significantly increased at 1262 ngFEU/ml. The significant difference between the groups according to this index after the treatment was 1.43-fold ($p<0.1$). Therefore, the results obtained indicate the positive effect of therapy with the use of quercetin in patients of the main group and the negative dynamics of D-dimer contents in patients of the control group.

During the initial determination of the blood serum endothelin level, it was determined that in the group of patients with COVID-19 with concomitant T2DM, it exceeds normal values. After treatment, a positive effect of quercetin on the level of endothelin was noted. In particular, blood serum endothelin values in patients of the main group had a tendency to decrease to 40.69±2.93 ng/ml, and in patients of the control group to 46.23±3.25 ng/ml, but they still exceeded norm indicators.

Primary capillaroscopy of the nail bed in patients with COVID-19 with concomitant T2DM was performed during the second-third day from the moment the patients were admitted to the hospital. Significant changes in some qualitative morphological parameters of capillaroscopy were observed ($p<0.05$) (Table 2).

In particular, studying capillary morphology in the main group revealed high frequencies: capillary branching in 8 (25.8%) patients, bushy capillaries in 8 (25.8%) patients, tortuous capillaries in 19 (63.3%) patients, pericapillary edema in 29 (96.7%) patients, and microhemorrhages in 13 (43.3%) patients (Table 2).

Examining capillaroscopy's qualitative morphological parameters in the control group upon hospital admission showed high frequencies: capillary branching in 10 (33.3%) patients, bushy capillaries in 10 (33.3%) patients, tortuous capillaries in 18 (60.0%) patients, pericapillary edema in 30 (100.0%) patients, and microhemorrhages in 15 (50.0%) patients (Table 2).

During re-examination after the performed treatment with quercetin use, in patients of the main group the indices of pericapillary edema significantly decreased in 19 (63.3%) people (non-parametric Pearson's test $\chi^2= 10.4167$ $p = 0.0012$, $p<0.05$) and manifestations of such microvascular complications as hemosiderin deposition decreased in 13 (43.3%) patients (non-parametric Pearson's test $\chi^2= 4.3439$ $p = 0.0371$, $p<0.05$). In the control group of patients, no significantly reliable ($p>0.05$) decrease in quality indices of capillaroscopy was found after the basic treatment (Table 2).

When studying the quantitative morphological parameters of the capillaries of patients with COVID-19 with concomitant T2DM during the initial examination,

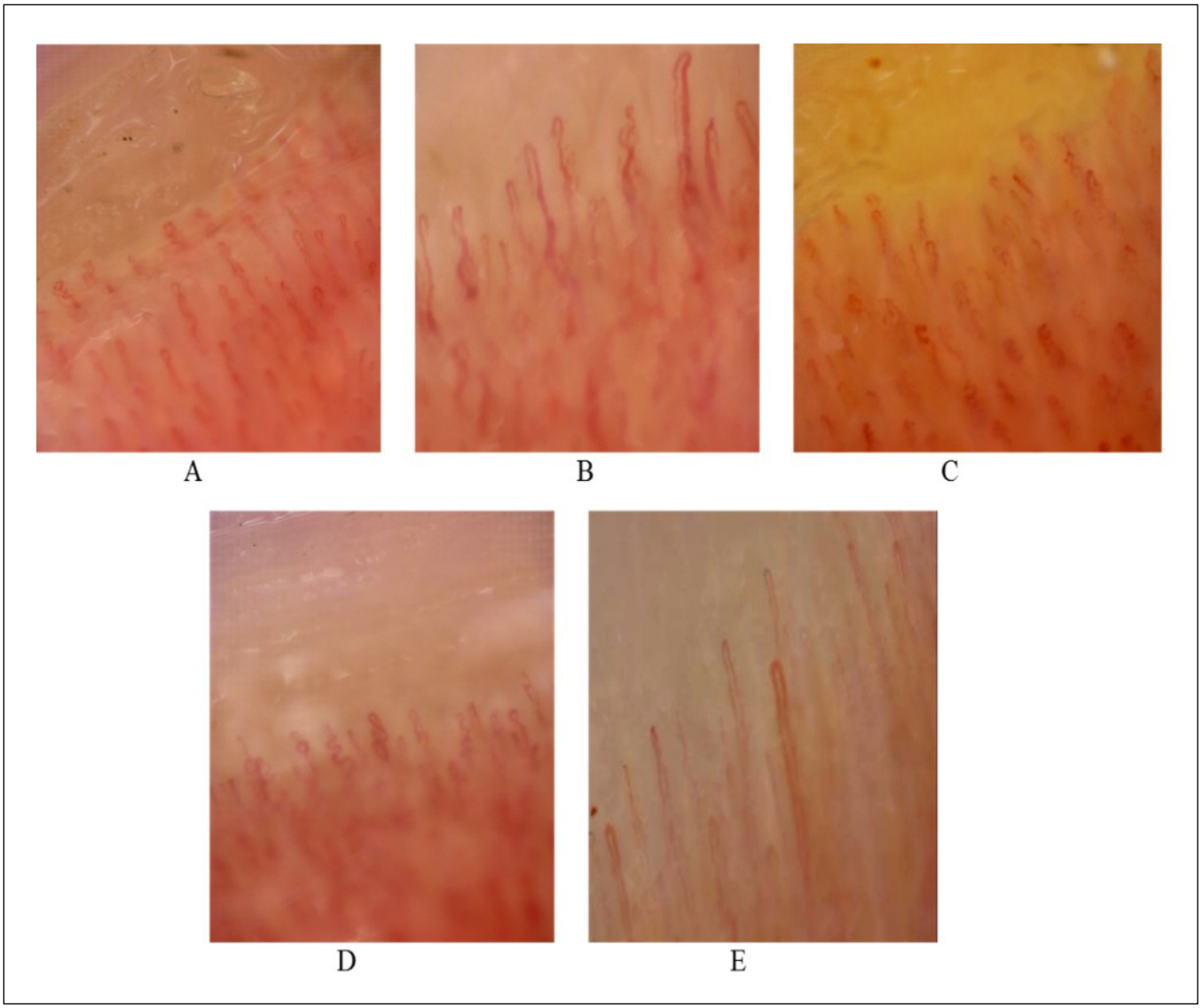

Fig. 1. Nailfold capillaroscopy abnormalities detected in patients with COVID-19 with T2DM (magnification 200×). A) pericapillary edema; B) capillary ectasia; C) microthrombosis; D) tortuous capillaries and microvascular disorders; E) low capillary density.

it was found that in patients of the main group there was a significant decrease in the length of the capillaries, a significant narrowing of the diameter of the arterial and an increase in the diameter of the venous capillary segment, and a significant increase in the diameter of the transitional capillary sections, the density of the capillary network significantly decreased (Table 3).

During re-examination after the treatment with quercetin, the length of the capillaries significantly increased in patients of the main group, the indices of the arterial diameter significantly increased and the indices of the diameter of the venous and transitional sections of the capillary decreased, the density of the capillary network significantly increased. In the control group of patients after the basic treatment, a significant increase in the length of the capillaries was also noted, the indices of the diameter of the venous and transitional sections of the capillary significantly decreased, and the intercapillary distance has significantly decreased. At the same time, in patients of this group, there was no reliably significant increase in the diameter of the arterial section of the capillary and an increase in the density of the capillary network (Table 3).

Representative images of capillary changes in patients diagnosed with COVID-19 with T2DM are represented in the Fig. 1.

DISCUSSION

We've determined in the main group, a positive dynamics was noted in the reduction of manifestations of respiratory insufficiency and increase in saturation in patients with RI of the I and II degrees. Such a reliable improvement in saturation may be due to the membrane-stabilizing,

antioxidant and endothelium-protective effects of quercetin. The results obtained correspond to the data of studies of the other authors [12, 15].

We've also determined a reliable positive effect of quercetin on the level of acute-phase indices, which are the key markers of disease progression and predictors of severity [2, 16, 17]. The data we've obtained, differ from the results published by the authors of the study of the quercetin effectiveness in patients with pneumonia associated with the coronavirus disease (COVID-19), in which there was no statistically confirmed difference between the study groups in terms of CRP and ferritin levels [12].

It should be noted that in our study, high levels of D-dimer in patients with COVID-19 with concomitant T2DM were accompanied by a severe course of the disease, which correlates with data from the literature [2, 18, 19]. Therapy with the use of quercetin had a positive effect (absence of an increase in the level of D-dimer) in patients of the main group. In our opinion, the use of quercetin allows more effective prevention of thrombotic complications of the coronavirus disease (COVID-19). The results obtained by us correspond to the data of studies regarding the effectiveness of quercetin in patients with pneumonia associated with the coronavirus disease (COVID-19) [12]. A positive influence of quercetin on the level of endothelin was also found, which was evidenced by a significant difference between the indices in patients of the main and control groups after the treatment.

Studying the microcirculatory bed in patients with COVID-19 and concomitant T2DM using nail plate capillaroscopy, revealed hemosiderin deposition, common abnormalities were pericapillary edema, capillary dilatation, tortuous and dilated capillaries. The density of the capillary network and the diameter of the arterial section of the capillary were significantly reduced. Such data have been confirmed by a number of scientists [14, 20]. After the treatment with quercetin, pericapillary edema and hemosiderin deposition significantly decreased in patients of the main group, capillary length and diameter indices of the arterial section of the capillary increased. This indicates an improvement in capillary blood flow in patients with COVID-19 with concomitant T2DM under the influence of quercetin treatment.

CONCLUSIONS

1. In patients with COVID-19 with concomitant type 2 diabetes who received quercetin for 10 days against the background of basic therapy, significantly more often, compared to patients in the control group, general weakness decreased (non-parametric Pearson's test χ^2= 5.7109, p = 0.017, p<0.05), body temperature normalized (non-parametric Pearson's test χ^2= 5.1923, p = 0.023, p<0.05), improved saturation indicators (Student's parametric test t=8.73, p<0.001) and the manifestations of respiratory insufficiency decreased, which shortened the length of stay of patients in the hospital by 2 days (parametric Student's criterion t=2.17, p=0.017, p<0.05).
2. The use of quercetin against the background of basic therapy in patients with COVID-19 and concomitant type 2 diabetes significantly reduced the level of acute phase parameters (interleukin-6, CRP and serum ferritin) (p<0.05), the growth of D-dimer level in blood serum slowed down (p<0.1), which reduced the manifestations of endothelial dysfunction, improved qualitative (decreased pericapillary edema and deposition of hemosiderin, p<0.05) and quantitative (increased density of capillaries and diameter of the arterial part of the capillary, p<0.05) parameters of capillaroscopy, and therefore capillary blood flow, in comparison with patients of the control group.

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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

RECEIVED: 10.03.2024
ACCEPTED: 01.08.2024

ORIGINAL ARTICLE

CONTENTS

Oral health status in relation to nutritional status amongst 6-12 years aged orphans in Baghdad city, Iraq

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ABSTRACT

Aim: We aimed to assess selected measures of oral health, namely, enamel defect, eruption of permanent teeth, and dental caries. The nutritional status of orphans was investigated by physical examination, and then the nutritional status was correlated with measures of oral health.

Materials and Methods: A total of 192 orphans aged of 6 and 12 living in all orphanages in Baghdad, Iraq, were studied. Enamel defect was derived from the WHO's modified developmental defects of enamel index, investigation of caries using Decay -Missing – Filled index (DMF) for permanent teeth, the decay-missing filled index for primary teeth (dmf) index. All permanent teeth were inspected for eruptive stage. According to body mass index (BMI) for age, subject's visits and birthday dates were recorded as day/month/year. Height was measured in centimetre and weight in kilogram. Data were then transfer data to BMI Excel calculator.

Results: The prevalence of enamel defect was low in the sample, and fully erupted teeth had high prevalence amongst orphans (more than 9 years old). A high percentage of caries existed amongst orphans, and the highest percentage of the sample was normal in weight.

Conclusions: Enamel defect was higher amongst normal weight then overweight children. The mean of permanent teeth eruption increased with increased BMI for age. A high caries prevalence existed amongst orphans.

KEY WORDS: children, orphan, enamel defect, oral health status, public health

Wiad Lek. 2024;77(9):1969-1978. doi: 10.36740/WLek/193200 DOI

INTRODUCTION

Orphans are defined by UNICEF and its international partners as children under the age of 18 who have lost one or both parents In 2016, Iraq had over 4.5 million orphans as a result of the violence that followed the 2003 war, as well as instability and population displacement, [1]. Good oral health is necessary for optimal physical and mental health and is thus a basic human right [2]. Enamel is generated by ameloblast cells, which are lost when a tooth erupts into the oral cavity; enamel cannot therefore regenerate itself. The main stages of amelogenesis are the period of matrix production, the secretory stage, and the period of maturation, which consists of mineral crystal growth and the loss of protein and water , the severity of developing enamel defects depends on the length and force of the insult, as well as the stage of enamel organ development at the time the lesion occurs [3]. The processes of tooth eruption are still not completely known, but active eruption appears as root formation starts. This phenomenon leads to the assumption that eruptive force originates from the periodontal ligament. The eruption force is provided by the periodontal ligament once the tooth has pierced the gingiva, but not whilst the tooth is still intraosseous, The fact that active tooth eruption occurs as root formation begins supports the concept that the periodontal ligament is the source of eruptive power; nonetheless, the mechanics of tooth eruption are yet unknown. Only once the tooth has broken through the gingiva can the periodontal ligament aid in tooth eruption, Pre-eruptive movements occur during crown creation and are very tiny that they could only be detected by vital staining experiments. Some authors divided eruption into three stages: Pre-eruptive movements, eruptive movements and post-eruptive movements [4]. Periodontal disease and tooth decay are the two most common oral health problems, and both are caused by plaque. If not treated, tooth loss can impair a person's ability to eat, speak, and feel good about themselves. Malnutrition poses a significant concern to some vulnerable communitiesPlaque has been connected to each of the most prevalent oral diseases, namely, tooth decay and gum disease. If left

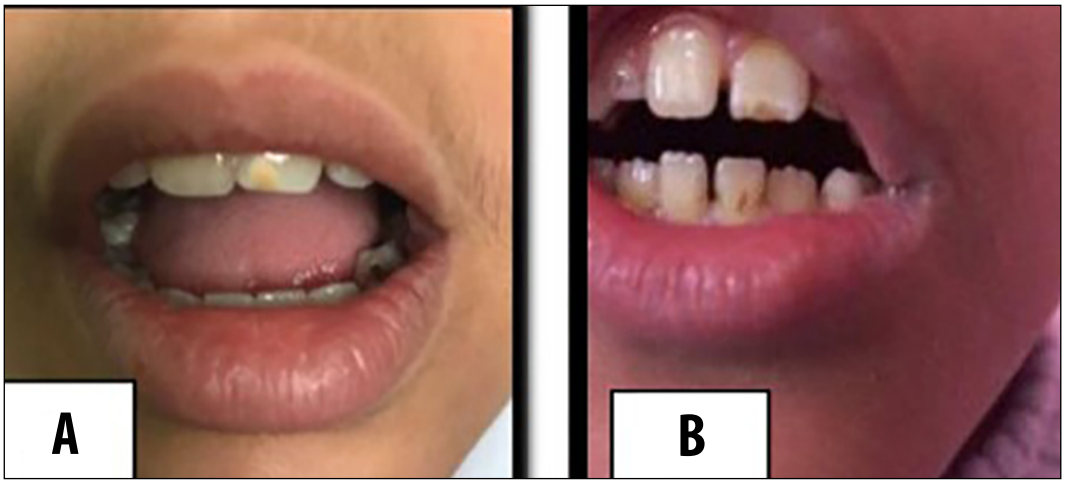

Fig. 1. Clinical appearance of developmental defect of enamel. A: Demarcated opacity; B: Hypoplasia.

untreated, tooth loss may occur, which is detrimental to one's self-esteem and ability to talk and chew food [5]. Nutrition refers to a living entity's utilisation of components from its surroundings to support its own essential activity [6]. Without sufficient nutrition, an organism is more likely to become unwell [7].

AIM

The aim of our research was to assess selected measures of oral health: enamel defect, eruption of permanent teeth, dental caries, and to investigate the nutritional status of orphans by their physical examination to link nutritional status to oral health outcomes.

MATERIALS AND METHODS

One hundred ninety-two children aged 6–12 years old were examined from all orphanages in Baghdad city, Iraq. Preceding the process of data collection, consent was obtained from the Directorate of Labor and Social Affairs/Department of Persons with Special Needs in Baghdad City to run the study. The research was conducted from November 2022 until the end of January 2023. A pre-study ethical approval was obtained from the scientific committee of the Department of Pedodontics and Preventive Dentistry and the ethical committee at the College of Dentistry/University of Baghdad to achieve the subject without obligation. In accordance with WHO's [8] requirements for oral examinations, children were examined in a setting designed for maximum effectiveness and cooperation. To acquire good illumination and provide the kids with the most comfort, the examiner used an aperture where sunlight entered as a source of light whilst standing behind the kids' heads. The tools used were several pairs of tweezers, a plane mouth mirror, containers, gauze, and a CPI probe. The criteria for enamel defect were derived from WHO's modified developmental defects of enamel (DDE) index [8], which was utilised in the study and registration of permanent teeth. They were classified as delimited opacity, diffuse opacity, and hypoplasia according to their clinical presentation (Fig.1).

All the permanent teeth of the children were examined regarding their eruptive stage in natural light by a dental mirror and the registration of teeth was performed as stated by the FDI notation system [9] (Fig.2).

The primary teeth were coded alphabetically, whereas the permanent teeth were coded numerically. The CPI probe can be used to confirm visual evidence of caries on the tooth surfaces Carious lesions were seen on all tooth surfaces that were compromised. When calculating tooth loss due to caries, the front teeth were counted as having four surfaces and the back teeth as having five retained roots were counted as five decayed surfaces for the posterior and four decayed surfaces for the anterior teeth (8) (Fig.3).

According to the body mass index (BMI) indicator, to achieve categorisation of BMI for age, subject's visits and birthday dates were recorded as day/month/year. After measuring height in centimetre and weight in kilogram, data were transferred to the BMI Excel calculator (summary report Paediatrics. 2007, Geneva, Switzerland) [10], <5% (underweight), 5%–85% (normal), 85–95% (at risk of overweight), and >95% (overweight or obese). According to age that ranged between 6-12 years old the average would be 9 years old, so the age groups was less or equal to 9 years old and the second group would be more than 9 years old. The statistical tests Student's t-test and one-way ANOVA were used. A p value of <0.05 was considered statistically significant.

RESULTS

This study included a total of 192 orphans aged 6-12 years. Children aged less or equal to 9 years old had

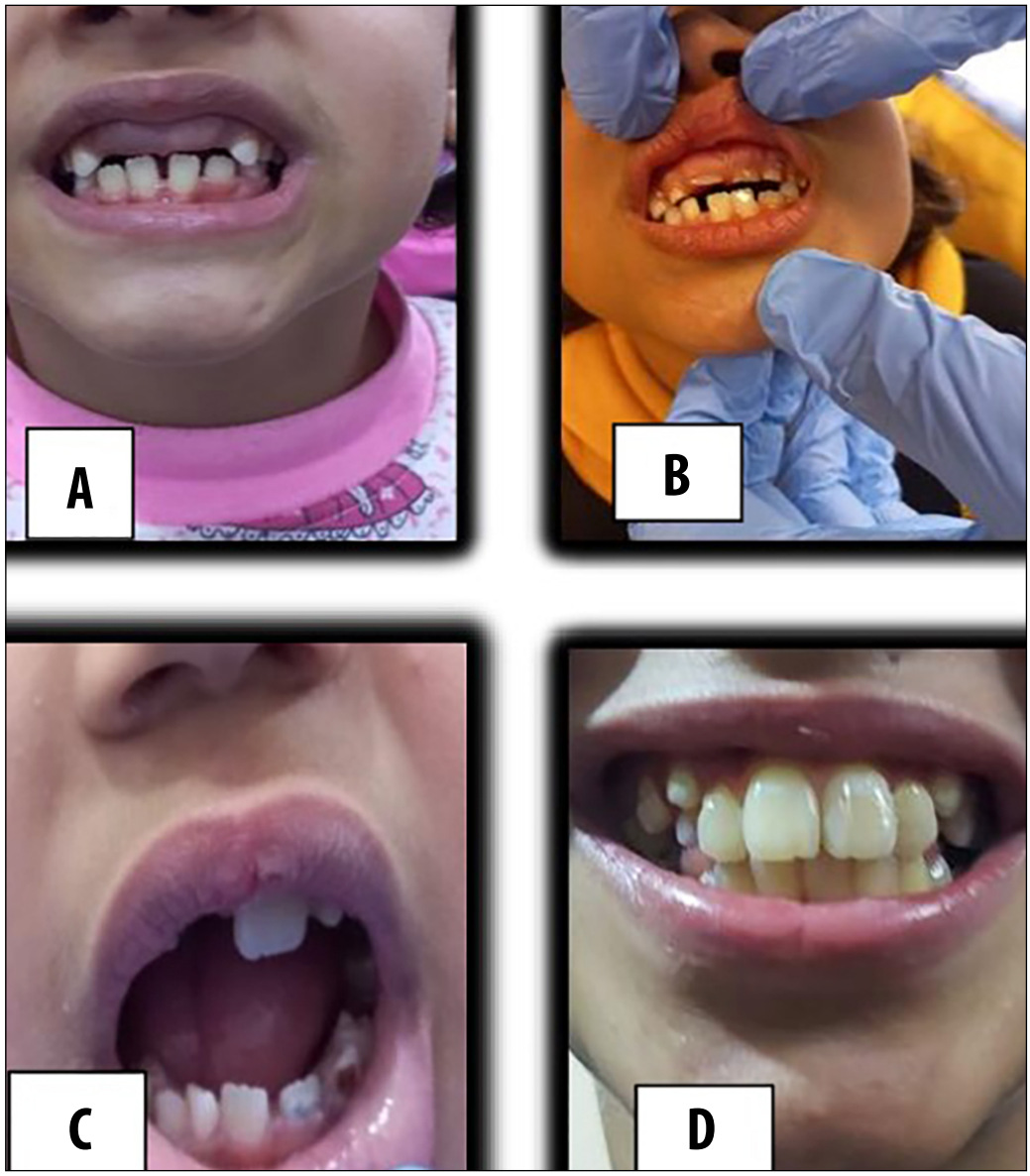

Fig. 2. Eruption stages of permanent teeth. A: stage 0; B: stage 1; C: stage 2; D: stage 3.

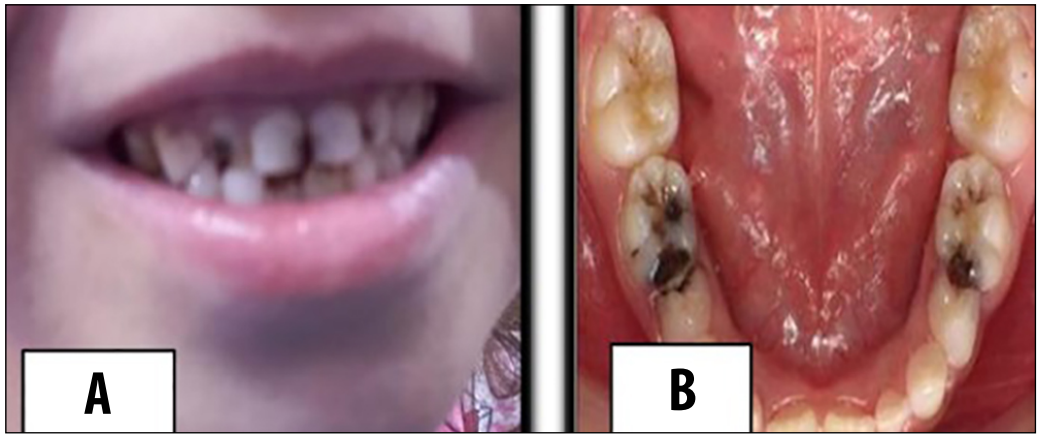

Fig. 3. Caries in primary teeth. A: anterior caries; B: posterior caries.

the same percentage (50%) as children more than 9 years old. Boys and girls constituted close proportions, with boys forming 46.88% while girls 53.13%. Children in governmental orphanages constituted 68.75% of the study sample, while in the private orphanages were 31.25%. Regarding BMI-age relation, the high

Table 1. Descriptive and statistical test of eruption (ER) scores with age and gender

	Variables	Group 1(<=9, Male)*		Group 2 (9+, F)**		T test	P value***
Age	ER0	15.229	0.559	5.458	0.516	12.836	0.000
	ER1	0.604	0.091	0.542	0.109	0.441	0.659
	ER2	1.771	0.163	0.792	0.105	5.065	0.000
	ER3	5.563	0.501	15.792	0.511	14.293	0.000
	Total teeth eruption	7.938	0.528	17.125	0.473	12.959	0.000
Gender	ER0	11.667	0.696	9.176	0.743	2.446	0.015
	ER1	0.667	0.122	0.490	0.077	1.219	0.225
	ER2	1.644	0.140	0.961	0.142	3.430	0.001
	ER3	9.022	0.659	12.137	0.747	3.127	0.002
	Total teeth eruption	11.333	0.665	13.588	0.686	2.346	0.019

* Group 1: according to gender: male , according to age equal or less than 9 years old. ** Group 2: according to gender: female, according to age: more than 9 years old. ***Significant at $P < 0.05$.

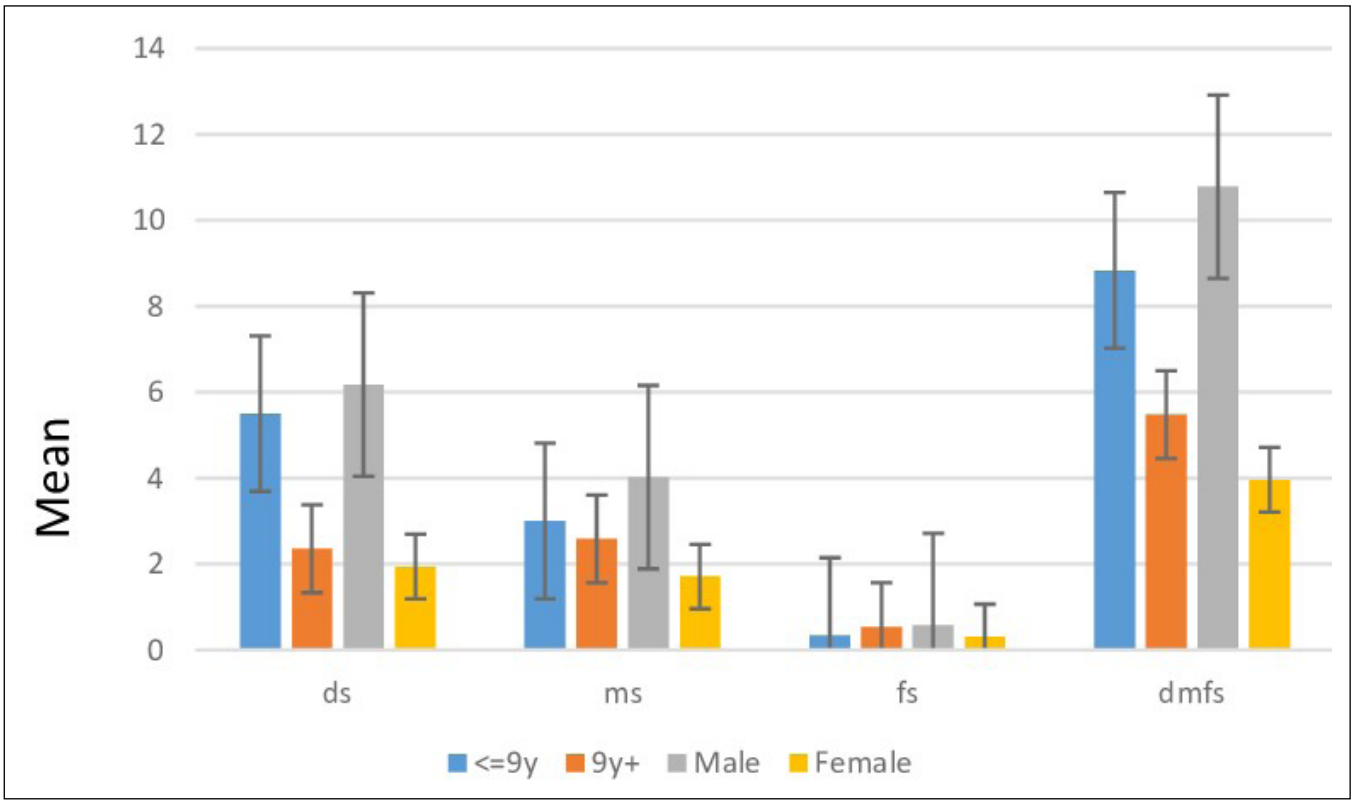

Fig. 4. Descriptive and statistical test of primary caries experience.

prevalence was for acceptable weight (74.48%), while underweight and overweight had the same percentage being the least percentages. The prevalence of enamel defect was 31.13% of the total sample. The demarcated opacity had the highest mean among the sample followed by diffuse opacity then hypoplasia had the least mean. According to age the highest percentage (66.67%) was amongst older children (more than 9 years old). According to gender, the higher percentage was for females that had DDE about 56.67%. It was found that the upper centrals were the most affected teeth by the DDE [11-21], followed by upper molars [16], respectively. Conversely, the upper left lateral incisor 42 and first premolar tooth 44 had the least percentage. According to age, children with eruption stage 0 were more amongst the youngest children less or equal to 9 years old with a high significant difference ($P< 0.01$), also stage 1 of teeth eruption was more amongst youngest children but with no significant difference stage 2 teeth eruption was more amongst younger

Table 2. Descriptive and statistical test of permanent caries experience

Variables.	Caries	Group 1 (<=9, Male)*		Group 2 (9+, F)**		T	P value***
		Mean	±SE	Mean	±SE		
	DS	**1.000**	**0.192**	**2.021**	**0.568**	**1.703**	**0.091**
Age 1: ≤9 years 2: >9 years	MS	0.438	0.157	0.333	0.113	0.539	0.591
	FS*	0.021	0.015	0.688	0.109	6.054	**0.000**
	DMFS*	1.458	0.239	3.042	0.582	2.517	**0.013**
Gender 1: male 2: female	DS	2.111	0.606	0.980	0.179	1.791	0.076
	MS*	0.178	0.087	0.569	0.163	2.115	**0.036**
	FS*	0.533	0.109	0.196	0.056	2.763	**0.007**
	DMFS	2.822	0.610	1.745	0.259	1.625	0.107

* Group 1: according to gender: male , according to age equal or less than 9 years old. ** Group 2: according to gender: female, according to age: more than 9 years old. ***Significant at $P < 0.05$.

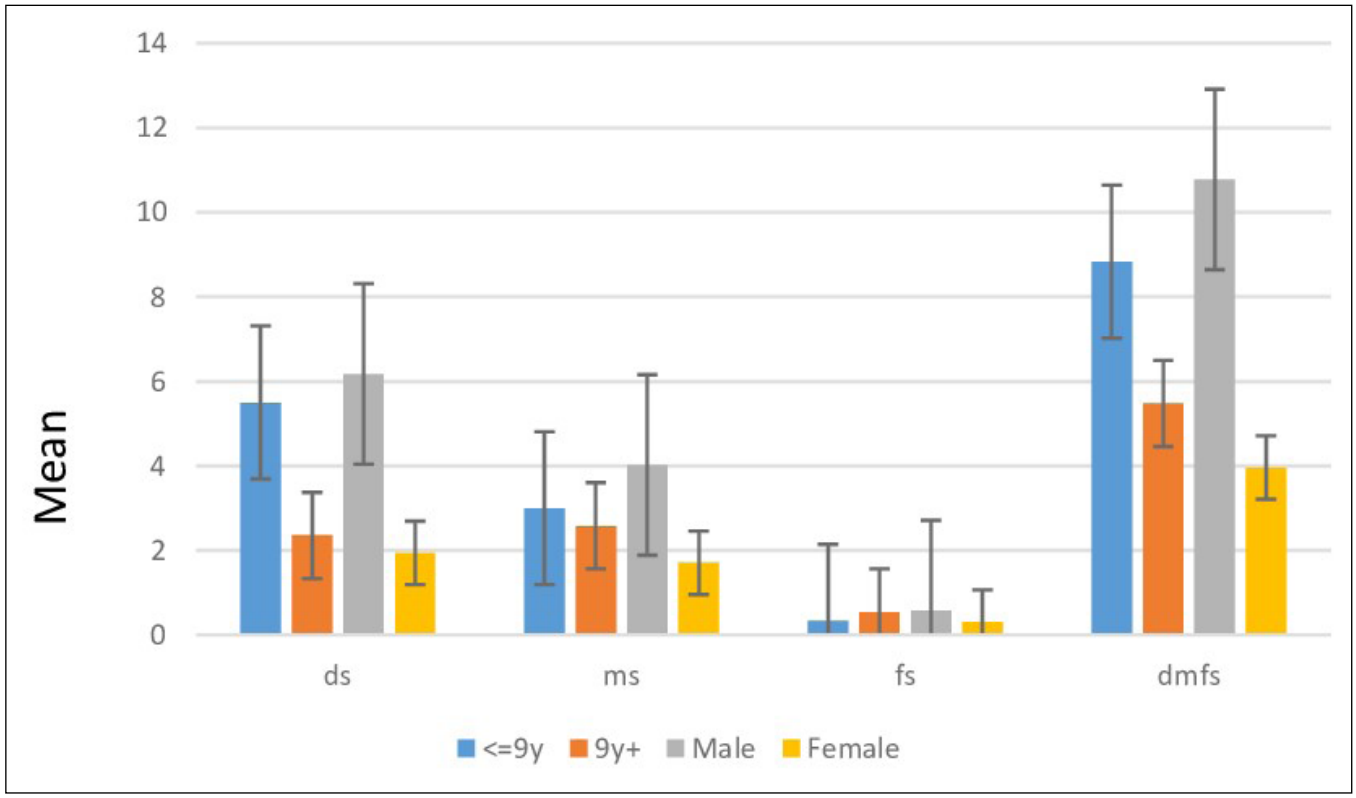

Fig. 5. Descriptive and statistical test of permanent caries experience.

children with significant difference between them. Conversely, stage 3 of teeth eruption had the highest mean amongst older children (more than 9 years old), with high significant difference. According to gender, stage 0 and 2 teeth eruption was greater amongst males than female, with a significant difference between them. Stage 1 was also greater amongst males, but with no significant difference statistically. Stage 3 teeth eruption had a higher mean amongst females than males, with a high significant difference (Table 1).

According to caries, there was high percentage of caries prevalence among the children of both private and governmental orphanages (88.54%). The high caries percentage was more among younger children (51.76 %). According to gender, caries percentage was more among males (51.76%) than female. Caries experience for the primary dentition according to age and gender groups, the decay in primar teeth fraction(d) mean value showed significant decline with advancing age with P<0.001 for both. In regard to missing teeth reflected by surfaces (m), the mean rank value higher among orphans at age 9 years or less (P=0.589), no

Table 3. Descriptive and statistical test of primary caries experience according to nutritional status

Variables.	Underweight		Normal		Overweight		Obese			
	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	F	P value
ds	4.500	1.232	3.713	0.520	4.938	1.340	4.235	1.621	0.267	0.849
ms	0.500	0.342	2.867	0.457	4.000	1.633	3.176	1.189	1.309	0.273
fs	0.625	0.287	0.476	0.110	0.313	0.151	0.059	0.059	0.797	0.497
dmfs	5.625	1.217	7.056	0.712	9.250	2.492	7.471	2.471	0.503	0.680

Table 4. Eruption of permanent teeth in relation to nutritional status

Variables.	Underweight		Normal		Overweight		Obese			
	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	F	P value
ER0	10.625	1.873	10.685	0.571	8.813	1.889	8.647	2.307	0.670	0.571
ER1	0.500	0.316	0.615	0.083	0.813	0.228	0.059	0.059	2.035	0.110
ER2	1.313	0.395	1.385	0.124	1.063	0.213	0.588	0.258	1.744	0.159
ER3	10.563	1.756	10.224	0.567	12.063	1.870	13.294	2.273	1.164	0.325
Total teeth eruption	12.375	1.791	12.224	.535	13.938	1.733	13.941	2.141	0.581	0.628

significant difference among the age groups in regards to filling in primary teeth fs was found. The mean rank of decay-missing-filled primary teeth reflected by surface (dmfs) showed significant difference. In regard to gender the Figure 4 illustrates higher and significant mean of ds, fs and dmfs among males compared to females of institutionalized orphans, high ms mean in males than females and significant elevated dmfs mean rank in males than females (P=0.000) as shown in fig. 4.

Table 2 showed caries reflected by surface DMFS for permanent teeth among age and groups, the DS mean showed progressed with advancing age as found with no significant difference among age groups. Results showed there was higher percentage of MS among younger children with no significant difference among the age groups. In regard to FS fraction, there was a significant high mean among older children. Significant increased DMFS mean rank was revealed in age group older than 9 years old for orphan groups. According to gender. High mean rank DMFT was found to be elevated in males than females although statistically not significant (Fig.5).

ORAL HEALTH STATUS IN RELATION TO NUTRITIONAL STATUS

Regarding the BMI for age index, the weight and height of children were recorded. We found that the highest percentage of the sample was normal in weight (74.48%), whereas the least belonged to the overweight and underweight group, which had the same percentage of 8.33%. In relation to BMI, the highest percentage was for children with normal weight followed by overweight then obese children. The least percentage was for thinnest children. The demarcated opacity had the highest mean amongst normal weight children with no significant difference, whereas underweight children had no demarcated enamel defect amongst them. Diffused opacity was found only amongst normal weight children, and is no diffuse opacity existed amongst underweight, overweight, and obese children. Hypoplasia had the highest mean (0.563) amongst overweight children, with no significant difference, followed by normal weight children. Obese children had the least mean amongst underweight orphans. According to BMI the higher percentage of caries was amongst normal-weight orphans, followed by overweight and then underweight. The lowest percentage was amongst obese children in this sample. According to caries experience in primary dentition according to BMI-age grades, the ds and ms mean had high value in the overweight group, with no significant difference amongst institutionalised orphans. Meanwhile, the mean of fs was higher amongst underweight orphans with a non-significant difference. The dmfs mean was higher amongst overweight children, with no significant difference amongst BMI-age groups of institutionalised orphans (Table 3).

According to BMI-age, caries reflected by surface (DMFS) for permanent teeth in institutionalised, results demonstrated a high mean of DS in overweight group, with no significant difference. Conversely the least mean in the obese group had no significant difference. With regard to the MS mean, the value in the obese group was high with no significant difference from BMI-age in the institutionalised orphans. By contrast, it was low

in the overweight group of orphans. According to FS, the highest mean was amongst overweight orphans, with no significant difference amongst BMI-age groups, there was high with no significant DMFS mean value in overweight group. The same DMF result was revealed for orphan groups. Permanent teeth eruption (mean and standard error) by BMI was found that the mean value of permanent teeth eruption increased with increased BMI for age, without statistically significant difference. Additionally, the mean of the un-erupted permanent teeth was the least amongst the thinnest group. Meanwhile, the obese group had the highest mean value of the erupted permanent teeth. Comparisons amongst the nutritional status categories in relation to the eruption stages, stage 0, stage 3, and the total eruption stages of permanent teeth eruption; there was a highly significant difference between the thinnest group and the other nutritional status groups (Table 4).

ORAL HEALTH STATUS IN RELATION TO RESIDENCE

The sample was more amongst governmental than private orphanages – 132 vs 60 children respectively. Study showed that enamel defect was found more amongst governmental than private orphan children, 70% vs 30% respectively. The relation between types of enamel defect and type of resident, ED1 was more amongst governmental orphanages, with no significant difference, whereas no ED2 was found amongst children in private orphanages and governmental had a significant higher mean. Nevertheless, ED3 had non-significant higher mean amongst private orphanages. Caries had large number amongst governmental and private orphanages with larger prevalence amongst governmental orphanages, 65.88% vs 34.12% respectively. The relation between primary teeth caries and resident, ds had higher mean in private orphanages, whereas ms had higher mean amongst children of governmental orphanages, and both had no significant difference. Regarding fs no one of children of private orphanages had filling in primary teeth so governmental orphanages had significantly higher mean of fs. According to dmfs the higher mean was amongst private orphanages with no significant difference. DS, MS, and DMFS had higher mean amongst children of private orphanages with only DS had significant difference statically. Meanwhile, FS had significant higher mean amongst orphans of governmental orphanages. Total permanent teeth eruption was more amongst children of private than governmental orphanages with no significant difference. With regard to eruption stages (ER0, ER1, and ER2) was more amongst children of governmental orphanages, and only with ER2 was the difference significant. Additionally, ER3 had higher mean amongst children of private orphanages with no significant difference.

DISCUSSION

In this study, the prevalence of the enamel defects for the total sample was 31.13%, which was higher than that reported by Idiculla et al. in India [11]. However, it was lower than that reported by Abd-alalmeer and Al-Haider in Iraq [12]. Studies have found varying estimates of the prevalence of enamel developmental defects. This discrepancy may be attributed to changes in sample size and age, as well as in technical assessment processes (such as the type of lighting used or whether or not the teeth were dried). Another possible explanation for the discrepancies between these studies is that they all used slightly different terminologies and diagnostic criteria [13]. Concerning gender, enamel defect was more common amongst females than males, with significant differences between them. This finding disagreed with that of Abd-alameer and Al-Haider [12]. Variations may exist amongst the studies regarding the sample size. Likewise, an individual may be vulnerable to develop DDEs amongst various groups of children. The age of 6–7 years reportedly falls within the mixed dentition stage. This stage is characterised by the eruption of permanent teeth (mandibular first molars, mandibular central incisors, and upper maxillary first molars) [11]. A significant correlation has been found between delayed eruption and male gender. A statistically significant correlation has also been found between the number of normal eruptions of permanent teeth in females and boys. According to numerous international studies [14, 15], females' permanent teeth began to erupt earlier than those of boys [15]. Herein, the percentage of caries prevalence amongst children in private and governmental orphanages is high at 88.54%, and they were comparablein value. This value was higher than that estimated by Sharma et al. [16], but lower than those estimated by Abdalmajeed [17] . This may be due to different sample size, age, and variations in the methods used for determining the prevalence of dental caries. Environmental factors affect caries etiology, such as the fluoride level in Baghdad's public water supply, which Ahmed et al. [18] estimated to be 0.14 ppm. Males demonstrated significantly higher caries experience for primary dentition than females amongst orphans. This finding may be related to the earlier shedding of deciduous teeth in females than it does in males [15], in contrast to the result of Ahmed et al. [18] but in accordance with that of Khare et al. [19].

Normal weight had the highest prevalence amongst orphan children, consistent with the finding of Abdalmajeed [17]. This may be an indication of improvement in nutritional status amongst Iraqi institutionalised orphan children throughout the current years. The present study revealed that the mean of BMI-age and age had a greater correlation amongst orphan children more than nine years' old. This result disagreed with that in Khare et al. [19], in which institutionalised orphans are found to have the non-significant very weak inverse relationship between BMI and age. Amongst the institutionalised, males had a lower mean BMI than females, with significant relation that agreed with [18]. However, the study failed to reach the level of significance. Caries experience with permanent dentition is found to be higher in the thinness group than in the acceptable weight group of institutionalised orphans. Most of them may be related to low economic background, so they are at high risk for dental caries [20]. Children who are underweight may have a lower DS because their permanent teeth come in later. High quantities of fatty acids may restrict the growth of cariogenic bacteria and reduce the amount of dietary fermentable carbohydrates in overweight/obese people. A fatty layer over plaque prevents sugar from fermenting. Conversely, excessive consumption of caries-protective diets such as high-fat items may account for the reduced prevalence of caries in overweight and obese individuals. In the primary and permanent dentitions of institutionalised orphans, the ms/MS ratio contained a greater proportion of dmfs/DMS than fs/FS. This finding indicated that if therapy was to be administered, it should focus on tooth extraction rather than the preservation of deciduous and permanent teeth. It describes the minimal dental care that these children received. This finding concurred with that of Gunawardane et al. [21]. Enamel defects were greater amongst children in governmental than private orphanages. The highest prevalence was diffuse opacity with a significant difference. Sample size, dental care (which may be more and better in governmental orphanages), and type of nutrition can be the reason for the difference. Eruption of permanent teeth stage 2 was greater amongst orphans of governmental than private orphanages with significant statistical relation. Sample size can explain this difference in addition to the possibility that governmental orphans had more dental care and fillings in primary teeth than those in private (fs had a high prevalence with a significant difference in governmental orphanages, whereas ds was more amongst the private) so this can keep normal space and better oral condition for permanent teeth eruption. Moreover, governmental orphans had a higher mean of BMI, height and weight which can explain the difference. In this study, caries had a larger number amongst governmental and private orphanages, with a larger prevalence amongst private orphanages. Regarding (ds) orphans were low amongst private and governmental, and this finding is in accordance with. The lower (ds) levels seen amongst governmental than private orphans may be partly due to the effectiveness of scheduled dietary control and regular oral-hygiene measures or to the duration of being an orphan because of the effect of neglect [22]. DS amongst orphans of governmental orphanages was less than in private orphanages, with significant differences between them. This result may be explained by low dental knowledge about teeth importance amongst private orphanages and seeking treatment in case of pain only. Amongst institutionalised orphans, caries experience in primary dentition (dmfs) was found to decrease with advanced age. The age difference was significant possibly because of the natural exfoliation of primary teeth. Conversely, caries experience in permanent teeth significantly increased with advancing age, consistent with the result of Abdalmajeed (2016) [17] owing to the eruption of the permanent teeth and establishment of the contact area. The severity of dental caries may continue to increase with age due to the accumulative and irreversible nature of dental caries [23]. Moreover, ds and DS were more amongst private than those in governmental orphanages, whereas fs/FS was more in governmental than private orphanages. Thus, governmental orphanages had more knowledge about dental care and more visit to dental care for orphan children. Furthermore, in relation to the fact that life condition in governmental orphanages is more organised and children may have lived there from age 6 to 12 consistently. Conversely, children in private orphanages can live only for a few months or years.

CONCLUSIONS

The prevalence of enamel defect was low in amongst orphan children. Enamel defect was higher amongst normal weight then overweight children, and teeth eruption (fully erupted teeth) was higher amongst older children (more than 9 years old). We further found that the mean value of permanent teeth eruption increased with increased BMI for age. Caries prevalence amongst children in private and governmental orphanages was high. The caries percentage of primary was amongst normal-weight orphans followed by overweight, whereas of permanent dentition was found to be high amongst the thinness group.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 12.12.2023
ACCEPTED: 11.09.2024

ORIGINAL ARTICLE

CONTENTS

Analysis of the intensity of carious infection of teeth in children permanently living in conditions of a pollated ecosystem

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ABSTRACT

Aim: Assessment of dental caries and its impact on the level of dental health of children aged 15–18 years of Upper Tysa region, who constantly live in conditions of combined negative effects of factors of natural and technological genesis.

Materials and Methods: During the dental examination, the incidence of dental caries in 360 children aged 15-18 years from various biogeochemical zones of the cross-border region of the Upper Tysa region was studied and assessed using the International Caries Detection and Assessment System — ICDAS II (International Caries Detection and Assessment System).

Results: It was found that the use of physiologically suboptimal drinking water from decentralized water resources is among the environmental determinants of the state of dental health of children in the Upper Potyssia. In lowland children in Hungary and Slovakia, caries was observed more often on the chewing surfaces than on the proximal ones (46/42; 64/54), and in Ukraine and Romania, on the contrary, the prevalence of the carious process was observed on the proximal surfaces (71/98; 70/ 94). This may be related to longer exposure to factors. In conclusion, the analysis of the ICDAS II indicator among children of the cross-border region of the Upper Tysa region indicates certain features that underlie the state of dental health in different regions, including climate-geographic zones and special biogeochemical provinces.

Conclusions: The obtained data can be used both to forecast the dynamics of dental morbidity in children living in these regions, and to develop differentiated tactics of treatment and preventive measures.

KEY WORDS: ICDAS II, children, Upper Tysa region, the intensity of tooth decay, the polluted ecosystem

Wiad Lek. 2024;77(9):1979-1988. doi: 10.36740/WLek/195165 DOI

INTRODUCTION

In recent decades, there has been an increase in the number of eco-dependent diseases, including dental diseases [1-8]. Long-term research confirms that long-term pollution of the area with toxic compounds leads to the fact that they accumulate in individual components of the ecosystem, which negatively affects living organisms [9-17].

The issue of ecosystem pollution in the Transcarpathian region is relevant today, as it is this region of Ukraine that suffers the most from floods, devastating deforestation and other environmental damage. The Upper Potyssia region covers the territory of a unique natural complex that provides the catchment area of the Tisza River from the city of Rakhiv (Ukraine) to the city of Dombrat (Hungary). Transcarpathia is almost completely correlated with Upper Potyssia, which according to the modern administrative-territorial division includes the Transcarpathian region of Ukraine, Eastern Slovakia, the northern districts of Sabolch of the Satmar county of Hungary, Satu-Mar and Baia-Mar counties of Romania. The region is demarcated by the state borders of Ukraine, Hungary, Romania, and Slovakia (Fig. 1) [18-20].

It is well known that Upper Tysa region, like the entire Transcarpathian region, is an endemic area with a biogeochemical deficiency of fluorine and iodine, which has a significant impact on dental health [21-24]. A real threat to the population, nature, and recreational resources for the Upper Potyssia is the critical ecological situation (Fig. 2), which has developed in large areas of the region and is associated with the pollution of the Tisza River basin by products of emergency emissions from mining enterprises in Romania (2000), waste with extremely high concentrations salts of heavy metals in the Tisza tributary to the Samos River, Baia Mare and Baia Borsha bridges, which, together with catastrophic floods (1998, 2001), led to the

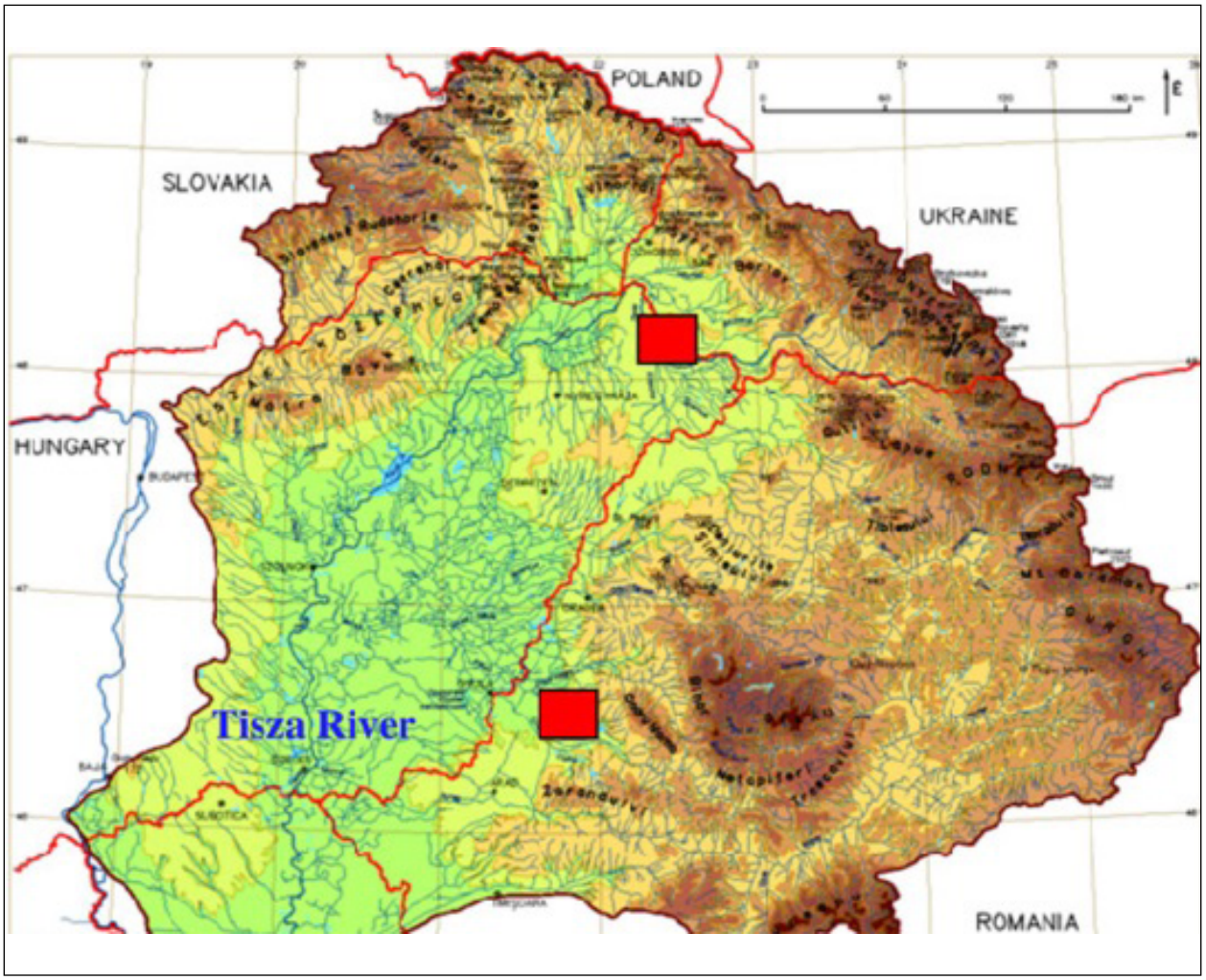

Fig. 1. Administrative-territorial map of the UpperTysa region.

accumulation of toxicants in the ecosystems of flooded areas [18-21]. Such pollution with heavy metal ions (HM), cyanides, radionuclides, pesticides, phenols, nitrogen oxides, nitrates determine the current state of many ecosystems, and the main danger for the biosphere in this case is caused by the ability of these toxicants to accumulate in living organisms.

The average level of providing the population of these territories of Transcarpathian with tap water is 32.2% [25], and there is practically no centralized water supply in rural settlements. Their water supply is mainly provided by physiologically suboptimal drinking water from domestic wells and boreholes. According to data from water resources departments of transboundary countries [26-29], a slightly better situation is observed with regard to centralized water supply - 57.72% of settlements in Hungary, 49.63% in Romania, and 51.56% in Slovakia, however, in neighboring countries, a significant share of use of physiologically suboptimal drinking water from decentralized water resources, which increases the risks of biological influence of environmental factors on the dental health of the population. Through drinking water and regional food products, toxicants, migrating through trophic chains, accumulate in the human body, which causes disruption of many metabolic processes, has a mutagenic, carcinogenic and embryotoxic effect, negatively affects the immune status of the human body, as well as the level of general somatic and dental health. Children are especially sensitive to the negative impact of the environment on the body, in which a tendency to deterioration of general somatic health by 23-42% is observed [17,22,24].

All this led to an urgent need for international and interregional and scientific cooperation in order to monitor the remediation and protection of the affected ecosystems of the Upper Potyssia in the program for the development of integration cooperation of scientists of the Carpathian region in the field of environmental protection «Mobilization, accumulation, distribution and bioremediation of heavy metals in polluted ecosystems of the upper basin of the Tisza River» (0103U007901, 2003-2004), «Development of a system of monitoring

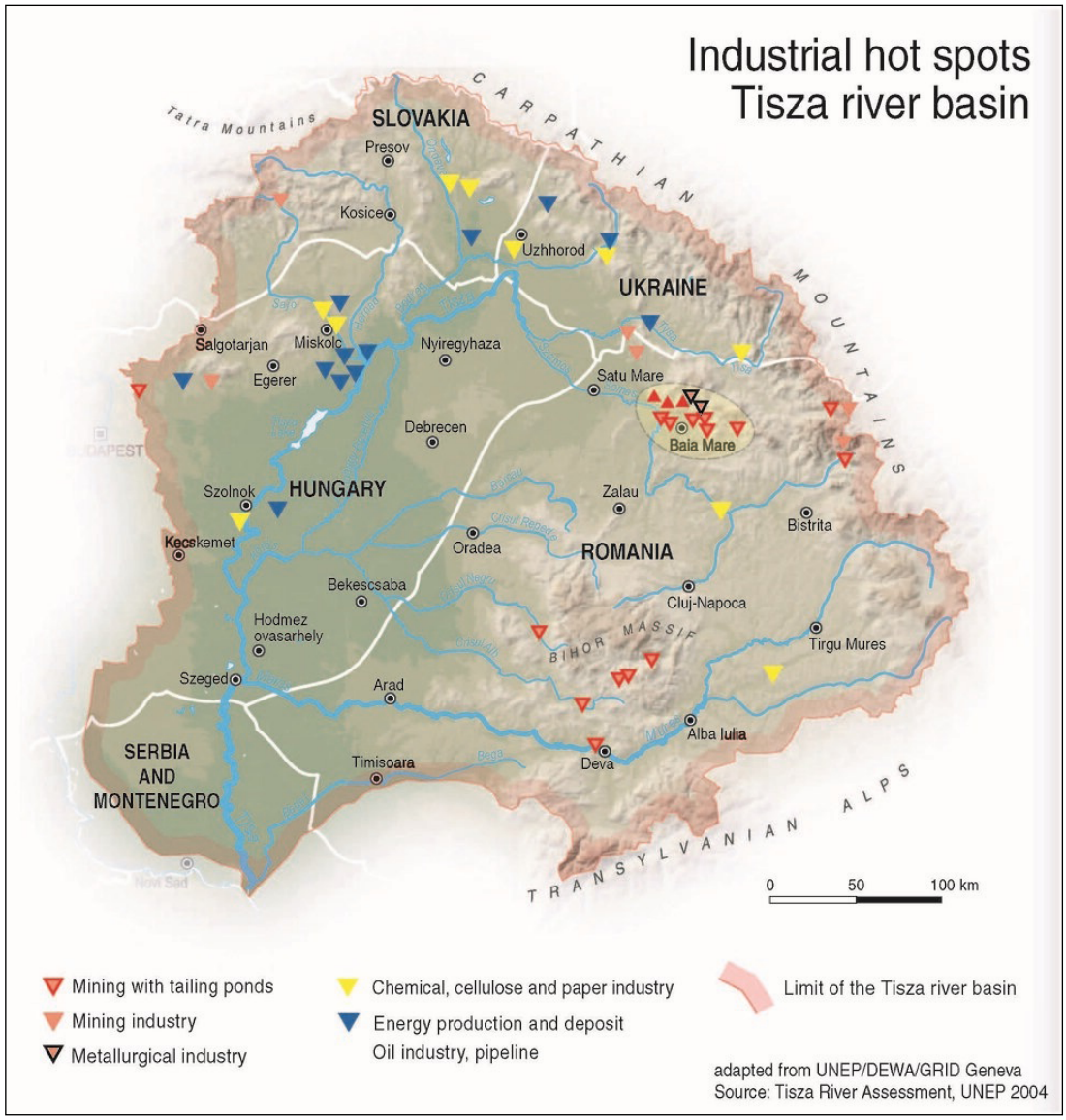

Fig. 2. The main pollutants of the Tysa River basin in Transcarpathian region.

studies of the state of environmental pollution in the border areas of the Coast region» (HU 2003/004-347-05-02-02, 2005-2006) [19], which was implemented with the support of the European Union. But most of the fundamental ecological and medical-biological issues of this plan remain open, which indicates the relevance of targeted scientific research and monitoring of the adaptation capabilities of the organism and the impact of polluted territories of the Upper Tysa region ecosystem on the formation of dental health of children who permanently live in this area.

An in-depth study of the mechanisms of the pathogenetic influence of adverse environmental factors on dental health will allow for the scientific justification and development of differentiated regionally targeted dental prevention programs, which will contribute to increasing the effectiveness of the primary prevention of dental caries and periodontal tissue diseases in children.

AIM

Assessment of dental caries and its impact on the level of dental health of children aged 15–18 years of Upper Tysa region, who constantly live in conditions of combined negative effects of factors of natural and technological genesis.

MATERIALS AND METHODS

To achieve the set goal during 2018-2022. a dental examination of children aged 15–18 years of the Upper Tysa region was carried out in each geographical and natural-climatic region (Table 1).

A total of 360 children were examined, of which 199 (55%) were boys and 163 (45%) were girls. As for their age, the average value was (14.17±1.06) years from the number of practically healthy children permanently living in this area. The coefficient of internal examination

Table 1. Geography of children's examination

Country	Climate-geographic zone		
	Lowland	Foothills	Mountain
Romania (Maramureş County)	Marmarosh - Sighetu	Baia Mare	Borşa
Ukraine, (Transcarpathian region)	Berehove and district	Khust city and district	Tyachiv city and district
Hungary	Zahon and settlements Sabolch - Satmar - Bereg region	Tokaj and settlements Borshod - Abay Zemplen region	Polgar and settlements Hajdu-Bihar region
Slovakia (Košice Region)	Mykhailivtsi	Sobrantsi	Strazhskoye

Table 2. The number of carious lesions on teeth in children in the lowland biogeochemical zone according to the ICDAS II system

	Ukraine		Hungary		Slovakia		Romania	
Code	1	2	1	2	1	2	1	2
0	183 (72)	183 (65,1)	210 (78)	210 (80,2)	203 (76)	203 (79,4)	192 (73,1)	192 (67,2)
1	6 (2,4)	7 (2,5)	3 (1,2)	2 (1,1)	7 (2,6)	6 (2,5)	6 (2,3)	7 (2,6)
2	11 (4,2)	12 (4,4)	9 (4,1)	9 (4,2)	12 (4,6)	9 (3,4)	11 (4,3)	13 (4,4)
3	24 (9,4)	27 (9,6)	15 (7,2)	11 (5,4)	12 (4,6)	12 (4,5)	22 (8,5)	25 (8,7)
4	16 (6,4)	19 (6,6)	11 (5,3)	8 (3,7)	13 (4,8)	9 (3,4)	17 (6,3)	19 (6,7)
5	8 (3,3)	21 (7,5)	6 (3,1)	9 (4,2)	12 (4,6)	11 (4,2)	8 (3,2)	18 (6,2)
6	6 (2,4)	12 (4,4)	2 (1,1)	3 (1,2)	8 (2,9)	7 (2,6)	6 (2,3)	12 (4,2)
Together with the affected surfaces	71 (28)	98 (35,0)	46 (22)	42 (19,8)	64 (24)	54 (20,6)	70 (26,9)	94 (32,8)

Notes: 1 — on chewing surfaces, abs. (%), 2 — on the proximal surfaces, abs. (%).

(Kappa index) was 0.83. Clinical examination data were recorded in a specially developed child's dental status assessment card based on WHO recommendations and a child's dental examination card for epidemiological studies. When making a diagnosis of caries, the generally accepted classification of ICD-10 was used. The study was carried out taking into account the main provisions of the GCP ICH and the Helsinki Declaration on Biomedical Research, the Council of Europe Convention on Human Rights and Biomedicine (2007) and the recommendations of the Committee on Bioethics under the Presidium of the National Academy of Sciences of Ukraine (2002) and the positive opinion of the Bioethics Commission of the Uzhhorod National University. Children and parents were informed about the purpose and methods of the study and, subject to obtaining written consent, the children were examined. Joint epidemiological surveys of the cross-border territories of Hungary, Romania and Slovakia were conducted in accordance with the agreement on cooperation and partnership with the Uzhgorod National University at the clinical bases of supporting higher educational institutions.

During the dental examination, the incidence of dental caries in children from different biogeochemical zones was studied and assessed using the International Caries Detection and Assessment System — ICDAS II (International Caries Detection and Assessment System), which is an evidence-based system for clinical visual caries detection and provides an opportunity to determine the stage and depth of the carious process — from the first carious changes in the enamel to the pronounced cavity in the dentin of the tooth. The data of clinical observations were recorded in the cards for the examination of the condition of the oral cavity in children for epidemiological studies, which allow the registration of the state of the hard tissues of the tooth with the help of six codes: three - for the assessment of carious changes in the enamel and three - for the assessment of carious changes in the dentin in the sequence of their growth severity [29].

The International Caries Classification and Management System (ICCMS — International Caries Classification and Management System) [29] is based on the international ICDAS II system, according to which three stages of carious lesions are distinguished: initial, moderate and extensive (extensive), and it is not about depth, but precisely about the stage of development of the pathological process, which allows for the application of certain algorithms for each stage, which cover diagnostic, preventive and therapeutic measures. In addition to direct classification (determining the stage

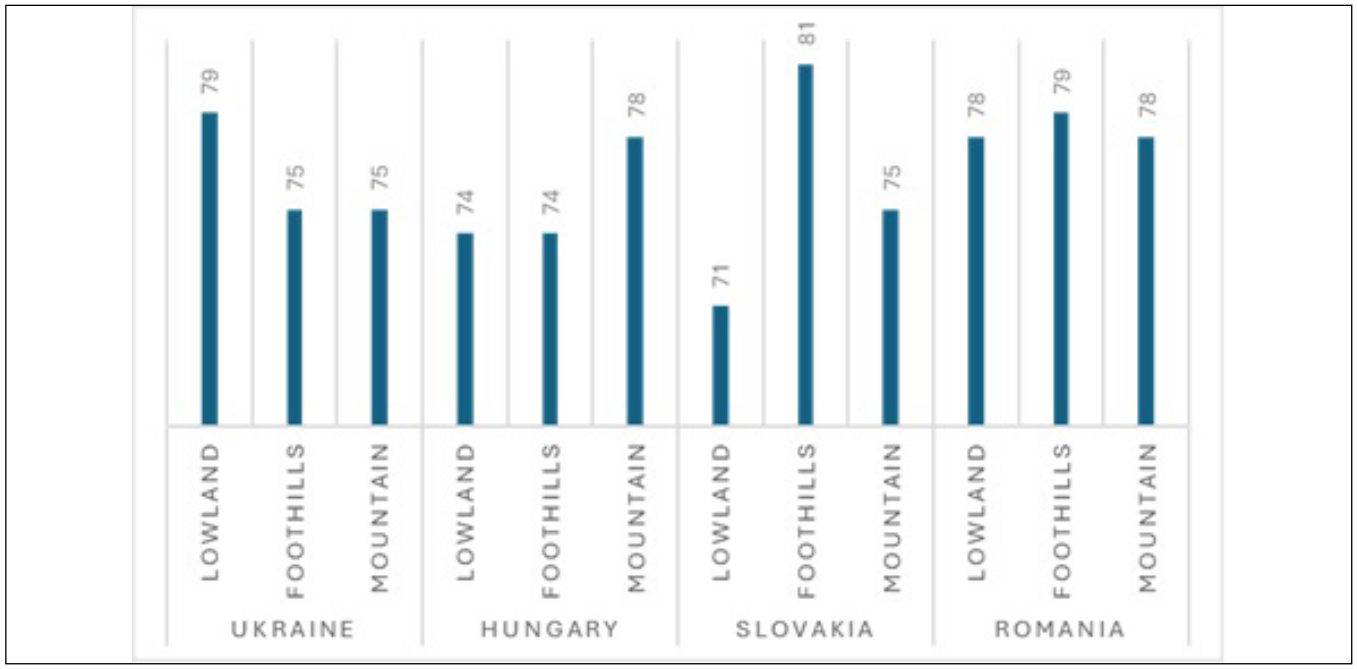

Fig. 3. Comparison of the depth of carious lesions in children of the Upper Tysa region according to the ICDAS II system.

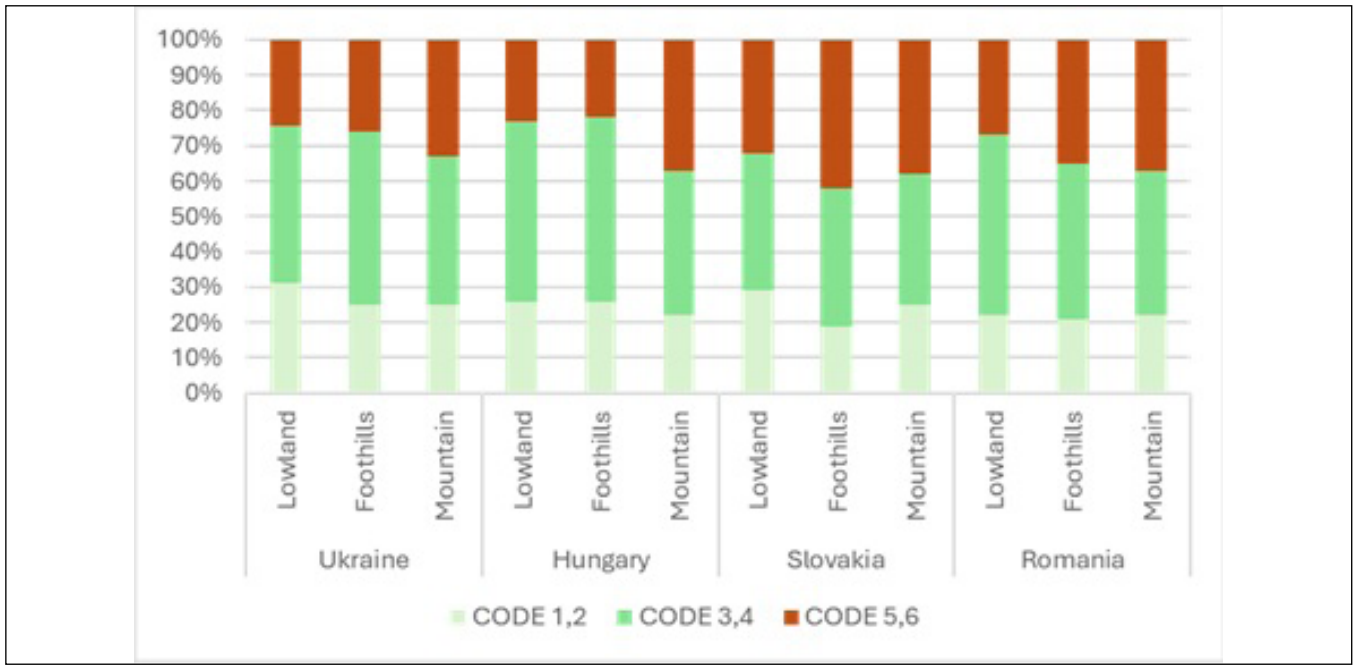

Fig. 4. The frequency of carious damage to the dentin of molars in children.

and activity of caries), the system includes determination of the individual risk level of the development and progression of the disease, decision-making and recommendations regarding tactics — preventive measures, disease control, tooth tissue preservation, and operative treatment.

The results of laboratory and clinical studies were processed by methods of variational statistics with determination of the mean value, its errors, Student's t-test for multiple comparisons, using Excel (MS Office 2018, Microsoft, USA) and STATISTICA 6.0 (StatSoft, USA). Differences in indicators at the level of significance $p < 0.05$ were considered statistically significant.

RESULTS

To assess dental caries, children were divided into three groups according to their biogeochemical habitat zones: lowland, foothills, and mountains. In lowland children in Hungary and Slovakia, caries was observed more often on the chewing surfaces than on the proximal ones (46/42; 64/54), and in Ukraine and Romania, on the contrary, the

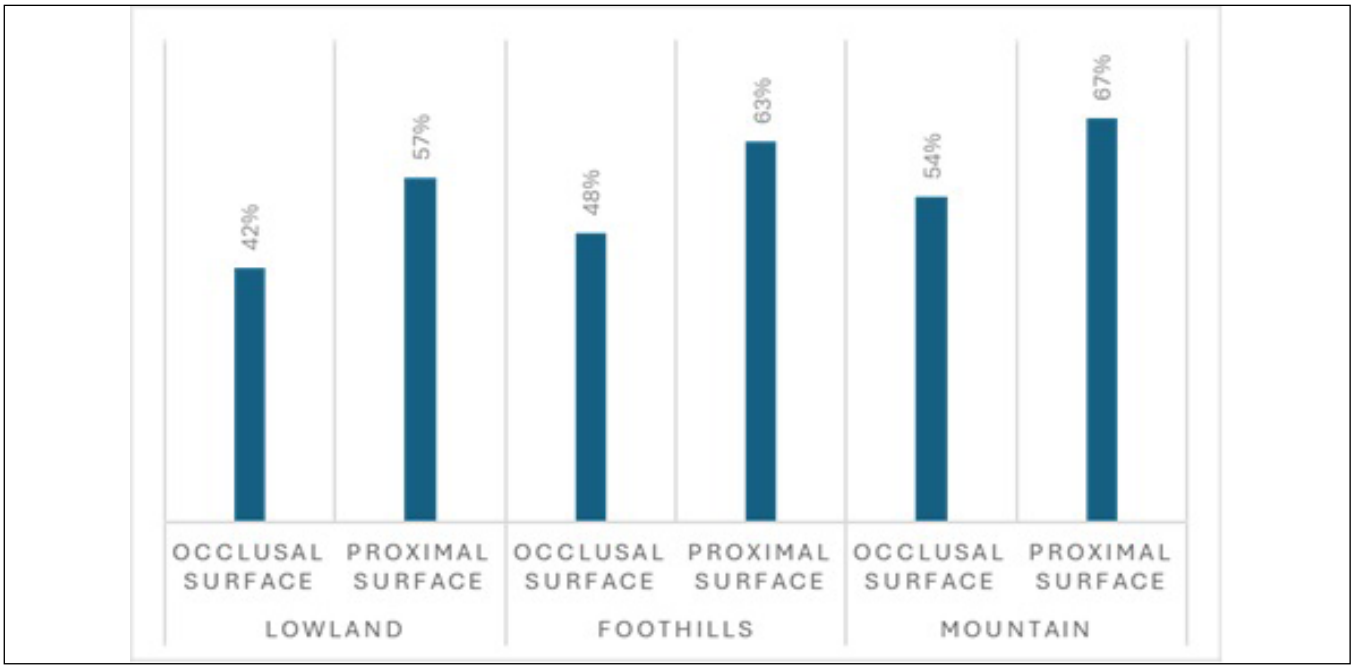

Fig. 5. The frequency of secondary carious lesions of molars in children of the Upper Tysa region according to the ICDAS II system.

prevalence of the carious process was observed on the proximal surfaces (71/98; 70/ 94) (Table 2). Regarding the distribution of carious lesions by depth, the average stage of carious lesions (codes 3, 4) among the examined children of the lowland zone of Ukraine and three neighboring countries (Hungary, Slovakia, Romania) was observed more often than the stages of deep and superficial carious lesions (codes 5, 6 and 1, 2).

Accordingly, 86 (51%) lesions (45 (51%), 46 (39%), 83 (51%)); 47 (28%) lesions (20 (23%), 38 (32%), 44 (27%)) and 36 (21%) lesions (23 (26%), 34 (29%), 37 (22%)) (Fig. 3, 4). The number of filled teeth in this group was 106 (9.5%). 78 fillings were located on the chewing surfaces of the teeth, 28 - on the proximal ones. Signs of secondary caries were observed on 33 (42%) fillings out of 78, located on the chewing surfaces of molars, and 16 (57%) fillings out of 28, on the proximal surfaces (Table 3, Table 4, Table 5, Fig. 5).

In the second group of the foothill zone (Table 3), the number of carious cavities on the proximal surfaces increased and slightly exceeded the number on the chewing surfaces (86/113, 71/114, 80/101), with the exception of Hungary (75/55). Regarding the distribution of carious lesions by depth, the average stage of carious lesions (codes 3, 4) among the examined children of the foothill biogeochemical zone of Ukraine and two neighboring countries (Hungary, Romania) was observed more often than the stages of deep and superficial carious lesions (codes 5, 6 and 1 , 2). Accordingly, 98 (49%) lesions (68 (52%), 79 (44%)); 52 (26%) lesions (29 (22%), 63 (35%)) and 49 (25%) lesions (33 (26%), 39 (21%)), except in Slovakia, where the stage of deep carious lesions prevails (77 (42%)/73 (39%)/35 (19%)) (Fig. 3, Fig.4). Also, 233 sealed teeth (19.2%) were found in this group of children. 94 teeth had fillings on the masticatory surfaces and 139 on the proximal surfaces. Signs of secondary caries were present in 45 fillings (48%) out of 94 on the chewing surfaces of molars and 88 fillings (63%) out of 139, which were localized on the proximal surfaces (Table 5, Fig. 5).

In the third group of the mountain zone (Table 4), the number of carious cavities on the proximal surfaces increased and significantly exceeded the number on the chewing surfaces (87/123, 77/99, 79/113, 83/122). Regarding the distribution of carious lesions by depth, the average stage of carious lesions (codes 3, 4) among the examined children of the mountainous biogeochemical zone of Ukraine and two neighboring countries (Hungary, Romania) was observed more often than the stages of deep and superficial carious lesions (codes 5, 6 and 1 , 2). Accordingly, 88 (42%) lesions (72 (41%), 84 (41%)); 70 (33%) lesions (65 (37%), 75 (37%)) and 52 (25%) lesions (39 (22%), 46 (22%)) except Slovakia, where the number of lesions corresponding to codes 5, 6 practically corresponds to the number of lesions corresponding to codes 3, 4, but prevail over the values of codes 1, 2 (73 (38%) ≈ 70 (37%)) > 49 (25%) (Fig. 3, 4). In this group, 273 filled teeth (29.3%) were observed. In 117 teeth, fillings were located on the chewing surfaces, in 156 - on the proximal ones. Out of 117 signs of secondary caries, 63 (54%) had fillings located on chewing surfaces and out of 156, 104 fillings (67%) were located on proximal surfaces (Table 5, Fig. 5).

DISCUSSION

The analysis of the structure of carious lesions in children aged 15-18 years, who live in the conditions of the combined negative effect of factors of natural and technological

Table 3. The number of carious lesions on teeth in children in the foothills climate-geographic zone according to the ICDAS II system

	Ukraine		Hungary		Slovakia		Romania	
Code	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
0	202 (70,2)	202 (64,2)	214 (74)	214 (79,3)	217 (75,2)	217 (65,4)	198 (71,1)	198 (66,1)
1	10 (3,5)	11 (3,6)	6 (2,2)	4 (1,5)	7 (2,6)	6 (1,9)	9 (3,3)	7 (2,4)
2	13 (4,4)	15 (4,7)	12 (4,1)	11 (4,2)	13 (4,5)	9 (2,6)	13 (4,7)	10 (3,4)
3	27 (9,5)	31 (9,8)	24 (8,2)	15 (5,4)	16 (5,6)	18 (5,5)	19 (6,8)	29 (9,6)
4	19 (6,7)	21 (6,7)	18 (6,3)	11 (4,2)	14 (4,8)	25 (7,6)	17 (6,1)	14 (4,6)
5	10 (3,5)	21 (6,7)	9 (3,1)	11 (4,2)	10 (3,6)	27 (8,2)	12 (4,3)	25 (8,5)
6	7 (2,3)	14 (4,4)	6 (2,2)	3 (1,3)	11 (3,7)	29 (8,8)	10 (3,7)	16 (5,4)
Together with the affected surfaces	86 (29,8)	113 (35,8)	75 (26,0)	55 (20,7)	71 (24,8)	114 (34,6)	80 (28,9)	101 (33,9)

Notes: 1 — on chewing surfaces, abs. (%), 2 — on the proximal surfaces, abs. (%).

Table 4. The number of carious lesions on teeth in children in the mountain climate-geographic zone by the ICDAS II system

3	**Ukraine**		**Hungary**		**Slovakia**		**Romania**	
Code	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
0	186 (68,1)	186 (60,2)	204 (72,3)	204 (67,2)	198 (71,7)	198 (63,4)	191 (69,7)	191 (61,1)
1	9 (3,2)	14 (4,4)	7 (2,6)	10 (3,4)	10 (3,6)	12 (3,9)	10 (3,7)	11 (3,6)
2	12 (4,4)	17 (5,5)	9 (3,5)	13 (4,4)	13 (4,7)	14 (4,6)	13 (4,7)	12 (3,9)
3	20 (7,4)	27 (8,6)	13 (4,7)	20 (6,6)	13 (4,7)	20 (6,5)	16 (5,8)	26 (8,3)
4	17 (6,3)	24 (7,7)	19 (6,6)	20 (6,6)	13 (4,7)	24 (7,6)	16 (5,8)	26 (8,3)
5	17 (6,3)	22 (7,3)	18 (6,5)	23 (7,5)	16 (5,7)	22 (7,2)	15 (5,6)	26 (8,3)
6	12 (4,4)	19 (6,3)	11 (3,8)	13 (4,4)	14 (4,9)	21 (6,8)	13 (4,7)	21 (6,6)
Together with the affected surfaces	87 (31,9)	123 (39,8)	77 (27,7)	99 (32,9)	79 (28,3)	113 (36,6)	83 (30,3)	122 (38,9)

Notes: 1 — on chewing surfaces, abs. (%), 2 — on the proximal surfaces, abs. (%).

genesis (Upper Tysa region), shows that in the lowland biogeochemical zone, the stage of medium carious lesions significantly prevails, in contrast to the stages of deep and superficial lesions .

Among children of the foothills and mountain climate-geographical zones, there is also a prevalence of medium carious lesions of molars, however, for these zones of Slovakia, the prevalence of the stage of deep carious lesions of molars is characteristic. In the course of the examination, it was established that the worst caries situation in children of the Upper Depression region of Ukraine and Romania, as evidenced by the high quantitative index of detected carious cavities, is primarily due to the peculiarity of the geomorphological surface of the region, its hydrological regime and the high degree of polyetiological pollution of the studied ecosystem. Also, the frequency of carious damage to the dentin of molars is the highest in the mountain biogeochemical zone, the maximum peak of the damage is observed precisely in the settlements of the Upper Tysa region of Ukraine and Romania, the minimum is in children of the lowland biogeochemical zone of Hungary.

Among children of the mountainous biogeochemical zone of the Upper Tysa region, a high prevalence of secondary carious lesion of molars is characteristic - 167 abs. values among all examined. Secondary caries is much more often observed when defects are localized on the proximal surfaces of molars in children. Regarding the distribution of carious cavities of molars by localization, in children of the Upper Tysa region, there is a tendency to damage the proximal surfaces of molars, the exception is the lowland biogeochemical zones of Hungary and Slovakia, where there is a prevalence of damage to the chewing surface of molars in children.

According to Hungarian colleagues Szőke J, Petersen PE [24], among children aged 15-18 years living in the territory of western and central Hungary for the period 2018-2022, there is a predominant surface lesion of the hard tissues of the teeth, which corresponds to codes 1,2 according to the system ISDAS II, CODE 1 is 31.2%, CODE 2 – 19.8% of 828 examined children. According to J. Szőke, in 2019, 42.6% of children aged 15 years have caries and intact teeth. Thus, for comparison, in the western regions

Table 5. Number of fillings and their analysis on children's teeth in different climatic and geographical zones

Indicators	Climate-geographic zone					
	Lowland		Foothills		Mountain	
	on occlusal surfaces Abs. (%)	on the proximal surfaces of Abs. (%)	on occlusal surfaces Abs. (%)	on the proximal surfaces of Abs. (%)	on occlusal surfaces Abs. (%)	on the proximal surfaces of Abs. (%)
secondary caries	33 (42)	16 (57)	45 (48)	88 (63)	63 (54)	104 (67)
the total number of fillings	78 (100)	28 (100)	94 (100)	139 (100)	117 (100)	156 (100)

Table 6. Comparative analysis of the indicator of the intensity of carious lesions of the hard tissues of the teeth among children of the cross-border of the Upper Tysa region depending on the determinant of iodine-fluorine devitsite

Indicators	Ukraine			Hungary			Slovakia			Romania		
	Climate - geographical zones											
	Lowland	Foothills	Mountain	Lowland	Foothills	Mountain	Lowland	Foothills	Mountain	Lowland	Foothills	Mountain
The ICDAS II index value, CODES	> 4	> 4	> 5	≤ 3	≤ 4	> 4	> 4	> 4	> 4	> 4	> 4	≥ 5

of Hungary in 1985, 15-year-old children had an average of 5 teeth affected by caries, and after 30 years, the caries rate decreased to 2.3 DMFT in 2017-2020. Answers to the questionnaire showed that 11.9% of 15-year-olds visited the dentist because of pain or discomfort in the mouth, and 40.5% were dissatisfied with the appearance of their teeth.

According to the data of Romanian researchers Stanciu, Ioana-Andreea; Tanase, Mihaela; Luca, Rodica, among the examined children aged 15-17 years in the central regions of Romania during 2020, there is a predominant superficial lesion of the hard tissues of the teeth, which corresponds to codes 1,2 according to the ISDAS II system, CODE 1 is 46.8%, CODE 2 is 28 .8% of the 1025 examined children, however, there is damage to the mantle dentin corresponding to codes 3,4: CODE 3 is 39.1%, CODE 4 is 14.4% of the total number of examined according to the ISDAS II system.

In conclusion, the analysis of the ICDAS II indicator among children of the cross-border region of the Upper Potyssia indicates certain features that underlie the state of dental health in different regions, including climate-geographic zones and special biogeochemical provinces (table 6) [23]. The obtained data can be used both to forecast the dynamics of dental morbidity in children living in these regions, and to develop differentiated tactics of treatment and preventive measures.

CONCLUSIONS

Analysis of the structure of carious lesions in children aged 15–18, who were born and constantly live in the conditions of the combined negative effect of factors of natural and technological genesis (Upper Tysa region), indicates that there is a continuing trend of the caries intensity indicator, a significant progression of the stage of medium carious lesions in the lowland biogeochemical zone, in contrast to the stages of deep and surface damage. During the survey, it was established that the worst caries situation is in children of the Upper Tysa region region of Ukraine and Romania, which is evidenced by the high quantitative index of detected carious cavities - 528 and 550, respectively, primarily due to the peculiarity of the geomorphological surface of the region, the peculiarities of the hydrological regime and the high degree of polyetiological pollution territory of the studied ecosystem.

It was found that the use of physiologically suboptimal drinking water from decentralized water resources is among the environmental determinants of the state of dental health of children in the Upper Tysa region.

The obtained indicators of dental health are closely correlated with the level of environmental and hygienic safety, and the degree of correlation is the highest for teenagers in the 16-17-year-old group. This may be related to longer exposure to factors.

Among children of the mountainous biogeochemical zone of the Upper Tysa region, a high prevalence of secondary carious lesion of molars is characteristic — 167 abs. Values among all examined. Secondary caries is much more often observed when defects are localized on the proximal surfaces of molars in children (208/141,

respectively). As for the distribution of carious cavities of molars by location, in children of the Upper Tysa region, there is a tendency to damage the proximal surfaces of molars, the exceptions are the lowland biogeochemical zones of Hungary and Slovakia, where the prevalence of damage to the chewing surface of molars in children is observed. The use of the ICDAS II index is a simple and informative method for assessing caries in children, which allows analyzing and differentiating the structure of carious lesions by depth, which is important in the development of using the ICDAS II index is a simple and informative method for assessing caries in children, which allows analyzing and to differentiate the structure of carious lesions by depth, which is important in the development of predictive algorithms of preventive protocols and makes them understandable and acceptable studies in the scientific environment, and to join international programs on this issue.

Further study of the pathogenetic relationships of the negative impact of factors of natural and technological genesis on the dental health of children who constantly live in conditions with the geochemical anomaly of fluorine-iodine deficiency will contribute to the development of differentiated, regionally adapted programs of stomatological endogenous prevention and increase its effectiveness.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 07.06.2024
ACCEPTED: 19.09.2024

ORIGINAL ARTICLE

CONTENTS

Specifics of barrier function impairment of the large intestine in patients with ulcerative collitis and joint damage

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ABSTRACT

Aim: To study the characteristics of barrier function impairments of the large intestine in patients with UC and concomitant joint damage.

Materials and Methods: At the clinical base of the Department of Therapy and Family Medicine, 80 patients with inflammatory bowel disease (IBD) were examined. Patients with IBD were divided into two groups. Group 1 (n=78) included patients with IBD and diagnosed with joint damage, namely spondylarthritis (SPA), and group 2 (n=40) included patients with IBD without joint damage. Patients were tested to determine the level of fecal calprotectin (FC), a1-antirpsin (a1-AT) and zonulin. Changes in the quantitative and qualitative composition of the colon microflora were assessed.

Results: Patients with UC have been diagnosed with large intestine dysbiosis (LID), which is more pronounced in patients of the 1st group. Stage 3 dysbiosis was 17,3 % ($p<0.01$) more likely to be found in patient of the 1st group, and stage 4 dysbiosis - 12,9 % ($p<0.05$) more likely. The level of zonulin, both in blood serum and in feces, was significantly higher in patients with UC compared to controls. In group I patients we found a significant increase in zonulin compared to group II patients (by 1.5 times in blood serum and by 1.4 times in feces, $p<0.01$). Identical results were observed when analyzing the levels of α1-AT in blood, feces, as well as its clearance (1.6 times ($p<0.01$), 1.3 times ($p<0.05$) and 1.4 times ($p<0.01$) accordingly).

Conclusions: Increased levels of zonulin and a1-AT in blood serum and feces was found in patients with UC, which indicates an increased permeability of the intestinal wall in these patients. At the same time, more pronounced changes indicating intestinal barrier function impairment were found in patients with UC and SPA. In patients with UC and SPA, a direct correlation between the change in intestinal permeability and LID severity was established (fecal zonulin levels correlate with LID stages III and IV- $r=0.94$; $p<0.01$ та $r=0.88$; $p<0.01$ accordingly).

KEY WORDS: inflammatory bowel disease, ulcerative colitis, joint damage, spondylarthritis, diagnostics, calprotectin, a1-antitrypsin, zonulin, dysbiosis

Wiad Lek. 2024;77(9):1989-1995. doi: 10.36740/WLek/195166 DOI

INTRODUCTION

Inflammatory bowel disease (IBD) - is a chronic inflammatory disease of the gastrointestinal tract (GI tract), which includes two clinical manifestations: Crohn's disease (CD) ulcerative colitis (UC). A recent study, conducted by the Center of disease prevention and control according to the 2015 National Health Survey has shown that IBD is actually a more widespread disease than was previously reported. Their analysis showed that IBD affects 3.1 million or 1.3% of the US adult population, approximately three times higher than previous estimates [1].

IBD is often associated with extraintestinal manifestations (EIMs). EIMs during IBD are so widespread that these diseases should be considered as systemic and not limited to the GI tract alone [2]. EIMs are a common phenomenon both in Crohn's disease and UC. Almost any organ system can be affected, including musculoskeletal, dermatological, renal, hepatopancreatobiliary, and pulmonary systems [3]. In general, peripheral arthritis, aphthous ulcers in the oral cavity, episcleritis, or erythema nodosum may be associated with active intestinal inflammation and may improve with standard treatment of intestinal inflammation. However, anterior uveitis, ankylosing spondylitis, and primary sclerosing cholangitis usually occur independently of disease outbreaks [4].

Arthropathy is common feature among patients with IBD, and these disorders are known as spondylarthritis (SPA). Depending on the initial symptoms, spondylarthritis is divided into axial and peripheral. Radiological signs of sacroiliitis are observed in approximately 15-27% of patients with UC [5].

The pathogenesis of EIMs in IBD is unclear, but is believed to involve immune and genetic factors. It is believed that the immune response can be induced at extraintestinal areas by the diseased mucosa of the GI tract due to common epitopes at different areas. Bacteria translocating across the permeable intestinal barrier elicit an adaptive immune response that ultimately fails to distinguish between bacterial epitopes and epitopes of joints and skin [1].

Thus, the study of the characteristics of barrier function impairments of the large intestine and its relation to joint damage in UC is currently extremely relevant.

AIM

The aim of the research to study the characteristics of barrier function impairments of the large intestine in patients with UC and concomitant joint damage.

MATERIALS AND METHODS

Complex evaluation and treatment of patients were conducted at the clinical base of the Department of Therapy and Family Medicine Faculty of postgraduate education of Uzhhorod National University. The clinical study included 118 patients with UC. Patients with UC were divided into 2 groups depending on joint damage or the lack of it:

- group I included of 78 patients with UC who have been diagnosed with joint damage, namely SPA. Group I consisted of 63 males (80.8 %) and 15 (19,2 %) females. Average age was 51.3±4.7 years.

- group II included 40 patients with UC without joint damage. Group I consisted of 11 males (27.5 %) and 29 (72.5 %) females. Average age was 48.9±5.3 years.

The control group included 30 practically healthy individuals (18 male (60.0%) and 12 (40.0%) female). Average age was 50.7±5.5 years.

All studies were conducted with patients consent. Written informed consent for appropriate diagnosis and treatment was obtained from all patients and controls, and all measures were taken to ensure data depersonalization. The research methodology complied with the Helsinki Declaration of Human Rights of 1975 and its 1983 revision, the Council of Europe Convention on Human Rights and Biomedicine, as well as Ukraine's legislation.

Exclusion criteria were age under 18 and over 75; Crohn's disease; lactose intolerance, gluten intolerance; celiac disease; surgical interventions on the intestines, including appendectomy for up to 6 months prior; colon cancer; doligosigma; diverticulosis of the colon; type 1 diabetes; type 2 diabetes (decompensated stage); mental illnesses that do not allow an adequate assessment of the patient's state of health and the signing of consent for diagnosis and treatment; pregnancy and lactation; HIV infection; oncological diseases.

UC was diagnosed according to IBD diagnostic standards. The diagnosis of UC was verified using endoscopic (rectomanoscopy, sigmoscopy, colonoscopy) and morphological testing. The activity of fecal calprotectin (FC) was determined by enzyme-linked immunosorbent assay (ELISA) using the Tecan Sunrise test system from ImmunDiagnostic (Germany).

SPA with predominant damage to the spine was diagnosed based on physical and clinical examination methods, as well as the results of imaging methods (radiological examination, computed tomography or magnetic resonance imaging) of the spine. Functional assessment of spinal mobility and muscle strength of the back and abdominal core muscles was performed to study the mobility of the spine (Shober test, spine extension, fingertip to floor distance test, functional tests to determine the strength endurance of the back extensor muscles and the abdominal core muscles), as well as the Harris test and Lekens index according to the recommendations of the American Academy of Orthopaedic Surgeons (AAOS, 2018), American College of Rheumatology (ACR), 2018, European League Against Rheumatism (EULAR), 2018. Examination of the spine included inspection, palpation, and objective pain assessment.

In order to determine the barrier function of the intestine, the level of α1-antitrypsin (α1-AT) in blood serum and feces was assessed by ELISA using the test system of Immuniagnostic AG (Germany), and its clearance was calculated based on the obtained values. The level of zonulin was also determined in blood serum and feces by ELISA using the test system of the company "Elabscience" (USA).

The qualitive and quantitative composition of the microflora of the large intestine was studied. For this purpose, feces from patients were collected in dry, sterile dishes and delivered to the bacteriological laboratory no later than 2 hours after collection. The material was placed on a standard set of selective and differential diagnostic nutrient media for growth and isolation of aerobic and anaerobic microorganisms using a tenfold dilution method. Changes in the quantitative and qualitative composition of the microflora of the large intestine were determined using the unified practical classification of intestinal dysbiosis by Kuvaeva-Ladodo (1991), according to which 4 degrees of dysbiotic disorders are distinguished.

The analysis and processing of the results of the examination of patients was carried out with the help of the STATISTICA 10.0 computer program (StatSoft Inc, USA) using parametric and non-parametric methods of evaluating the obtained results.

RESULTS

Clinical symptoms indicating intestinal damage in examined patients with UC were evaluated (Table 1).

Table 1. Clinical manifestations of intestinal damage in examined patients

Clinical manifestation	Patients with IBD			
	Group 1 (n=78)		Group 2 (n=40)	
	Abs. number	%	Abs. number	%
Frequency of bowel movements:				
- 1-5 times a day	49	62.8 %**	15	37.5 %
- 6-10 times a day	22	28.2 %	18	45.0 %**
- more than 10 times a day	7	9.0 %	7	17.0 %*
Pain along the colon	32	41.0 %	20	50.0 %
Meteorism	38	48.7 %*	12	30.0 %
Feeling of incomplete bowel movement	20	25.6 %*	5	12.5 %
Impurities in the stool:				
- blood	38	48.7 %	32	80.0 %**
- mucus	68	87.2 %	38	95.0 %

Note: the difference between the indicators in patients by groups is significant: * - $p<0.05$; ** - $p<0.01$.

The results show that clinical symptoms indicating UC are more pronounced in patients of the II group (without joint damage), namely, the frequency of diarrhea in the patients of the II group was on average 6-10 times a day, while in the patients of the I group the frequency of defecation was increased up to 5 times a day. Also, blood was significantly more often detected in the feces of group II patients (80.0% of patients - $p<0.01$).

After assessing the quantitative and qualitative composition of microorganisms in the feces of examined patients with UC in combination with joint damage, the degree of severity of colon dysbiosis in these patients was determined – Fig. 1.

As per the obtained results, it has been shown that patients with UC of the I group have more pronounced dysbiotic changes in the colon. At the same time, 17.3% ($p<0.01$) of patients of the I group were more often diagnosed with dysbiosis of the III degree, as well as 12.9% - with dysbiosis of the IV degree ($p<0.05$). In patients with UC of the II group (without SPA), when assessing the severity of dysbiotic changes, the II degree of severity was more often diagnosed.

The degree of activity of the pathological process was evaluated using the fecal calprotectin indicator in patients with UC – Fig. 2.

A significant increase in the level of FC in patients with UC of both groups was observed - up to 524.71±5.26 mkg/l in patients of the I group and up to 648.77±6.43 mkg/l in the examined subjects of the II group, while the normal range (23.15±0.66 mkg/l) was observed in the control group ($p<0.001$). It should be noted that a greater degree of activity was observed in patients of the II group with UC (Table 2).

The level of zonulin, both in blood serum and in feces, was significantly higher in patients with UC compared to controls (Table 2). Additionally, in patients with UC and SPA, a significant increase of this biomarker of intestinal lesions was established compared to the data in patients of the II group (by 1.5 times in blood serum and by 1.4 times in feces - $p<0.01$). Identical results were also observed when analyzing α1-AT levels in blood, feces, as well as its clearance (by 1.6 times ($p<0.01$), 1.3 times ($p<0.05$) and 1.4 times ($p<0.01$), respectively). The obtained results point at impairments in the intestinal barrier function in examined UC patients. At the same time, more pronounced changes were found in patients with UC and EIMS, namely SPA.

A correlational analysis was performed to determine the relationship between the degree of disease activity according to the FC level, the severity of large intestine dysbiosis (LID) and indicators of impaired intestinal permeability in these patients – Tables 3, 4.

Correlation analysis indicates a relationship between the change in the zonulin level in blood and feces and the severity of LID and the degree of inflammation according to the level of FC mainly in patients with UC and SPA. At the same time, the obtained results indicate a high specificity and sensitivity to changes in the level of zonulin in feces, for determining the impairment of the intestinal barrier function in patients with IBD. In patients of the II group, a correlation was established mainly between the change in the level of zonulin in feces and the studied parameters.

The a1-AT indicator, which also indicates increased permeability of the intestinal wall and impaired barrier function, correlates with the degree of activity of the pathological process in the intestine in patients with UC according to the FC level. A relationship between the severity of LID and the level of α1-AT mainly in feces was also observed, especially in cases of UC and concomitant SPA.

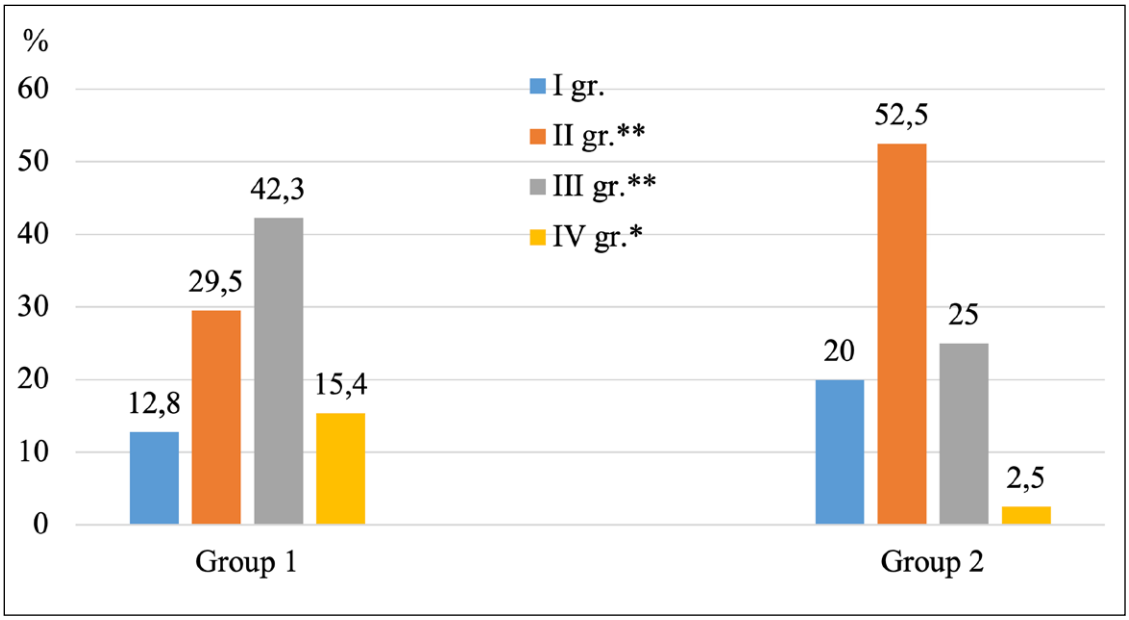

Fig. 1. Dysbiosis manifestation of the large intestine in examined patients with UC.
Note: the difference between the indicators in patients by groups is significant: * - $p<0.05$; ** - $p<0.01$.

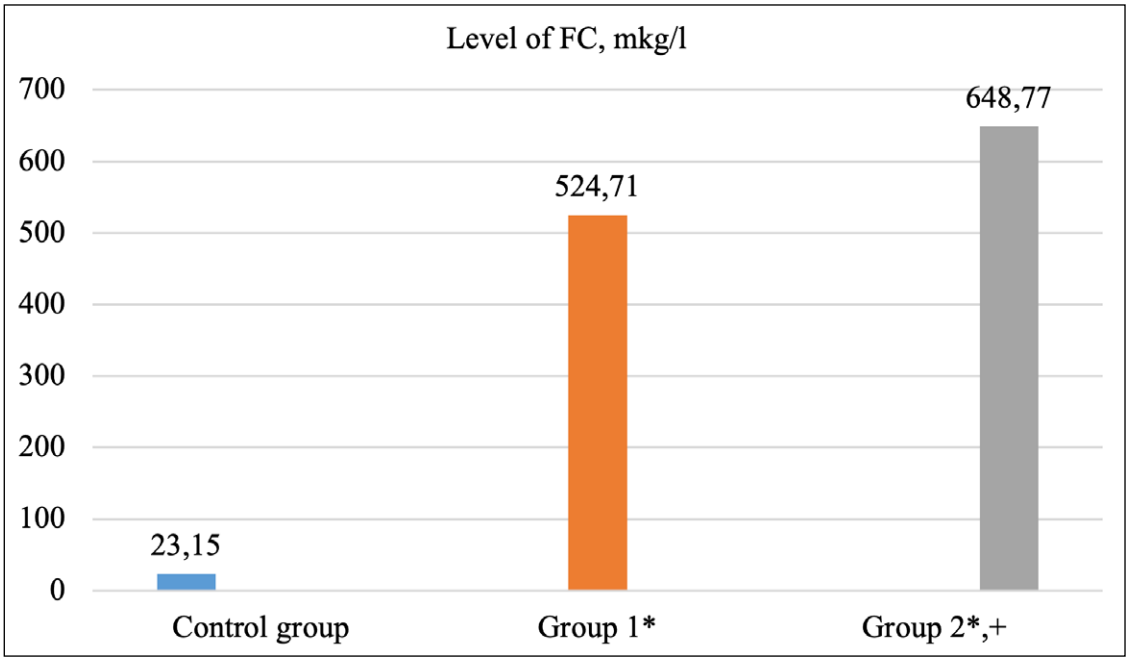

Fig. 2. The level of fecal calprotectin in the examined subjects.
Note: the difference between the values in patients with UC and the control group is significant: * - $p<0.001$;
the difference between the indicators in patients by groups is significant: + - $p<0.01$.

Therefore, an impairment of the intestinal barrier function is indicated by increased levels of zonulin and α1-AT in the blood and feces of patients with UC. The analysis of the obtained data makes it possible to assert the role of increased intestinal permeability in the progression of EIMS in UC, namely joint damage. At the same time, both the severity of LID and impaired intestinal permeability directly affect EIMS in patients with IBD, which should be taken into account in the diagnosis and treatment of these patients.

DISCUSSION

Several pathways by which the microbiota may contribute to EIMS in UC have been previously discussed. Due to increased permeability of the intestinal barrier, components of the microbiota, such as lipopolysaccharides, bacterial antigens or metabolites, can move from the intestine to extraintestinal organs or cause systemic inflammatory reactions [6]. Dysbacteriosis can lead to the activation of intestinal immune cell populations

Table 2. Indicators of biomarkers of intestinal barrier impairment in the examined subjects

Indicator	Control group (n=30)	Examined patients	
		Group 1 (n=78)	Group 2 (n=40)
zonulin:			
in blood serum, mg/dl	13.44±0.21	129.17±1.88***++	87.91±1.15**
in faeces, mg/dl	16.23±0.44	194.56±2.41***++	138.44±1.75**
α1-AT:			
in blood serum, mg/dl	118.77±2.08	388.12±3.46**++	244.17±2.26**
in faeces, mg/dl	13.69±0.15	39.55±0.38**+	29.56±1.29**
clearance α1-AT, ml/day	16.32±0.51	81.69±1.74***++	57.84±1.07**

Note: the difference between the indicators of the control group and the examined patients is statistically significant: * - p<0.05; ** - p<0.01; *** - p<0.001; between the indicators of patients in group 1 and group 2, the difference is statistically significant: + - p<0.05; ++ - p<0.01.

Table 3. Comparison of zonulin levels with the level of FC and severity of large intestine dysbiosis in examined patients

Indicator	Examined patients			
	I group		II group	
	Indicator			
	zonulin		zonulin	
	serum	feces	serum	кал
FC	r=0,90; p<0,01	r=0,96; p<0,01	_	r=0,96; p<0,01
LID II	r=0,76; p<0,01	r=0,78; p<0,01	r=0,80; p<0,01	r=0,82; p<0,01
LID III	r=0,82; p<0,01	r=0,94; p<0,01	_	r=0,84; p<0,01
LID IV	_	r=0,88; p<0,01	_	_

Table 4. Comparison of a1-AT levels with FC level and severity of large intestine dysbiosis in examined patients

Indicator	Examined patients		
	I group		II group
	Indicator		
	a1-AT		a1-AT
	serum	feces	feces
FC	r=0,80; p<0,01	r=0,86; p<0,01	r=0,78; p<0,01
LID II	_	r=0,80; p<0,01	r=0,70; p<0,05
LID III	_	r=0,84; p<0,05	_

that eventually migrate to other organs. Preliminary data suggest an increase in clostridia in patients with IBD and arthritis. Patients with SPA had decreased faecal microbial diversity (increased Ruminococcus gnavus and Dialister), but these patients did not suffer from IBD [7-9].

Arthritis is the most common EIMS of IBD, affecting axial joints, peripheral joints, or both. SPA affects both genders equally, with a higher prevalence in cases of Crohn's disease involving the colon than in cases of UC, and may occur before, concurrently with, or after the onset of IBD [10]. Our results indicate that UC is more often combined with SPA in males.

Currently the only biomarker approved and recommended by the European Crohn's and Colitis Organization (ECCO), is the commonly used fecal calprotectin. Inflammation in IBD leads to an acute phase response, detectable in serum and blood, characterized by increased concentrations of proteins involved in coagulation and fibrinolysis, such as fibrinogen and plasminogen; components of the complement system; proteinase inhibitors, including α1-antitrypsin, α1-antichymotrypsin; transport proteins such as haptoglobin and ceruloplasmin; and a number of other proteins, such as C-reactive protein (CRP) or ferritin. A Czech study examining 40 IBD patients for fecal and serum zonulin levels showed that both were elevated in patients with active Crohn's disease, which may be explained by the fact that zonulin is considered the best marker of increased permeability of the intestine [11].

Therefore, one of the important steps in understanding the role of intestinal permeability was the discovery

of zonulin [12]. Zonulin regulates intestinal permeability by modulating intracellular tight junctions. Zonulin, as an analogue of Vibrio cholerae toxin - zonula occludens (47 kDa protein), increases intestinal permeability in the small intestine and participates in the development of innate intestinal immunity. Circulating zonulin is considered as a potential biomarker of intestinal permeability [13]. A decrease in the intestinal barrier function may be related to the secondary activation of zonulin [14].

Various research is being conducted to study the relationship between zonulin and microbiocenosis, systemic inflammation and intestinal barrier dysfunction. A study by Zak-Golab A. et al. (2013) indicates a relationship between the level of zonulin and changes in the intestinal microbiocenosis, as well as systemic microcellular inflammation in patients with diabetes [13]. With the presence of inflamation, intestinal dysbacteriosis can "influence" epithelial cells to produce increased amounts of zonulin in the intestinal lumen and in the bloodstream which can lead to an impairment of the intestinal barrier function, that enables external antigens to enter the bloodstream and cause an excessive immune response, which, in in turn, leads to further permeability [15]. Results of a study by Caviglia GP et al. (2019) show, that serum zonulin levels are highly sensitive for assessing intestinal permeability in patients with IBD [16]. An increase in the concentration of zonulin in blood serum is associated with an increase in the functional activity of circulating neutrophils and an increase in the number of CD3+CD8+ cells, NK cells and a decrease in the number of CD19+ cells in patients with UC [17].

Therefore, our studies has shown that intestinal barrier function impairment is present in patients with UC, which is more pronounced in patients with EIMS and depends on the severity of LID.

CONCLUSIONS

1. In patients with UC, an increase in the level of zonulin and a1-AT in blood serum and feces was found, which indicates an increased permeability of the intestinal wall in these patients. At the same time, more pronounced changes indicating disturbances in the barrier function of the pocket were found in patients with UC and SPA. 2. In patients with UC and SPA, a direct correlation between the change in intestinal permeability and the severity of LID was established (the level of zonulin in feces correlates with LID III and IV - $r=0.94$; $p<0.01$ and $r=0.88$; $p<0.01$ respectively).

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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".

CONFLICT OF INTEREST
The Author declare no conflict of interest

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RECEIVED: 10.06.2024
ACCEPTED: 11.09.2024

ORIGINAL ARTICLE

CONTENTS

Iron deficiency and heart failure with preserved ejection fraction

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ABSTRACT

Aim: We aimed to assess the prevalence of ID in patients HFpEF and its relation to functional capacity and quality of life.

Materials and Methods: We included in the analysis 121 consecutive outpatients newly diagnosed of HFpEF and tested with iron-related parameters. Patients were subdivided in two groups according to the presence of ID (n = 76, mean age 65.3 ± 7.1 years) or without ID (n =45, mean age 61.6 ± 7.4 years). Physical examination, routine laboratory tests, serum ferritin, transferrin saturation (TSAT), hs CRP, N-terminal proB-type natriuretic peptide (NT-proBNP), standard transthoracic echocardiogram examinations, functional capacity and quality were performed and assessed.

Results: Among all tested patients with iron-related parameters, 63% (76) met the European Society of Cardiology criteria for ID. Additionally, 29% (22) were found to have coexisting anemia. Patients with ID had more pronounced HF symptoms, higher NT-pro-BNP, hs CRP, ferritin and lower TSAT values and more severe diastolic dysfunction.

Patients with HFpEF and ID performed worse functional capacity during the 6MWT and had lower quality of life with Minnesota Living with Heart Failure Questionnaire.

Conclusions: ID as one of the most common comorbidities in HFpEF significantly impairs the functional capacity and quality of life.

KEY WORDS: heart failure with preserved ejection fraction, iron deficiency

Wiad Lek. 2024;77(9):1996-2001. doi: 10.36740/WLek/195167 DOI

INTRODUCTION

Nowadays, the heart failure with preserved ejection fraction (HFpEF) is considered to be one of the major issue in healthcare system, due to its increasing prevalence among aged adults, high mortality and morbidity rates [1].

The diagnosis of HFpEF remains very challenging since there is a lack of universal diagnostic criteria with modest sensitivity and specificity, gaps in our understanding of the disease's pathophysiology, heterogeneity of HFpEF populations and presence of multiple noncardiac comorbidities contribute to this [2].

One of the common comorbidity in patients with HFpEF that affects up to 59% of individuals is iron deficiency [3]. Moreover, the recent studies have found much higher values of ID prevalence in patients with decompensated HFpEF as compared to compensated [4].

The pathophysiology of ID in HFpEF is complex and multifactorial. The reduction of iron intake such as poor appetite, impaired gastrointestinal absorption, co-administration of proton pump inhibitors, the increased iron losses due to anticoagulants or gastric ulceration and comorbidities such as chronic kidney disease, inflammatory activity etc. may contribute to ID [5,6].

The process of iron absorption and mobilization is regulated by hepcidin, its chronically elevated level in the setting of proinflammatory conditions such as HF, respectively impeding iron homeostasis and resulting in functional and absolute ID [7].

Recent study reveals the significant association between baseline hepcidin concentrations and the long-term risk of all-cause mortality, nonfatal cardiovascular events persisted after adjusting for established cardiovascular risk factors, medications, plasma hs CRP, plasma ferritin and other potential confounding factors [8].

Therefore, additional studies are required to assess the prognostic value of higher circulating hepcidin concentrations in predicting the future risk of adverse cardiovascular outcomes.

Since iron is *essential* in erythropoiesis, it also has a major role in mitochondrial energy production and many other cellular processes in myocardium and skeletal muscle, thus the likelihood of ID having an impact on exercise capacity is very high [9]. Thus, even in the absence of anaemia ID per se can be harmful.

The presence of ID in patients with HFpEF contributes to symptoms such as fatigue, tiredness, breath-

lessness, reduced exercise tolerance, increased time to recover after exercise, impaired health related quality of life [10].

Moreover, it has been associated with worse clinical outcomes, higher risk of hospitalizations and all-cause mortality rates [11].

Also, a recent study have been shown that, among patients with chronic HF who were assessed for anemia and ID at baseline and 12 months, anemia developed in 16% of nonanemic patients and resolved in 23% of anemic patients [12].

Further investigations are required to understand the pathophysiology and potential distinct treatments for patients with HFpEF and ID phenotype.

AIM

We aimed to assess the prevalence of ID in patients HFpEF and its relation to functional capacity and quality of life.

MATERIALS AND METHODS

We included in the analysis 121 consecutive outpatients newly diagnosed of HFpEF and tested with iron-related parameters in central city hospital between September 2023 and April 2024**.** Patients were subdivided in two groups according to the presence of ID (n = 76, mean age 65.3 ± 7.1 years) or without ID (n =45, mean age 61.6 ± 7.4 years). The average age was 63.4 ± 11.1 years, with females comprising 68 % of the patients.

Physical examination, routine laboratory tests such as complete blood count (CBC), fasting blood glucose, serum lipids, urea, creatinine, ALT, AST, uric acid, serum ferritin, transferrin saturation (TSAT), hs CRP, N-terminal proB-type natriuretic peptide (NT-proBNP) were performed and assessed.

The ESC and ACC/AHA/HFSA guidelines define ID as a ferritin concentration of <100 ng/mL, or 100-300 ng/mL plus a transferrin saturation (TSAT) <20%.

Anaemia was defined, using the World Health Organisation (WHO) criteria, as a haemoglobin of <12.0 g/dL in women and <13.0 g/dL in men.

There was no statistically significant difference between the study groups and subject controls regarding age, gender or smoking status.

The diagnosis of HFpEF was made according to current HF guidelines: symptoms ± signs of HF, elevated levels of B-type natriuretic peptide and relevant structural heart disease including LV hypertrophy and/or left atrial (LA) enlargement, and/or evidence of diastolic dysfunction on echocardiography.

Standard transthoracic echocardiogram (2D and Doppler) examinations were performed using commercially available equipment (GE Healthcare, Chicago, IL, USA) according to the current guidelines. Cardiac morphology was assessed in standard four- and two-chamber views. The biplane Simpson method was used to determine the left ventricular ejection fraction. The degree of diastolic dysfunction was stratified to one out of four grades [Grade I (impaired relaxation), Grade II (pseudonormal), Grade III (reversible restricted), and Grade IV (fixed restricted)].

Functional capacity was measured by the 6-min walking test (6MWT), which determining the submaximal exercise capacity.

Quality of life was assessed with the Minnesota Living with Heart Failure Questionnaire (MLHFQ). This test covers physical, emotional, and social issues with 21 items, each ranging from 0 to 5, with higher scores denoting worse quality of life.

Statistical analyses were carried out in SPSS 22.0 Statistical Package Program for Windows (SPSS Inc., Chicago, Illinois).

Continuous variables were presented as the mean ± standard deviation (SD) and were compared using an independent samples t test. The differences between groups were checked by Chi-square test for categorical variables and by independent t-test for continuous variables.

The results were analyzed with a 95% confidence interval at a significance level of $p < 0.05$ or with a 99% confidence interval at a high significance level of $p < 0.01$.

RESULTS

Among all tested patients with iron-related parameters, 63% (76) met the European Society of Cardiology criteria for ID. Additionally, 29% (22) were found to have coexisting anemia.

Patients with ID had more pronounced HF symptoms (paroxysmal nocturnal dyspnea – 74% vs 43%; $p < 0.05$, peripheral edema – 35% vs 19%; $p < 0.05$, worse New York Heart Association class (Class ≥ II-III) – 73% vs 54%; $p < 0.05$).

The values of SBP, DBP and HR did not significantly differ among HFpEF patients with and without ID ($p > 0.05$) (Table 1).

The fasting glucose, creatinine, lipid profile, uric acid, AST, ALT, TSH values were almost similar in both groups, but haemoglobin values - significantly lower mainly in female HFpEF patients with ID than in male (11.5 ± 1.7 g/dl vs 12.9 ± 1.8 g/dl respectively; $p < 0.05$).

HFpEF patients with ID had higher ferritin and lower TSAT values compare with HFpEF patients without ID mainly in female (224.3 ± 69 ng/mL,165.7 ± 72 ng/mL vs 99.2 ± 48 ng/mL,104.6 ± 55 ng/mL and 13.65 ± 4.1%, 17.92 ± 5.6% vs 28.75 ± 4.8%, 33.15 ± 5.2% respectively; $p < 0.05$).

Table 1. Baseline characteristics of HFpEF patients with ID vs. without ID

	HFpEF patients with ID (n = 76)		HFpEF patients without ID (n = 45)		p value
	Female (n = 47)	Male (n = 29)	Female (n =25)	Male (n = 20)	
Heart rate (beats/min)	86 ± 19	89 ± 17	77 ± 14	79 ± 18	0.36
Systolic BP (mmHg)	130.7±22.5	133.6±27.4	132.5±23.1	134.2±29.5	0.25
Diastolic BP (mmHg)	80.3±11.4	81.2±9.6	75.3±12.5	78.5±10.9	0.17
Haemoglobin (g/dl)	11.5 ± 1.7	12.9 ± 1.8	12.7 ± 2.1	13.6 ± 1.5	0.02
Ferritin (ng/mL)	224.3 ± 69	165.7 ± 72	99.2 ± 48	104.6 ± 55	0.03
TSAT (%)	13.65 ± 4.1	17.92 ± 5.6	28.75 ± 4.8	33.15 ± 5.2	0.04
Creatinine (mg/dl)	1.09±2.7	1.1±2.8	0.97 ± 2.3	1.05 ± 2.5	0.19
Fasting glucose (mmol/l)	6.6 ± 2.1	6.7 ±2.7	6.1 ± 2.8	6.2 ±2.5	0.06
LDL-C (mmol/l)	3.53±2.16	3.72±2.49	3.27±3.52	3.68±3.45	0.62
HDL-C (mmol/l)	1.01±1.59	0.96±1.57	0.85±1.96	0.93±1.73	0.08
Triglyceride (mmol/l)	1.79±1.31	2.4±1.52	1.12±1.83	2.1±1.94	0.11
Uric acid (μmol/L)	358±29.3	432±26.5	336±34.5	420±38.2	0.84
TSH (mIU/mL)	3.5 ± 2.2	3.1±2.5	3.2±2.46	3.0±2.76	0.12
AST (U/L)	24.8 ± 4.67	29.3±5.17	23.3 ± 4.32	28.1± 5.26	0.36
ALT (U/L)	34.2±6.29	35.8±7.50	32.5±6.31	34.2±7.14	0.45
NT-proBNP (pg/ml)	567±23.4	569±28.5	347 ±33.8	350±37.2	0.03
hs CRP (mg/L)	3.2 ±1.2	2.9±1.3	1.7±1.2	1.9 ±1.1	0.04
LV diastolic dimension (mm)	52.9 ± 4.8	53.6 ±4.3	49.1 ±5.2	50.9 ±4.1	0.03
LV mass (g)	179 ± 55	187± 59	164±62	175±44	0.12
LV mass index (g/m^2)	78 ± 11	91 ± 14	57±12	80±13	0.04
Mitral annular e' (cm/s)	7±2	7±1	9±2	9±1	0.02
E/e ' ratio	13±2	12±2	10±1	10±2	0.06
LA volume index (ml/m^2)	27.5±2.8	29.7±3.2	24.8±3.3	26.1±2.5	0.04
EF, %	60.2±9.3	61.4±7.2	63.7±8.5	65.3±9.4	0.29

With regard to NT-proBNP as a biomarker of heart failure, the group analyses revealed higher values among all patients with ID vs without ID (567±23.4 pg/ml,569±28.5 pg/ml vs 347 ±33.8 pg/ml, 350±37.2 respectively; p <0.05).

The significant increased of serum hsCRP values were also observed in HFpEF patients with ID than in without ID (3.2 ±1.2 mg/L, 2.9±1.3 mg/L vs 1.7±1.2 mg/L, 1.9 ± 1.1 mg/L respectively; p < 0.05), indicating greater systemic inflammation.

An inflammatory biomarker (hs CRP) and NT-proBNP have also been associated with ID.

Furthermore, high values of hs CRP and NT-proBNP were inversely correlated with TSAT (r=-0.29, r=-0.25 respectively; p < 0,05).

It was been found that HFpEF patients with ID had more pronounced LV diastolic dysfunction.

Thus, ID patients had lower lower mitral annular lateral e' velocity (7±2 cm/s, 7±1 cm/s vs 9±2 cm/s, 9±1 cm/s respectively; p < 0.05), an increased LV diastolic dimension (52.9±4.8 mm, 53.6±4.3 mm vs 49.1±5.2 mm, 50.9±4.1 mm respectively; p < 0.05) and an increased LV mass index (78±11 g/m^2, 91±14 g/m^2 vs 57±12 g/m^2, 80±13 g/m^2 respectively; p < 0.05) as compare to HFpEF patients without ID.

The levels of left atrial volume index were also elevated in patients with HFpEF and ID than in without ID (27.5±2.8 ml/m^2, 29.7±3.2 ml/m^2 vs 24.8±3.3 ml/m^2, 26.1±2.5 ml/m^2 respectively; p < 0.05).

There was no clinically significant difference between the EF values among study groups (p < 0.05).

Patients with HFpEF and ID performed worse functional capacity during the 6MWT. The distance walked by HFpEF patients with ID was significantly lower than that walked by patients with normal iron status (398 ± 125 m vs. 461 ± 137 m; respectively; p < 0.05)(Fig.1).

In addition, the number of patients who had to discontinue the 6MWT before its completion was greater among patients with HFpEF and ID compared to patients without ID (6 vs 1). The worse exercise capacity

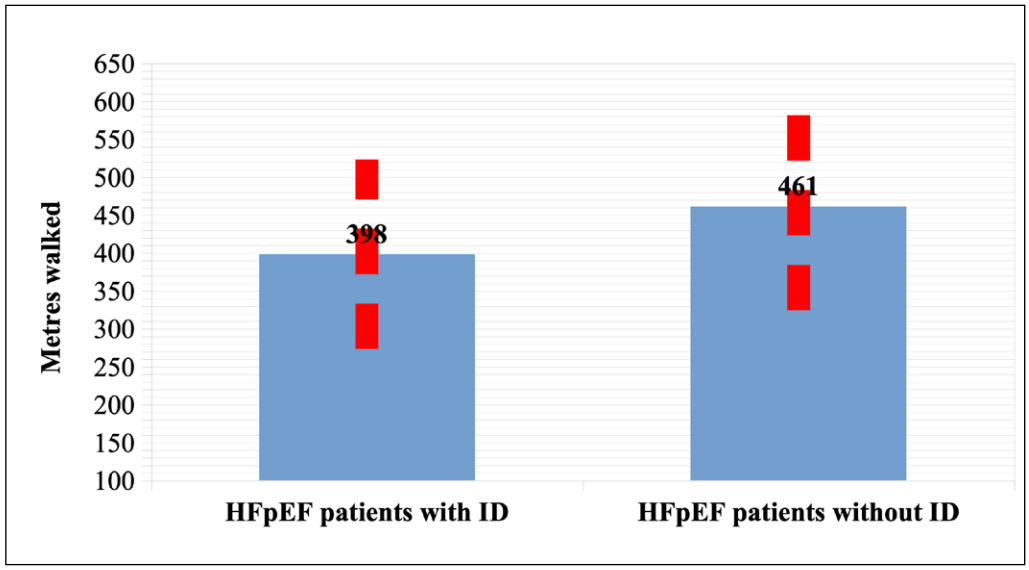

Fig. 1. Functional capacity (6MWT).

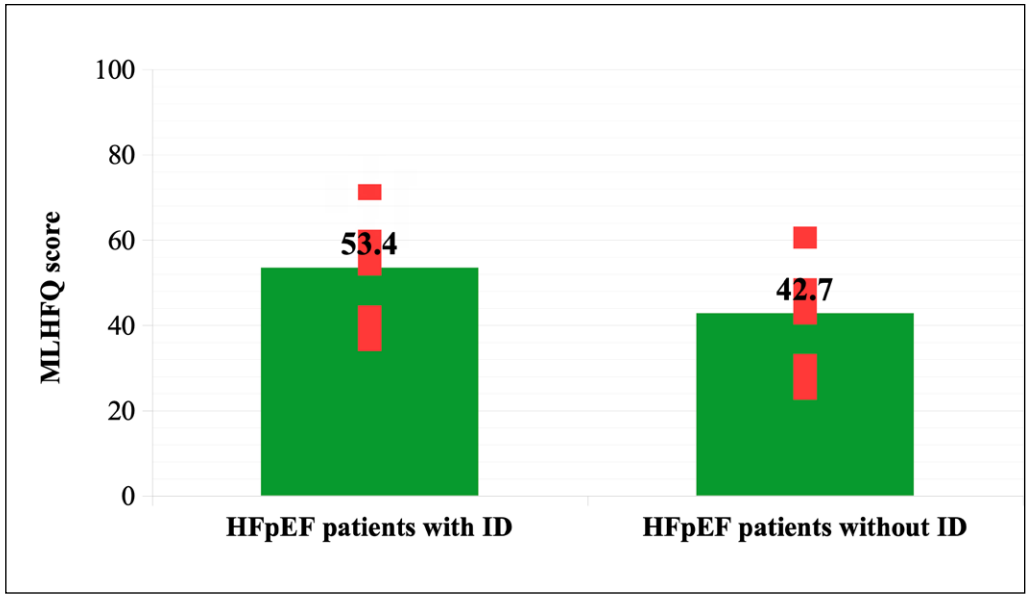

Fig. 2. Quality of life (MLHFQ).

was also observed in HFpEF patients without anemia ($p < 0.05$).

The assessment of quality of life with the Minnesota Living with Heart Failure Questionnaire (MLHFQ) revealed significant higher global score in patients with HFpEF and ID compared to normal iron status (53.4±19.6 vs 42.7±20.3 points respectively; $p < 0.05$)), reflecting worse quality of life (Fig.2).

The most affected dimensions evaluated with MLHFQ in patients with HFpEF and ID were physical, social and personal.

DISCUSSION

Almost 50% of patients with HF are ID with a slightly higher prevalence in patients with HFpEF compared to their counterparts with midrange (HFmrEF) or reduced ejection fraction (HFrEF)[13].

ID affects a lot of physiological processes such as altered mitochondrial function and cardiac energetics, reduces exercise capacity (because of impaired cardiac and skeletal muscle function), reduced cardiac performance during exercise or higher heart rates in patients with HF [14].

Therefore, nowadays the prevalence of ID has been a growing area of interest for prediction of CV incident.

The largest observational study supports that low serum ferritin values have been associated with incident HF, worse diastolic function, higher LV filling pressure but were not associated with measures of LV size or mass or measures of LV systolic function after adjustment for multiple risk factors and other confounders [15].

A 50% lower plasma ferritin level is associated with a higher risk for incident HF overall (hazard ratio [HR],

1.20; 95% confidence interval [CI], 1.08-1.34) and a higher risk for incident HFpEF (HR, 1.28; 95% CI, 1.09-1.50; p = 0.002) both after adjustment for demographics and clinical risk factors [16].

The recent studies had shown the lowest mortality rate for those HFpEF patients with a serum ferritin <100 ng/mL and a TSAT >20% and highest for those with a serum ferritin >100 ng/mL with a TSAT <20% [17].

Univariable analysis revealed the lower TSAT, but higher serum ferritin, were associated with a higher all-cause and CV mortality [18].

TSAT better predicted all-cause mortality, as well as long-term risk for HF hospitalizations despite several iron metabolism biomarkers demonstrating strong association with severe outcomes [19].

Thus, it is important to incorporate additional biomarker data, beyond ferritin and TSAT, when assessing for ID in HF patients [20].

CONCLUSIONS

ID as one of the most common comorbidities in HFpEF significantly impairs the functional capacity and quality of life.

Nevertheless, further prospective investigations is warranted to investigate the potential mechanisms and future treatment possibilities.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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RECEIVED: 15.06.2024
ACCEPTED: 28.09.2024

ORIGINAL ARTICLE

CONTENTS

Differentiated approach to management of patients with irritable bowel syndrome and ulcerative colitis in non-alcoholic fatty liver disease

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ABSTRACT

Aim: To determine the main clinical and laboratory features and severity of colon dysbiosis in irritable bowel syndrome (IBS) and IBD in patients with NAFLD.

Materials and Methods: 80 patients with NAFLD were examined. Patients were divided into two groups. Group 1 (n=40) included patients with NAFLD in combination with ulcerative colitis (UC), and group 2 (n=40) included patients with NAFLD and IBS (clinically manifested by diarrhoea). At patients diagnosed the level of faecal calprotectin (FC) and a1-antirpsin (a1-AT). Changes in the quantitative and qualitative composition of the colon microflora were assessed.

Results: In both groups of examined patients, a decrease of *Bifidobacteria* and *Lactobacilli*, as well as *Enterococcus* and *E. coli* with normal enzymatic properties was found compared with the control group. In patients with NAFLD and IBD, an increase in the level of FC was found in 23.8 times compared with the control group. As expected, there was an increase in the level of a1-AT in the blood serum, faeces and its clearance in patients of group 1.

Conclusions: In patients with NAFLD, both UC and IBS have similar clinical symptoms. An effective biomarker for differentiating and choosing treatment tactics in patients with NAFLD and UC is the determination of the level of FC.

KEY WORDS: non-alcoholic fatty liver disease, inflammatory bowel disease, ulcerative colitis, irritable bowel syndrome, diagnostics, calprotectin, a1-antitrypsin, colon dysbiosis

Wiad Lek. 2024;77(9):2002-2007. doi: 10.36740/WLek/195168 DOI

INTRODUCTION

Non-alcoholic steatohepatitis (NASH) and benign steatosis are on the histologic spectrum of non-alcoholic fatty liver disease (NAFLD), a clinicopathological condition [1]. Experts estimate that a quarter of the world's population has NAFLD. The incidence of non-alcoholic steatohepatitis (NASH) is projected to increase by 56% over the next 10 years [2-4]. Such a rapid increase in the number of people with NAFLD is associated with the pandemic of obesity and type 2 diabetes [5].

In the pathogenesis of NAFLD, a significant role is assigned to the disturbance of the functional state of the colon and, as a result, the accumulation of microbial waste products and endotoxins. NAFLD and steatohepatitis are associated with increased intestinal barrier permeability and translocation of bacteria or bacterial products into the bloodstream. An experimental study has shown that intestinal epithelial barrier and intestinal vascular barrier disorders are early events in the pathogenesis of NAFLD [6].

Numerous studies have shown a strong association between inflammatory bowel disease (IBD) and NAFLD. In the study by Bessissow et al, the prevalence of NAFLD was 33.6% in patients with IBD. Also, study found that the prevalence of NAFLD was 8.2% in patients with IBD compared to patients without NAFLD. Many possible pathophysiological hypotheses have been proposed to explain this relationship, including disease-specific risk factors such as chronic inflammation, steroid exposure, drug hepatotoxicity, malnutrition, and altered gut microbiota [7-9].

Another global challenge of the 21st century is the COVID-19 pandemic. Many patients, whose number is constantly growing, have persistent gastrointestinal symptoms that they attribute to COVID-19. SARS-CoV-2, the virus that causes COVID-19, replicates in the gut, and acute COVID-19 is associated with changes in the gut microbiome. Gastrointestinal symptoms are present in half of patients with acute COVID-19, persist 6 months after COVID-19 in 10-25% of patients, and are rated as

the most distressing symptom in 11% of all patients. These symptoms include heartburn, constipation, diarrhoea and abdominal pain and decrease over time. The cause of prolonged gastrointestinal symptoms of COVID-19 is unknown, and hypotheses include the SARS-CoV-2 virus itself infecting the gastrointestinal tract; COVID-19, which may be accompanied by changes in the gut microbiome, a profound systemic inflammatory response and critical illness; and/or the impact of pandemic stress on gastrointestinal function and symptom perception, which may be unrelated to either SARS-CoV-2 or COVID-19 [10].

Thus, the study of the peculiarities of early diagnosis of conditions manifested by bowel lesions (irritable bowel syndrome (IBS), IBD) in patients with NAFLD and a differentiated approach to the management of such patients is currently extremely relevant.

AIM

The aim of the research to determine the main clinical and laboratory features and severity of colon dysbiosis in IBS and IBD in patients with NAFLD.

MATERIALS AND METHODS

At the clinical base of the Department of Procedure of Internal Diseases, 80 patients with NAFLD were examined. The examined patients with NAFLD for the period 2020 to 2024 were treated in the gastroenterological and endocrinological departments of the Municipal Non-Profit Enterprise «Andriy Novak Transcarpathian Regional Clinical Hospital» of the Transcarpathian Regional Council. Among the examined patients, there were 46 (57.5%) men, with an average age of 40.7±5.5 years; there were 34 (42.5%) women, with an average age of 39.5±4.8 years. The control group included 30 healthy individuals (18 (60.0%) men and 12 (40.0%) women). The average age was 44.3±4.7 years.

All studies were conducted with patient consent. Written consent was obtained from all patients and control subjects for appropriate diagnosis and treatment, with all measures taken to ensure data anonymity, and the methodology was in line with the Helsinki Declaration of Human Rights of 1975 and its 1983 revision, the Council of Europe Convention on Human Rights and Biomedicine, and Ukrainian legislation.

The exclusion criteria were as follows: age under 18 years and over 75 years, liver damage due to viral (hepatitis B, C, D viruses), alcohol etiology; Wilson-Conovalov disease; haemochromatosis; lactose intolerance, gluten intolerance; intestinal surgery, including appendectomy for up to 6 months; colon cancer; doligosigma; colon diverticulosis; type 1 diabetes mellitus; type 2 diabetes mellitus (decompensation stage); pulmonary tuberculosis; psychiatric diseases that do not allow adequate assessment of the patient's health status and signing an informed consent for diagnosis and treatment; pregnancy and lactation; acute myocardial infarction, stroke (in the history of up to 6 months); systemic autoimmune diseases; HIV infection; oncological diseases.

Patients with NAFLD were divided into two groups. Group 1 (n=40) included patients with NAFLD in combination with ulcerative colitis (UC), and group 2 (n=40) included patients with NAFLD and IBS (clinically manifested by diarrhoea).

The diagnosis of NAFLD (metabolic-associated fatty liver disease (MAFLD) or steatotic liver disease associated with metabolic disorders) was made in accordance with the criteria of the unified clinical protocol (Order of the Ministry of Health of Ukraine of 06.11.2014 No. 826) and the EASL-EASD-EASO clinical guidelines for the diagnosis and treatment of these patients. The degree of liver damage was determined using online calculators NAFLD fibrosis score (NFS), Fibrosis 4 calculator (FIB-4), fibrotest, FibroIndex, Forns, APRI, and liver elastometry results. All patients underwent an ultrasound examination of the abdominal cavity according to the generally accepted methodology.

The diagnosis of UC was established according to the standards for the diagnosis of IBD. In all patients, the diagnosis of UC was verified using endoscopic (rectoromanoscopy, sigmoscopy, colonoscopy) and morphological methods of investigation.

The diagnosis of IBS was made on the basis of the IV Rome criteria and the clinical guidelines of the Ukrainian Gastroenterological Association for the management of patients with irritable bowel syndrome.

The activity of faecal calprotectin (FC) was determined by ELISA using the Tecan Sunrise test system, ImmunDiagnostic (Germany). The level of α1-antitrypsin (α1-AT) was determined in blood serum and faeces by ELISA using a test system from Immundiagnostic AG (Germany), and its clearance was calculated based on the obtained values.

To study the species and quantitative composition of the colon microflora, faeces were collected in dry sterile dishes and delivered to the bacteriological laboratory no later than 2 hours after collection without the use of preservatives. The material was sown on a standard set of selective and differential diagnostic nutrient media for the isolation of aerobic and anaerobic microorganisms by the method of tenfold dilution

Table 1. Clinical manifestations of intestinal lesions in the examined patients

Clinical manifestation	Patients with NAFLD	
	Group 1 (n=40)	Group 2 (n=40)
Diarrhoea	100.0 %	100.0 %
Frequency of bowel movements:		
- 1-5 times a day	12.5 %	92.5 %***
- 6-10 times a day	70.0 %***	7.5 %
- more than 10 times a day	17.5 %	0
Meteorism	47.5 %	65.0 %*
Pain along the colon	40.0 %	70.0 %**
Feeling of incomplete bowel movement	30. 0 %	75.0 %**
Impurities in the stool:		
- blood	55.0 %	0
- mucus	90.0 %***	10.0 %

Note: the difference between the indicators in patients by groups is significant: * - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$.

(10^{-1}-10^{-9}). Changes in the quantitative and qualitative composition of the colon microflora were determined using the unified working classification of intestinal dysbiosis by Kuvaeva-Ladodo (1991), according to which 4 phases of dysbiotic disorders are distinguished.

The analysis and processing of the results of the examined patients were performed with the help of the computer program STATISTICA 10.0 (StatSoft Inc, USA) using parametric and non-parametric methods of evaluation of the results.

RESULTS

Clinical changes indicating intestinal lesions in patients with NAFLD were evaluated (Table 1).

At all patients with NAFLD were diagnosed with diarrhoea. However, in patients of group 2, the frequency of stools in the vast majority of patients did not exceed 5 per day, while in patients of group 1, diarrhoea occurred more often up to 10 times per day (in 70.0% of cases). Mucus and blood impurities in the faeces are mainly diagnosed in patients with NAFLD and UC. However, flatulence is 27.5% more common in patients with NAFLD and IBS. Pain along the colon, as well as a feeling of incomplete emptying, were significantly more often diagnosed in patients with IBS in group 2.

A microbiological examination of the faeces was conducted to determine the peculiarities of the quantitative and qualitative composition of microorganisms in the colon lumen in the examined patients with NAFLD in combination with IBS or UC – Table 2.

In both groups of examined patients, a decrease of *Bifidobacteria* and *Lactobacilli*, as well as Enterococcus and *E. coli* with normal enzymatic properties was found compared with the control group. This was accompanied by an increase in the number and percentage of pathogenic and opportunistic microflora in both study groups of patients with NAFLD. However, in patients of group 1, more pronounced changes in the microbial composition of faeces in the colon were found.

A detailed analysis of the microbial composition of faeces made it possible to establish a difference in the data obtained, namely, in patients of group 1 with NAFLD and UC, an increased number of haemolytic form of *E.coli, Enterobacter, Citrobacter, Staphylococcus, Klebsiella, Clostridium, Candida* were significantly more often detected in the faeces. Therefore, the data obtained indicate pronounced dysbiotic changes in the colon in patients with NAFLD and UC, which is manifested by a decrease in normal microflora (*Bifidobacterium, Lactobacillus*) and an increase and activation of opportunistic microflora (mainly gram-negative forms of facultative anaerobes that acquired aggressive properties).

The level of faecal calprotectin and a1-AT in the examined patients with NAFLD was determined to study the severity of inflammation and intestinal barrier permeability in these patients (Table 3).

In patients with NAFLD and UC, an increase in the level of FC was found in 23.8 times compared with the control group, while in patients with NAFLD and IBS, the level of FC did not actually differ from the norm.

Interesting results were obtained regarding changes in the level of a1-AT. As expected, there was an increase in the level of a1-AT in the blood serum, faeces and its clearance in patients of group 1. However, patients

Table 2. Quantitative and qualitative composition of the colon microflora in the examined subjects

Indicator	Examined patients	
	Group 1 (n=40)	Group 2 (n=40)
Bifidobacterium:	Control group 100.0 % (8.55±0.07)	
frequency (%)	65.0 % **	75.0 %*+
lg colony forming units (CFU)/gr	5.71±0.06**	6.95±0.07**+
Lactobacillus:	Control group 100.0 % (6.74±0.09)	
frequency (%)	70.0 % **	80.0 % *+
lg CFU/gr	4.96±0.05**	5.58±0.07*+
E.coli (with normal enzymatic properties):	Control group 93.3 % (7.99±0.10)	
frequency (%)	75.0 % **	82.5 % *
lg CFU/gr	4.87±0.08**	6.76±0.07*+
E.coli (haemolytic form):	Control group 0 % (0.75±0.04)	
frequency (%)	22.5 %+	10.0 %
lg CFU/gr	4.74±0.08***++	2.93±0.06**
Enterococcus:	Control group 90.0 % (7.52±0.04)	
frequency (%)	55.0 %**	70.0 %*++
lg CFU/gr	5.12±0.09**	6.76±0.11*+
Enterobacter:	Control group 23.3 % (1.18±0.05)	
frequency (%)	50.0 %**+	40.0 %*
lg CFU/gr	3.04±0.05**+	2.12±0.04*
Citrobacter:	Control group 26.7 % (1.54±0.03)	
frequency (%)	60.0 %**+	35.0 %
lg CFU/gr	3.06±0.12*+	1.81±0.11
Staphylococcus:	Control group 26.7 % (3.31±0.07)	
frequency (%)	65.0 % **++	30.0 %
lg CFU/gr	4.99±0.14*+	4.08±0.12*
Klebsiella:	Control group 16.7 % (1.44±0.06)	
frequency (%)	45.0 %**+	30.0 %*
lg CFU/gr	3.86±0.10**+	2.75±0.14*
Clostridium:	Control group 16.7 % (4.56±0.16)	
frequency (%)	45.0 %**	27.0 %*
lg CFU/gr	5.82±0.12*+	4.58±0.14
Proteus:	Control group 10.0 % (0.55±0.03)	
frequency (%)	32.5 %**+	22.5 %*
lg CFU/gr	2.24±0.08***+	1.71±0.05**
Candida:	Control group 3.3 % (2.97±0.11)	
frequency (%)	20.0 %**	15.0 %*
lg CFU/gr	4.77±0.07**+	3.65±0.05*

Note: differences between the indicators of the control group and patients of groups I and II are significant: * - $p<0.05$; ** - $p<0.01$; ** - $p<0.001$; differences between the indicators of patients of groups I and II are significant: + - $p<0.05$; ++ - $p<0.01$.

with NAFLD and IBS also had a slight increase in the level of a1-AT. This indicates a violation of intestinal permeability and barrier function of the colon not only in patients with NAFLD and UC, but also in patients with a combination of NAFLD and IBS.

DISCUSSION

It is known that the disruption of the connection between the intestine and the liver is characterised by a number of pathogenic mechanisms, including a weakening of the intestinal barrier and increased intestinal permeability,

Table 3. Indicators of biomarkers of intestinal lesions in the examined patients

Indicator	Examined patients		
	Контрольна група (n=30)	Group 1 (n=40)	Group 2 (n=40)
FC, mkg/l	26.17±0.95	623.44±3.74***+++	35.71±0.42
α1-AT:			
in blood serum, mg/dl	124.15±1.85	397.50±3.81**++	171.23±1.56*
in faeces, mg/dl	14.22±0.16	40.06±0,50**+	23.88±0.31*
clearance α1-AT, ml/day	17.41±0.42	83.45±2.12**++	29.45±0.23*

Note: the difference between the indicators of the control group and the examined patients is statistically significant: * - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$; between the indicators of patients in groups I and II, the difference is statistically significant: + - $p<0.05$; ++ - $p<0.01$; +++ - $p<0.001$.

which leads to endotoxemia and inflammation, as well as changes in the profile of bile acids and levels of metabolites produced by the intestinal microbiome [11, 12]. It is particularly relevant and important to study changes in the functional activity of the intestine in patients with NAFLD and such common conditions as IBS and UC.

Calprotectin or the S100A8/S100A9 complex is located in the cytosol of neutrophilic granulocytes and is defined as an acute phase protein. Calprotectin is associated with a number of inflammatory conditions, including rheumatoid arthritis, psoriasis, and cardiovascular diseases. During inflammatory reactions, calprotectin is produced in increased amounts by neutrophils as a body's response to stress, performing its pro-inflammatory functions. Faecal calprotectin is used in clinical practice as a biomarker of active intestinal inflammation in patients with inflammatory bowel disease. Given the pathogenetic role of hepatic neutrophil infiltration and systemic inflammation in NAFLD, circulating calprotectin may reflect the involvement of neutrophilic inflammation in the pathogenesis of NAFLD. However, only a limited number of experimental studies have been conducted to investigate the role of calprotectin as a biomarker in patients with NAFLD, and the results have been controversial [13, 14].

Calprotectin levels are elevated as a sign of intestinal inflammation in obese patients with NAFLD. This is directly proportional to body weight, waist circumference and waist-to-height ratio. It is believed that FC, which is an easy-to-use and inexpensive biomarker, can be safely used to demonstrate the presence of intestinal inflammation in obesity [15].

Thus, the group of patients with NAFLD and symptoms of bowel lesions requires a detailed analysis of not only clinical manifestations, but also a mandatory element at the stage of research is to determine the level of FC, which makes it possible to diagnose IBD and differentiate it from functional bowel lesions.

CONCLUSIONS

In patients with NAFLD, both UC and IBS have similar clinical symptoms. An effective biomarker for differentiating and choosing treatment tactics in patients with NAFLD and UC is the determination of the level of FC.

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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".

CONFLICT OF INTEREST
The Authors declare no conflict of interest

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RECEIVED: 09.06.2024
ACCEPTED: 28.09.2024

ORIGINAL ARTICLE

CONTENTS

Peculiarities of neurovegetative regulation in children and adolescents with sarcopenia according to heart rate variability indicators

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ABSTRACT

Aim: To find what extent the state of neurovegetative regulation in children and adolescents with sarcopenia differs from their peers without signs of sarcopenia.
Materials and Methods: The sarcopenic index and hand grip strength, which were simultaneously in the range below the 25th percentile of the reference values of these indicators for the corresponding age and gender, were considered the criteria for sarcopenia. A bioelectrical impedance analyzer, "TANITA MC-780 MA" (Japan), was used to obtain the SI indicator. The functional state of autonomic regulation was assessed using the HRV-scanner hardware and software complex (Biosign, Germany).
Results: After processing the examination results according to our algorithm, it became clear that there were no children with signs of autonomic dysfunction in the GWS and BWS subgroups. At the same time, in the GS and BS subgroups, the percentage of children with autonomic dysfunction was 69% (30 out of 42 people) and 74% (28 out of 38 people), respectively.
Conclusions: Registration of a standard 5-minute rhythmocardiogram against the background of spontaneous breathing should be supplemented with a 1-minute breathing test in the diaphragmatic breathing mode with an imposed rhythm of 6 breathing movements per minute, which allows for assessing the presence or absence of parasympathetic dysfunction.

KEY WORDS: autonomic dysfunction, autonomic nervous system, muscle mass, deep breathing

Wiad Lek. 2024;77(9):2008-2014. doi: 10.36740/WLek/195169 DOI

INTRODUCTION

The absolute values of heart rate variability (HRV) parameters and their combinations often do not make it possible to make a reasonable conclusion about the features of the functional state of the patient's autonomic system. In this regard, the methodological approach to HRV analysis proposed by R.D.Beise and M. Hofer, who founded the private research company Biosign in 2000 [1], known for developing HRV-scanner hardware and software complexes, deserves attention. The essence of these scientists' innovative approach to the HRV analysis is that they reduced the significant number of heart rate variability parameters to three groups of indicators that characterize the ANS's tone, dynamics, and flexibility [2].

The tone of the ANS is estimated based on the average values of the R-R interval during the studied period or the heart rate (HR), which are inversely related to each other. The dynamics of ANS are judged by indicators SD1 and SD2, which are obtained by analyzing the Poincaré plot. They are measured in ms and describe the dispersion of heartbeats in a Poincaré diagram. SD1 expresses the width of the point cloud and is more sensitive to rapid high-frequency changes in heart rate, while SD2 describes the length of the point cloud and quantifies long-term HR. At the same time, SD1 is associated with rapid changes in heart rhythm, which mainly depends on parasympathetic activity. SD2 reflects long-term heart rate changes dependent on sympathetic and parasympathetic activity. The SD2/SD1 ratio indicates the balance between short-term and long-term heart rate variability. If this ratio is high (SD2 is significantly greater than SD1), this may indicate increased sympathetic nervous system activity. The flexibility of ANS regulation is judged by the range of heart rate fluctuations (HR). Parameters such as the standard deviation of the duration of R-R intervals, known as SDNN, the coefficient of variation, or the total power of the heart rate spectrum are suitable for this purpose.

For a comprehensive assessment of HRV based on the tone, dynamics, and flexibility of ANS regulation, the HRV-scanner software converts the results of HRV calculations into rank values from 0 to 100% based

on the reference values of the parameters obtained during the examination of healthy people of the appropriate age. For example, a 10% flexibility score means that only 10 out of 100 healthy individuals have less flexibility than the subject, and the vast majority of 90% achieve a better value for this parameter. Diagrams 1 and 2 are a graphic illustration of this method of assessing the functional state of the ANS.

However, applying the described technique may lead to incorrect conclusions if the patient's breathing rate changes. The reason for this is the well-known phenomenon of sinus respiratory arrhythmia [3]. In particular, the high frequency of the examinee's breathing (above 12 respiratory movements in 1 minute) minimizes physiological respiratory sinus arrhythmia. It leads to the formation of a low HRV, which is mistakenly considered a manifestation of autonomic dysfunction. Such a situation in children is often associated with chronic psychoemotional stress [4]. Therefore, the authors of the methodology suggest that you must complement the standard 5-minute ECG recording with uncontrolled breathing with the following 1-minute deep breathing test with a frequency of 6 breathing movements per minute. Comparing the results of standard and deep breathing tests allows you to find the reason for the reduced HRV.

AIM

In this context, we formulated the task of finding out to what extent the state of neurovegetative regulation in children and adolescents with sarcopenia differs from their peers without signs of sarcopenia.

MATERIALS AND METHODS

The study included 80 healthy children aged 10 to 14 years who underwent rehabilitation at the Transcarpathian Regional Children's Sanatorium «Malyatko» and had signs of sarcopenia. The control group consisted of 40 children of the same age without signs of sarcopenia.

The sarcopenic index (SI, kg/m2) and hand grip strength (GS, kg) of the Handexer Grip Strength Tester digital hand dynamometer (USA), which were simultaneously in the range below the 25th percentile of the reference values of these indicators for the corresponding age and gender, were considered the criteria for sarcopenia. [5,6]. A bioelectrical impedance analyzer, "TANITA MC-780 MA" (Japan), was used to obtain the SI indicator. Height (L, m) was measured using a GIMA height meter (Italy).

The functional state of autonomic regulation was assessed using the HRV-scanner hardware and software complex (Biosign, Germany). This complex allows you to register and analyze the patient's ECG in the 1st standard lead during different time intervals and functional tests and obtain a number of heart rate variability parameters that characterize the activity of different parts of the ANS. A feature of this device that distinguishes it from other similar HRV analyzers is the possibility of simultaneous quantitative assessment of breathing frequency (HR, min-1) and the effect of breathing on heart rate (HR, %) (Fig.1, Fig. 2)

In our study, all examined children underwent a standard 5-minute ECG recording in the mode of uncontrolled spontaneous breathing followed by a 1-minute deep breathing test, during which the ECG was recorded in the mode of controlled deep diaphragmatic breathing with a frequency of 6 respiratory movements per minute. Both tests were performed in the sitting position of the examinee after 10 minutes of adaptation to the conditions of the examination and informing him about the specifics of the diagnostic procedure.

Tone (T, %), dynamics of various divisions of the ANS (D1, % and D2, %), and its flexibility (F, %) were estimated based on the primary HRV parameters according to a special software algorithm that takes into account the reference curves of these parameters for persons of a given age and sex. In addition, the state of the vegetative balance was judged by the D2/D1 ratio.

The main (with sarcopenia) and control (without sarcopenia) groups were divided into subgroups by sex, which included 38 boys and 42 girls and 22 boys and 18 girls, respectively. Statistical processing of the obtained data was conducted after testing the hypothesis about the normality of the distribution of indicators in subgroups using the Student's test for independent samples. The difference between subgroups was considered probable at the significance level of $p<0.05$.

RESULTS

A comparison of indicators of neurovegetative regulation between subgroups of children with sarcopenia and without signs of sarcopenia, obtained according to the results of a standard 5-minute registration, showed the same type of differences in both girls and boys (Table 1). Thus, in the subgroup of girls with sarcopenia (GS), statistically significantly lower values of tone (T), dynamics of the parasympathetic link of the ANS (D1), and flexibility of autonomic regulation were obtained (F). At the same time, pronounced sympathicotonia was observed in the GS subgroup according to the D2/D1 ratio. Similar differences occurred between the subgroups of boys with sarcopenia (BS) and without signs of sarcopenia (BWS).

Table 1. Indicators of neurovegetative regulation obtained as a result of standard 5 min registration (M±SD)

Indicators (M±SD)	Girls (n=60)		Boys (n=60)	
	With sarcopenia GS (n=42)	Without sarcopenia GWS (n=18)	With sarcopenia BS (n=38)	Without sarcopenia BWS (22)
T,%	65,3±10,4**	89,9±15,3	65,3±10,4**	82,4±12,3
D1,%	68,3±14,8**	92,4±10,7	61,8±14,3**	90,1±14,5
D2,%	76,3±12,5*	86,4±14,8	76,3±12,5*	79,3±14,9
D2/D1, од.	5,91±1,24***	2,91±0,82	6,04±1,83**	3,11±0,93
F, %	66,7±16,8**	93,5±13,1	67,3±14,9*	89,2±12,4
ЧД, хв$^{-1}$	17,3±4,8*	11,7±3,4	18,1±6,2*	13,2±3,6
ВД, %	12,4±6,8***	61,5±7,6	17,3±8,7***	58,7±13,0
SI, кг/м^2	4,3±9,8*	5,9±0,6	4,4±0,7*	6,2±0,8
GS, кг	17,2±2,8*	21,7±3,6	18,5±3,7**	24,5±3,9

* - p<0,05; ** - p<0,01; *** - p<0,001.

Table 2. Indicators of neurovegetative regulation obtained as a result of the deep breathing test (M±SD)

Indicators (M±SD)	Girls (n=60)		Boys (n=60)	
	With sarcopenia GS (n=42)	Without sarcopenia GWS (n=18)	With sarcopenia BS (n=38)	Without sarcopenia BWS (22)
T,%	75,3±12,2**	92,2±14,7	75,3±12,5**	85,7±13,7
D1,%	79,6±15,8	90,4±13,7	81,8±13,6	89,2±13,2
D2,%	60,2±11,9*	76,6±10,8	56,3±10,2*	69,2±12,6
D2/D1, од.	4,66±1,35***	2,35±0,89	5,44±1,73**	2,47±0,94
F, %	74,8±17,2	91,5±15,8	77,3±18,4	68,9±16,5
ЧД, хв$^{-1}$	6,0	6,0	6,0	6,0
ВД, %	22,1±5,8***	71,5±7,9	27,4±9,9***	68,1±12,6

* - p<0,05; ** - p<0,01; *** - p<0,001.

Respiratory rate (RR) was likely to be higher in children with sarcopenia compared to children without signs of sarcopenia, both among girls and boys. No significant differences were found between the subgroups regarding the impact of breathing on HRV (BI). However, the reason for this may not be the absence of the influence of breathing but the insufficient accuracy of measuring this indicator with the given number of observations, as indicated by its relatively large root mean square deviation.

Using rank values of indicators of neurovegetative regulation, expressed as a percentage of the reference values of these parameters, allows for identifying children with signs of autonomic dysfunction in the examined subgroups. Since there are currently no generally accepted criteria for autonomic dysfunction based on the proposed parameters, we accepted as such a criterion the simultaneous finding of at least 3 of the 4 ranking indicators of neurovegetative regulation in the range below the 25th percentile for this age category. After processing the examination results according to this algorithm, it became clear that there were no children with signs of autonomic dysfunction in the GWS and BWS subgroups. At the same time, in the GS and BS subgroups, the percentage of children with autonomic dysfunction was 69% (30 out of 42 people) and 74% (28 out of 38 people), respectively, illustrated in Fig. 3.

Taking into account the fact that children with signs of sarcopenia probably had a higher respiratory rate during standard registration, it can be assumed that low values of tone and dynamics of neurovegetative regulation in them are associated with excessive activation of the sympathetic link of the ANS. This, in turn, probably leads to reciprocal suppression of the parasympathetic link of the ANS and worsens the functional state of neurovegetative regulation. However, in the case of a decrease in the functional tension of the sympathetic link, it is quite possible to return to the optimal state of autonomous regulation. To discover this possibility, we used a deep breathing test, in which the subject makes controlled breathing excursions for 1 minute with a frequency of 6 breathing movements per minute. This breathing mode is a potent stimulator of the parasympathetic link of the ANS and can significantly increase the physiological

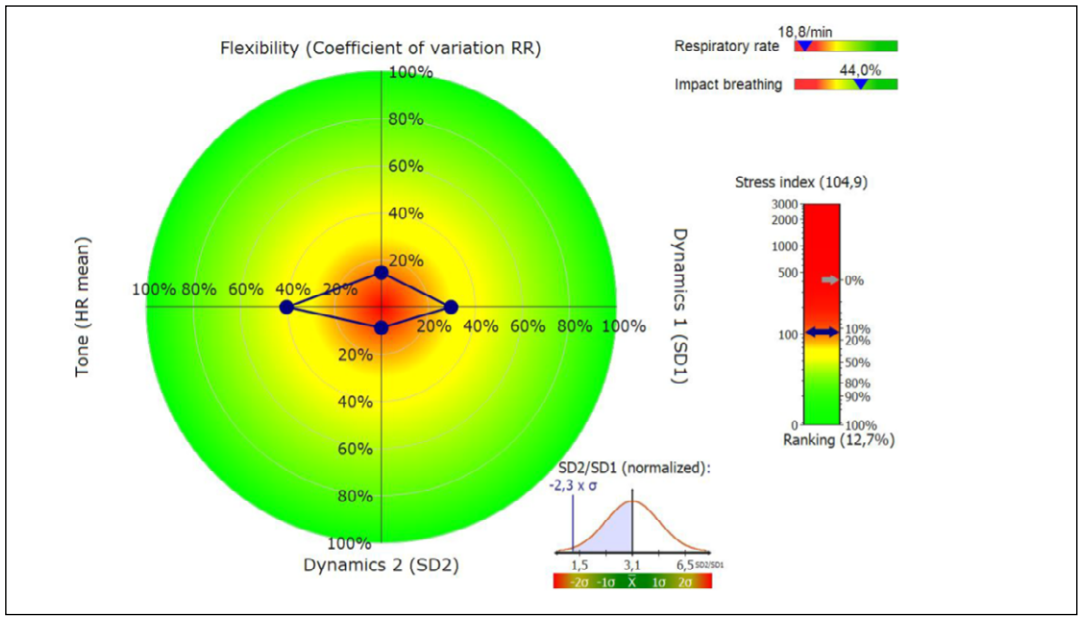

Fig. 1. An example of a visual representation of the results of a standard 5-minute registration of HRV of the examined P. (13 years old).

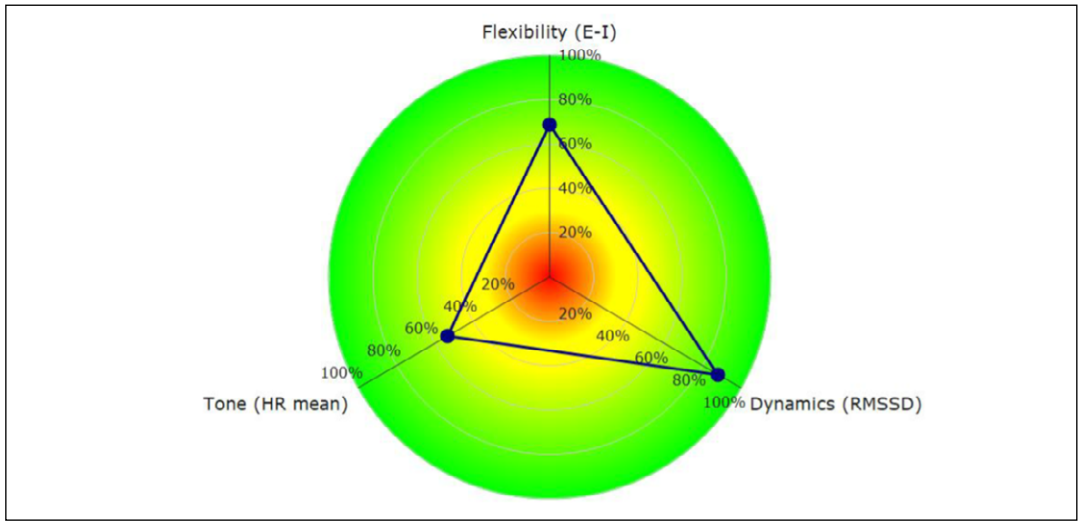

Fig. 2. An example of a visual representation of the results of the deep breathing test of the examined P. (13 years old).

sinus respiratory arrhythmia. However, this ability is preserved only in those examined who do not have parasympathetic autonomic dysfunction of organic origin. Thus, the breathing test helps to distinguish functional changes in parasympathetic regulation from pathological changes associated with morphological damage to the structures of the ANS.

Table 2 presents the data obtained from the deep breathing test in all four subgroups. In contrast to the standard 5-minute recording in the spontaneous breathing mode, the studied subgroups did not statistically significantly differ in many parameters of neurovegetative regulation. In particular, no difference was found between the D1 and F indicators between the subgroups of GS and GWS and BS and BWS. Probable differences were preserved in these subgroups according to T and D2/D1 indicators. The analysis of the presence of children with signs of autonomic dysfunction in the subgroups of GS and BS according to the results of the breathing test showed that their number significantly decreased compared to the standard 5-minute registration (Fig. 4) and amounted to 52 % (22 out of 42 people) and 39%, respectively (15 out of 38 people).

DISCUSSION

Sarcopenia is a pathological condition of the musculoskeletal system, which is characterized by a loss of muscle mass and strength and is traditionally associated with adults and older people. However, in recent years, the opinion that it can also develop in children, especially against the background of other diseases or pathological conditions, is increasingly common in the literature [7]. Sarcopenia can have a significant negative impact on a child's physical,

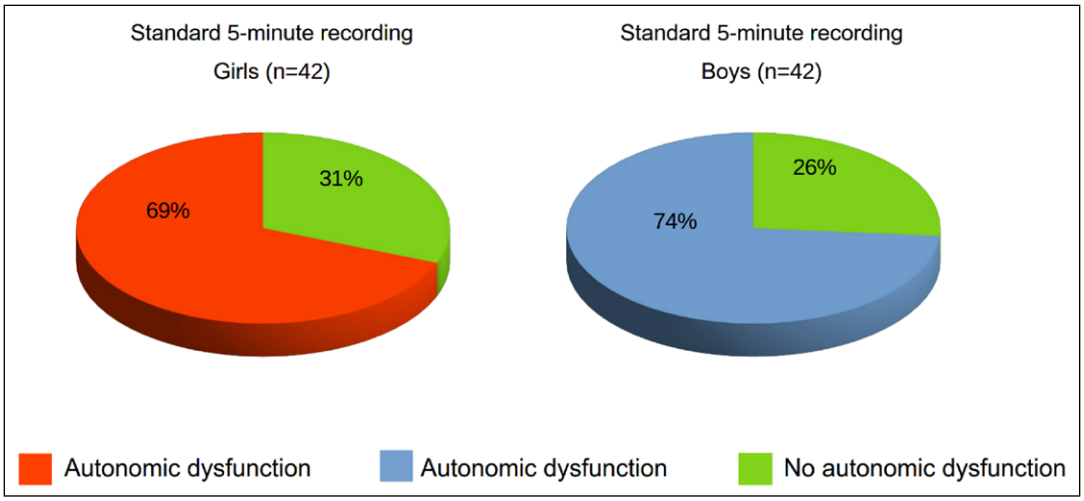

Fig. 3. The ratio of children with autonomic dysfunction and without signs of autonomic dysfunction according to the results of a standard 5-minute recording.

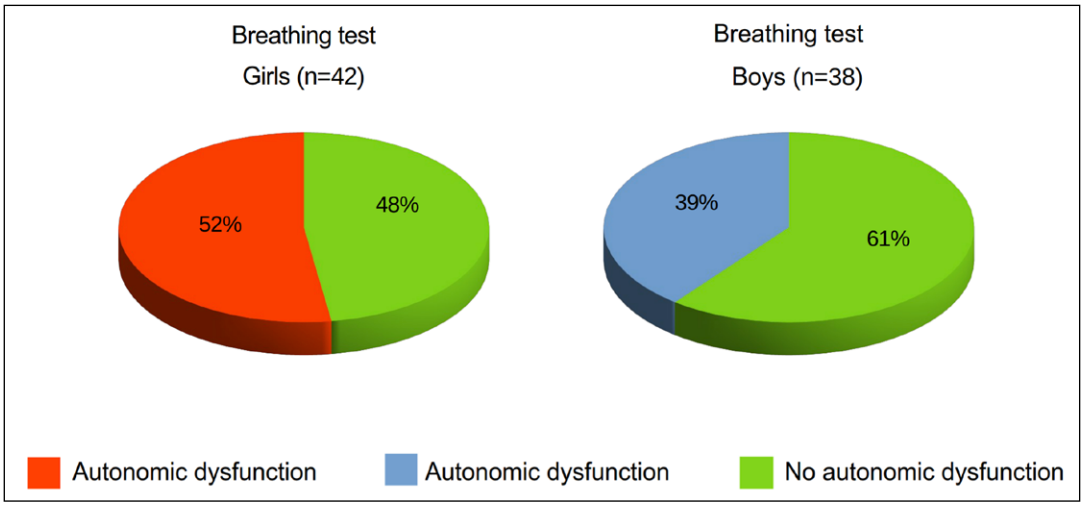

Fig. 4. The ratio of children with autonomic dysfunction and without signs of autonomic according to the results of the breathing test.

psychological, and social development, as skeletal muscle plays an important role in metabolism and overall health throughout the life cycle. New evidence suggests that prenatal (maternal diet during pregnancy and genetic defects) and postnatal factors (physical activity, hormones, dietary protein, and obesity) influence the acquisition of muscle mass and strength early in life [8]. As a result, low muscle mass and strength contribute to many adverse health outcomes in childhood. In particular, studies have demonstrated an inverse relationship between muscle mass and strength with individual and clustered metabolic risk factors [9]. The literature also consistently reports low muscle mass and strength are associated with decreased bone parameters during growth, increasing the risk of osteoporosis in old age[10]. In addition, increased muscle mass is associated with improved neural development in the early years of life [11].

Despite the growing interest in childhood sarcopenia, this concept still needs to be implemented in terms of identification criteria [12]. Advances in research in this area will allow practitioners to recognize sarcopenia better, not only in pediatric patients with chronic diseases but possibly also in otherwise healthy children. In our opinion, it became possible to identify children with signs of sarcopenia due to the widespread introduction into clinical practice of non-invasive and highly informative bioimpedance analysis of the component composition of the body. This method makes it possible to quantitatively characterize the total content of skeletal muscles in the subject's body and their content in individual regions of the body. It is the appendicular mass of skeletal muscles, related to the square of height, that is considered an informative sarcopenic index. If you combine this indicator with the results of standard hand dynamometry, you can get a fairly accurate criterion of sarcopenia. In our study, the finding of both parameters in the range below the 25th percentile of their reference values for a given age and gender is considered a criterion. Subgroups of girls and boys with sarcopenia, separated in this way, demonstrated significant differences in the functional state of the ANS compared to their peers without signs of sarcopenia. These differences were reduced to a decrease in the tone of neurovegetative regulation, a decrease in the dynamics of the parasympathetic link

of the ANS, and the flexibility of autonomic regulation in general. Such features of the ANS in this contingent, in our opinion, significantly reduce the adaptive reserve of the children's body and are a risk factor for the occurrence of metabolic disorders, cardiovascular pathology, and growth retardation.

The results of our study showed that the use of a standard 5-minute ECG recording to calculate HRV leads to overdiagnosis of autonomic dysfunction in children with sarcopenia. The reason for this, in our opinion, is insufficient consideration of the effect of breathing frequencies on HRV indicators. In many of the examinees, a high respiratory rate neutralizes the physiological impacts of sinus respiratory arrhythmia and artificially lowers HRV. At the same time, if you record ECG in the diaphragmatic slow breathing mode with an imposed rhythm of 6 respiratory movements per minute, the activation of the parasympathetic link can significantly increase HRV. This approach to diagnosing autonomic dysfunction in the HRV-scanner hardware and software complex uses a 1-minute deep breathing test. With its help, our study found that standard registration overestimated the number of individuals with autonomic dysfunction among girls and boys by 17% and 35%, respectively.

CONCLUSIONS

1. In children and adolescents with sarcopenia, there is a high probability of the formation of autonomic dysfunction, which is manifested in a decrease in the tone, dynamics, and flexibility of neurovegetative regulation.
2. For the instrumental diagnosis of autonomic dysfunction, it is advisable to study heart rate variability with the help of modern hardware and software complexes, particularly the HRV scanner (Biosign, Germany).
3. Registration of a standard 5-minute rhythmocardiogram against the background of spontaneous breathing should be supplemented with a 1-minute breathing test in the mode of diaphragmatic breathing with an imposed rhythm of 6 breathing movements per minute, which allows assessing the presence or absence of parasympathetic dysfunction.

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We would like to express our sincere gratitude to Reinhard Beise and BioSign GmbH for their generous donation of the HRV-Scanner Study, which made it possible to conduct our research aimed at supporting the well-being of children affected by the ongoing war in Ukraine. Your contribution has played a crucial role in providing these children with the opportunity to benefit from biofeedback methods, enabling them to better cope with stress and

improve their health. We deeply appreciate your support in making a positive difference in their lives.
This work is a continuation of previous research conducted within the framework of the research program "Informativeness of indicators of the body composition in the diagnosis, treatment and prevention of diseases of internal organs" (state registration number 0122U201421).

CONFLICT OF INTEREST
The Authors declare no conflict of interest

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RECEIVED: 10.05.2024
ACCEPTED: 22.09.2024

ORIGINAL ARTICLE

CONTENTS

Clinical and diagnostic features of Crohn's disease in young children

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ABSTRACT

Aim: To highlight the distinctive features of CD in young children, based on personal clinical experience in observation and treatment, with the objective of improving the accuracy of diagnosis and the effectiveness of treatment.

Materials and Methods: The study involves the results of treatment 11 young children with CD. The diagnosis was based on the combination of the following data: clinical manifestations of the disease and its course, as well as the results of laboratory, instrumental, and step biopsy with morphological studies.

Results: In young patients with CD, combined lesions of the small and large intestines predominate, manifesting as gastrointestinal disorders against the background of a severe general condition, intoxication, and extraintestinal complications. Young children with CD often show signs of asthenic syndrome, developmental delay, and high inflammatory activity. All the children in the study showed low serum iron levels, dysproteinemia, and high calprotectin levels.

Conclusions: 1. CD in young children has such clinical features as combined lesions of the small and large intestines (45.45% of cases), high activity of the disease. frequent extraintestinal manifestations of the disease, and developmental delay. All young children with CD upon admission to the hospital were in severe condition, exhibiting signs of intoxication, diarrhea, and blood in their stools. 2. Laboratory findings are characterized by serum iron deficiency, dysproteinemia, and high levels of calprotectin. 3. Knowledge of the clinical and diagnostic features of CD is essential for specialists to provide appropriate medical care.

KEY WORDS: Crohn's disease (CD), children, early age, clinical picture, diagnosis

Wiad Lek. 2024;77(9):2015-2019. doi: 10.36740/WLek/195170 DOI

INTRODUCTION

Crohn's disease (CD) in young children poses a substantial problem for these vulnerable patients. It is a gastrointestinal disease of unclear etiology, characterized by transmural (affecting all layers of the intestinal wall) segmental granulomatous inflammation, leading to intestinal (local) complications and extraintestinal (systemic) manifestations [1, 2]. Currently, the approaches to diagnosing and treating CD remain a topic of ongoing discussion among experts globally. CD is among the conditions that present significant challenges for practicing physicians. The increasing mortality rate among children with CD, including infants, is concerning and has attracted significant attention from both international and domestic researchers [3]. In recent years, there has been a noted increase in the incidence of CD in European countries. The early-onset incidence (new cases per year) in children is 4.37 per 100,000, and the prevalence is 14 per 100,000 [4]. According to a population-based Scottish study, over the past four decades, the incidence of early-onset CD and ulcerative colitis has tripled – in children under 5, the frequency increased from 0.7 (1981–1985) to 2.0 per 100,000 per year (2008–2013) [5]. Experts estimate that the approximate number of patients with CD in Ukraine is 30.33 per 100,000 of the population, with 48% experiencing moderate to severe inflammatory activity, though the exact number of patients is unknown due to the absence of a registry [6]. In the last decade, there has been a trend toward an increase in early-onset cases, with the incidence of CD in childhood having tripled [7, 8]. However, to this day, CD in young children remains one of the least studied pathologies. Global research offers only incomplete and controversial data on the clinical presentation, diagnostic potential, and treatment strategies for CD in young children.

AIM

Aim was to highlight the distinctive features of CD in young children, based on personal clinical experience in observation and treatment, with the objective of improving the accuracy of diagnosis and the effectiveness of treatment.

MATERIALS AND METHODS

The study included the results of examination, treatment, and observation of 11 children with CD aged from 11 months to 3 years. Patient data were collected from medical history, review of outpatient case records, hospital discharge reports, and other relevant sources. The diagnosis was based on a combination of the following data: assessment of clinical manifestations of the disease and its course, and the results of laboratory, instrumental, and morphological studies. The instrumental methods of examination in children included: endoscopic examination with biopsy (fibrogastroduodenoscopy, fibrocolonoscopy, sigmoidoscopy), barium X-ray examinations of the gastrointestinal tract, double-contrast CT scan of the abdomen, MRI of the abdomen and pelvis, and morphological examination of clinical biopsy material. Biopsies should be multiple (or stepped), which involves taking samples from 5 areas along the large intestine, including the rectum and ileum. The gold standard for examining children with perianal lesions is rectal examination under anesthesia. When symptoms of CD appear at an early age, it is necessary to exclude the presence of primary immunodeficiency [9]. Thus, an immunological marker for suspected CD is antibodies to Saccharomyces cerevisiae. CD is verified by the morphological examination of clinical biopsy material [10, 11].

The activity of CD was assessed using the PCDAI (Pediatric Crohn's Disease Activity Index), as presented in Table 1 [12].

The study applied commonly accepted methods for statistical data processing in medical and biological research. Non-parametric methods were used due to the non-representative sample and the preference for analyzing qualitative rather than quantitative characteristics. Numerical indicators are presented in absolute values and in percentage ratios.

Compliance with bioethical principles was ensured.

RESULTS

While CD primarily affects the terminal ileum (a synonym for the disease is terminal ileitis), in young children, combined involvement of the small and large intestines predominates. Among the studied patients, 5 children exhibited combined involvement of the small and large intestines, which accounted for 45.45% of cases. In 2 (18.18%) young children, there was involvement of the ileum, in 3 (27.27%) patients only the large intestine was affected, and in 1 (9.09%) patient, there was involvement of the jejunum, which corresponded to the data presented by Aloi M et al. [13]. Perianal complications, known as 'perianal Crohn's disease', were observed in 3 children in the study group: in 2 children with involvement of the large intestine and in 1 child with involvement of the ileum, accounting for 27.27%.

Upon admission to the hospital, the main manifestations of CD in young children were abdominal pain, vomiting, abdominal distension, the presence of purulent-hemorrhagic and mucous discharge from the rectum, fistulas in the buttock and perineal areas, rectal erosion, perianal dermatitis, loose stools, fever, and more. All young children with CD admitted to the hospital were in serious condition with signs of intoxication. Common symptoms in young children with CD included anemia, weakness, fever of unknown origin, fatigue, weight loss, loss of muscle mass, developmental delay, stunted growth, and more. More than half of the patients, namely 6 children (54.54%), had extraintestinal manifestations of CD: lesions of the oral mucosa in the form of canker sore, eye involvement such as iridocyclitis, and erythema nodosum. The analysis of the disease history data revealed that patients had previous complaints and changes in their general condition ranging from 1 month to 2 years.

Based on the nature of bowel movements, weight, height, the presence of perianal and extraintestinal manifestations, as well as laboratory changes (levels of Ht, ESR, and albumin), CD in the majority of young children in the study group was active. The high activity of CD in these young children, as assessed by the PCDAI (Pediatric Crohn's Disease Activity Index) scores, corresponded to moderate and severe forms of the disease.

All patients had inflammatory changes in their blood tests, including elevated ESR, leukocytosis, and high levels of C-reactive protein. It is noteworthy that all young children with CD in the study group had iron deficiency (low serum iron levels) and dysproteinemia. The level of fecal calprotectin, an inflammatory protein that indicates the degree of intestinal inflammation, was also elevated. The method of measuring fecal calprotectin has been implemented in practical healthcare as a screening tool to differentiate between inflammatory and non-inflammatory bowel diseases. In patients with CD, the level of calprotectin increased by 3-4 times or more (the normal range is 50 mcg/g) [14].

Upon admission to the hospital, the studied group of patients had elevated average values of the leukocyte intoxication index (LII) and the hematological index of intoxication (HII).

Antibodies to Saccharomyces cerevisiae, which serve as an immunological marker when CD is suspected, were detected in 6 young children, accounting for 54.54%.

Symptoms such as abdominal pain, weight loss, diarrhea, and asthenic syndrome were predominant

Table 1. Pediatric Crohn's Disease Activity Index (PCDAI)

Criteria	Presence	Scores
Abdominal Pain	None	0
	Mild intensity	5
	Severe	10
Bowel Movements, Frequency, Consistency	0-1 time/day, liquid, no blood present	0
	2-5 times/day, with minor blood presence	5
	≥ 6 times/day	10
Weight	No weight loss	0
	Weight loss of 1-9%	5
	Weight loss ≥ 10%	10
Height	Below 1st percentile	0
	From 1st to 2nd percentiles	5
	Below 2nd percentile	10
Abdominal Tenderness	No tenderness	0
	Tenderness or palpable mass	5
	Severe tenderness	10
Perirectal Manifestations	None	0
	Fistula, inflammation	5
	Abscess	10
Extraintestinal Manifestations	None	0
	One manifestation	5
	More than 2 manifestations	10
Hematocrit (children under 10 years)	≥33%	0
	28-32%	2.5
	≤28%	5
Hematocrit (girls 11-18 years)	≥34%	0
	29-34%	2.5
	≤29%	5
Hematocrit (boys 11-14 years)	≥35%	0
	30-34%	2.5
	≤30%	5
Hematocrit (boys 15-18 years)	≥37%	0
	32-36%	2.5
	≤32%	5
ESR (mm/hr)≤	≤ 20	0
	20-50	2.5
	≥50	5
Albumin (g/dl)	≥3.5	0
	3.1-34	5
	≤3	10
PCDAI Interpretation	No activity (remission)	≤ 10 scores
	Mild to moderate	11-30 scores
	Severe	30-100 scores

in young children. Among patients under one year of age, low body weight was present in 100% of cases, and growth delay occurred in 50% or more of the children. In children aged 1-3 years, disturbances in weight and height indicators were observed in 82.82% of cases (9 patients), and diarrhea in 63.64% (7 children). Blood in the stool was noted in 72.73% of the young children.

Key diagnostic methods for CD in children include endoscopic examinations. Children with CD exhibit aphthous lesions on a normal or inflamed intestinal mucosa, In one child, the jejunum was affected. During rectosigmoidoscopy and fibrocolonoscopy, there were signs of focal infiltration, swelling, hyperemia with the formation of a cobblestone appearance of mucosa in the affected segments of the colon, enhanced or absent vascular pattern, multiple ulcers with debris, and the presence of liquid hemorrhagic content and/or blood clots in the intestinal lumen [15].

Today, methods of MRI and CT with bowel filling using water, such as hydro-MRI and hydro-CT, have become widely used. They enable highly accurate assessment of bowel wall thickness, the presence of intestinal ab-

scesses, stenoses, and effusions. These methods have expanded the diagnostic capabilities for CD in young children and in the early stages of the disease [16].

In 2014, the Porto Criteria for diagnosing CD were published (by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition). They emphasize that even among the methods of additional diagnosis for CD, there is none that can guarantee the detection of CD in a child with 100% certainty. Therefore, clinicians must be aware that the diagnosis of CD should be verified by morphological methods of investigation [17].

According to the results of the morphological examination of biopsies taken during diagnostic procedures, the characteristic changes in the small intestine were as follows: uneven lymphoplasmacytic infiltration, infiltration by segmented neutrophils, eosinophils, and focal lymphoproliferative changes in the lamina propria of the small intestine; lymphocytic infiltration, areas of fibrinoid necrosis of the vessel wall, ulcerative defects with proliferative inflammatory changes, and fibrosis of the muscle layer; lymphoproliferative changes in the serous membrane, and the presence of ulcers with smooth edges extending into the subserosal layer. The morphological changes in the biopsy material of the colon were as follows: infiltration and lymphoplasmacytic infiltration of the lamina propria of the mucous membrane, areas of mucosal fibrosis, follicle formation in the mucous membrane, focal angiomatosis, areas of hyperplasia of intramural ganglia, ulcerative and proliferative inflammatory defects of the mucosal and serosal layers; muscle layer fibrosis; thickening of the intestinal wall due to pronounced fibrotic changes, presence of ulcers with smooth edges extending to the subserosal layer.

The results of morphological examination of biopsy samples from the esophagus, stomach, and duodenum, taken during esophagogastroduodenoscopy (EGD), in some small children revealed chronic atrophic duodenitis and erosions of the gastric mucosa, changes characteristic of chronic gastroduodenitis, and the presence of gastropathy.

Diagnostic search for CD must include mandatory testing for yersiniosis, which should be excluded in patients with CD.

In the treatment of CD in young children, it is crucial to use a combined approach, integrating conservative and surgical treatments. Surgical and medical treatments for CD should complement each other. The key to effective treatment is timely diagnosis of the pathology and determining appropriate medical care for the child, which should be based on multidisciplinary approaches in both diagnostic and therapeutic strategies.

DISCUSSION

Clinical monitoring and diagnosis of CD in young children must be comprehensive and multidisciplinary, involving pediatricians, gastroenterologists, immunologists, pediatric surgeons, morphologists, and others in the diagnostic process.

Our own experience of observing children with CD aged 11 months to 3 years reveals differences in the clinical presentation and laboratory findings in this pathology. In young patients, combined lesions of the small and large intestines predominate, manifested by gastrointestinal disorders against the background of a generally severe condition, intoxication, and extraintestinal complications. Asthenic syndrome and developmental delay are typical for young children with CD. High activity of the inflammatory process is also common in young children [4].

Regarding laboratory features, all the children from the study had low serum iron levels, decreased albumin levels, elevated gamma globulin levels, and fecal calprotectin levels increased by 3-4 times or more. Antibodies to Saccharomyces cerevisiae, which serve as an immunological marker when CD is suspected, were detected in half of the patients (54.54% of cases). Yersiniosis was excluded in all children with CD, which we consider a mandatory step in the diagnostic search.

The primary diagnostic methods for this group of patients are endoscopic examinations with biopsy sampling and morphological verification of the diagnosis.

Given the high number of cases of late diagnosis and unsatisfactory treatment outcomes of CD in young children, the issue requires the development of a unified strategy and treatment approach.

CONCLUSIONS

1. CD in young children has such clinical features as combined lesions of the small and large intestines (45.45% of cases), high activity of the disease process according to the PCDAI index, frequent extraintestinal manifestations of the disease (54.54% of cases), and developmental delay (100% of cases). All young children with CD upon admission to the hospital were in severe condition, exhibiting signs of intoxication, diarrhea, and blood in their stools.
2. Laboratory findings in young children with CD are characterized by serum iron deficiency, dysproteinemia, and high levels of calprotectin.
3. Knowledge of the clinical and diagnostic features of CD in young children is essential for specialists involved in the treatment of such patients to provide appropriate medical care.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 27.05.2024
ACCEPTED: 18.09.2024

ORIGINAL ARTICLE

CONTENTS

Effect of some immunological markers on the level of anti-mullerian hormone (AMH) in women infected with *Toxoplasma gondii*

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ABSTRACT

Aim: To study effect of *T. gondii* infection on levels of immunological parameters for possible effects that may appear in infected women in the future, and to study direct correlation of infection in raising AMH value.

Materials and Methods: 80 blood samples were collected and divided into two equal groups. Of unmarried women, their ages ranged from 20-25: 1st group was from infected with Toxoplasma who visited health institutions, 2nd group was from whom were not infected as a control group. Toxoplasma infection was diagnosed using the LAT test and the ELISA test. Detection of human IL-2, IL-10 and IL-12 (pg/ml) ELISA KIT, Assay Max Human was conducted according to manufacturing company.

Results: An increase in the level of human IL-2, IL-10 and IL-12 (pg/ml) in infected women compared to control group. A positive correlation of the human IL-2, IL-10 and IL-12 (pg/ml) with AMH level, which supports possibility of adopting AMH level as an indicator of cases of polycystic ovary cysts and infertility in future.

Conclusions: It was conducted to determine the relationship between the levels specific parameters such as IL-2, IL-12, and AMH and their effect on women with Toxoplasmosis, where the relationship was positive between the immune parameters, its levels increased in the event of infection, which increases possibility of using it as indicators of infection and also to predict the incidence of polycystic ovary syndrome or occurrence of infertility cases, there is a positive correlation between IL-2, IL-12 with level of AMH.

KEY WORDS: Toxoplasmosis, immunological, IL-2, IL-12, anti-mullerian hormone

Wiad Lek. 2024;77(9):2020-2026. doi: 10.36740/WLek/193199 DOI

INTRODUCTION

An internal parasite belonging to the felidae family, *Toxoplasma gondii* uses humans as its intermediate host. In most cases, patients' feelings are asymptomatic [1]. *T. gondii* is a parasite that mostly affects the central nervous system in mammals, birds and reptiles. It can also infect the skeletal muscles and reproductive system. This parasite is harmful to people who receive organ transplants and causes acquired toxoplasmosis [2]. Additionally, it results in congenital toxoplasmosis in the fetuses of a number of domestic and wild animal species, including pigs, sheep and goats [3]. The virulence and pathogenicity of *T. gondii* strains are categorized into three genotypes. The first genotype is primarily isolated from human hosts, while the second genotype is observed in severe cases of infection [4], when a host ingests *T. gondii* tissue cysts, sometimes referred to as bradyzoites, from an intermediate host that has already been infected, infection results. Inflammation results from *T. gondii* entering the small intestine and changing into the rapidly dividing tachyzoite form [5]. The parasite's life cycle depends on two aspects of the immune response triggered by this technique. *T. gondii* infects immune cells and replicates inside of them. The parasite spreads throughout the body by invading immune cells and then moving on to new hosts in the brain, muscles and other organs [6]. Chronic infection is distinguished by the change of parasites to a bradyzoite transcriptional programme and the elimination of the bulk of tachyzoites [7]. This process entails the formation of a cyst wall rich in saccharides, which is critical for the survival of the parasite during its passage through the gastrointestinal tract of the subsequent host [8]. Thus, the opportunity for transmission is restricted, as the parasite destroys the host prior to this transition occurring in the absence of a robust immune response [9]. Interleukin-2 (IL-2) and interleukin-12 (IL-12) are required for the initiation and expansion of innate and adaptive immune responses in cells such as epithelial or endothelial cells [10]. Inflammasomes regulate the

activity of numerous IL-2 cytokines, multi-protein interactions, and resistance to microbial infections [11]. Innate immune cells, including monocytes, dendritic cells, and macrophages generate cytokines of this nature. Nonhematopoietic immune complexes regulate the intracellular cysteine protease-mediated cleavage and subsequent activation of immature forms of these cytokines [12]. It is a protein that is manufactured by the granulosa cells found in the ovarian follicle, and it is considered one of the best tests used to detect ovarian reserve [13]. Its percentage decreases in women as they age, becoming very few and cannot be noticed or detected when menopause is reached, which makes it Also an indicator to predict the approaching of this stage [14]. Sertoli cells generate ovarian reserve hormone, a significant contributor to the process of gender differentiation from the moment of fetal gender differentiation until puberty. Ovarian reserve hormone is a member of the transforming growth factor β family. Granulosa cells additionally generate anti-mullerian hormone (AMH) from the moment of implantation until the cessation of ovarian function [15]. AMH is secreted from granulosa cells during the development of primary eggs in follicles up to 6 mm in diameter, and its expression gradually decreases with follicle development. Serum AMH levels are associated with other clinical features such as cycle duration, average ovarian size, and hormone levels [16]. Testosterone and androstenedione in the blood, therefore AMH is a potential biomarker for polycystic ovarian morphology and PCOS, and can be measured to avoid the need for ultrasound examinations in the early follicular phase [17]. In comparison to healthy women, women infected with the parasite exhibit greater resistance to insulin in the bloodstream as age and weight increase. Research has demonstrated that hyperinsulinemia is progressively more significant in the intricate development of polycystic ovary syndrome. This is supported by the identification of a significant correlation between insulin resistance and abnormalities in ovarian function, and it is evident that the level of insulin resistance increases with the severity of the condition [18]. There is a lower possibility of spontaneous ovulation in PCOS individuals. This suggests that severe hypomenorrhea or amenorrhea is coupled with moderate or severe insulin resistance. Measuring the level of AMH in serum is informative and necessary during IVF treatment cycles [19]. Since it is closely related to the number of oocytes obtained after stimulation and the number of eggs retrieved, it is useful in predicting suboptimal and excessive ovarian responses upon stimulation. Most reports indicate a weak predictive value of AMH on the pregnancy rate in the new cycle of IVF treatment, so it has been suggested that it gives some prediction of pregnancy, especially when fresh or frozen embryo transfers are performed in the field [20]. AMH has recently been studied as a potential new clinical measure for ovarian reserve and gonadotropin responsiveness. Several big prospective studies have been published in recent years, showing highly fascinating new data about the application [16]. In assisted reproductive technologies, the clinical utility of AMH measurement in predicting both the quantitative and qualitative ovarian response is noteworthy. The AMH hormone is a Sertoli cell-specific protein in males. It is secreted by the testicles via the gonads beginning in the eighth week of pregnancy, and at a significant level until puberty. When the development of the Sertoli cells is complete, the synthesis of the AMH hormone diminishes compared to the condition in women. The regulation of testicular activity appears to be AMH's sole physiological function in adult males. AMH is a marker of Sertoli cell function, as it is released in both serum and semen by adult males. AMH can be found in adult males. Finding out about its measurement could be helpful in learning more about spermatogenesis.

AIM

The aim of our research was to study the effect of T. gondii infection on levels of immunological parameters for possible effects that may appear in infected women in the future, and to study the direct correlation of infection in raising AMH value.

MATERIALS AND METHODS

Eighty blood samples were collected and divided into two groups (40 in each group). The unmarried women were aged 20–25 years. The first group consisted of women infected with toxoplasma and visiting medical institutions, and the second group consisted of women who were not infected as a control group. Toxoplasma infection was diagnosed using the LAT test and the ELISA test. Sterile plain tubes were used to collect blood samples, which were numbered, and some information was recorded on a special form. Following a five-minute separation of the blood using a centrifuge set at 3000 rpm, the serum was placed in numbered plastic containers and stored at -20°C until various immunological and serological tests were conducted. Detection of human IL-2, IL-10 and IL-12 (pg/ml) ELISA KIT the Assay Max Human was conducted according to the manufacturing company using the same procedure. AMH was measured by a blood sample drawn from the vein using a small needle, and the examination was completed.

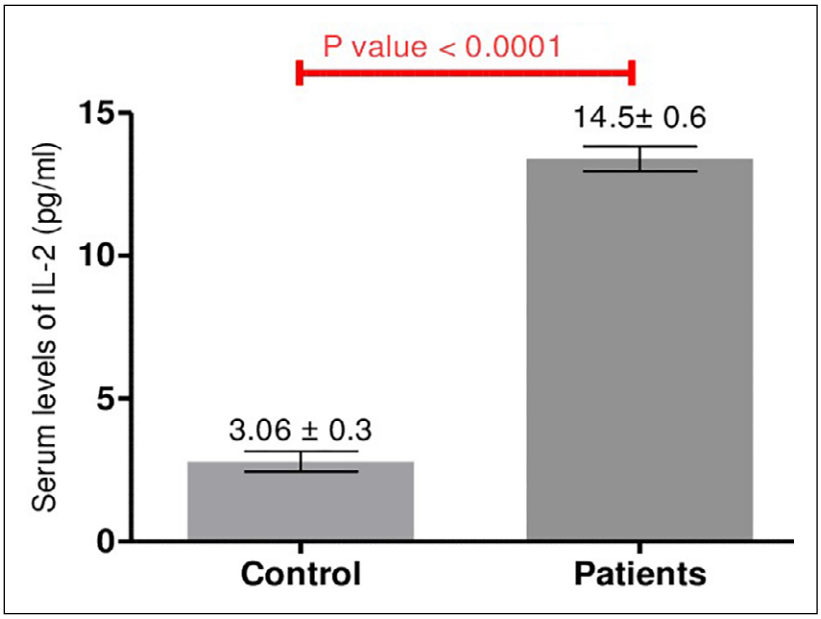

Fig. 1. Concentration of IL-2 (pg/ml) in patients infected with T. gondii compared with the control group (p-value ≤ 0.001).

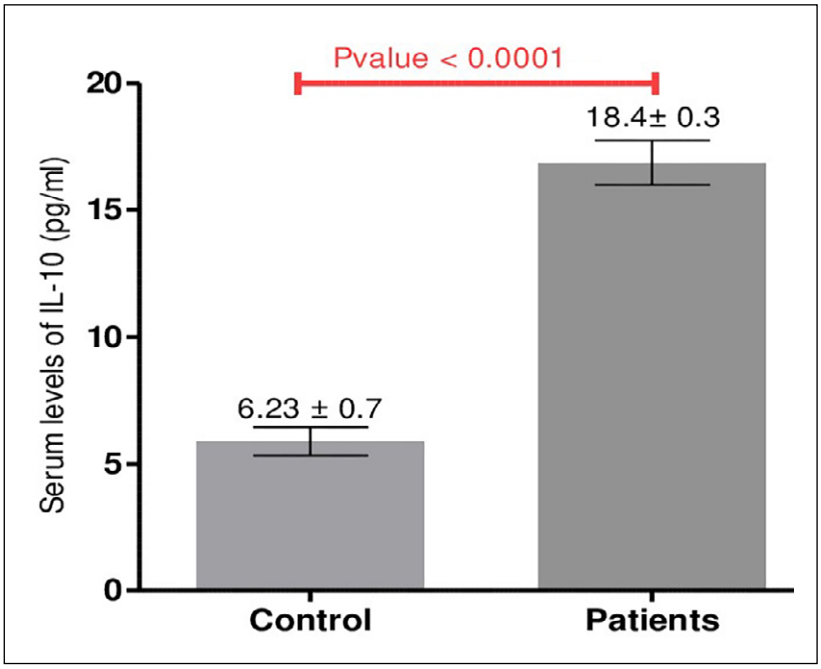

Fig. 2. Concentration of IL-10 (pg/ml) in patients infected with T. gondii compared with the control group (p-value ≤ 0.001).

STATISTICAL ANALYSIS

The mean ± standard error (SE) is used to represent the data. Patient and control group data were compared using a Student`s t-test. The relationships between variables were determined using both (r) and Pearson's correlation. A p-value ≤ 0.001 was considered significant.

RESULTS

Human IL-2 level estimation (pg/ml) KIT ELISA (Fig.1). The current study found that the concentration of IL-2 (pg/ml) in patients with *T. gondii* infection was substantially higher (P≤ 0.001) at 14.5±0.6 pg/ml in comparison to the control group's – 3.06±0.3 pg/ml.

Human IL-10 level estimation (pg/ml) KIT ELISA (Fig.2). The results of the current investigation showed that the concentration of IL-10 (pg/ml) in patients with T. gondii infection was considerably (P≤ 0.001) elevated to 18.4±0.3 pg/ml compared with 6.23±0.7 in the control group.

Human IL-12 level estimation (pg/ml) KIT ELISA (Fig.3) in the current investigation shows that the concentration of IL-12 (pg/ml) in *T. gondii*-infected patients was considerably (P≤0.001) higher at 17.32±0.3 pg/ml than in the control group at 5.04±0.5 pg/ml.

As shown in fig. 4, the concentration of AMH (pg/ml) in patients with *T. gondii* infection was substantially higher (P≤ 0.001) at 5.2±0.1 pg/ml in comparison to the control group's level of 2.5±0.04 pg/ml.

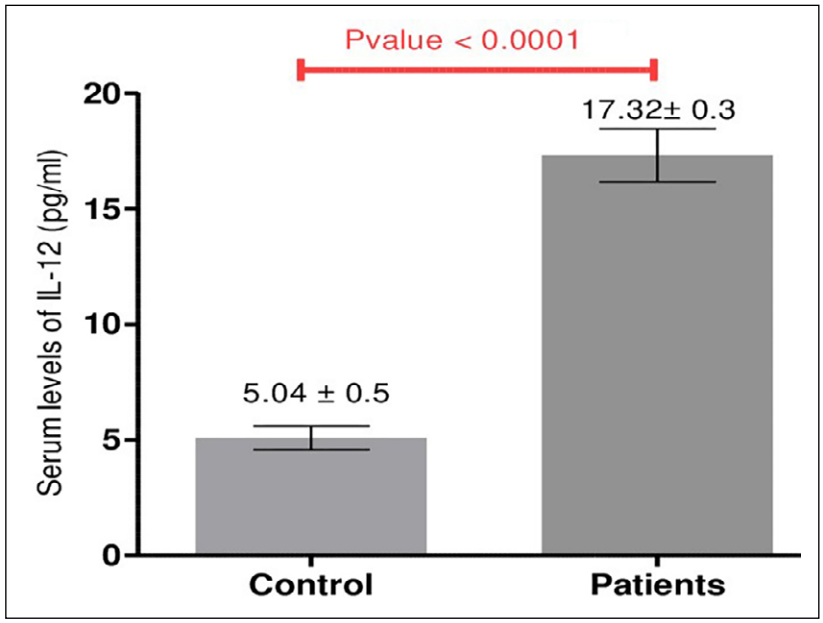

Fig. 3. Concentration of IL-12 (pg/ml) in patients infected with T. gondii compared with the control group (p-value ≤ 0.001).

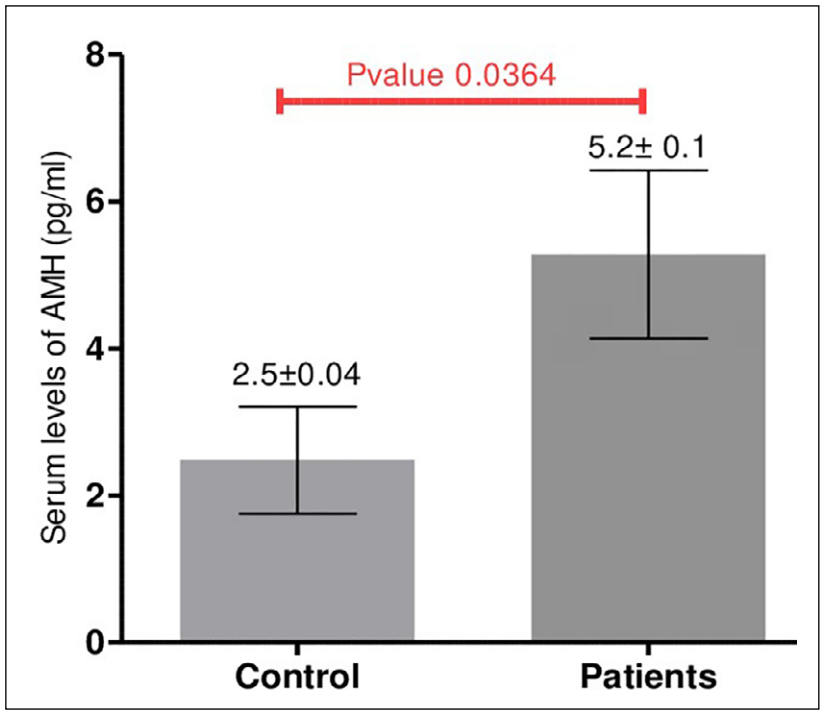

Fig. 4. Concentration of AMH (pg/ml) in patients infected with T. gondii compared with the control group (p-value ≤ 0.001).

DISCUSSION

The study's findings revealed a significant increase in the serum levels of AMH, IL-2, IL-10 and IL-12 in individuals infected with *T. gondii* parasites as compared to the control group. These findings could be attributed to IL-2, IL-10, and IL-12 production aiding in host control of *T. gondii* infection, as we previously demonstrated that *T. gondii* infection increases the synthesis of IL-2 transcripts and activation of primary human monocytes [21]. The current study's findings revealed a considerable increase in the blood serum of those infected with the *T. gondii* parasite as compared to the control group. The reason for this increase was that positive CD4 and CD8 T lymphocytes produce interferon-gamma (IFN-g) naturally, as well as natural killer cells (NK), which are activated when the infection begins by stimulating dendritic cells (DCs) and neutrophil leukocytes for the early production of IL-12, which stimulates natural killer cells to produce interferon (IFN-γ) and is produced in high concentrations by T-lymphocytes during chronic toxoplasmosis infection to prevent reactivation of the parasite's tissue cyst and stimulate macrophagous cells provide antigen presentation, increase the effectiveness of lysosomes in macrophage cells [22], stimulate the effectiveness of natural killer cells,

inhibit the effectiveness of Th2 helper T lymphocytes, and also produce IL-12 positive CD4 T lymphocytes. It works to stimulate the production of IFN-γ from CD8-positive T-lymphocytes; both IL-12 and IFN-g are of fundamental importance in generating host resistance against the toxoplasmosis parasite, and thus, cellular immunity has a role in controlling the infection [24]. The current investigation found a considerable rise in IL-12 in women with polycystic ovarian syndrome. The reason for the increase is that androgen levels rise, which causes macrophages to change into proinflammatory macrophages, which causes the emission of more proinflammatory cytokines and worsens the clinical signs of polycystic ovarian syndrome. One is said to be resistant to obesity. Clinical PCOS is characterized by insulinemia and a change in macrophage polarisation from an anti-inflammatory to a pro-inflammatory M1 state [23]. The current study's findings are in line with another study that found that IL-12 concentrations increased significantly in pregnant women experiencing acute and chronic infections, as well as in women who had abortions. The primary host response to *T. gondii* may involve the generation of derived IL-12, as the level of IL-10 can limit the production of IFN-γ via IL-12 synthesis in host cells. Macrophages, which then increase the number of natural killer cells, and IFN-γ helps start the acute infection that causes the parasite to make IL-10 in the host. This stops the production of IL-12 and lowers the amounts of IFN-γ and IL-2 [24]. The results of the study demonstrated a statistically significant increase in the concentrations of IL-12 and IL-10 in the serum of women infected with toxoplasmosis. These levels of IL-12 and IL-10 stimulate the secretion of the disease from monocytes, which in turn encourages the production of IFN-γ, a key immune response mediator. Against the parasite, IFN-γ controls the inside of the parasite cells in a cell-autonomous manner. In addition, IFN-γ prevents parasitic reproduction in the human body by increasing tryptophan degradation in fibroblasts. Conversely, IL-10 secretion is necessary to maintain normal pregnancy and reduce risks of spontaneous miscarriage [2]. Ovarian reserve analysis is performed to measure the levels of AMH present in the body, which is a protein that is manufactured by granulosa cells located in the ovarian follicle. There is a direct relationship between its percentage and the woman's level of fertility, the higher the percentage, the higher the number of eggs in the body [8]. It is worth noting that its highest levels are usually recorded when a woman reaches approximately 25 years of age. Then, its levels begin to decrease after reaching the age of thirty. It is noteworthy that an AMH analysis can be performed on any day of the menstrual cycle, meaning that its levels remain constant and are not affected during it [18]. Examining the ovarian reserve means trying to predict a woman's ability to reproduce by inferring the number and quality of her remaining eggs. At the time of a female's birth, the ovary contains approximately 2 million eggs, and when she reaches puberty, her egg reserve becomes 400,000 eggs. At the age of thirty, their number decreases [20]. To approximately 25 thousand eggs, so as women age, their chance of conceiving decreases due to the decrease in the number and quality of eggs. Eggs may also be affected by some genetic and environmental factors, some medical conditions, early menopause, and smoking, which are among the most important analyses that predict the number and quality [20]. Ovarian reserve hormone AMH is secreted by small follicles that are less than 4 mm in size in the ovaries. For follicles larger than 8 mm, hormone release first declines and eventually ceases. Because the AMH test's value is constant, it may be performed on any day of the cycle. It is regarded as a crucial fertility test because it shows how many eggs are still in the ovaries based on the analysis of blood samples [25]. Leukemia patients with toxoplasmosis, which is considered a major risk factor for their lives, revealed that the patient's serum levels of IgM and IgG infected with the *T. gondii* parasite were high. At the same time, the concentration of IL-12 in the cells was constant [22]. Hematopoietic cells, along with transplanted or moved cells, increased the production of IFN-γ and NK cells. These cells work together to stop leukemia from growing in the body. It shows that IL-12 can be permanently produced in hematopoietic cells, including transplanted and transferred cells. It also increased the production of IFN-γ and activated natural killer cells, all of which may work together to stop the growth of leukemia in living organisms.

CONCLUSIONS

The current study was conducted to determine the relationship between the levels specific parameters such as IL-2, IL-12, and AMH and their effect on women with toxoplasmosis, where the relationship was positive between the immune parameters. Its levels increased in the event of infection, which increases the possibility of using it as indicators of infection and also to predict the incidence of polycystic ovary syndrome or the occurrence of infertility cases. There is a positive correlation between IL-2, IL-12 with level of AMH.

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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

RECEIVED: 23.12.2023
ACCEPTED: 11.09.2024

ORIGINAL ARTICLE

CONTENTS

In silico study of new isatin- sulfonamide derivatives as carbonic anhydrase inhibitors

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ABSTRACT

Aim: To evaluate compound I, II, III, and IV's anticancer properties that have just been produced. These substances were created with the specific purpose of targeting solid tumors' carbonic anhydrase enzyme.

Materials and Methods: The chemical synthesis involved the use of 4-aminobenzenesulfonamide, Ethyl 4-aminobenzoate, isatin and its derivatives, absolute ethanol, DMF, glacial acetic acid. Docking studies were conducted using the MOE software program version 2015.10.

Results: Since acetazolamide and the sulfanilamide group shared the same pharmacophore, they were chosen. When compared to acetazolamide, compounds II and III produced a maximum score and an irreversible relationship.

Conclusions: Using the Molecular Operating Environment (MOE) software, the binding model and two values the RMSD and S.score—are computed for newly synthesized compounds. When compared to acetazolamide, the theoretically generated compounds showed promise results with these proteins and good binding affinities with the receptor active pocket (S. score: - 6.89, -7.12, -6.75).

KEY WORDS: in silico, cancer, carbonic anhydrase inhibitor, sulfonamide, isatin

Wiad Lek. 2024;77(9):2027-2032. doi: 10.36740/WLek/193997 DOI

INTRODUCTION

As the leading cause of death worldwide and a major impediment to efforts to extend life expectancy, cancer remains a major global health concern. In 112 out of 183 countries, prior to reaching 70 years old, cancer is among the most common causes of death according to data from (WHO) for 2019. Furthermore, in 23 other nations, it ranks as the third or fourth most common cause of death [1-2]. Antitumor medication resistance in malignant cells is primarily accountable for the increased incidence of cancer treatment failure, creating new challenges for the healthcare system [3]. The discovery of novel treatments is required due to the issues with significant toxicity, drug resistance, and poor of selectivity with current chemotherapeutic medications [4]. Bicarbonate ion is produced reversibly by zinc-containing metallo enzymes known as carbonic anhydrases (CAs) [5]. All human CAs (hCAs) are members of the α-class, and humans have at least 15 different (CA) isoforms with varying molecular characteristics, tissue distribution, and subcellular localization [6]. Human cells currently express twelve α family catalytically active (CA) isoforms [7]. Since (hCA) XII expression limits in normal tissues and plays a critical role in pH regulation in a number of cancers (including breast, brain, colorectal, and others), this isozyme has gained interest as a target for antineoplastic therapy development [8]. The most researched class of (hCA) inhibitors (CAIs) is the sulfonamides and related bioisosteres, such as sulfamates and sulfamides, which have been used clinically for more than 70 years to treat obesity, glaucoma, and epilepsy as well as acts as diuretics [9]. Many structurally unique sulfonamide derivatives have finally been found to exhibit strong anticancer activity both in vivo and in vitro [10]. Medicinal chemists are interested in heterocyclic compounds because of their unique chemical properties and range of biological activity [11]. Its possible use as a treatment for a variety of diseases, such as cancer, has been studied [12]. Several naturally occurring alkaloids with indole as their fundamental ring have been found to be therapeutically active drugs, indicating that the indole nucleus is a particularly active nucleus in the pharmacy sector [13]. Isatin's nucleus, sometimes referred to as indole quinone or indanedione, may be viewed as a preferred scaffold for the creation of physiologically active substances [14]. The isatin scaffold could be decorated to produce a variety of biological

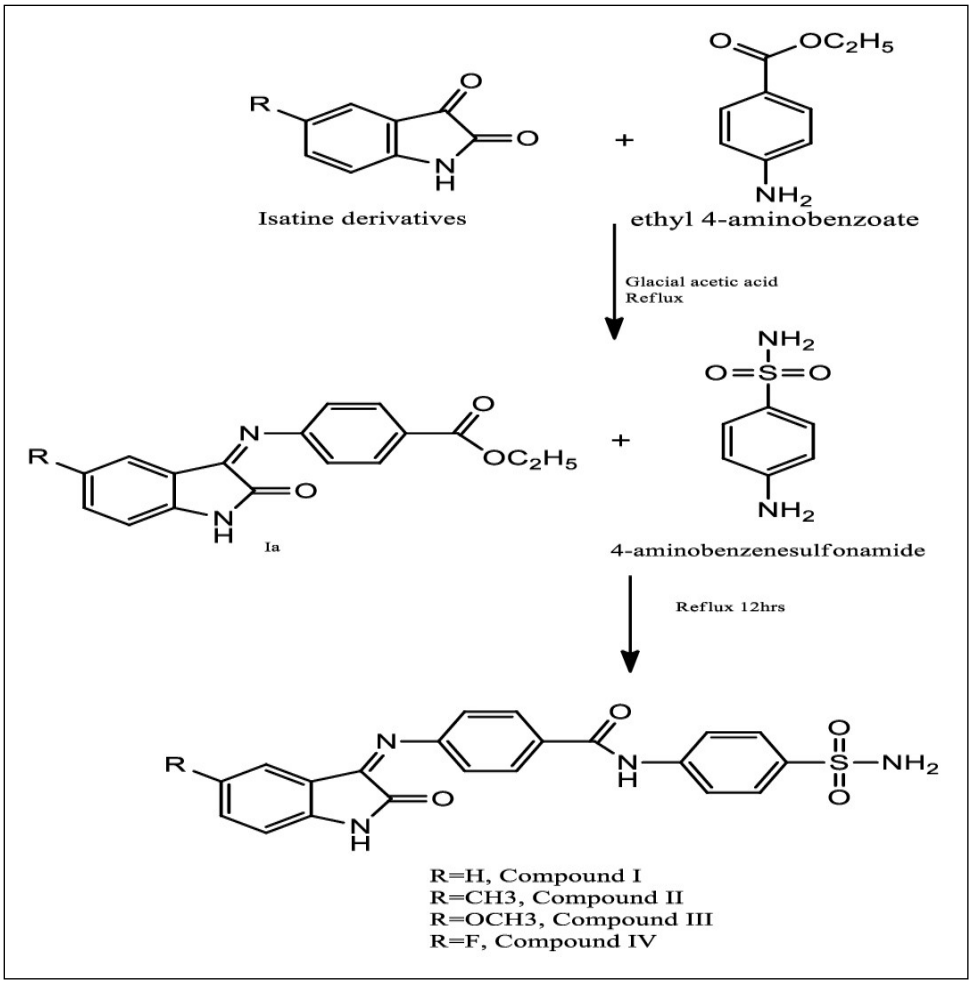

Fig.1. Synthesis of intermediate and final product.

effects, including anti-oxidant, anti-cancer, HIV reverse transcriptase inhibition, neuroprotective, anti-fungal, anti-bacterial, and anti-diabetic properties [15]. Isatin is a viable tail scaffold for creating compounds with promising carbonic anhydrase inhibitory activity profiles against tumor-associated CA isoforms IX and XII [16-17]. Depending on this background benzene sulfonamide as a zinc anchoring moiety linked to an isatin tail through ethyl p-aminobenzoate were created and combined to function as CAIs.

AIM

To evaluate compound I, II, III, and IV's anticancer properties that have just been produced. These substances were created with the specific purpose of targeting solid tumors' carbonic anhydrase enzyme.

MATERIALS AND METHODS

CHEMICAL SYNTHESIS

The following scheme explains the design of a new compounds pathway for it compounds derived from sulfonamide

THE SOFTWARE AND SYSTEM OF THE COMPUTER

We utilize Chem Draw professional Software Pro 12.0 and Molecular Operating Environment (MOE) 2015 both of which is downloaded.

LIGAND AND RECEPTOR PREPARATION WITH MOLECULAR DOCKING PROCEDURE

Chem Draw Professional (12.0) was used to precisely draw the ligand molecular structures. Following that, the ligand is protonated in a three-dimensional shape, partial charge is added, energy is minimized, and the results are saved. We extract the receptor from (MOE), which is the crystal structure of genetically altered CA XII (PDB: 1JCZ /chain A).

The following steps are used to prepare the target protein:

The remaining chain sequences were removed, leaving only the ones implicated in the protein function. The minor molecules were eliminated. Additionally, molecules of water were eliminated. Fixing the potential of the protein atoms and determining its active site comes first, as adding hydrogen conceals bonds.

Table 1. Binding properties of newly synthesized compounds with CA XII (PDB: 1JCZ/ chain A)

Compound	Docking S- scores in ΔG (Kcal/mol)	RMSD	Number of binding sites	Molecules that involve in binding
ACTAZOLAMIDE	-5.82	2.094	3	Zn 3:901, Thr199, Lys67
I	-6.64	1.321	4	Zn 3:901, Thr199, His96, His94
II	-6.89	1.678	5	Zn 3:901, Thr199, His96, His94, Ser132
III	-7.12	1.921	6	Zn 3:901, Thr199, His119, His96, Ser132, Leu198
IV	-6.75	1.211	7	Zn 3:901, Thr200, Thr199, His94, Lys67, Ser132, Leu198

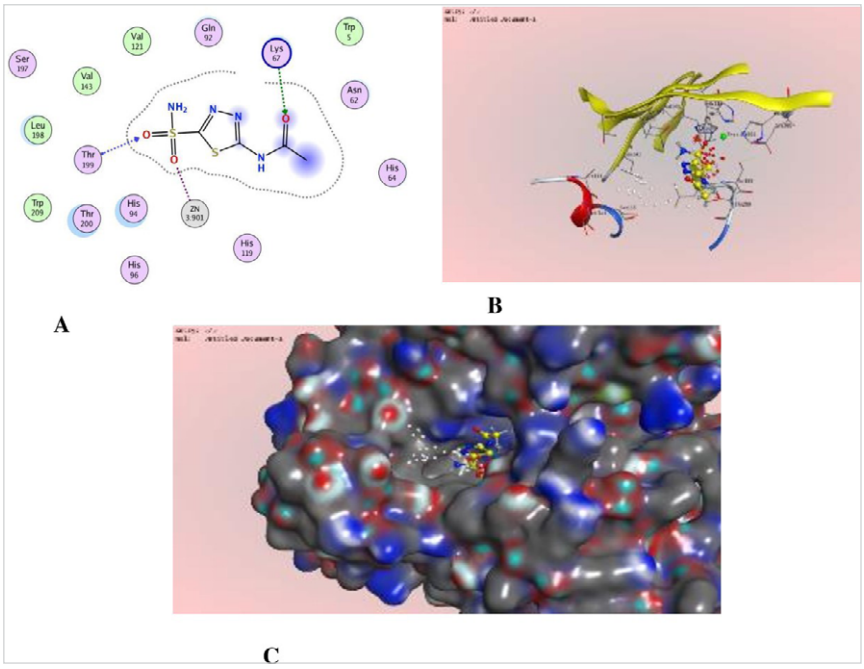

Fig. 1. Acetazolamide with Carbonic anhydrase XII (PDB code: 1JCZ), where (A) explain the 2D picture of binding Acetazolamide with active site, (B) explain the 3D picture of binding Acetazolamide with an active site and (C) explains the 3D picture of entrance and binding with whole protein.

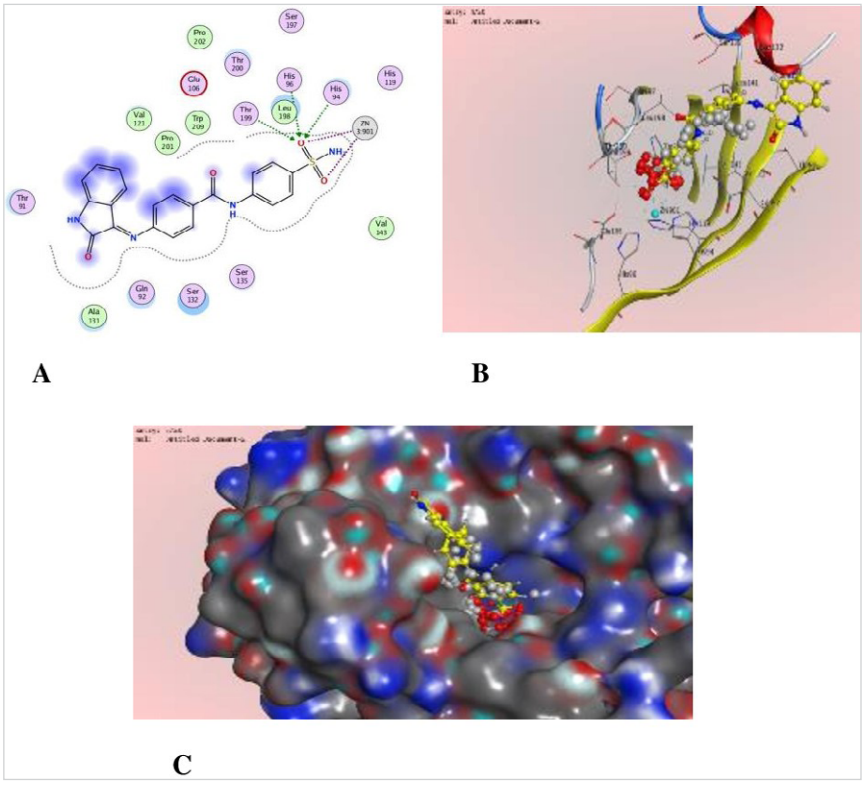

Fig.2. Compound I with Carbonic anhydrase XII (PDB code: 1JCZ), where (A) explain the 2D picture of binding Compound I with active site, (B) explain the 3D picture of binding Compound I with an active site and (C) explains the 3D picture of entrance and binding with whole protein.

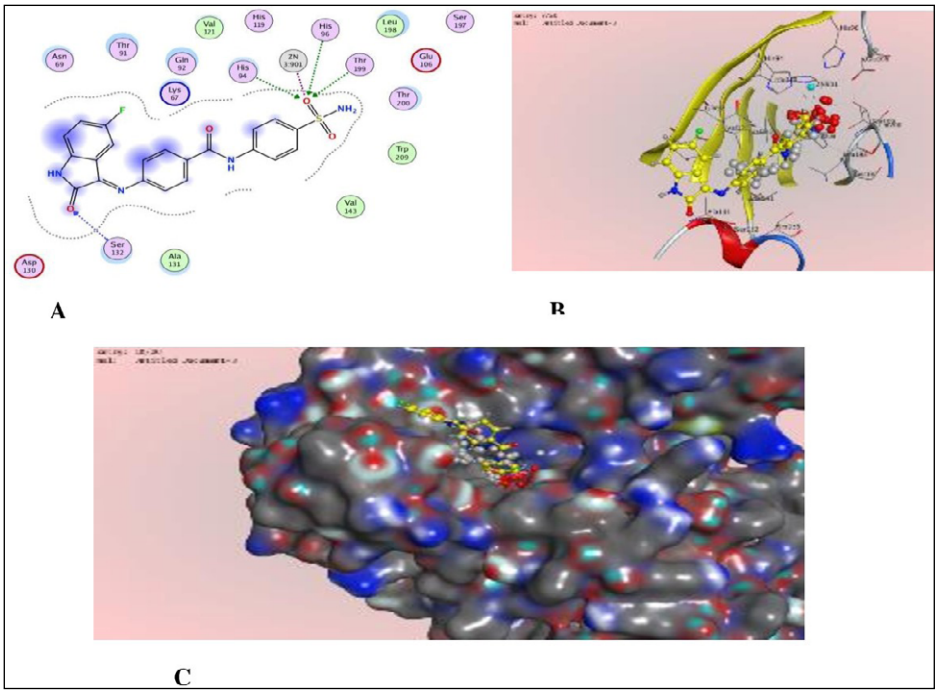

Fig. 3. Compound II with Carbonic anhydrase XII (PDB code: 1JCZ), where (A) explain the 2D picture of binding Compound II with active site, (B) explain the 3D picture of binding Compound II with an active site and (C) explains the 3D picture of entrance and binding with whole protein.

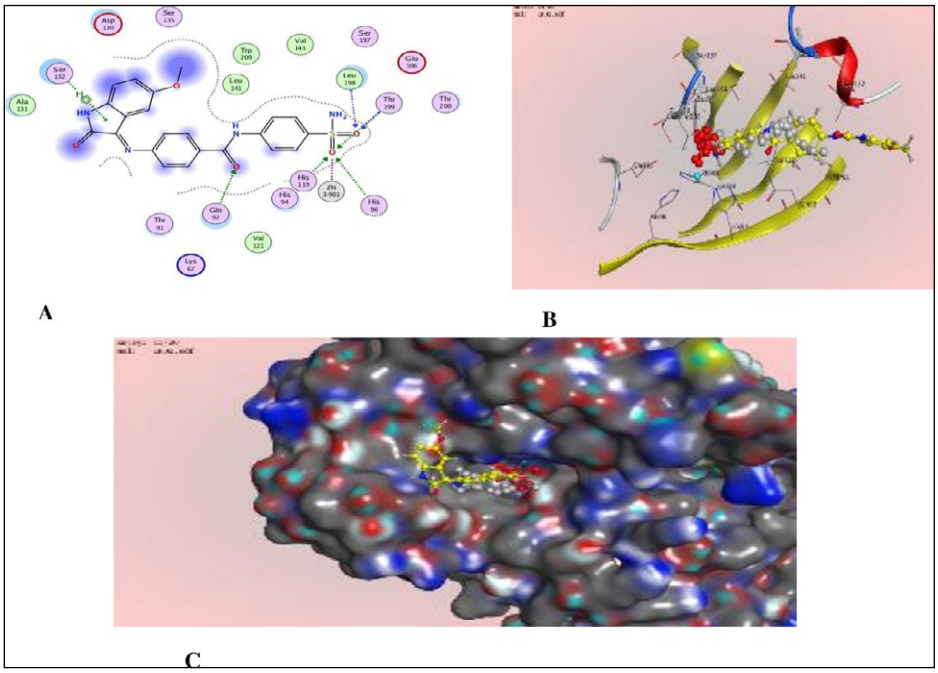

Fig.4. Compound III with Carbonic anhydrase XII (PDB code: 1JCZ), where (A) explain the 2D picture of binding Compound III with active site, (B) explain the 3D picture of binding Compound III with an active site and (C) explains the 3D picture of entrance and binding with whole protein.

The final step involves loading the previously generated ligand from saved data into MOE and starting the docking process [18].

RESULTS

MOLECULAR DOCKING AND VIRTUAL SCREENING

The goal of molecular docking is to use computer-based techniques to prepare for the ligand-receptor complex [19]. The software also offers capabilities for comparing the binding modes of several ligands to the same protein target and for displaying and analyzing the docking data [20]. Molecular Operating Environment (MOE) explains binding specificity of new synthesized compounds to carbonic anhydrase XII enzyme in the same manner as the active site of acetazolamide. The developed compounds' inhibitory actions were graded depending on the value of S. Score and Rmsd (Root mean square deviation), which is showing distance average between the atoms of the pose and original ligand for the site of the anti-cancer that studied, and the similarity in amino acids that entering in the inter-

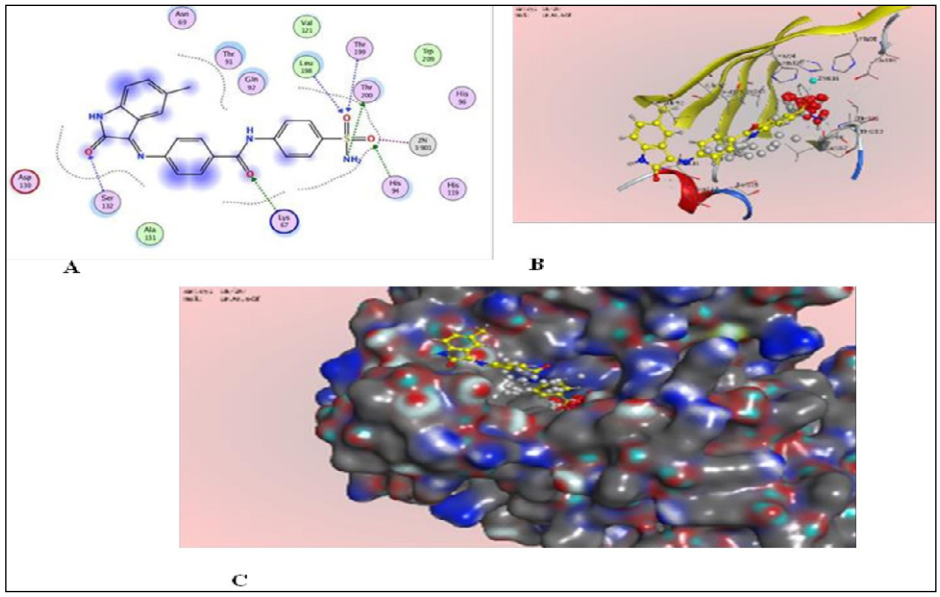

Fig.5. Compound IV with Carbonic anhydrase XII (PDB code: 1JCZ), where (A) explain the 2D picture of binding Compound IV with active site, (B) explain the 3D picture of binding Compound IV with an active site and (C) explains the 3D picture of entrance and binding with whole protein.

action on the same active site. Interaction between acetazolamide and its interaction site, consisting of Zn 3:901, Thr199, and Lys67 (Table 1).

DISCUSSION

The compounds' effectiveness as anticancer drugs against the carbonic anhydrase XII enzyme were assessed by the Molecular Operating Environment docking results; most of the compounds that were examined had good binding affinity with target proteins in comparison to acetazolamide. With the highest s. score (–7.12), compound III demonstrates how the methoxy substitution improves the orientation of the proposed ligand in the receptor pocket. In comparison to other molecules, the compound IV with methyl substitution created more hydrogen bonds with a number of significant amino acid residues in the protein, giving it a more stable orientation and a stronger binding affinity. Compound II with a fluoro substituent exhibits a high S. score (–7.46) and good rmsd. On the other hand, the inhibitor lacking substitution exhibits a reduced s. score (–6.64) and a low Rmsd (1.321).

CONCLUSIONS

This work aims to quantify and enhance the in-Silico interaction between sulfonamide derivative chemicals and the carbonic anhydrase enzyme. To evaluate the effectiveness of newly synthesized compounds as carbonic anhydrase XII inhibitors, use the Molecular Operating Environment (MOE). When compared to acetazolamide, most of the compounds revealed an enhanced binding affinity with the target proteins. All compound has greatest S. score especially compound III had the greatest S. Score (-7.12), suggesting that the methoxy alteration improves the recommended ligand's orientation within the receptor pocket. This displayed the substitution of isatin improves binding interaction and improves flexibility binding with carbonic anhydrase XII.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 26.05.2024
ACCEPTED: 01.10.2024

ORIGINAL ARTICLE

CONTENTS

Molecular study of FAM20A gene and biochemical analysis for amelogenesis imperfecta patients

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ABSTRACT

Aim: This study aimed to diagnose Amelogenesis Imperfecta patients if have an isolated type or are related to a syndrome such as enamel renal syndrome.

Materials and Methods: This case-control study included (60 patients and 20 controls). DNA extraction from the blood sample then used the Conventional PCR reaction and Agarose Gel Electrophoresis. The generated PCR fragments were subjected to Sanger sequencing. Geneious software showed the genotypes after aligning with a reference sequence in the Gene Bank. In addition, biochemistry analyses are performed by using a spectrophotometer.

Results: The FAM20A gene was presented with three genotypes (TT, TC, CC) and two alleles (T and C), no significant variations were found. There was a positive correlation between the TT genotype with Amelogenesis Imperfecta; this means that patients whom caring TT genotype have a risk for Amelogenesis Imperfecta than other genotypes and non-significant relation regarding serum creatinine, potassium, and calcium while the serum urea and alkaline phosphatase have significant results.

Conclusions: patients who carry for TT genotype have a higher risk for Amelogenesis Imperfecta than other genotypes and no significant relation between creatinine, potassium, and calcium, while the serum urea and alkaline phosphatase show a significant relation.

KEY WORDS: Amelogenesis Imperfecta, nephrocalcinosis, enamel renal syndrome, FAM20A

Wiad Lek. 2024;77(9):2033-2042. doi: 10.36740/WLek/194014 DOI

INTRODUCTION

Amelogenesis imperfecta (AI) is a term used to describe a group of genetically based developmental conditions that impact the composition and clinical look of enamel in all or nearly all of the teeth in a roughly equal way. These conditions may also be linked to morphologic or biochemical alterations in other parts of the body [1]. Amelogenesis imperfecta and nephrocalcinosis (NC) together may indicate pleiotropism or a contiguous gene syndrome. According to one theory, there may be an underlying anomaly in the interstitial architecture that causes the kidney's dystrophic calcification and the teeth's aberrant enamel formation [2]. Further studies are needed to determine the involvement of dental proteins in phosphate and calcium metabolism and renal function since another idea proposes that many of the proteins previously believed to be specific to tissue may also be found in non-dental tissues [3]. The autosomal recessive disorder known as enamel renal syndrome (ERS) is rare and poorly characterized. Numerous terms with comparable features have been suggested, including AI and NC syndrome [4, 5]. These conditions are believed to be signs of a single sickness caused by underlying abnormalities in the FAM20A gene [6]. Clinically, typical oral characteristics include hypoplastic (AI), postponed tooth eruption, calcification in the pulp, hyperplastic dental follicles, various degrees of gingival hyperplasia, and the presence of calcified nodules [7]. Other renal diseases, such as NC, have also been described as frequent findings, especially in early adulthood [8]. Additionally, a condition called NC and other renal disorders have been reported often, especially in adolescents and young adults. It follows that even those with oral symptoms but no-renal issues who have balletic FAM20A mutations are thought to eventually develop NC [9]. Because FAM20A encodes proteins that are produced in suprabasal cells in the gingiva, odontoblasts and dental pulp, as well as in ameloblast throughout the secretory and maturation phases of enamel formation, these proteins are essential for gingival homeostasis and enamel development. It has been demonstrated that many FAM20A variants, including it end gain, missense, frame shift, and splice-site mutations, are present in individuals with the ERS phenotype [10-14].

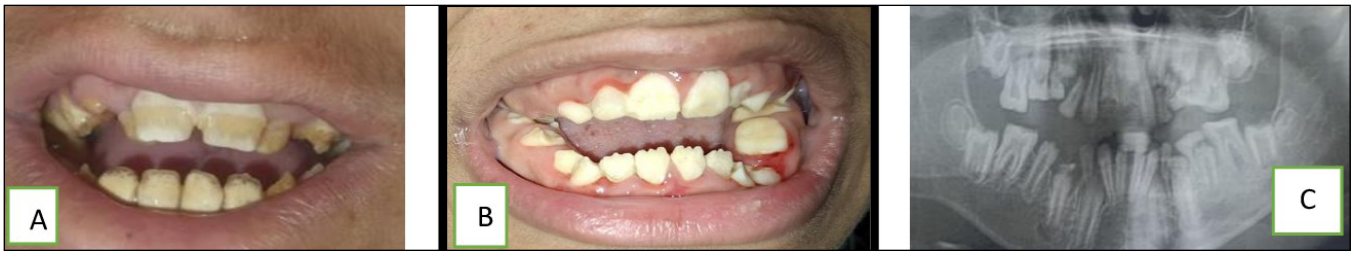

Fig. 1. A: clinical appearance of AI patient; B: AI patient with gengival enlargment; C: radiological appearance of AI patients in this study.

Table 1. Primers developed for the study

Primer	Sequence (5′→3′ direction)	primer size, bp	Product size, bp	TM (°C)
		FAM20A		
Forward	AAGGACCCCACAGGTGTTTT	20	711	60.64
Reverse	TGTGTCTCAGTTTCCCTGTCTG	20		60.33

AIM

This study aimed to diagnose patients with amelogenesis imperfecta if have an isolated type or are related to a syndrome such as enamel renal syndrome and this study represents the first study to take this number of patients with molecular and biochemical analyses.

MATERIALS AND METHODS

STUDY DESIGN AND PARTICIPANTS

The resident population of Al Salam Township in Diyala City was assessed for AI and enamel renal condition in a cross-sectional study conducted between April 25, 2022, and October 16, 2023. A study sample of sixty individuals with AI, diagnosed clinically as shown in (Fig.1-A and 1-B) and radiologically by take extraoral radioghraph as shown in (Fig. 1-C) and enamel kidney condition and 20 patients as a control group were then included in the study, the age of the sample was arranged between 21-45 years for both genders.

BLOOD COLLECTION

Five milliliters of blood were split into two tubes for our study: two milliliters were placed in an EDTA tube and frozen at -20°C, while a gel tube containing three milliliters was centrifuged for five minutes. After that, the serum was transferred to a biochemical lab in a tube that had been prepared for testing.

BIOCHEMICAL LABORATORY METHOD

Human creatinine, urea, calcium potassium and alkaline phosphatase levels in the serum samples were determined using the spectrophotometer.

STUDY PRIMERS

The University Code of Student Conduct (UCSC) programs double-checked the primers, which were created with Primer 3plus, V4, and had their reference sequences verified by the National Center for Biotechnology Information (NCBI) database. Alpha DNA Ltd. (Canada) produced and lyophilized them. All primer sequences used in the tests for this investigation are included in (Table 1).

PREPARING OF PRIMERS

For each assay in this study, the required primers, as shown in table 1, were prepared as follows: after dissolving the lyophilized sample in nuclease-free water according to the manufacturer's instructions, a stock solution with a concentration of 100µM was prepared and stored at -20°C. Diluting 10µL of each primer stock solution in 90µL of nuclease-free water yielded a working solution with a concentration of 10µM, which was maintained at -20°C until use.

DNA SEQUENCING AND GENOTYPING

DNA EXTRACTION FROM THE BLOOD SAMPLE

Using the *Easy Pure*® Genomic DNA Kit (Trans Gen, biotech EE101-01), one milliliter of whole blood was centrifuged for 1 minute/12000 rpm or 5 minutes/5000 rpm. After removal of the supernatant, blood was resuspended in 500 µl TE buffer and stored at -20 °C until ready for evaluation.

DNA CONCENTRATION AND PURITY ASSESSMENT

TE buffer was used as a blank solution before a Nano-drop 2000c spectrophotometer (Thermo

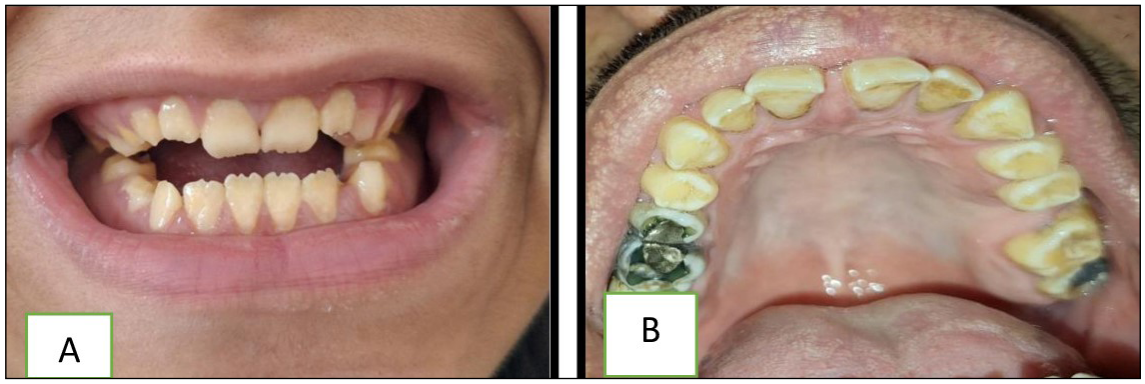

Fig. 2. A: female amelogenesis imperfcta patient; B: male amelogenesis imperfcta patient.

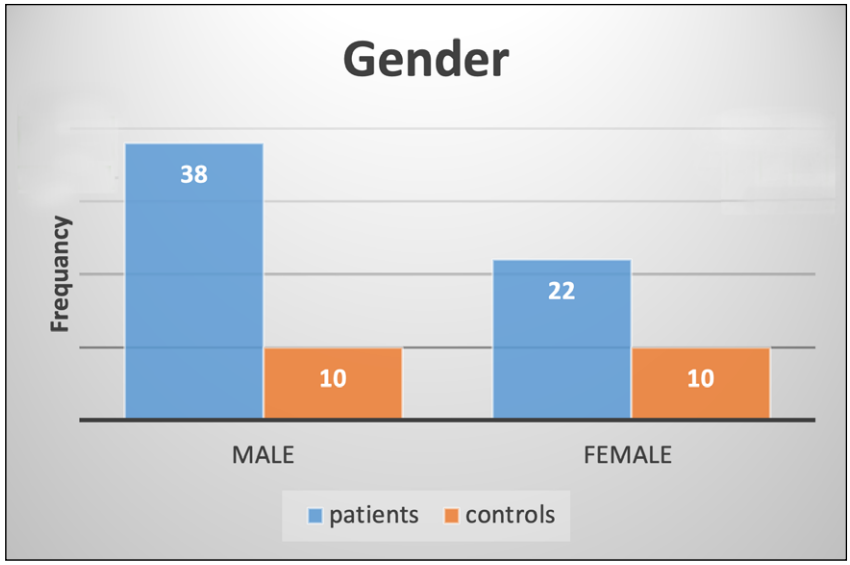

Fig. 3. Distribution of patients into groups according to gender.

Fisher Scientific) with basic computer control and data logging software was used to determine the concentration of DNA samples that demonstrated acceptable integrity. To find the concentration in ng/µl, two microliters of the isolated DNA were put into the Nano-drop.

CONVENTIONAL PCR REACTION

When running the PCR, the reaction was tuned by testing four annealing temperatures: 56, 58, 60 and 62°C. An annealing temperature of 58 °C was found to be optimal for producing clear and sharp bands in agarose gel, hence it was used in the current study. This protocol employs 2xEasyTaq® PCR Super Mix. All PCR reactions were carried out in a 25 µl final volume and according to the manufacturer's instructions.

AGAROSE GEL ELECTROPHORESIS

Extracted DNA and amplified PCR fragments were separated on an agarose gel and then examined under UV light after ethidium bromide staining.

DNA LOADING AND ELECTROPHORESIS

A mixture of 3 µl loading dye and 7 µl isolated genomic DNA (or PCR product) was loaded into the wells of the gel. Following the loading of all wells, the electrical power was turned on at 100 volts (5V/cm^2) for 60 minutes. This caused DNA with a negative charge to migrate from the cathode (-) to the anode (+) poles.

AGAROSE GEL STAINING AND UV VISUALIZATION

After staining the electrophoretic gels with ethidium bromide, which was made by adding 70µl of the 10 mg/ml ethidium bromide to 300 ml of D.W., the gel was stained by soaking in the solution for 20-30 minutes, and then the gel was placed into the gel documentation system to view the DNA bands at a 365 nm wavelength. Special software was used to save the photos captured by the device on the computer [15].

DNA SEQUENCING

The generated PCR fragments were subjected to Sanger sequencing using an ABI3730XL automated

Table 2. Comparison between patients and control groups by age

Groups	Mean	Std. Deviation	mean P-value	p-value
Patients	31.63	8.42	1.09	0.4 NS
Control	30.00	7.90	1.78	

Table 3. Comparison of data between patient and control groups for urea, creatinine, calcium, potassium and alkaline phosphatase

Groups		Urea	Creatinine	Calcium	potassium	ALP
Patients	Mean	37.61	0.66	9.75	4.46	58.11
	Std. Deviation	8.88	0.11	0.87	0.33	8.88
	Std. Error of Mean	1.15	0.01	0.11	0.055	1.15
Control	Mean	30.67	0.61	9.48	4.37	67.85
	Std. Deviation	6.33	0.15	0.62	0.49	11.45
	Std. Error of Mean	1.41	0.03	0.14	0.11	2.56
	p-value	0.002**	0.07 NS	0.1 NS	0.4 NS	0.001**

** - significant; NS non-significant.

Table 4. Pearson correlation between patients and controls according to age for urea, creatinine, calcium, potassium and alkaline phosphatase

		Age	Urea	Creatinine	Calcium	Potassium	ALP
Age	Pearson Correlation	1	0.385	0.244	0.181	-0.158	0.128
	Sig. (2-tailed)		0.000	0.029	0.108	0.160	0.260
Urea	Pearson Correlation		1	0.541	0.019	0.075	0.051
	Sig. (2-tailed)			0.000	0.866	0.506	0.651
Creatinine	Pearson Correlation			1	-0.119	0.393	0.081
	Sig. (2-tailed)				0.293	0.000	0.475
Calcium	Pearson Correlation				1	-0.014	-0.022
	Sig. (2-tailed)					0.899	0.845
Potassium	Pearson Correlation					1	-0.162
	Sig. (2-tailed)						0.151
ALP	Pearson Correlation						1
	Sig. (2-tailed)						-

DNA sequencer (Macrogen Corporation, Korea). After matching the genotypes with the reference sequence in the GenBank, clever software displayed the genotypes.

STATISTICAL ANALYSIS

The IBM SPSS Statistics 26 application was utilized to determine how various factors affected the study's parameters. The T-test and one-way ANOVA were used to statistically compare means. A meaningful comparison between percentages (0.05 and 0.01 probability) was made using the chi-square test. Graph Pad Prism 9 was used to create the figures in this investigation.

RESULTS

DISTRIBUTION OF STUDIED SAMPLES ACCORDING TO GENDER

The study sample consisted of 60 AI patients of both sexes: there were 22 (36.7%) females and 38 (63.3%) males (Fig.2A-B).

Control group with comparable age and gender makes up the second group. These twenty-odd healthy-looking people 10 males and 10 females showed no signs of illness or noticeable anomalies.

There are no significant results when comparing patients and control individuals with a p-value equal to 0.2 (Fig.3.).

DISTRIBUTION OF SAMPLES ACCORDING TO AGE GROUP

Table 2 shows the association status between the two groups of AI patients and control individuals according to age. The outcome showed that the observed frequency distributions in the two samples among various age groups showed non-significant differences at P>0.05. The mean age of AI patients is 31.63 ± 8.42 and the mean for control individuals was 30.84 ± 7.90 (Table 2).

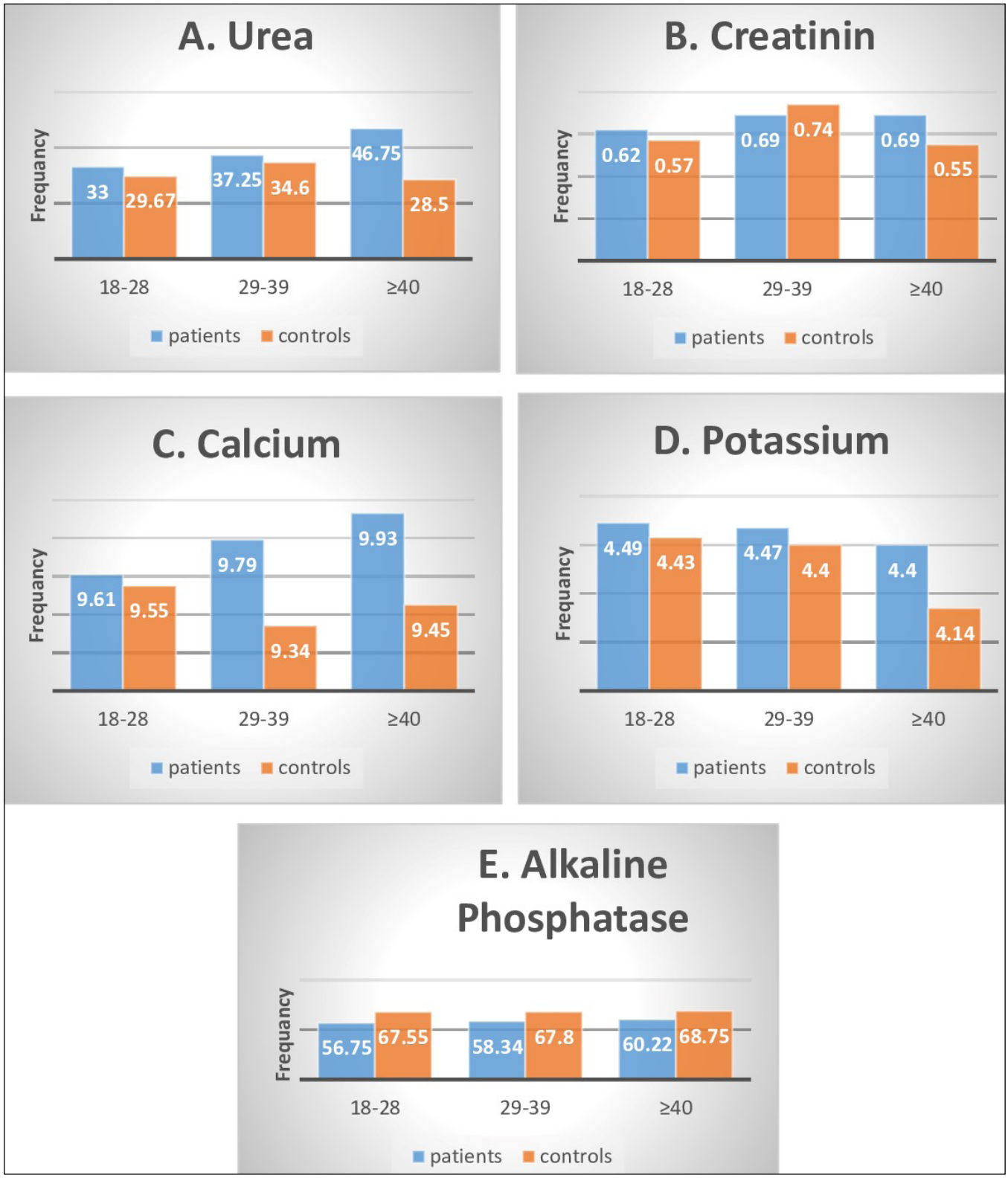

Fig. 4. Comparison between patients and controls for (A) urea, (B) creatinine, (C) calcium, (D) potassium, (E) alkaline phosphatase.

Figure 4 shows the comparison between age groups in amelogenesis imperfecta patients and healthy individuals, so when observed non-significant between two different age groups for biochemical markers (creatinine= 0.4, calcium=0.6, potassium = 0.005, and alkaline phosphatase=0.5) while the P-value for urea= 0.001 represent significant result (Fig.4 A-E).

Table 3 investigates the serum urea in the patient and control group with the mean and SD. The value was evident, with a mean value of 37.61, while the mean

Fig. 5. FAM20A gene sequencing was received from the Gene Bank for both cases and controls. Samples are represented by the query, while the subject is represented by the data from the NCBI database.

value of serum urea for the control group was 30.67 and significant difference was found between them. On the other side, the serum creatinine for AI patients was equal to 0.66 and that was non-significant when compared with control individuals who have a mean of serum creatinine equal to 0.61. Another test taken in this study was serum calcium and this test recorded a mean for the patient of 9.75 and had non-significant relation with the control individual who has a mean equal to 9.48. On the other hand, we investigate the serum potassium and found that its means for patient and control groups equal 4.46 and 4.37 respectively, and the relation between them was non-significant. The last analysis performed, the alkaline phosphatase biochemical analysis, showed a significant correlation between the mean value for patients 58.11 and the mean value for controls 67.85 (Table 3).

Table 4 presents the results of Pearson correlation between patients and control groups depending on age for urea, creatinine, calcium, potassium and alkaline phosphatase. Regarding t the testing of the relationships between age factors and biochemical markers, the results showed a non-significant difference among them at $P>0.05$, while highly significant differences were obtained between the levels of each factor's tests independently at $P<0.001$, (i.e., among age factors, and biochemical markers).

FAM20A GENE

Two alleles (T and C) and three genotypes (TT, TC, and CC) were found for the FAM20A gene. The genotypes in the sick and healthy groups were found to be compatible with the Hardy-Weinberg equilibrium (HWE),

and there were no discernible variations between the predicted and observed genotype frequencies in blood samples (Table 5).

Inspecting of FAM20A gene genotypes and allele frequencies in patient groups and control group revealed that the homozygous genotype frequency of T-allele showed non-significant variation in patients compared to control (35 vs. 58.4%), and there was a positive correlation (etiological factor) between TT genotype with AI, which means that patients whom caring TT genotype have a risk for AI than other genotypes, although patients had a decreased frequencies of the T allele (93 vs. 77%) and an increased frequency of the C allele (27 vs. 23%) compared with the control group (Table 6).

FAM20A GENE SEQUENCING RESULTS

In the current investigation, the targeted area of the FAM20A gene of AI and control was amplified using a PCR reaction on 80 blood samples. Amplification of this region was confirmed by agarose gel electrophoresis. Using ethidium bromide dye, the FAM20A gene PCR amplified products were electrophoresed on an agarose gel and the resulting figure shows that all samples had a successful double band amplified fragment size of 220 bp. DNA samples (PCR product) were obtained by a certain company (Macrogen). Analysis has been done on the sample DNA sequencing data. Alignment was performed using the NCBI nucleotide alignment tool, Nucleotide BLAST (Basic Local Alignment Search Tool), whereby the software Geneious Provision: 7.0.0 comparing the DNA sequence acquired in this investigation with the sequence of the human FAM20A reference gene NG_029809 (Table 7, Fig.5).

DISCUSSION

There are a few limitations to this study because AI is an uncommon condition. The purpose of this study was to investigate the relationships between biochemical analysis and AI. The patients' ages varied from 21 to 45 years, and there was no discernible difference in age across the groups (p=0.4) , so the comparison between the two groups is more accurate because the biochemical analyses changed as a result of age [16]. Regarding gender, the findings can be the consequence of disparities in the sample size and ethnic variation. The gender distribution in the patient as well as control groups was not identical. Of the 60 patients with AI of both sexes in the research sample, 38 (63.3%) were men and 22 (36.7%) were women, while Vani et al. [17] found 33 (47.82%) were women and 36 (52.17%) were men.

SERUM UREA

When comparing patients with renal illness with the control group, there were extremely significant increases in the levels of urea and creatinine [18], so when comparing the AI patients' thoughts about having enamel renal syndrome to the healthy control group, the current study demonstrated a significant prevalence of blood urea within the normal range. This finding is consistent with the data published by Ergun and Ataol [19], which demonstrates that the patient's clinical examination revealed inadequate enamel thickness and that the serum urea level in the laboratory was normal. This outcome is consistent with Nitayavardhana et al. [7], which investigated the diagnostic oral profile of recessive FAM20A mutation-induced enamel renal disease and came to the same conclusion.

SERUM CREATININE

The current study found no differences between the levels of serum creatinine in AI patients suspected of having enamel renal syndrome when compared with healthy control group and this was represented by the no significance of the result and this matches with another research [7, 19, 20], while Roomaney et al. [8] found that 7.2% of patients have elevated serum creatinine.

SERUM CALCIUM

Since calcium makes up a majority of bones and teeth, it is not unexpected that abnormalities in the metabolism of calcium are linked to the majority of chronic illnesses, such as osteoporosis, renal disease, and periodontal tissues [21]. In this study, the result of serum calcium was within the normal range for AI patients suspected to have renal enamel syndrome and non-significant difference when compared with healthy individuals, and this result same as the result of Vogel et al. [22]. Also, this result matches to some extent the results of other studies [7, 19, 20].

SERUM POTASSIUM

Obtel et al. [23] found low serum potassium concentrations in patients who displayed severe enamel wear, which is not similar to our result that found non-significant relation between patients and healthy individuals.

ALKALINE PHOSPHATASE (ALP)

ALP is linked to mineralization, promoting and/or accelerating the mineralization process. Amelogenesis

Table 5. Numerical and percentage frequencies of FAM20A gene genotypes and their Hardy-Weinberg equilibrium (HWE) in the control group and patients group.

FAM20A	Patient group		Control group	
	Observed	Expected	Observed	Expected
Wild TT	35	36.038	12	12.800
Hetero TC	23	20.925	8	6.400
Mutant CC	2	3.038	0	0.800
Total	60	60	20	20
p-value	0.4		1.00	

Table 6. Genotype and allele frequencies of the FAM20A gene in control and patient groups in blood samples.

Genotype FAM20A	Patient group (n=60)	Control group (n=20)	P-value	OR	CI 95%
TT	35 (58.4%)	12 (60%)	--	1.00	(Reference)
TC	23 (38.3%)	8 (40%)	0.9	0.98	0.3492 to 2.7828
CC	2 (3.3%)	0 (0%)	0.7	1.6	0.0790 to 39.2411
Total	60	20	--	--	--
		Allele Frequency			
T	93 (77%)	32 (80%)	--	1.00	(Reference)
C	27 (23%)	8 (20%)	0.7	1.1	0.4791 to 2.8148

Table 7. Sequencing ID at gene bank, score, expects, and compatibility of DNA sequences obtained

Score	Expect	Identities	Gaps	Strand
1055 bits (571)	0.0	579/582(99%)	3/582(0%)	Plus/Plus

may be impacted by a potential pathway of phosphate movement through cells and matrix, similar to what has been proposed for the bone [24]. Enzymes like alkaline phosphatase are currently useful biochemical indicators for detecting of chronic periodontitis [25]. Ali et al. found that the ALP enzyme level was significantly higher than the non-periodontitis group [26]. In this study, there is a significant relationship between patients' alkaline phosphatase and control alkaline phosphatase, and this disagrees with Vogel et al. [22]. The increased activity of ALP indicates that the pathological destructive process has affected the alveolar bone, which means that the periodontal disease has significantly advanced [27]. Ozdas et al. discovered that blood ALP levels in AI subjects are statistically significantly aberrant, and the results are consistent with our investigation even in the occurrence of an additional condition [28].

FAM20A

Ding et al. identified autosomal recessive mutations in FAM20A as the cause of ERS [6], and also Kantaputra et al. found a relationship between a mutation in FAM20A and ESR [14]. Wang et al. found that FAM20A, a putative Golgi kinase with a kinase homology domain that locates in the Golgi, is important for regulating biomineralization processes, and those polymorphisms in FAM20A result in ERS [11].

CONCLUSIONS

Patients who carry for TT genotype have a higher risk for amelogenesis Imperfecta than other genotypes and have no significant relation between creatinine, potassium and calcium, and the biochemical analysis done for serum urea and alkaline phosphatase shows a significant relation between the patients and the control individuals.

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This investigation was carried out by local laws and the ethical guidelines provided in the Declaration of Helsinki. The local ethics committee provided ethical approval and provided the research protocol, informed permission forms, and subject information, University of Baghdad (Ref. 445, Jan 2022).
Contributions from MTI were gathering of data, statistical analysis, findings, discussion, draft, and final writing. The primary concept and critical revision were brought to by SSA.

CONFLICT OF INTEREST
The Authors declare no conflict of interest

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RECEIVED: 12.06.2024
ACCEPTED: 02.10.2024

ORIGINAL ARTICLE

CONTENTS

Correction of dysmenorrhea in teenage girls with autonomic dysfunction syndrome

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ABSTRACT

Aim: The aim of our study was detection and correction of dysmenorrhea in teenage girls with autonomic dysfunction syndrome.

Materials and Methods: The research group consisted of 37 teenage girls, aged 12-16 years, who were undergoing rehabilitation treatment with signs of dysmenorrhea and vegetative dysfunction syndrome. The study lasted for 2 years (2020-2021). A study of the variability of the heart rate at rest and under the conditions of an orthostatic test was carried out using the "Cardiolab" complex by the method of spectral analysis.

Results: Analyzing the initial state of the autonomic nervous system in girls with menstrual cycle disorders, the primary predominance of the sympathetic nervous system in 33.3% of girls was revealed. Before treatment, autonomic reactivity was characterized by a hypersympathetic type in 44.4% of girls, by an asympathetic type in 22.3% of children. Spectral analysis at the beginning of treatment showed that high frequencies (HF) prevail in most girls (66.0%), which indicates an increased influence of the parasympathetic part of the autonomic nervous system, and 18.5% of girls have a balanced state of the autonomic nervous system. Predominance in the spectrum of LF and VLF components was found in 15.5%, which indicates the predominance of central humoral-metabolic ergotropic effects on heart rate variability. After the course of treatment an improvement in the condition of teenage girls was observed. Menstruation became less painful or painless at all, the duration and frequency of menstruation (from 1 to 3 days, on average 1.8±0.1) and blood loss decreased. The girls felt better, did not complain of dizziness and headache.

Conclusions: According to the results of the study, it was found that the proposed course of treatment for teenage girls with a menstrual cycle disorder with signs of autonomic dysfunction contributed to the normalization of the cycle, improvement of the general condition of the girls.

KEY WORDS: dysmenorrhea, teenage girls, heart rate variability

Wiad Lek. 2024;77(9):2043-2046. doi: 10.36740/WLek/196162 DOI

INTRODUCTION

Protecting the reproductive health of the population is one of the most important tasks today. According to the reports of researchers from different countries, dysmenorrhea occupies the main place in the structure of gynecological diseases. It accounts for more than 60% of all teenage visits to a gynecologist [1,2]. Most girls have characteristic signs of autonomic dysfunction syndrome, which has recently been observed more and more often among children [3,4]. Deterioration of the environmental situation, the consequences of the COVID 19 pandemic, a significant decrease in the number of children engaged in sports are the reasons for the prevalence of autonomic dysfunction syndrome [5-7]. In 33.3% of children, dysfunction of the autonomic nervous system persists for many years despite treatment, in 17-20% of children they progress [8].

The prevalence of autonomic nervous system disorders, according to some authors, ranges from 54.6% to 72.6%. This is especially true for teenage girls, since the hormonal changes of the body during puberty contribute to more frequent manifestations: «marbling» of the skin, cyanosis of the nasolabial triangle or extremities, thermoregulation disorders, «intestinal colic», heart rhythm disturbances or repolarization processes on the ECG. The «peak» of manifestations of vegetative dysfunction falls on the puberty period, which is accompanied by violent emotional manifestations, psychosomatic disorders, in particular, menstrual cycle disorders. According to many authors, teenage girls with dysmenorrhea and manifestations of autonomic dysfunction due to impaired autonomic regulation of the heart and blood vessels have functional changes in the cardiovascular system [5-7]. Changes in indicators of the cardiovascular system in response to physical exertion are a reliable indicator of adaptive reactions, especially when studying the mechanisms of heart rate regulation [2].

The analysis of heart rate variability will allow to assess the vegetative balance, functional reserves of the body and to fully determine the interaction of all organs and systems and the level of participation of all regulation

mechanisms in the vital activity of the body [8]. It is important to determine the total power of the heart rhythm spectrum.

The complex of medical measures should take into account the adaptive capabilities of the body.

The use of various physical factors contributes to the effectiveness of treatment due to the optimization of the regulatory function of the autonomic nervous system [8].

AIM

The aim of our study was detection and correction of dysmenorrhea in teenage girls with autonomic dysfunction syndrome.

MATERIALS AND METHODS

The research group consisted of 37 teenage girls, aged 12-16 years, who were undergoing rehabilitation treatment in the village of Polyana with signs of dysmenorrhea and vegetative dysfunction syndrome. The study lasted for 2 years (2020-2021).

Using the spectral analysis method, clinical and functional studies were carried out before and after treatment.

To determine the effect of the course of treatment on the manifestations of dysmenorrhea and the functional state of the autonomic nervous system, a study of the variability of the heart rate at rest and under the conditions of an orthostatic test was carried out using the «Cardiolab» complex by the method of spectral analysis.

Spectral analysis is the most accurate method for quantitative assessment of periodic processes in the heart rhythm. The following types of oscillations are distinguished in the structure of the heart rhythm: high-frequency - (High Frequency – HF component), frequency range of 0.4–0.15 Hz – illustrate the influence of the parasympathetic part of the nervous system on heart rate modulation; low-frequency oscillations (Low Freqyency – LF component), the frequency range of 0.15–0.04 Hz illustrates the influence of the sympathoadrenal system; very low frequency - (Very Low Freqyency – VLF), frequency range 0.04–0.015 Hz, associated with humoral-metabolic and cerebral ergotropic effects [8-10].

During the study, the following indicators were determined: total power of the spectrum (TP) (ms^2), content of HF, LF and VLF frequencies, integral indicator – LF/ HF.

Mathematical processing of the obtained research results was carried out using the MS Office Excel computer program, as well as the Statistics 6 software package.

RESULTS

37 teenage girls who were being treated with signs of dysmenorrhea and autonomic dysfunction syndrome took part in the study. The study lasted for 2 years (2020-2021). Girls complained of irregular and painful menstruation, headache, dizziness, general weakness and emotional lability at the beginning of treatment. Menarche appeared in all studied girls at the age of 11-13 years, which corresponds to the age norm. Menstrual cycle pathology was manifested: 22 (59.3%) girls had irregular menstruation, 10 (25.9%) had painful menstruation of normal duration, and 5 (14.8%) had painful and prolonged menstruation. The average duration of the menstrual cycle was 59 ± 14.5 days, the duration of bleeding was 4.6 ± 1.4 days, according to the nature of the blood loss, profuse discharge. The average duration of the menstrual cycle is 27.7 ± 11.6 days.

Analyzing the initial state of the autonomic nervous system in girls with menstrual cycle disorders, the primary predominance of the sympathetic nervous system in 33.3% of girls was revealed. Before treatment, autonomic reactivity was characterized by a hypersympathetic type in 44.4% of girls, by an asympathetic type in 22.3% of children.

Spectral analysis at the beginning of treatment showed that high frequencies (HF) prevail in most girls (66.0%), which indicates an increased influence of the parasympathetic part of the autonomic nervous system, and 18.5% of girls have a balanced state of the autonomic nervous system.

Predominance in the spectrum of LF and VLF components was found in 15.5%, which indicates the predominance of central humoral-metabolic ergotropic effects on heart rate variability (Table 1).

After the course of treatment, which included 10 procedures of electrophoresis, physiotherapy, magnetic therapy and massage of the collar zone, an improvement in the condition of teenage girls was observed. Menstruation became less painful or painless at all, the duration and frequency of menstruation (from 1 to 3 days, on average 1.8±0.1) and blood loss decreased. The girls felt better, did not complain of dizziness and headache.

During treatment, activation of the sympathoadrenal system was observed, as evidenced by an increase in low frequencies in 41.4% of patients, a balanced state of the autonomic nervous system in 37.9%, and activation of the parasympathetic division of the autonomic nervous system in 20.7%.

The dynamics of the HRV spectral analysis data revealed an increase in the total spectral power of TR from 4259±587.8 to 4461±743.0, the absolute values of the power of the LF and VLF components were higher than observed before treatment, a decrease in the HF component was recorded (Table 2).

Table 1. HRV indicators of teenage girls with signs of dysmenorrhea and autonomic dysfunction syndrome at rest

Parameters	Before treatment (n=37)	After treatment (n=37)
TP, ms^2	4259,0±587,8	4461,0±743,2
VLF, ms^2	786,9±69,7	1120,0±207,4 *
LF, ms^2	918,5±103,0	1146,0±156,3
HF, ms^2	2369,0±465,4	2085,5±424,0
LF/HF	0,71±0,09	0,76±0,09 *
VLF, %	23,9±1,7	24,9±1,5
LF, %	24,7±1,4	27,6±2,0
HF, %	46,7±2,7	42,6±2,5

Note: * $p < 0.05$ - significant difference of the indicator before and after treatment.

Table 2. HRV indicators of teenage girls with signs of dysmenorrhea and autonomic dysfunction syndrome during an orthostatic test

Parameters	Before treatment (n=37)	After treatment (n=37)
TP, ms^2	2249,0±209,0	2149,0±224,0
VLF, ms^2	854,3±94,4	892,5±103,9 *
LF, ms^2	824,7±74,1	857±98,0
HF, ms^2	271,5±45,5	272,9±53,8
LF/HF	4,47±0,4	4,31±0,46 *
VLF, %	38,0±2,0	39,0±2,5
LF, %	38,9±2,1	38,4±2,5
HF, %	12,2±1,2	13,2±2,0

Note: * $p < 0.05$ - significant difference of the indicator before and after treatment.

An adequate reaction of the segmental structures of the sympatho-adrenal system during the orthostatic test was noted, in the HRV spectrum this was reflected in the form of an increase in the spectral power (VLF) of waves from 786.9±69.7 to 854.3±94.4 ms^2, a decrease in the spectral power high-frequency (HF) waves from 46.7±2.7 to 12.2±1.2 ms^2.

CONCLUSIONS

Thus, the proposed course of treatment for adolescent girls with a menstrual cycle disorder with signs of autonomic dysfunction contributed to the normalization of the cycle, improvement of the general condition of the girls. Positive dynamics can be observed according to spectral analysis data.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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RECEIVED: 05.06.2024
ACCEPTED: 03.10.2024

REVIEW ARTICLE

CONTENTS

Nowadays and the future of the 3d digital technologies in modern orthodontics

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ABSTRACT

Aim: To investigate the usage trends of different 3D digital technologies in modern orthodontics during the previous eight years to identify their future prospects.
Materials and Methods: A systematic literature search on PubMed revealed 258,059 publications concerning digital technologies in modern orthodontics. Amongst 125 eligible articles, we chose 37 high quality articles. The quality assessment was performed according to the Jadad scale for randomised controlled trials and controlled clinical trial studies.
Conclusions: Our investigation revealed that in the near future, significant changes brought by AI will be implemented into visualization technologies (intraoral scanners and synthesized from CBCT 3D cephalometric landmarks detection), design technologies (3D digital virtual setup software), and different manufacturing technologies such as 3D printers. By fully embracing digital technologies orthodontic clinics and laboratories can digitally replicate their entire operations and ensure a standardized exchange of information along the worldwide orthodontic workflow from diagnostics to therapy. CBCT has a leading position amongst all digital imaging technologies in orthodontics, as it provides a complete and accurate representation of anatomical structures in all three planes. The digital approach improved diagnostic precision, streamlined treatment planning, and eliminated the need for messy and uncomfortable impression materials.

KEY WORDS: orthodontics, modern technologies, digitalization, accuracy, three-dimensional imaging

Wiad Lek. 2024;77(9):2047-2056. doi: 10.36740/WLek/195140 DOI

INTRODUCTION

Modern orthodontics is undergoing a significant transformation, propelled by the integration of cutting-edge tools, techniques, and technology, leading to a noticeable transition from manual to digital procedures. Over the past 10 years, the creation of new innovative, completely automated, and accelerated prototyping methods has revolutionized the approach to making three-dimensional orthodontic diagnostic measurements. The presented changes must be incorporated within orthodontics to ensure this field harmonizes with the state-of-the-art approach. Simultaneously it is valid to state the added value that the conducted research makes to the existing database of knowledge [1]. Three-dimensional technologies have been widely used in orthodontics for the last ten years. Their implementation has significantly changed approaches for orthodontists. 3D technologies replicate anatomical structures to present three-dimensional anatomy and the bone tissue and soft tissue examination more precisely [2]. In dentistry, 3D-scanning technology increases the accuracy of orthodontic diagnostics in general while 3D-printing technology provides an opportunity to manufacture personalized orthodontic appliances and new changes to traditional manufacturing approaches. The most popular 3D technologies in modern orthodontics are digital vizualization technologies such as: 3D cephalometry, digital models, 3D Photography, CBCT (cone beam computerized tomography), 3D imaging for Indirect-Direct bonding, aligner fabrication, Digital Smile Design, CAD/CAM, Facial 3D WL scans, Intraoral scanning, Orthognathic Surgery 3D Planning—Surgical Splint Manufacturing, Different professional software programs (Invisalign Clincheck Pro, Maestro 3D, Ormco Insignia, 3Shape OrthoAnalyzer) that integrate data and provide 3D volumetric data sets that have a great potential for research and planning in both orthodontics and orthognathics. These methods are better than 2D x-rays or solid records since they are easier to keep, prone to fewer handwriting errors, and provide a 3D evaluation of craniofacial structures [1,2]. The future of digital orthodontics is expected to be significantly

spread by 3D technologies as a study into its potential and solutions for current issues continues.

AIM

To investigate the usage trends of different 3D digital technologies in modern orthodontics during the previous eight years to identify their future prospects.

MATERIALS AND METHODS

This systematic review was performed according to the PRISMA statement [3] The framework of this systematic review according to PICO [4] was: Population: orthodontic patients; Intervention: CAD/CAM, scanning oral cavity, 3D cephalometrics, 3D technologies; Comparison: analogue technologies, 3D-printing or no intervention; Outcomes: efficiency and accuracy. The PICO question of this study was as follows: Which 3D technologies are commonly used nowadays and will become the most popular in future orthodontic practice and following clinical trials to get the most accurate diagnostic and manufacturing technologies compared to analogue technologies?

STUDY DESIGN

A systematic literature review approach to gather and analyze relevant literature on 3D technologies usage in orthodontics from 2016 to 2023.

SEARCH STRATEGY

Figure 1 illustrates a systematic literature search conducted on PubMed using keywords "Diagnostics" and "Digital orthodontics" or "Digital technologies" or "CAD" or "CAM", or "3D scanning" or "3D printing" or "clear aligners" or "virtual planning" resulting in the identification of 258,059 publications. These publications were then screened based on inclusion and exclusion criteria, including years 2016-2023, availability of free full text, language (English only), and focus on human subjects.

INCLUSION AND EXCLUSION CRITERIA

The inclusion criteria for selecting studies from the systematic literature search conducted on PubMed using specific keywords included publications between 2016 and 2023, availability of free full text, English language publications, studies focusing on human subjects, and content directly addressing the 3D technology usage in orthodontics for diagnostic accuracy improvements. The following exclusion criteria were applied: abstract and author debates or editorials; lack of effective statistical analysis; papers not related to practical implementations of scanners in orthodontics.

QUALITY ASSESSMENT

According to the PRISMA statements, the evaluation of methodological quality indicates the strength of evidence provided by the study because methodological flaws can result in biases [3]. The quality assessment was performed using the Jadad scale for randomised controlled trials and controlled clinical trials studies [4]. Evaluation of whether the study was randomized, double-blinded with appropriately described methods to determine the risk of bias. Cook criteria were used for quality assessment for those reviews [5].

DATA EXTRACTION

Following the inclusion criteria. titles and abstracts were independently selected by two authors (K.K. and K.S.). The full text of each identified article was then analyzed to verify whether it was suitable for inclusion and amongst 125 eligible articles we chose 37 high-ranked quality articles. Characteristics of the included studies have been presented in Table 1.

REVIEW AND DISCUSSION

Table 1 illustrates study characteristics including the current review from 2016 to 2023, examining the application of 3D technologies in orthodontics. The trend shows steady growth, with elevation from 2019 (4351 publications) and onwards. Results showed a significant increase in publication counts, nearly doubling from 2019 to 2023, indicating an escalation in scholarly activity or an expansion of the discipline. Results show a range of study designs, such as experimental and cohort studies, clinical and comparative studies, systematic reviews, in vitro studies, and cross-sectional studies, and reflect a multidisciplinary approach to comprehending the applications and implications of 3D technologies in orthodontics. As far as sample sizes are concerned, these vary significantly across studies, ranging from small-scale experiments with as few as two participants to larger cohort sizes exceeding 200 individuals, reflecting the diverse scopes and methodologies of the research conducted. Most studies included in this review focus on recent advancements, highlighting the ongoing relevance and evolving nature of research related to 3D technologies.

Figure 1 illustrates a systematic literature search conducted on PubMed. Figure 2 shows the result of the

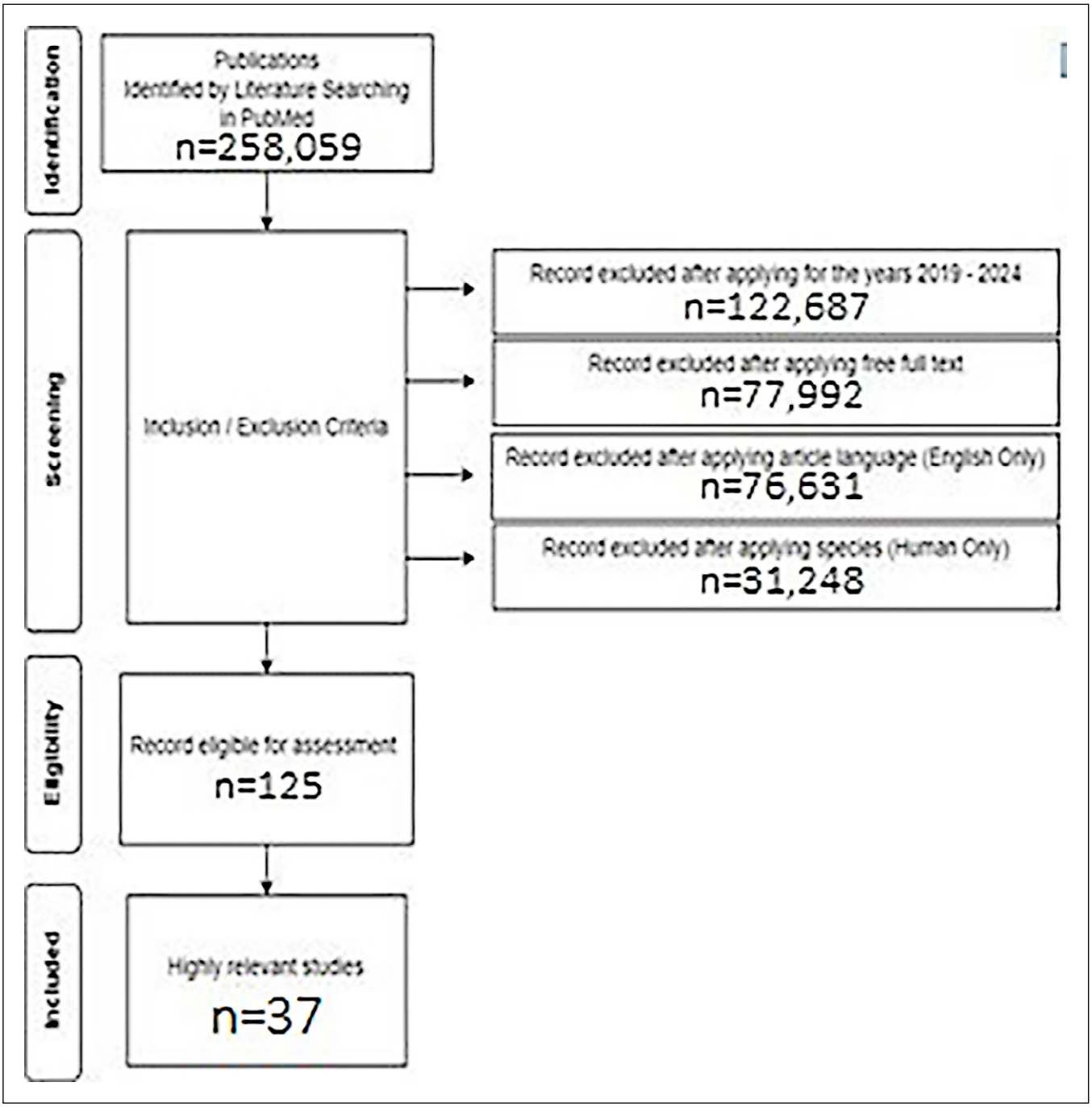

Fig. 1. PRISMA flow diagram.

current review. There was a significant upward surge in publication over the past five years. Table 2 provides a comprehensive overview of the distribution of various 3D diagnostic and manufacturing technologies in orthodontics production, as identified in a systematic literature review. 3D scanning emerges as the most frequently mentioned technology, cited in fourteen instances across multiple studies [19–42]. CAD/CAM follows with five mentions [34-38] while CBCT appears four times [21–24]. Additionally, the review encompasses a diverse range of other 3D diagnostic and manufacturing technologies, collectively referenced 30 times [6–20], [19–23], [28–31], [38–42]. This comprehensive overview underscores the multifaceted application of 3D diagnostic tools in digital orthodontics, providing valuable insights into the evolving landscape of modern diagnostic methods.

Future of digital visualization and photogrammetry: Digital imaging and diagnostics brought significant advancements to orthodontics. CBCT is used to make 3D images with different fields of view (FOVs) of the head in a 1:1 ratio. The virtual head of the patient is attained by the superimposition of digital dental models, CBCT, and facial scanning for diagnosis, treatment

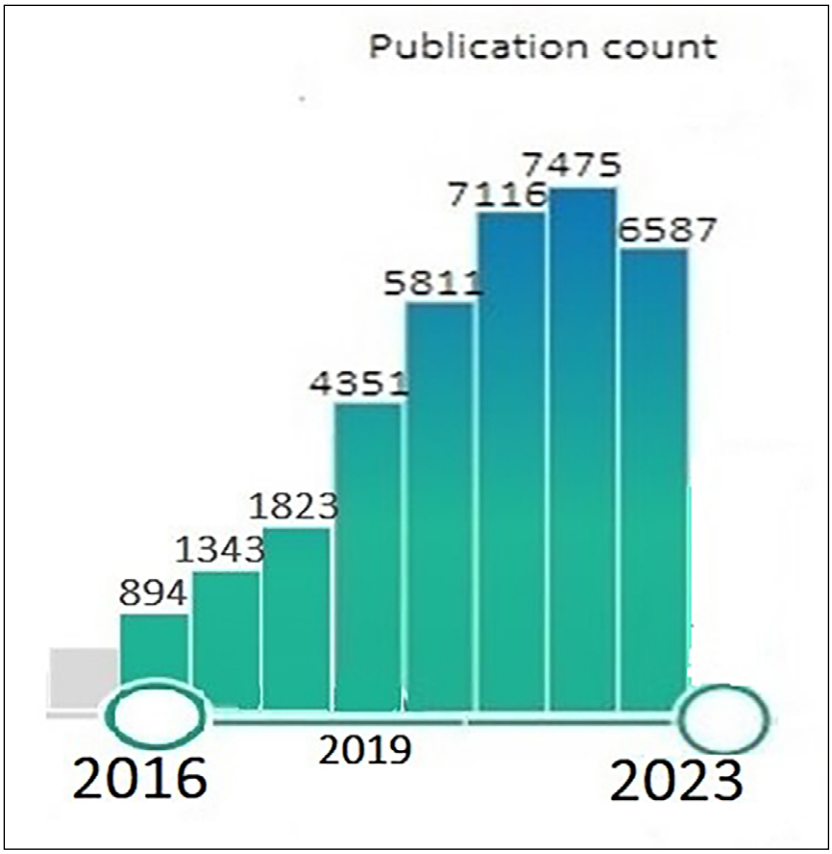

Fig. 2. Publication trend.

plans, and computer-aided design (CAD) and computer-aided manufacturing (CAM) procedures. Software like Dolphin, Anatomage, and 3Shape can be used for diagnosis and treatment planning. Orthodontists have started to utilize 3D CBCT to conquer the insufficiency of 2D radiographic records. A systematic review by Sam et all [20] assessed the reliability of different 3D cephalometric landmarks in CBCT imaging. Their conclusion showed that further research is required to evaluate the reliability of 3D cephalometric landmarks when evaluating 3D craniofacial complexes. Serafin [18] and the authors described their point of view about the accuracy of automated 3D cephalometric landmarks detection, using deep learning compared with manual tracing for cephalometric analysis of 3D medical images. Their conclusion revealed that deep learning algorithms showed excellent accuracy results for automated 3D cephalometric landmarking. In the last two years, promising algorithms have been developed and improvements in landmark annotation accuracy have been made. De Queiroz et all [20] discussed Artificial Intelligence in 3D cephalometrics and evaluated studies that assessed the level of agreement between AI, regardless of system, with the human registration for annotating cephalometric landmarks in digital imaging examinations (2D or 3D). The study conclusion revealed that AI allows to identify landmark placement more precisely on both 2D and 3D images.The study by Jedli´nski et all [19] focused on systematical review and synthesize available controlled trials investigating the accuracy and efficacy of intraoral scanners for orthodontic purposes to provide clinically useful information and to direct further research in this field. The authors' conclusions were next: plenty of data available on the accuracy and efficacy of different scanners. Scanners of the same generation from different manufacturers have almost identical accuracy. Due to those reasons future similar research will not introduce much to orthodontics. The challenge for the coming years is to find new applications of digital impressions in orthodontics. A systematic review by Kustrzycka et all [23] revealed that the substrate type impacts the general accuracy of intraoral scans. The experienced operator influences accuracy, whereas more experienced ones and smaller scan sizes make more accurate scans. A conventional impression technique in a full-arch recording provides the lowest deviation. Angelone et all [24] wrote a systematic review about intraoral scanners, which are routinely used for the reconstruction of 3D dental models for orthodontic treatments and planning and can also be implemented beyond the scope of orthodontic interventions, thus providing alternative diagnostic tools for the detection of oral cavity pathologies/anomalies (e.g., caries, dental wear, periodontal diseases, oral cancer, infections) to traditional methods or available gold standards (e.g., radiographic modalities). They

Table 1. Study Characteristics

Author's	Publication Year	Study Design	Sample Size
Venezia et al. [6]	2023	Observational, retrospective, cross-sectional or comparative study ggff	80
Liu et al. [7]	2021		100
Abu-Arqub et al. [8]	2023		160
Stamm et al. [9]	2022		1
Adel et al. [39]	2021		40
Grassia et al. [40]	2023		4
Schott et al. [41]	2019		31
Beri et al. [42]	2022		45
Patano et al. [10]	2022	Systematic Review	11
Koletsi et al. [11]	2021		7
Shrivastava et al. [12]	2023		6
Baxmann et al. [13]	2023		48
Tartaglia et al. [14]	2020		39
Rossini et al. [15]	2016		35
Torres et al. [16]	2023		4
Desai et al. [17]	2023		4
Serafin et al. [18]	2023		15
de Queiroz et al. [19]	2023		40
Sam et al. [20]	2019		13
Antonacci et al. [21]	2023		16
Jedliński et al. [22]	2021		16
Kustrzycka et al. [23]	2020		13
Angelone et al. [24]	2023		25
Mai.H. et al. [25]	2020		6
Maiet al. [26]	2017		5
Thawri et al. [27]	2023		36
Ishida,Y et al. [28]	2021	In vitro study	2
Hoffman et al. [29]	2022	In vitro study	24
Saccomanno et al. [30]	2022	Survey study	120
Meade et al. [31]	2023	Cohort study	30
Plattner et al. [32]	2023	Clinical Study	40
Felter et al. [33]	2018		16
Choi et al. [34]	2023		1
Bachour et al. [35]	2020		23
Koller et al. [36]	2022		37
Schwärzler et al. [37]	2023		46
Jaber et al. [38]	2021		40

concluded that the differences between the scanners are mainly due to the 3D imaging principle, the different wavelengths used, the image acquisition principle, and the scanner wand. More research is needed to test their performance levels in the context of their differences, which appear to be established only in the case of dental caries. Mai et all [25] reviewed comparisons of the accuracy of mobile device–compatible face scanners for facial digitization with that of systems for professional 3D facial scanning. Overall, mobile device–compatible face scanners did not perform as well as professional scanning systems in 3D facial acquisition, but the deviations were within the clinically acceptable range of <1.5 mm. Significant differences between results when 3D facial scans were performed on inanimate facial objects and when performed on the faces of living

Table 2. Frequency Distribution of technologies in orthodontics

Digital Technologies in Orthodontics	Frequency	Author's
3D diagnostic and treatment technologies	30	[6]–[20], [19]–[23], [28]–[31], [38]–[42].
CAD/CAM	5	[34-38]
Cone-beamed computer tomography (CBCT)	4	[21–24]
Various 3D scanning technologies	14	[19]–[24], [28]–[31], [38]–[42].
Various 3D printing technologies	11	[6], [8–10], [26, 27, 31, 32, 37], [40-41]

participants were found; thus, caution should be exercised when interpreting results from studies conducted on inanimate objects. Network meta-analysis made by Antonacci et all [22] determined the accuracy of various face-scanning technologies in the market, concerning the different dimensions of space (x, y, and z axes). The main attention was paid to the technology types and the best procedures for high-quality scan acquisition. They found out that limiting the movements of the patient and scanner allows for more accurate facial scans with all the technologies involved. Active technologies such as laser scanners, structured light, and infrared structured light have accuracy comparable to static stereophotogrammetry while being more cost-effective and less time-consuming. Beri et all [42] made a study protocol including photogrammetry accuracy with 3D scanning and conventional impression method for craniomaxillofacial defects by using software analysis. This article's conclusion provided an update on defect data acquisition, editing, and design using open-source and commercially available software in digital workflow in modern orthodontics and maxillofacial prosthodontics. A comparative study made by Jaber et all [38] assessed the dimensional accuracy and reliability of dental digital models prepared by direct intraoral scanning and indirect scanning of the plaster models compared to the plaster models as the gold standard. Both direct and indirect scanning techniques are accurate and reliable for digital model preparation and can be considered an alternative to traditional plaster models used in clinical orthodontics diagnostic applications. The intraoral scanning technique can be considered a valid alternative for indirect scanning of the plaster models to prepare digital working models during the digital design and fabrication of orthodontic appliances such as clear aligners.

Designing in modern orthodontics: Three-dimensional scanning and modeling revolutionized how orthodontic information is captured and analyzed. Orthodontists now use intraoral scanners to create virtual 3D models of a patient's teeth, allowing for a detailed examination of tooth positioning, occlusion, and jaw relationships. Hence, these digital models serve as the foundation for treatment planning and facilitate efficient communication among the orthodontic team. With CAD orthodontists can design customized orthodontic appliances, such as clear aligners or lingual braces, using digital models of the patient's teeth. The case report of Choi et all [34] describes the use of the CAD/CAM virtual orthodontic system in a skeletal Class III adult patient undergoing orthognathic surgery and orthodontic treatment with maxillary first premolar extractions to provide treatment effectively while reducing the number of brackets requiring rebonding and shortening the overall treatment time. Conclusions were the following: ideal tooth alignment and occlusion can be achieved by an individualized orthodontic treatment plan and optimal prescriptions of customized brackets using the CAD/CAM virtual orthodontic system. In addition, this system can contribute to efficient orthodontic treatment in surgical cases. However, the evaluation of customized bracket bonding accuracy and additional detailing procedures related to functional occlusion are needed to maximize the advantages of the system. A research study by Adel et all [39] evaluated the accuracy of three different 3D digital model registration software packages for linear tooth movement measurements, regarding a 3D digital virtual setup. Compare and Geomagic software packages consistently showed maximum accuracy in measuring the amount of tooth movement in the maxillary arch compared to the reference standard. Comparison of the software showed the highest agreements in the mandibular arch. None of the three studied software packages showed poor agreement with the Digital Setup across all tooth movement measurements. Buccolingual tooth movements showed the highest agreement amongst linear measurements. Grassia et all [40] in a research study assessed the accuracy (trueness and precision) of orthodontic models obtained from crowded and spaced dentition finalized for the production of clear aligners. Four 3D printers featuring different technologies and market segments were used for this purpose and they concluded that the accuracy of orthodontic models generated for clear aligners can be affected by different 3D printer technologies and anatomical characteristics of dental arches.

Digital manufacturing in orthodontics: After CAD procedures CAM orthodontic appliances are precisely

manufactured using 3D printers or milling machines, ensuring a perfect fit for each patient and enhancing treatment efficiency. Venezia et all [6] made a comparative study that evaluated the accuracy of orthodontic models for the production of clear aligners generated with four 3D printers featuring different technologies and belonging to different market segments. They concluded that the accuracy of orthodontic models generated for clear aligners can be influenced by different technologies/market segments of the 3D printers used. An original article by Koller et all [36] investigated the digital construction, the CAD/CAM production, and the intraoral positioning accuracy of custom-manufactured novel 3D CAD/CAM titanium retainers. Based on the results, the present study shows a high level of congruence between the 3D virtual planning and the final intraoral position of the fabricated novel 3D CAD/CAM titanium retainers. A prospective randomized clinical study made by Schwärzler et all [37] compared transfer accuracy and immediate loss rate of hard versus soft transfer trays utilizing a CAD/CAM workflow. Study results concluded the following: CAD/CAM technology for indirect bracket bonding is a reliable method; low rate of immediate loss with both hard and soft resin; soft resin is more favorable than hard resin for accuracy and usability; indirect bonding of molar brackets is less accurate than of incisor brackets. An observation study made by Al Mortadi et all [6] shows how to create a removable orthodontic appliance digitally using an intraoral scan. For the maxillary and mandibular arches, an intraoral scan was performed. The virtual Hawley retainer was created using 3Shape Orthodontics Appliance Designer. It is made up of a base plate and two alloy components, Adam Clasps and Fitted Labial bow. After the alloy components were combined using cold-cured acrylic, the design of the base plate was adjusted to accommodate their insertion. Using computer-aided design (CAD) and computer-aided manufacturing (CAM) technology, this innovative method offers an alternate manufacturing process for removable appliances. The method explained provides a forerunner to digital manufacture of other orthodontic appliances.

Prospectives of digital technologies in orthodontics: As a field, digital orthodontics has changed the game by providing accurate, effective, and patient-friendly tooth alignment treatment methods. Early in the course of treatment, patients benefit from increased comfort and convenience as digital impressions take the place of traditional molds. We noticed that studies focused on CAD/CAM and scanner development and their implementation into solving any challenging aspects of orthodontic diagnosis and therapy aspects. On the other hand, there are plenty of data available about accuracy and efficacy. In future studies, scanners and CAD/CAM technologies should serve only as tools for clinical phenomena observation. The challenge for the coming years is to find new applications of digital impressions and imagining in orthodontics. Additionally, by using virtual simulations, patients and orthodontists may both see the anticipated result, improving communication and guaranteeing that alignment objectives are successfully accomplished. Modern material science and manufacturing techniques have produced smaller, stronger aligners that can precisely apply force to shift teeth into the correct position. Furthermore, the incorporation of functionalities such as SmartTrack technology improves the alignment and consistency of aligner therapy, guaranteeing maximum comfort and effectiveness during the course of treatment. The planning and execution of orthodontic treatments is being completely transformed by artificial intelligence (AI), which provides previously unheard levels of accuracy and efficiency. Artificial intelligence (AI)-powered software platforms may create extremely accurate treatment plans that are customized to each patient's specific dental anatomy and needs by evaluating enormous volumes of patient data and utilizing machine learning algorithms to move teeth into the correct position. Furthermore, the incorporation of functionalities such as SmartTrack technology improves the alignment and consistency of aligner therapy, guaranteeing maximum comfort and effectiveness during the course of treatment. The planning and execution of orthodontic treatments is being completely transformed by artificial intelligence (AI), which provides previously unknown levels of accuracy and efficiency. AI-powered software platforms may create extremely accurate treatment plans that are customized to each patient's specific dental anatomy and treatment goals by evaluating enormous volumes of patient data and utilizing machine learning algorithms. Moreover, AI systems are always learning and changing in response to actual results, which helps them to improve treatment plans and outcomes over time. In addition to increasing the effectiveness of orthodontic treatments, this iterative method gives orthodontists the ability to provide patients with individualized care and achieve better clinical results. The field of orthodontics is constantly developing due to scientific and technical advancements, and straightening teeth has a bright future. With advancements in digital orthodontics, transparent aligner technology, expedited treatment approaches, and AI-powered treatment planning, obtaining a beautiful, straight smile is now more feasible and fun than in the past.

CONCLUSIONS

We conclude that CBCT has a leading position amongst all digital imaging technologies in orthodontics, as it provides a complete and accurate representation of anatomical structures in all three planes. The digital approach improved diagnostic precision, streamlined treatment planning, and eliminated the need for messy and uncomfortable impression materials. Instead of traditional dental impressions, orthodontists use digital scanners to capture accurate 3D images of the patient's teeth and jaw. By fully embracing digital technologies orthodontic clinics and laboratories can digitally replicate their entire operations and ensure a standardized exchange of information along the worldwide orthodontic workflow from diagnostics to therapy. Our investigation revealed that in the near future, significant changes brought by AI will be implemented into visualization technologies (intraoral scanners and synthesized from CBCT 3D cephalometric landmarks detection), design technologies (3D digital virtual setup software), and different manufacturing technologies such as 3D printers. The reproducibility of direct scanning is comparable to indirect scanning although a slight difference can be noticed (0.02 mm).

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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

RECEIVED: 07.06.2024
ACCEPTED: 29.09.2024

REVIEW ARTICLE

CONTENTS

Individual and molecular risk factors for the development of rheumatoid arthritis

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ABSTRACT

Rheumatoid arthritis is a chronic autoimmune disease of the joints of unknown etiopathogenesis. It affects between ~0.5 and 1% of the total population and occurs 2–3 times more often in women than in men. Several antibodies have been identified in the serum of patients with RA, including rheumatoid factors (RF), anti-citrullinated protein antibodies (ACPA) and anti-carbamylated protein antibodies. These autoantibodies can form immune complexes in the joints, leading to inflammation and damage to the articular cartilage. A characteristic symptom of advanced RA is persistent inflammation of the synovium, which usually affects peripheral joints in a symmetrical way. The exact aetiology of RA is unclear,it is known to be a multifactor disease in which a complex interplay between the host and the environment determines the overall risk of being susceptible to the disease, as well as its persistence and severity. Below we present the RA risk factors including main individual risk factors as hormonal factors, hereditery factors, epigenetic factors as well as the risk of concomitant environmental factors of RA as diet, cigarettes and alcohol abuse. Environmental contaminants, socio-economic factors and molecular mechanisms of RA known so far.

KEY WORDS: rheumatoid arthritis, RA, molecular risk factors, individual risk factors

Wiad Lek. 2024;77(9):2057-2069. 10.36740/WLek/193976 DOI

INTRODUCTION

DEFINITION AND EPIDEMIOLOGY OF RA

Rheumatoid arthritis is a chronic autoimmune disease of the joints of unknown etiopathogenesis. It affects between ~0.5 and 1% of the total population and occurs 2–3 times more often in women than in men. Several antibodies have been identified in the serum of patients with RA, including rheumatoid factors (RF), anti-citrullinated protein antibodies (ACPA) and anti-carbamylated protein antibodies. These autoantibodies can form immune complexes in the joints, leading to inflammation and damage to the articular cartilage. Depending on the presence of RF and ACPA in the serum of patients with RA, the disease can take one of the following two forms: seropositive RA, which is the most common and is characterized by the presence of RF and/or ACPA, or seronegative RA, which is characterized by the absence of both RF and ACPA [1].

A characteristic symptom of advanced RA is persistent inflammation of the synovium, which usually affects peripheral joints in a symmetrical way [2, 3].

Although the exact aetiology of RA is unclear, it is known to be a multifactor disease in which a complex interplay between the host and the environment determines the overall risk of being susceptible to the disease, as well as its persistence and severity. The risk factors for the development of RA can be broadly defined as host-related and environment-related. Host-related factors associated with the development of RA can be further divided into genetic, epigenetic, hormonal, reproductive and neuroendocrine factors and co-existing host-related factors. In contrast, environment-related risk factors include smoking and other airborne exposures; microflora and infectious agents; diet and socio-economic factors.

Based on many years of observation of patients with rheumatoid arthritis, it can be concluded that early diagnosis and initiation of treatment with disease-modifying antirheumatic drugs (DMARD) are of great prognostic importance and lead to quick remission of the disease and definitely improve treatment outcomes for patients with RA [4-6].

AIM

This review is an attempt to present the issue of individual and molecular risk factors for the development of rheumatoid arthritis in the light of available domestic and foreign literature.

REVIEW AND DISCUSSION

MAIN INDIVIDUAL RISK FACTORS FOR RA

An epidemiological correlation has also been noted between an increased future risk of developing RA and apparently unrelated comorbidities. The population of patients already diagnosed with RA are more likely to develop other comorbidities, however, they differ in terms of the risk of their occurrence. The three most common comorbidities in RA include anxiety disorders (62.1%, 95% CI: 43.6%; 80,6%), hypertension (37.7%, 95% CI: 29,2%; 46,2%) and depression (32.1%, 95% CI: 21.6%; 42.7%) [7].

It seems that there is a particular link between psychiatric disorders and RA. The co-existing posttraumatic stress disorder increases the risk of RA both in men and in women . This is thought to be due to the disruption of neuroendocrine-immunological mechanisms caused by chronic stress . Studies on the co-existence of RA with other diseases have shown that depression is one of the factors increasing the risk of developing RA. Depression increases this risk by 28-68% [8-10], whereas the use of antidepressants in patients with depression may protect against the development of RA. This may be related to systemic inflammatory mechanisms. Co-occurrence of RA with other rheumatic diseases (such as psoriatic arthritis), gastrointestinal diseases (such as inflammatory bowel disease) or dermatological diseases (such as alopecia areata, vitiligo) supports the hypothesis that these diseases have an immunological aetiology. There is an interesting inversely proportional relationship between schizophrenia and the development of RA. The epidemiological data, after having been re-verified in two updated meta-analyses, confirmed a significant protective effect of schizophrenia on the development of RA (OR 0,48–0,65) [11, 12].

There are many controversies concerning the relationship between atopy and allergic diseases and the risk of developing RA. High-quality cohort studies suggest a positive relationship between atopy and RA. A positive correlation has also been found between acute and chronic diseases of the upper and lower respiratory tract and an increased risk of seropositive and seronegative RA.

Other identified risk factors for RA include autoimmune diseases, such as Hashimoto's thyroiditis and Graves' disease, type 1 diabetes 1, alopecia areata, vitiligo , inflammatory bowel disease , multiple sclerosis. Long-term cohort studies suggest that sleep disorders (including obstructive sleep apnoea) and other sleep disorders not related to apnoea represent additional risk factors for RA [13-15]. Recent studies have shown the aforementioned oral mucosal immunity, together with oral and/or intestinal dysbiosis and chronic infections have been closely implicated in the etiology and pathogenesis of RA. The results of a prospective observational study by American and Canadian scientists indicate a high incidence of overweight and obesity (up to 69% in the population of patients with early RA (disease duration <3 years). The conducted study showed that increased body weight is a factor independent of other variables. risk of poorer response to treatment (taking into account demographic factors, smoking, duration of arthritis, baseline disease activity). It turns out that these people are less likely to achieve lasting remission compared to patients with a normal body mass index [48]. They are strongly associated with additional environmental factors, such as smoking tobacco and exposure to occupational dust, which affect the condition of the mucosa. An additional risk factor has been shown to be immunoglobulin IgA, as an early factor in the development of the disease.

HORMONAL FACTORS

Due to the fact that the risk of RA in women is about 2-3 times higher than in men, and also due to the more severe and aggressive progression of the disease with a high inflammatory activity and a high degree of disability [16], it can be assumed that there is a direct relationship between hormonal factors and susceptibility to RA. This is also confirmed by observations that in pregnant women there are frequent remissions of the disease, whereas the second peak in the incidence of RA in women occurs during menopause, which supports the hypothesis that hormonal factors play a significant role in the progression of RA. A decrease in estrogen and/or progesterone levels in the postpartum period and menopause predisposes to an increased incidence of RA. However, there are many controversies concerning the role of hormonal factors in the etiopathogenesis of this disease. In general, estrogens are considered pro-inflammatory, unlike progesterone and androgens, which have an anti-inflammatory effect and whose levels are reduced in men and women with RA. Nonetheless, it is worth noting that the effect of estrogens is more complex, as they may also have anti-inflammatory action in many cells and tissues. The assessment of the role of estrogens as pro-inflammatory or anti-inflammatory factors must also take into account many other factors, such as their concentration in the serum and tissues, dominant cell types and estrogen receptors, as well as the stage of life. Despite controversies, multicentre studies suggest that breastfeeding may protect against RA. The number of births, age during the first period, age during the first pregnancy, the use of oral

contraceptives and hormone replacement therapy play a significant role too. A cohort study published in Oxford Rheumatology (August 2024) showed that oral contraceptives use was associated with a decreased risk of RA in users. In contrast menopausal hormone therapy use was associated with an increased risk of RA. Age at menarche >14 years is associated with a greater RA risk compared with menarche at 13. Based on reports, it is suspected that the earlier the 1st pregnancy, is connected with the lower the risk of RA.

Therefore, it is believed that a sudden drop in estrogen levels during early menopause, the postmenopausal period , the postpartum period and the use of anti-estrogenic agents (such as selective estrogen receptive modulators and aromatase inhibitors) can be identified as risk factors for RA.

RA is also associated with disorders in the neuroendocrine system. It is thought that cortisol and adrenal androgens have anti-inflammatory effects and that their deficiency may trigger activation of an inflammatory process in the synovium. There is also a correlation between hormonal balance disorders and changes in the sympathetic and sensory systems, which likewise contribute to the development of RA. Any disturbances of the sympathetic system have an effect on the progression of RA, due to the shift of sympathetic signalling from anti-inflammatory beta and A2 receptors to inflammatory alpha and A1 receptors.

Another important endocrine mediator with immunomodulatory properties is vitamin D. Research in recent years has shown that there is a close relationship between vitamin D and the immune system. Vitamin D produces an anti-inflammatory effect due to its influence on macrophages, dendritic cells and FLS lymphocytes (which have a vitamin D receptor), indicating that it may potentially protect against RA [17, 18]. Nonetheless, the true role of vitamin D as a factor protecting against RA remains unclear. A large randomized controlled trial (RCT) failed to demonstrate a preventive effect of daily supplementation of calcium and vitamin D on the risk of RA. Instead, more robust evidence confirms the association between vitamin D deficiency and a poorer prognosis in patients with RA, which manifests itself with increased activity of the disease, impaired functions and poorer health-related quality of life [. Another interesting aspect is the relationship between exposures to UVB radiation and the risk of developing RA, suggesting that people who get more sunshine are less likely to develop RA. Research is ongoing to confirm the role of vitamin D as a protective factor. There are reports that there is a relationship between vitamin D deficiency and the occurrence of neuropathic pain. The possible pathomechanism of the phenomenon includes the neuroactive and indirectly neuroprotective effect of vitamin D, as well as its influence on the expression of nerve growth factor genes. Vitamin D deficiency may affect the nervous system, causing non-specific musculoskeletal pain [49].

More and more studies suggest that obesity is another risk factor for the development of RA . A number of meta-analyses have shown a positive association between obesity and RA, as well as an impact of the body mass index (BMI) on the risk of RA. There is some evidence indicating that this association is stronger in women and in seronegative patients (although it remains an open research question). As regards the existing relationship between obesity and RA, it should be assumed that metabolic and endocrine mechanisms play a significant role here. Increased secretion of pro-inflammatory cytokines and adipokines by adipocytes, as well as disorders of sex hormone metabolism due to excessive transformation in the adipose tissue, may increase the production of estrogens catalysed by the aromatase enzyme.

Although it can be hypothesized that the known systemic, metabolic and immunological effects of obesity (especially of visceral obesity) may predispose to autoimmune diseases, including RA , conflicting research findings indicate that further research is needed to understand the role of obesity in the development of RA, all the more so because weight loss may be an effective prophylactic intervention in people with an increased risk [19]. People with elevated cholesterol levels and disturbed lipid metabolism are much more likely to suffer from rheumatoid arthritis. Therefore, it can be presumed that the use of statins may have a role in protecting against the occurrence of RA. Possibly, this effect is caused by the lowering of lipid levels in the blood and the anti-inflammatory action of statins. However, the role of statins in the context of RA has not been fully understood, as studies on this issue have not as yet provided a clear answer.

HEREDITARY FACTORS

Hereditary factors play a significant role in the risk of developing rheumatoid arthritis (RA), which is confirmed by research involving families and twins. The risk of developing of RA in the identical twin of a patient with RA amounts to 9–15%, which is as much as four times higher than in the case of dizygotic twins and considerably much higher than in the general population [relative risk (RR) of [19-22, 25–35]. Similarly, the relative risk in first-degree relatives ranges from 2 to 5, which is comparable in men and women . In addition, the risk of RA increases 1.5–3-fold in offspring of parents

suffering from other immune-related inflammatory diseases, such as systemic lupus erythematous, Sjögren's syndrome, ankylosing spondylitis or Hashimoto's thyroiditis. Multicentre studies have shown that the contribution of genetic factors to the development of the disease amounts to 50–65% [23]. Recent studies suggest that this contribution is much higher in patients with seropositive RA (50%) than in patients with seronegative RA (20%) [24].

At present, over 100 loci are thought to be associated with an increased risk of RA in various ethnic populations. The major histocompatibility complex (MHC) locus was the first to be identified and remains the most studied region, accounting for approximately one-third of the genetic susceptibility to the disease [25]. In most populations, certain human leucocyte antigen (HLA) loci, such as HLA-DRB1, are strongly associated with RA. The risk varies depending on allele and ancestry, and is higher in the case of HLA-DRB1*0101/*0401*/0404 in Caucasians, in the case of HLA-DRB1*0405/*0901 in Asians and in the case of HLA-DRB1*1402 in native American Indians [26, 27].

An extremely important discovery was the identification of a sequence of five amino acids (QKRAA, QRRAA, RRRAA) in positions 70-74 of the third hyper-variability region of the third DRß1 chain, encoded by the HLA-DRB1 gene. This sequence was highly conserved in RA risk alleles and was therefore given the name of the shared epitome (SE). The SE hypothesis has played a key role in understanding the aetiology of RA. The presence of SE is more strongly associated with seropositive RA than with seronegative RA. In addition, it is associated with more severe progression of the disease , extra-articular symptoms and radiographic damage.

Subsequent studies extended the SE hypothesis by establishing that much of the MHC region-related risk of RA is associated with six amino acids in four HLA molecules: HLA-DRB1, HLA-B, HLA-DPB1 and HLA-A .However, only 30% of the genetic risk of RA comes from the MHC region. Therefore, a lot of effort has been invested to examine genes other than HLA. One of the best-studied non-genetic HLA variants which are associated with the function of immune cells is the single nucleotide polymorphism (SNP; R620W) in the PTPN22 gene, encoding a protein tyrosine phosphatase involved in antigen receptor signalling in B and T cells. This SNP has been widely replicated and remains the second strongest genetic risk factor for RA, with an OR just below 2. SNP R620W has only been associated with seropositive RA. Evidence in support of this observation comes from studies which indicate that it also increases the citrullination of peptides.

Interestingly, SNP R620W is absent in East Asian (e.g. Japanese) populations, which instead have common genetic variants in PADI4, the gene encoding peptidyl-arginine deiminase (PAD, peptide citrullination enzyme), associated with an increased risk of RA (OR 1,31 per copy allele). Other loci and genes involved in inflammatory pathways which are slightly less important in the etiopathogenesis of RA include CTLA4 , STAT4 , TNFAIP3, TRAF1-C5, IL2/21 , CD40, IL2RA/IL2RB IL6R, CCL21 (lymphocyte chemokine) and many other loci and genes. In the case of RA, new genes come under scrutiny and are being studied in other fields, such as oncology. For example, the human tumour suppressor gene RNASET2 has been found to be linked with the development of RA in Asian populations. This gene encodes T2 ribonuclease - an enzyme involved in the development of cancer. It has recently been found that this gene regulates the innate immune response (e.g. macrophage function), which is important for the development of RA [55, 56].

Importantly, in addition to susceptibility to the disease, some genes other than HLA have been found to be linked with the severity of the disease and with the differences between the seropositive and seronegative RA.

EPIGENETIC FACTORS

In recent year, more and more attention has been paid to the role of epigenetic factors in the development of RA. Epigenetic mechanisms give rise to hereditary changes in gene expression without requiring changes in the sequence of the deoxyribonucleic acid (DNA) [28, 29]. In addition, epigenetic modifications can be triggered by various external factors, such as drugs, smoke or diet, therefore they may constitute a link between genetic and environmental interactions. The key epigenetic changes include DNA methylation, histone modifications and non-coding RNAs, all of which have been proven to predispose to RA. Recent epigenomic studies have revealed differences in gene methylation in blood samples of patients with RA. The studies suggest the existence of two areas within the HLA locus (containing 9 genes) or in their close vicinity (1 gene), which suggests that the genetic risk of RA may be associated with this region and may be partially dependent on the methylation of DNA.

Recent findings have demonstrated that there are two distinct groups of patients with RA, which are characterized by specific methylome and transcriptome signatures in the hip, knee, and MCP (metacarpophalangeal) joints. This discovery explains the differences in disease severity in various joints. Another mechanism of epigenetic regulation are non-coding RNAs, including microRNAs (miRNA, approximately 22 nucleotides) and long non-coding RNAs (lncRNA, over 200 nucleotides).

Both types have been analysed for their role in susceptibility to RA, its severity and effectiveness of treatment. MiRNA-155, miRNA-146a, miRNA-223 and miRNA-124a have proved to be crucial for the pathogenesis of RA. Specific gene targets of these miRNAs have been identified, confirming their significance in the development of that disease [28].

The presence of citrullinated histones is considered an early factor triggering the production of autoantibodies and considered an important element of the pathogenesis of RA. Gene expression can also be modified by the process of histone phosphorylation. This applies especially to histone. The so-called citrullinated peptides (containing the amino acid citrulline) are target antigens of the autoimmune reaction, which results in the production of antibodies directed against them. Citrulline is not a typical amino acid, it is produced after the protein synthesis stage by modifying the amino acid arginine. This conversion, known as citrullination, is catalyzed by enzymes from the peptidyl arginine deaminase (PAD) family. This process affects the characterization of important protein parameters, such as net charge, conformation and antigenicity. Citrullination takes place in healthy tissues, but is also thought to be part of or a result of general inflammatory processes. An increased amount of citrullinated proteins was also observed in synovial fluid. Amino acid modification results in the creation of completely new epitopes. The patients' immune system then reacts with the newly created epitopes, producing ACPA (anti-citrullinated protein antibodies). This immune response, rather than the presence of citrullinated proteins itself, is characteristic of RA.

THE ROLE OF CONCOMITANT ENVIRONMENTAL FACTORS OF RA DEVELOPMENT

Multicentre studies have confirmed the significant impact of the environment on the development of RA. Environmental factors can be divided into several main categories, which have been described as the main determinants of RA and which depend on the diet, the use of stimulants such as alcohol and smoking, as well as socio-economic factors.

DIET

The epidemiological relationship between diet-related factors and RA is still widely studied. It can be concluded that diet-related changes in the intestinal microbiome has an effect on the risk of development of RA [30, 31]. It has been demonstrated that autoimmunity is strongly influenced by nutrition . Multicentre trials have shown that the Mediterranean diet based on the daily consumption of extra virgin olive oil, fish and omega-3 fatty acids protects against the development and clinical symptoms of RA. High consumption of sodium and red meat, which is typical for the Western diet, is associated with an increased risk of polyarthritis or RA. In addition, the above diet is associated with an increased risk of obesity, which is considered a significant health risk, especially in terms of the potential risk of developing RA. A diet rich in dietary fibre and reduced consumption of carbohydrates may improve the composition of the intestinal microflora in patients with RA. There have also been reports that the consumption of coffee may protect against RA, however, a Mendelian randomization analysis does not confirm a causal relationship between the consumption of coffee and the development of RA. Large prospective trials confirm that the Mediterranean diet and the use of products such as fruit and vegetables rich in vitamin C and antioxidants reduce the risk of developing RA. The Mediterranean diet which is especially rich in fruit, vegetables, olive oil and fish protects against the occurrence of RA. The studies confirming this fact are still ongoing.

CIGARETTES AND ALCOHOL

There is a close association between several harmful airborne factors and the development of RA. One of them is smoking cigarettes. Smoking accounts for 20-15% of the total risk of RA and for up to 35% of the risk of RA with a positive ACPA result [32, 33]. There is a clear relationship between the number of cigarettes smoked and the response of the body [32-34]. It has been shown that cessation of smoking gradually reduces the risk of developing RA and that after 20-30 years it returns to the level found in never-smokers . In addition, there is an association between passive exposure to cigarette smoke in the prenatal period, in childhood and in adulthood [33] and the occurrence of RA. Epidemiological studies have shed light on the pathogenesis of RA, demonstrating that smoking is responsible for the citrullination of proteins in situ (i.e. ACPA) and the ultimate development of RA [50, 51].

It has been proven that it is the cigarette smoke, and not tobacco or nicotine, which appears to have a key role in the development of RA, causing chronic inflammation of the respiratory tract. The positive correlation between exposure to cigarette smoke and the occurrence of RA is associated with a significant effect of smoking on immune responses, increased oxidative stress of the body, disruption of apoptosis and provocation of inflammatory processes. This in turn

causes citrullination and epigenetic changes, such as methylation of DNA. It has been shown that there is a gene-environment interaction between smoking and the HLA-DRB1 SE genotype. There is evidence of an association between cigarette smoking and the incidence of RA, as well as the severity of the clinical progression of RA and response to DMARDS drugs.

Low to moderate consumption of alcohol is associated with a reduced risk of RA, particularly in a dose-, time and gender-dependent manner. This association is more pronounced in the case of ACPA-positive RA. There are reports of a synergistic effect of alcohol and tobacco smoking, where the positive association between smoking and the occurrence of RA decreased with increasing consumption of alcohol. There have been reports of a three-way interaction between alcohol, smoking tobacco and HLA-DRB1-SE as regards the risk of developing ACPA-positive RA.

ENVIRONMENTAL CONTAMINANTS

The first identified contaminant is silica, especially in people working in mining and construction. The relationship between silica pollution and the occurrence of RA is associated with an increase in the titre of IL-1alpha, IL-1-beta, IL-2,IL-4, IL-6 i IL-10, as well as TNF-alpha, which indicates that exposure to silica stimulates the immune system and triggers an inflammatory reaction [35]. In addition, textile dust and inorganic dusts such as asbestos and cement, have been found to be risk factors for RA. Other pollutants contributing to the development of the disease also include particulate matter, ozone, carbon monoxide, nitrogen dioxide, sulphur dioxide and lead . In this case, the lungs are an organ that initiates autoimmunity, playing a significant pathophysiological role. Damage to the respiratory tract caused by exposure to the above substances leads to systemic inflammation, increased oxidative stress and epigenetic modifications. Air pollution increases the likelihood of developing RA. Fine PM2.5 particles are most closely associated with ACPA titres. It is interesting to note that there is a relationship between air pollution and the severity and exacerbation of RA, as exposure to high levels of air pollutants has been found to be associated with increased CRP levels and a higher risk of exacerbation of the disease.

SOCIO-ECONOMIC FACTORS

A lower socio-economic status appears to increase the risk of RA, although it is probably more strongly linked with a poor prognosis concerning the progression of the disease. It has been demonstrated that a lower educational level is independently associated with RA, in particular with the seropositive form. A low educational level of parents of children in the household as well as other poor socioeconomic status indicators in early stages of life have also been found to be associated with more severe RA in adults, further supporting positive epidemiological observations [38]. Studies suggest that socioeconomic deficit may be an identifiable risk factor for RA, possibly resulting from unmeasured environmental exposure connected with, among other things, infections or low-quality diet.

It is also worth noting that a low socio-economic status may be more common in people performing physical work, which is associated with an increased incidence of RA too. This relationship is also seen in the case of the father's profession and may be attributable to various factors. Physical workers are often exposed to the adverse effects of the earlier-described harmful inhaled substances such as textile dusts, inorganic dusts, silica and other earlier-mentioned substances, which are important risk factors for RA. Interestingly, it has been shown that long-term physical work is associated with an increased risk of both seropositive and seronegative RA, with interaction with HLA-SE in the former case . Other occupational factors such as stress, conflicts at work and shift work have also been found to increase the risk of RA.

MOLECULAR MECHANISMS OF RA DEVELOPMENT ASSOCIATED WITH ERAD RESPONSE TO UNFOLDED PROTEINS

Most secretory and transmembrane proteins fold and mature in the endoplasmic reticulum (ER). The flux of proteins entering the ER is dynamic and regulated. Adapting to this load requires quality control mechanisms that monitor the levels of unfolded proteins and prevent their accumulation due to the risk of aggregation [3]. To prevent the accumulation of abnormally folded proteins in the ER, proteins that fail quality control are retranslocated to the cytosol, where they are ubiquitinated. Abnormal and potentially toxic proteins are eliminated by proteasomes through the ER-associated degradation (ERAD) mechanism. The ERAD process involves the recognition of substrates in the ER membrane, their translocation to the cytosol, ubiquitination and delivery to the proteasome for degradation. Therefore, a wide range of misfolded proteins require a rapid and highly processable control mechanism. When these quality control mechanisms are compromised or when cells are subjected to a stress that alters the physiology of the secretory pathway, such as viral infection or in response to disease [9, 10], the balance between

folded and unfolded proteins in the ER is upset, leading to the accumulation of misfolded proteins, a condition termed ER stress.

Endoplasmic reticulum ER-associated degradation and the reaction of unfolded proteins are two key quality control mechanisms in the cell. ER-associated degradation is responsible for transferring misfolded proteins in the ER to cytosolic proteasomal degradation, while unfolded protein response (UPR) is activated in response to accumulation of misfolded proteins . When the balance between folded and unfolded proteins in the ER is upset, resulting in the toxic accumulation of misfolded proteins, chronic ER stress begins to dominate the abnormal cell function.

Eukaryotic cells then respond to ER stress and accumulation of unfolded proteins in the endoplasmic reticulum by activating the misfolded protein response - UPR [39]. One of the transmitters of the UPR in mammals is the PERK kinase, which, following ER stress, causes attenuation or global inhibition of protein synthesis via phosphorylation of the eIF2α factor. However, there is a group of genes termed the ER-adaptosome, which is induced in the transcription process under chronic ER stress. Eukaryotic cells with developed ER induce different translating mRNAs than normal cells. Therefore, it has been suggested that UPR-related risk factors may impair adaptation to chronic stress, increasing the risk of rheumatoid arthritis.

In addition to its key role in cell growth and function, UPR modulates the risk of diseases such as diabetes, hepatic steatosis, inflammatory bowel disease, cancer and others. The role of ER stress in rheumatoid arthritis has been suggested many times. Nowadays, the involvement of the UPR in RA patients is identified as an important contribution to both disease initiation, progression and response to treatment [40, 41]. Thus, how adaptation to chronic ER stress is modulated in rheumatoid arthritis cells and whether the lack of adaptation can be exploited in the treatment of patients with rheumatoid arthritis should be the subject of future studies to determine the response pathway of unfolded proteins as a modulator of rheumatoid arthritis initiation, progression and therapy (Table 1).

PERK KINASE-DEPENDENT ADAPTIVE CELLULAR RESPONSE PATHWAY UPR

Eukaryotic cells respond to ER stress by activating a signaling pathway called the unfolded protein response - UPR. The UPR is a collection of signaling pathways that can prevent the effects of ER stress by integrating the control of mRNA translation with the regulation of gene transcription. When, ER stress persists despite activation of these feedback responses, the UPR will initiate apoptosis [42]. In mammalian cells, the UPR consists of three main branches: inositol-requiring enzyme-1 **(**IRE1), protein kinase like endoplasmic reticulum kinase (PERK) and activating transcription factor 6 (ATF6), each of which is activated as a factor in the level of misfolded proteins and consequently activates the corresponding signaling cascades. As a consequence of induced ER stress, the trans-membrane factor ATF6 moves from the ER to the Golgi apparatus, where it is cleaved in a manner that releases the N-terminal domain of ATF6 (ATF6(N)). ATF6(N) moves into the nucleus and acts as a transcription factor. PERK is activated by oligomerization, and once activated, phosphorylates the translation initiation factor eIF2α (eIF2α). This reduces translation initiation, leading to a global decrease in protein synthesis. Paradoxically, eIF2α phosphorylation increases the synthesis of selected transcripts, some of which contain short overlapping open reading frames in their 5'UTR, such as ATF4, a transcription factor that coordinates the transcription of genes that determine cell fate after ER stress. The third UPR factor, IRE1, is both a kinase and an endonuclease. When activated, it digests the transcription factor XBP1 (XBP1) mRNA, removing a 26-nucleotide intron. This non-canonical splicing causes a shift in the reading frame, yielding a spliced form of XBP1 (XBP1s). XBP1s is a very potent transcription factor that consistently induces ER expansion (Fig. 1) [10].

An analysis of the Kyoto Encyclopedia of Genes and Genomes (KEGG) database showed that the group of genes under expression during chronic ER stress is enriched with those that encode proteins involved in ER function, including genes listed for the PERK-dependent UPR [43]. ER adaptosome genes (including 35 ER protein processing pathway genes) are known targets of UPR-induced transcription factors, including ATF6, which exhibits protective functions during chronic ER stress. One of these factors is a transmembrane glycoprotein termed wolframine, which is a regulator of ER calcium levels and plays a key role in cell homeostasis. Since ATF4 induction requires PERK activation and is essential for maximal ATF6 induction, it has been suggested that sustained PERK activity during chronic ER stress is crucial to this process. Recent scientific reports indicate that endoplasmic reticulum stress is an important etiological factor in various human diseases, including conditions associated with the development of inflammation. In particular, the study of the PERK cell signaling pathway has promising potential for understanding the pathological mechanisms involved in rheumatoid arthritis. Therefore, current ongoing studies aim to use mRNA expression (UPR genes) as molecular

Tab. 1. Genes of unfolded proteins response (UPR) pathway involved in development of rheumatoid arthritis

Gene	Function
PERK	protein kinase like endoplasmic reticulum kinase
ATF6	activating transcription factor 6
ATF6(N)	N-terminal domain of ATF6
eIF2α	translation initiation factor 2α
XBP1	X-box binding protein 1
ATF4	activating transcription factor 4
BAX	proapoptotic Bcl-2 associated X-protein
BCL-2	antiapoptotic B-cell CLL/lymphoma 2 protein
eIF2B	translation initiation factor 2B
eIF3	transcription initiation factor 3
eIF4	transcription initiation factor 4
NRF2	nuclear erythroid related factor 2

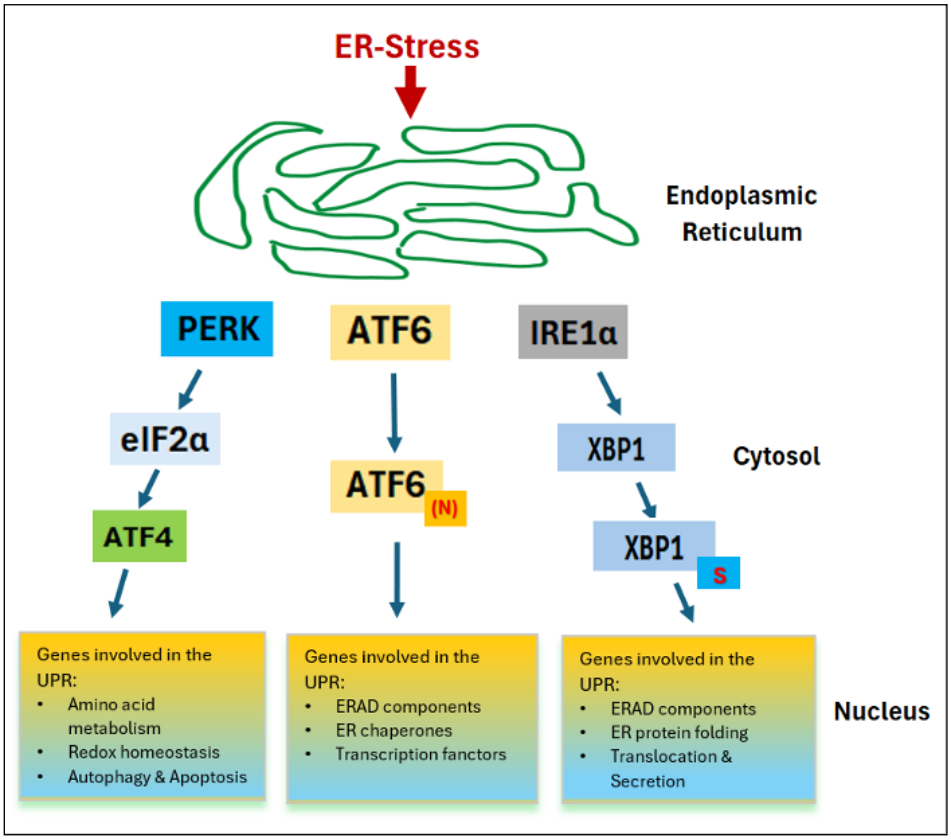

Fig. 1. Pathway of unfolded proteins response (UPR) to ER-stress, key components: protein kinase like endoplasmic reticulum kinase (PERK); activating transcription factor 6 (ATF6); N-terminal domain of ATF6 (ATF6(N)); translation initiation factor eIF2α (eIF2α); transcription factor XBP1 (XBP1); mRNA of XBP1 after removing a 26-nucleotide intron (XPB1s); activating transcription factor 4 (ATF4).

markers to determine global changes in the translation of chronic ER stress-specific factors in patients with rheumatoid arthritis.

UPR GENE EXPRESSION PROFILING AS A RISK MODULATOR IN RA PATIENTS

A major gene involved in the pathogenesis of RA is the PERK gene, which is involved in the unfolded protein response UPR pathway. Studies have shown that PERK gene expression is elevated in RA patients, leading to increased activation of the UPR pathway. This chronic ER stress may contribute to the development and progression of RA by inducing inflammatory cells to release cytokines, further perpetuating the inflammatory response. Given the role of PERK in the pathogenesis of RA, researchers have investigated the use of PERK inhibitors as a potential strategy for targeted molecular therapy. PERK inhibition has been shown to reduce inflammatory cytokine production and reduce joint inflammation in animal models of RA . Moreover, increased expression of autophagy-related genes, including Beclin-1, has been observed in RA patients,

suggesting a potential link between ER stress, mediated by PERK, and autophagy in the pathogenesis of this disease [44]. Additionally, administration of GRP78/BiP, a protein involved in the UPR pathway, has been shown to have potential therapeutic benefits in RA. However, further studies are needed to fully understand the complex relationship between PERK gene expression and RA pathogenesis.

It has also been shown that phosphorylation of eukaryotic initiation factor 2 (p-eIF2) may be a critical factor in the regulation of protein synthesis and may play a key role in the inflammatory response. In RA, *p-eIF2* gene expression has been shown to be involved in the inflammatory and proliferative processes of the disease [46]. Studies have shown that *p-eIF2* gene expression is increased in RA synovial membrane fibroblasts, which are cells lining the joints and contribute to inflammation and joint tissue degradation. Several studies have been conducted on the correlation between RA and *p-eIF2* gene expression. One study showed that RA patients exhibit differential expression of genes encoding cytokine/chemokine-dependent immunity compared to healthy individuals. Takigawa et al. study showed that the ATF6 factor protein, which regulates *p-eIF2* gene expression, simultaneously increases the expression of genes associated with inflammatory response in RA [58].

Another gene that has been identified as involved in the development and progression of RA is transcription factor 4 [45]. ATF4 is a transcription factor that is involved in regulating the cellular response to stress and maintaining cellular homeostasis. It is maintained constitutively at low concentrations, but can be rapidly induced to high levels under stress conditions. The findings have provided insight into the relationship between *ATF4* gene expression and the development of RA. A study by Krabben et al. analyzed gene expression in RA and found that *ATF4* was induced to high levels in the synovial membrane. These findings suggest that ATF4 may be involved in the pathogenesis of RA and may serve as a potential molecular marker of the disease [57].

Although the exact cause of the development of RA is unknown, genetic and environmental factors are thought to play an important role in the development of the disease. Accordingly, recent studies have focused on the role of apoptosis as a mechanism of programmed cell death in the pathogenesis of RA, and the *BAX* gene has emerged as a potential predisposing factor for increased disease risk. *BAX* is a proapoptotic gene that may play a key role in regulating cell death in a wide variety of autoimmune diseases [53, 54]. The aim of another study was to analyze the expression of apoptosis-related proteins in the synovial membrane of patients with rheumatoid arthritis. Consequently, they showed that the level of a potential gas pedal of apoptosis, which is BAX, was higher in healthy controls than in RA patients [59].

It appears that this characteristic imbalance in apoptosis-related proteins may contribute to the survival of autoreactive lymphocytes, leading to chronic inflammation in the synovial membrane. A study by Hilbers et al. examined the expression of the anti-apoptotic gene *BCL-2* in peripheral blood, synovial fluid lymphocytes and synovial tissues of RA patients [46]. The results indicated that *BCL-2* expression was not increased in lymphocytes or synovial tissues from RA patients. Instead, reduced *BCL-2* expression was observed, suggesting that impaired apoptosis may contribute to the pathogenesis of RA [46]. However, another study found that interleukin-17 increased *BCL-2* expression in synoviocytes in RA, suggesting a potential role for *BCL-2* in the inflammatory response in RA. Overall, the role of *BCL-2* gene expression in RA remains unclear and requires further research to fully understand its potential impact on the disease. To summarize previous studies, endoplasmic reticulum-associated ERAD degradation and the response to unfolded UPR proteins are two key mechanisms for controlling translation quality in the cell. Therefore, it has been suggested that *BCL-2* is involved in the regulation of ERAD protein synthesis, while *BAX* gene activity may be associated with UPR activation in response to the accumulation of misfolded proteins during chronic inflammation in RA patients.

PERK INHIBITORS IN TARGETED THERAPY AS A NEW TREATMENT STRATEGY FOR RA

Repression of protein translation by PERK kinase in response to chronic ER stress is reversed by an adaptive process that restores cellular homeostasis. Phosphorylation of eIF2α by PERK and other kinases attenuates translation initiation through repression of the multisubunit transcription factor eIF2B. This results in inhibition of the translation initiation complex and leads to global repression of protein synthesis. Since cells cannot survive under long-term repression of translation, homeostatic mechanisms are involved in the gradual restoration of protein synthesis during chronic ER stress. One such mechanism, described in another studdy, is the conversion from classical and efficient CAP-dependent mRNA translation at the 5′ end (7-methylguanosine with a 5′,5′ triphosphate bond) and driven by the eIF4E factor, to a less favorable mechanism that relies on ribosome recruitment by the eIF3 initiation factor. At the same time, this adaptation process is dependent on the continued repression of eIF2B activity.

PERK kinase interacts with its molecular targets in UPR signaling pathways in a cell type- and physiological context-dependent manner, demonstrating significant tissue specificity [47]. For example, PERK has been found to be essential for the progression of melanoma with BRAF mutations, but plays a lesser role in tumors without BRAF mutations . PERK regulates cellular redox through direct phosphorylation and activation of NRF2. PERK interacts with circadian oscillations (so-called clock genes) by inducing miRNA, which inhibits gene expression in a manner that affects Burkitt's lymphoma progression. Not surprisingly, a number of pharmaceutical companies have taken to synthesizing high-affinity PERK inhibitors. Glaxo Smith Kline developed the GSK2606414 (GSK414) inhibitor; Amgen developed the AMG PERK 44 inhibitor; and Eli Lilly developed Ly4. Our laboratory has also developed specific PERK inhibitors for the treatment of neurodegenerative disorders, including glaucoma (referred to as PERKi). The potential for the use of PERK kinase inhibitors in targeted therapy as a new strategy for treating diseases underlying disorders of protein biosynthesis encourages research into different tissue types and combinations with other drugs to evaluate its role as an effective molecular therapeutic target.

Analysis of the transcriptome in model cells subjected to long-term ER stress identified a set of 567 genes that were induced at the transcriptional level and translated into specific proteins known as the ER-adaptosome . Interestingly, 35 of these genes encode proteins that function in the ER as involved in folding, glycosylation, transport and degradation. Since the ER protein processing pathway involves a total of about 141 genes (according to the KEGG database; PATHWAY:ko04141), it should be noted that a relatively large subset of genes specifically in this category were up-regulated during adaptation to chronic ER stress. Consistently, specific mRNAs were upregulated by both changes in translation efficiency (61 genes) and mRNA abundance (105 genes). Therefore, the term ER-adaptosome has been proposed to describe a group of target-induced genes during chronic ER stress.

Although the exact cause of RA is unknown, previous studies have clearly shown that genetic factors play a significant role in the development of the disease. Our original study analyzed the expression of ER protein processing pathway genes in blood samples from patients with rheumatoid arthritis compared to control subjects. Gene expression was analyzed for *PERK, BCL-2, p-eIF2, ATF4* and *BAX*, as well as the endogenous housekeeping gene (*GAPDH*). Referring to the literature data, we found that the expression of ER stress genes (*PERK, BCL-2, p-eIF2, ATF4* and *BAX*) was significantly higher in RA patients than in controls. According to the literature, the disease is more common in women than in men [2]. Interestingly, our study showed that the expression levels of PERK-dependent UPR genes in patients divided by gender were higher compared to controls in both men and women, suggesting that the UPR response to misfolded proteins is a global process involved in the pathogenesis of RA.

CONCLUSIONS

In conclusion, knowledge of epigenetic and molecular factors in the development of RA provides an opportunity to better understand the etiopathogenesis of this disease, as well as the prospect of creating targeted therapy for the treatment of RA in both its early and advanced forms. Literature reports confirm the influence of both individual and molecular factors on the development of RA. The conclusions drawn from the analysis of the above-mentioned literature are consistent with my clinical observation of patients, expressed both by clinical symptoms, an increase in the concentration of inflammatory markers, and the results of imaging tests. Analyzing the cause of RA at the cellular level. The exact cause of RA is not fully understood, but it most likely involves a complex interaction between UPR chronic stress factors (ER-adaptosome), and genetics, as well as environmental factors. As shown, a key function in the regulation of chronic ER stress is played by PERK kinase, which may be a promising molecular target for RA therapy. Although the current findings are promising, further studies are needed to determine the safety and efficacy of PERK kinase inhibitors for the treatment of RA patients.

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CONFLICT OF INTEREST
The Author declare no conflict of interest

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RECEIVED: 05.08.2024
ACCEPTED: 03.10.2024

REVIEW ARTICLE

CONTENTS

Legal regulation of biomedical research: key principles and their implementation

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ABSTRACT

Aim: To analyze the key principles underlying the legal regulation of biomedical research and evaluate their practical implementation in contemporary legal frameworks.

Materials and Methods: This study employs a comprehensive literature review, including academic publications, legal documents, and international guidelines on biomedical research ethics. Qualitative analysis identified core principles of legal regulation. Comparative legal analysis assessed principle implementation across jurisdictions. Case studies of biomedical research projects provided practical insights. A systematic review of ethical committee reports and policy documents highlighted implementation challenges and best practices.

Conclusions: Ethical principles in biomedical research form a complex framework essential for protecting participants' rights while advancing science. A shift from paternalistic models to patient autonomy is evident, yet implementation challenges remain, particularly in balancing information disclosure with potential negative impacts. Evolving biomedical technologies necessitate ongoing principle refinement. Future efforts should focus on improving complex medical information communication, ensuring informed decision-making, addressing vulnerable populations' needs, while maintaining balance between individual rights and societal benefits. Attention is paid to the principle of informed consent, which is fundamental to modern bioethics and medical practice, reflecting the transition from a paternalistic model to a model emphasizing patient autonomy.

KEY WORDS: biomedical research, human rights, informed consent, personal autonomy, ethical principles

Wiad Lek. 2024;77(9):2070-2076. doi: 10.36740/WLek/195161 DOI

INTRODUCTION

In light of the rapid development of biomedical technologies and their growing impact on society, the issue of legal regulation of biomedical research is becoming particularly relevant. These studies, aimed at expanding the boundaries of scientific knowledge and improving the quality of people's lives, at the same time give rise to a number of ethical and legal challenges. In particular, they relate to ensuring the rights and freedoms of research participants, preserving their dignity and inviolability of private life [1].

Legal regulation of biomedical research is based on a number of key principles designed to harmonize the interests of scientific progress and protection of human rights. These principles cover a wide range of aspects: from ensuring the autonomy of the will of research participants to preserving the confidentiality of the obtained data. They form the foundation for the development of regulations and ethical codes in the field of biomedical research, defining the limits of permissible intervention in the human body and psyche [2].

The implementation of these principles in practice requires the creation of effective mechanisms of control and supervision over the conduct of biomedical research. This involves not only the legislative establishment of relevant norms, but also the formation of a system of ethical committees, the development of informed consent procedures, the establishment of clear criteria for assessing risks and potential benefits from research. Thus, legal regulation in this area should be flexible and adaptive, capable of responding to new challenges that society faces in connection with the development of biomedical technologies [3].

AIM

The aim of this research is to analyze the key principles underlying the legal regulation of biomedical research and evaluate their practical implementation in contemporary legal frameworks.

MATERIALS AND METHODS

This study employs a comprehensive review of relevant literature, including academic publications, legal documents, and international guidelines on biomedical research ethics. A qualitative analysis of these sources was conducted to identify and examine the core principles of legal regulation in biomedical research. Additionally, comparative legal analysis was used to assess how these principles are implemented in various jurisdictions, with a focus on national legislation and international conventions. Case studies of notable biomedical research projects were also analyzed to provide practical insights into the application of these legal principles. The research methodology also included a systematic review of ethical committee reports and policy documents to understand the challenges and best practices in implementing these principles.

REVIEW AND DISCUSSION

Legal regulation of biomedical research is based on a number of fundamental practical principles. Let's take a closer look at one of the key ones among them.

The principle of respect for individual autonomy is the cornerstone of the modern approach to biomedical research. According to J. Hans, this principle reflects a paradigm shift in the role of patients and research participants in modern biomedicine. The essence of this principle is the recognition of a person's capacity for independent, independent thinking and decision-making regarding participation in research and assessment of its potential consequences. A critically important aspect of the implementation of this principle is the creation of conditions under which the research participant is protected from any form of psychological pressure or manipulation, including covert ones. For example, it is unacceptable to create artificial interest or use other methods of indirect influence on a person's decision. Ensuring true freedom of choice is not just an ethical norm, but a necessary prerequisite for the legitimacy and validity of biomedical experiments with human participation [4].

The historically formed paternalistic model of domestic medicine was based on the presumption that the right to make decisions belongs exclusively to medical specialists, while the patient's opinion was considered incompetent and often ignored. This trend still persists in the Ukrainian health care system, where there is a certain resistance to the active involvement of patients in the decision-making process regarding their treatment or participation in medical research.

However, it is important to realize that such practices, which disregard the principle of individual autonomy, are not only ethically questionable, but also potentially threaten the fundamental interests of the patient or research participant. By placing a person in a subordinate position, we not only violate his moral rights, but also create a situation where his vital interests can be ignored or misinterpreted.

Therefore, the transition from a paternalistic model to a model based on respect for individual autonomy is not only an ethical imperative, but also a necessary condition for ensuring quality and safe medical care and conducting ethically based biomedical research [5].

The principle of respect for human dignity is the cornerstone of the ethical system that regulates social interactions. This complex concept covers a wide range of moral and ethical aspects of interpersonal relations in society. It manifests itself through a number of key behavioural patterns, such as:

1. Manifestation of benevolence in communication and actions;
2. Demonstration of respect for the diversity of thoughts and actions of others;
3. Compliance with norms of correctness in interaction;
4. Cultivation of politeness as a basic form of social communication.

This principle serves as a fundamental guideline for the formation of a healthy society where every individual is valued and respected regardless of their status or beliefs [6]. In the context of medicine in general, and especially in the field of biomedical research involving humans, the principle of respect for human dignity acquires special importance. It defines the nature of the interaction between the healthcare professional (or researcher) and the patient (or research participant) while maintaining all the above-mentioned characteristics. It is important to emphasize that compliance with this principle is not only desirable, but absolutely necessary: without it, it is impossible to conduct ethically acceptable biomedical research, as well as to build an effective health care system in general [1].

However, despite the obvious importance of this principle, its implementation in modern society often faces serious challenges. Particularly alarming is the tendency to objectify the human body, when it or its parts are viewed as a commodity or a thing. Clear examples of this are the discussions on the commercialization of organ and tissue donation, or the problem of prostitution. Such phenomena lead to a dangerous shift in public consciousness, where the human body begins to be equated with other objects of the material world, which contradicts the fundamental principle of respect for human dignity [7].

When conducting biomedical research involving a person, it is critically important to realize that the

object of research is not just biological material, but a whole person with his life and health. This concept is enshrined as the highest value in the Constitution of Ukraine and key international legal acts. The principle of respect for human dignity requires a special, respectful attitude towards a person and his bodily integrity. It is important to emphasize the universality of this principle: human dignity is an integral characteristic of every person, regardless of their individual characteristics or social status. This universality means that respect for human dignity cannot be conditioned or limited by factors such as ethnicity, skin color, religious beliefs, socio-economic status, health status or any other external characteristics. Thus, in the context of biomedical research, this principle provides an equal and ethical approach to all participants, regardless of their individual characteristics [8].

The principle of utility in biomedical research is a multidimensional concept that encompasses potential benefits both for the direct participants of the research and for society as a whole. A central aspect of this principle is a careful analysis of the relationship between the potential risks and the expected benefits of the research. Assessing the admissibility of such a ratio requires a comprehensive approach, which includes:

1. Comprehensive analysis of all aspects of research;
2. Systematic consideration of alternative methods and approaches;
3. Detailed study of all available information related to the study.

It is critical that assessments of potential harm consider not only the obvious physical and psychological risks to participants, but also all possible forms of negative impact. This may include social, economic, legal and other aspects that may affect the well-being of research participants or society as a whole. Thus, the principle of usefulness requires a multifactorial analysis and a balanced approach to assessing the ethical acceptability of biomedical research [9].

The principle of utility, which is fundamental in the regulatory regulation of biomedical research involving human subjects, requires a careful balancing of potential risks and expected benefits. The analysis of relevant international and national legal norms allows us to identify key criteria for assessing the ethics and legitimacy of such research. These criteria include the uniqueness and scientific significance of the research, its ethical optimization, scientific validity, a positive balance of risk and benefit, full awareness of both researchers and participants about possible consequences. Compliance with these criteria ensures not only scientific progress, but also protects the rights and safety of research participants, which is a key aspect of modern bioethics [10].

The principle of voluntary informed consent is a cornerstone of modern bioethics and medical practice. This principle not only protects against "medical tyranny" and ensures personal freedom, but also promotes an informed decision by the research participant, aware of the potential consequences of medical intervention or lack thereof. The concept of informed consent is a relatively new phenomenon in medical ethics. Historically, many physicians have taken a paternalistic approach, preferring to withhold from patients full information about their health and treatment. This practice was based on the belief that such information could harm the patient or complicate the treatment process. However, modern medical science and health care practice recognize the principle of informed consent as fundamental. It has become one of the key criteria for observing the rights of research participants and patients in general. This reflects a significant shift in medical ethics from paternalism to a model that emphasizes patient autonomy and their right to full information and participation in decisions about their own health and treatment. [11].

In the context of biomedical research, the principle of informed consent requires that the potential participant be provided with sufficient information to make an informed decision about participation. This information should include a detailed description of the aims, objectives and methodology of the study, a clear explanation of potential risks and expected benefits, a description of alternative options (especially in the case of therapeutic studies), as well as an explanation of the participant's right to ask questions and refuse participation at any time. what stage Information should be provided in a standardized, understandable form and be as complete as possible. Exceptions allowing incomplete disclosure of information are possible only when it is necessary to achieve the purpose of the study, under the condition of minimal undisclosed risks and with the guarantee of further full information of the participants. Such exceptions should be used with extreme caution in order not to violate ethical standards and the rights of research participants [11].

The principle of truthfulness in biomedical research is based on the need for honest and open dialogue between researchers and participants, while recognizing the difficulty of balancing full disclosure and maintaining some degree of confidentiality. This principle recognizes that while truthfulness is the foundation of social cooperation and trust, full disclosure of all information is not always appropriate or ethical. Understanding the difference between telling the truth and telling the whole truth is key, as well as recognizing the need for confidentiality in certain situations. The

ethical approach to truthfulness in research is based on two fundamental principles: the prohibition of lying and the limitation of revealing the truth only to those who have the right to it, which requires careful ethical analysis in each specific case [8].

In the context of biomedical research, the patient's right to information to provide informed consent is fundamental. However, a particularly complex ethical and legal situation arises in the case of placebo studies. The key question is whether the use of a placebo can be considered wrongful deception of the research participant. The decisive factor here is the method of informing about participation in such a study. If a participant is told that he will receive a drug that is potentially effective in this case and has no harmful side effects, this cannot be clearly interpreted as deception. With this wording, the participant receives enough information to provide informed consent, without violating the principle of truthfulness. Thus, the key is to provide the participant with all the necessary information without hiding important details, but also without revealing those aspects that could affect the reliability of the research results [6].

The principle of justice in the context of biomedical research is one of the most complex and important ethical aspects. The selection of research participants must be guided by the fundamental legal concepts of equality, impartiality and independence. However, the implementation of this principle is often complicated by the subjective understanding of the very concept of "justice", which can lead to social and interpersonal conflicts. Justice as a category serves as a measure of social reality, determining what should be preserved and what should be changed. It covers a wide range of relationships between the individual and society, various social groups, and deeply characterizes human activity. This concept requires a balance between the practical activities of individuals or groups and their social status, between rights and obligations, work and reward, personal achievements and their social recognition. Any violation of this balance is perceived by society as a manifestation of injustice.

In the context of biomedical research, the principle of equity should ensure equal access to research participation, fair distribution of risks and potential benefits, and protection of vulnerable populations from exploitation. This requires careful ethical analysis and ongoing monitoring to ensure that research does not exacerbate existing social inequalities or create new ones [12].

The principle of preserving medical secrecy is fundamental in medical ethics and legal practice. It prohibits the disclosure of professional information obtained during the study without the explicit permission of the participant. The importance of this principle is underscored by statutory protections, which in many cases give healthcare professionals the right not to disclose confidential information, even in court proceedings. This principle means that a doctor or researcher has no right to disclose any information about health status, disease characteristics or other medical data obtained confidentially from a patient or research participant without their express consent. This strict adherence to confidentiality is critical because unauthorized disclosure of medical information can lead to serious negative consequences for the individual, including social stigmatization, discrimination, psychological trauma, and disruption of personal and professional relationships. Preserving medical confidentiality not only protects the individual's privacy, but also promotes the establishment of trusting relationships between the doctor/researcher and the patient/participant, which is a necessary condition for effective treatment and conducting quality medical research [13].

According to European standards, information that a research participant provides to a doctor during a biomedical study is classified as "sensitive" data. As a general rule, such information is considered confidential and cannot be disclosed without the express consent of the participant.

However, there are exceptions to this rule. In certain situations, the disclosure of such information may be necessary to protect the interests of the state or society as a whole. This dichotomy between the protection of individual privacy and the potential need for disclosure in the public interest creates a complex ethical and legal dilemma. Such a situation emphasizes the importance of constant rethinking and improvement of both theoretical foundations and practical mechanisms for implementing the principle of preserving medical confidentiality. This requires a careful balance between the right to privacy, the need to ensure trust in the doctor-patient relationship, and the potential public interest that may justify the disclosure of confidential information in exceptional cases.

The importance of maintaining the confidentiality of medical information is most clearly manifested in cases where patients' trust in the health care system is undermined. A case in point is the situation with teenagers infected with sexually transmitted diseases (STDs). When the legislation obliged medical professionals to inform parents of minors about cases of STDs, this led to unforeseen negative consequences. Infected teenagers, fearing disclosure of information to their parents, avoided seeking medical help. This not only left them without proper treatment, but also contributed to the further spread of infections, which in some countries led to epidemic outbreaks.

The loss of trust in the health care system due to privacy violations has transformed into a serious public health problem. Only after the revision of the legislation and the restoration of the principle of confidentiality did the situation begin to improve. The guarantee of medical confidentiality encouraged young people to seek medical help, which significantly contributed to curbing the spread of STDs. This example clearly demonstrates how compliance with the principle of confidentiality in medicine not only protects the rights of individual patients, but also plays a key role in ensuring the effectiveness of the health care system and preserving public health as a whole [5].

The principle of privacy in the context of biomedical research is a fundamental ethical and legal norm. It categorically prohibits researchers from interfering in the private lives of research participants without their express consent, even if such interference is motivated by scientific interests. This principle is based on the understanding of man not only as a social being, but also as a unique individual with his own individuality. Recognition and protection of this individuality, as well as the right to privacy, are key aspects of respect for human dignity. A society that values and protects the privacy of its members thereby recognizes for them a certain degree of personal freedom. This freedom includes the right to control information about oneself, to decide who and to what extent can access personal data and aspects of an individual's life.

It is important to emphasize that respect for privacy and ensuring the inviolability of personal life are not just ethical norms, but necessary conditions for the functioning of a democratic society and the rule of law. They create a basis for the realization of other fundamental rights and freedoms, promote the development of individual autonomy and protect against unjustified interference by the state or other entities. In the context of biomedical research, compliance with the principle of privacy is particularly important, as such research often involves access to sensitive personal information. This requires researchers not only to obtain informed consent, but also to be constantly vigilant in maintaining data confidentiality and respecting the personal boundaries of research participants [3].

The concepts of "personal integrity" and "private life" are integral components of the broader concept of "individual freedom". These concepts are not just interconnected, but also logically follow from the principle of individual freedom. True individual freedom includes two key aspects:

1. Protection against illegal and violent state interference in a person's private life.
2. Guarantees of protection of life, honor, dignity and personal safety of every member of society.

It is important to note that the right to privacy is not absolute. Its limitation is possible, but only in cases clearly provided for by law. This ensures a balance between individual rights and public interest. The autonomy of the individual from the state, society or any social group is based on the guarantee of confidentiality of certain aspects of a citizen's private life. Without such a guarantee, true autonomy is impossible.

The legislation of Ukraine is aimed at:

1. Consolidation of legally defined procedures for the realization of privacy rights.
2. Creation of mechanisms to prevent violations of these rights.
3. Establishment of a procedure for the protection and restoration of violated rights and freedoms.
4. Determination of permissible limits of interference in private life by other persons, society and the state.

Thus, the legal system of Ukraine strives to create a comprehensive privacy protection mechanism that would ensure a balance between individual rights and public interests, while guaranteeing the fundamental freedoms of every citizen [13].

The principle of non-interference in private life is based on an understanding of the potential harm that may arise from the disclosure of personal information. In the context of biomedical research, two types of particularly sensitive information can be distinguished:

1. Personal information: data about private life, marital status, personal beliefs, etc.
2. Professional information (medical confidentiality): medical data, diagnoses, examination results.

Disclosure of these types of information without an individual's consent can lead to serious negative consequences, including social stigmatization, discrimination, psychological trauma, and disruption of personal or professional relationships.

When publishing research results, it is important to distinguish between two types of data:

1. Statistically summarized data: This information does not contain individual characteristics of the study participants. Its publication usually does not require a separate permission, as it does not carry the risk of identifying specific individuals.
2. Specific clinical cases: Such data may contain detailed personal and medical information. For their publication, it is necessary:

a) Obtain explicit permission from the research participant.
b) Take steps to anonymize data to minimize the risk of personal identification.

Adherence to these principles ensures a balance between the scientific value of the research and the protection of the privacy of the participants. This is not only

an ethical requirement, but also an important condition for maintaining public trust in medical research and the health care system as a whole [14].

CONCLUSIONS

1. Legal regulation of biomedical research is based on key ethical principles, such as respect for individual autonomy, respect for human dignity, utility, voluntary informed consent, truthfulness, fairness, medical confidentiality and privacy.
2. The implementation of these principles requires the creation of effective control and supervision mechanisms, including the formation of a system of ethical committees, the development of informed consent procedures and the establishment of clear criteria for assessing risks and potential benefits from research.
3. Particular attention is paid to the principle of informed consent, which is fundamental to modern bioethics and medical practice, reflecting the transition from a paternalistic model to a model emphasizing patient autonomy.
4. Preserving the confidentiality of medical information and respecting the privacy of research participants are critically important aspects that not only protect the rights of individuals, but also contribute to maintaining public trust in medical research and the health care system as a whole.
5. Legal regulation in the field of biomedical research should be flexible and adaptive, able to respond to new challenges associated with the development of biomedical technologies, while ensuring a balance between scientific progress and protection of the rights and safety of research participants.

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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

RECEIVED: 02.06.2024
ACCEPTED: 22.09.2024

REVIEW ARTICLE

CONTENTS

Human rights in the context of transhumanist medicine: ethical and legal aspects

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ABSTRACT

Aim: To analyze the concept of transhumanism as a new category that extends beyond traditional legal frameworks and its implications for human rights, personal freedom, and the boundaries of technological intervention in human biology.

Materials and Methods: The study employs a comprehensive literature review and analysis of scholarly works on transhumanism, bioethics, and related legal and philosophical concepts.

Conclusions: Transhumanism is a modern philosophical and scientific concept that proposes a radical rethinking of human nature through the use of advanced technologies aimed at overcoming human biological limitations. The development of transhumanist ideas creates new ethical, legal and social challenges, in particular regarding human rights, personal freedom, and the limits of technological intervention in the human body and consciousness.

KEY WORDS: human rights, law, medicine, transhumanism, bioethics, somatic rights

Wiad Lek. 2024;77(9):2077-2082. doi: 10.36740/WLek/195164 DOI

INTRODUCTION

In the modern world, where technological progress is rapidly changing our understanding of the limits of human capabilities, transhumanism appears as a philosophical current that seeks to use the achievements of science and technology to improve the physical and cognitive abilities of a person. This concept finds a particularly vivid embodiment in medicine, where advanced technologies open new horizons for treating diseases, prolonging life, and expanding human potential. However, along with incredible opportunities, transhumanist medicine raises complex ethical and legal issues, especially in the context of fundamental human rights [1].

Human rights, which have traditionally been considered inalienable and universal, face new challenges in the era of transhumanism. The question of how to protect the dignity and autonomy of the individual in the face of radical biotechnological interventions is becoming more and more relevant. Does a person have the right to genetic modification? How to ensure equal access to human enhancement technologies? Can society limit individual choices regarding biotechnological improvements? These and many other issues require careful analysis and rethinking of traditional concepts of human rights.

The ethical and legal aspects of transhumanist medicine cover a wide range of issues, from issues of informed consent and privacy to more global issues of social justice and equality. It is important to find a balance between the desire for technological progress and the need to protect fundamental rights and freedoms. This requires not only a review of existing legal norms, but also the development of new ethical principles that will take into account the unique challenges of the transhumanist era. The study of these aspects is critically important for the formation of a responsible approach to the development and implementation of transhumanistic technologies in medicine, which will ensure the protection of human rights in conditions of rapid technological progress [2].

AIM

The aim of the research is to analyze the concept of transhumanism as a new category that extends beyond traditional legal frameworks and its implications for human rights, personal freedom, and the boundaries of technological intervention in human biology.

MATERIALS AND METHODS

The basis of the research will be general scientific methods, such as analysis and synthesis, which will allow to divide the problem into component parts and form a holistic understanding of the relationships between

transhumanism, medicine and human rights. The comparative legal method will be used to compare different legal approaches to the regulation of transhumanistic technologies in medicine in different countries. The historical method will help to trace the evolution of human rights concepts and their transformation under the influence of technological progress. Special methods of legal science will also be applied, in particular the formal-legal method for analyzing legal norms, and the method of legal modeling for forecasting possible scenarios of the development of legal regulation in this area. In addition, it is planned to use sociological research methods, including a survey of experts in the field of bioethics, medicine and law, to obtain current opinions on the ethical dilemmas of transhumanist medicine.

REVIEW AND DISCUSSION

In the light of modern discussions about the future of humanity, the concept of transhumanism deserves special attention as a new category that goes beyond the traditional legal field. The issue of human evolution in the context of rapid technological development is becoming one of the most urgent and discussed in modern society. Rapid changes in all spheres of life, acceleration of scientific and technological progress and global challenges in the socio-economic, political and environmental spheres prompt representatives of the humanities to search for new philosophical and scientific approaches to understanding the key problems of humanity [3].

Transhumanism is one of the most controversial futurological teachings of our time. Proponents of this direction consider transhumanism as a rational worldview based on a critical analysis of scientific achievements and perspectives. This philosophy recognizes not only the possibility but also the desirability of fundamental changes in human nature through advanced technology. The purpose of such transformations is to overcome the limitations inherent in human existence, including suffering, aging and death, as well as a significant expansion of the physical, cognitive and psychological capabilities of a person [4].

The transhumanist approach offers a radically new perspective on the potential for human development, raising both admiration and serious ethical and philosophical questions. This concept presents society with the difficult task of rethinking traditional ideas about human nature, human rights, and the limits of technological intervention in human biology. In the context of legal regulation and ethical norms, the ideas of transhumanism create a new space for discussions and require careful analysis by legal scholars, philosophers and representatives of other humanitarian disciplines [5].

With the development of modern technologies such as genetic engineering, cryonics and artificial intelligence, the scientific community is faced with a number of new challenges that have the potential to change our traditional understanding of human nature and essence. As noted by D. Kovba and E. Hrybovod, these technological innovations lead to the transformation of the theory of humanism, expanding its classical principles, and in some cases even completely rethinking them. The concept of transhumanism arises against the background of a new scientific and technological revolution and, in the process of forming its institutional and conceptual structure, becomes an integral part of the modern information society. This idea reflects humanity's desire for technological improvement and overcoming biological limitations. The emergence of the phenomenon of transhumanism creates a need for its comprehensive analysis, especially in the context of the search for ethical, value, legal and political mechanisms. These mechanisms should determine the expediency and possibilities of using the latest technologies, including radical modifications of the human body, which could potentially lead to the emergence of a "post-human". Such an analysis is critically important for understanding and regulating the consequences of technological progress for humanity as a whole [6].

Modern reality shows a growing tendency towards "cyborgization" of man - the process of integration of natural and artificial elements. V. Yemelin singles out two main directions of this phenomenon. The first direction - medical - focuses on restoring lost functions of the body with the help of artificial implants or microchips. According to the scientist, approximately one in ten residents of developed countries already have some kind of synthetic prostheses or implants, including pacemakers, defibrillators, artificial heart valves and joints, not to mention cosmetic implants. The second direction is aimed at expanding the capabilities of a healthy person through the integration of various technical additions that directly interact with the human body. Today, such technologies are most actively implemented in the military industry, where they are used to increase the physical and cognitive capabilities of servicemen. This tendency to merge the biological and the technological raises important questions about the future of humanity and the limits of technological intervention in the human body [7].

In modern scientific discourse, the issue of transhumanism is actively discussed by Ukrainian researchers in various aspects. Thus, N. Krokhmal and I. Galchenko investigate the philosophical and social aspects of

transhumanism, considering it as a new stage in the development of humanity [8]. O. Kravchenko analyzes the ethical challenges of transhumanism in the context of biotechnological human improvement [9]. V. Lukyanets focuses on the study of transhumanist ideas in the context of post-non-classical science [10]. D.Sapronov examines the legal aspects of transhumanism, in particular the issue of regulation of new technologies [11]. O. Kostenko investigates the influence of transhumanist ideas on the development of criminal law [12].

The issue of transhumanism is also actively discussed by foreign scientists who consider a wide range of issues related to this topic. N. Bostrom, one of the leading theorists of transhumanism, analyzes the ethical and philosophical aspects of the technological improvement of man [13]. J. Hughes examines the social and political consequences of transhumanist technologies [14]. A. Sandberg focuses on cognitive improvement and its impact on society [15]. New Vita-More examines transhumanism through the prism of art and design [16]. F. Fukuyama, from a critical point of view, analyzes the potential risks of transhumanist ideas for human nature and democracy [17].

Modern American researchers emphasize the important role of bioethics in the context of the demographic aging of European society, where there is a quantitative predominance of elderly people. They emphasize the need to recognize the moral significance of the population aging process and the ethical challenges associated with it in many modern societies [18]. These scientists offer an innovative approach to solving the problems of gerontosophy and social inequality through the application of biotechnology in combination with the ethical principles of transhumanism. In their opinion, bioethics, together with other disciplines, has the potential to influence demographic trends and contribute to the formation of policy decisions aimed at improving the quality of life of the elderly and the aging process in general. This approach forms a new concept of "good citizenship" in an aging society, going beyond traditional ideas about health care. This concept involves the active participation of bioethics in the formation of public discourse and policy on aging, with the aim of creating a fairer and more inclusive society for people of all age categories [19].

In the medical field, the "concept of solidarity" has become widespread, which acts as a tool for ensuring justice and equality in health care issues. This concept encompasses a number of advanced medical innovations and practices. Key components of this concept include:

1. Integrated healthcare information systems that ensure effective exchange of medical data.
2. Biobanks are specialized repositories of biological materials and related information that contribute to the development of medical research.
3. Personalized medicine, which involves an individual approach to diagnosis and treatment based on the patient's genetic and other characteristics.
4. Organ, tissue, cell and blood donation programs, which play a critical role in providing vital resources for medical interventions.

Together, these elements form the basis for a more equitable and efficient health care system that seeks to ensure equal access to health care and resources for all members of society. [20].

Yu. Melyakova and S. Zhdanenko single out a special sphere of anthropological freedom - the freedom of pleasure. According to them, this form of freedom occupies a leading place among all possible freedoms available to the posthuman. This concept of freedom of pleasure goes beyond the traditional understanding of human rights and freedoms, reflecting new aspects of human existence in the context of transhumanist ideas. It is not just an opportunity to receive pleasure, but rather a fundamental right to seek and realize the various forms of pleasure that may become available thanks to technological progress and the expansion of human capabilities. This concept emphasizes the importance of individual choice and self-realization in a potential post-human society [21].

The Association of Transhumanists in its international forums and official declarations defines one of the key tasks of transhumanism as "increasing the level of human happiness", which includes, in particular, the fight against suffering. A number of means and technologies are offered to achieve this goal. Among the recommended methods of increasing satisfaction, the following are distinguished:

1. Pharmacological means: anxiolytics to reduce anxiety and fear, analgesics to relieve pain, entactogens and antidepressants to temporarily suppress negative emotions, doping and nootropics to improve cognitive functions.
2. Promising technologies that, according to the scientific community, can significantly increase the level of human satisfaction: telepresence systems, brain-computer interfaces, neuroprosthetics and brain modeling, technologies for transferring human consciousness to a synthetic medium, such as an artificial body (avatar).

These approaches reflect the desire of transhumanists to radically improve the human experience and overcome the biological limitations associated with negative emotions and physical discomfort. However, they also raise important ethical questions about the nature

of human happiness and the limits of technological intervention in the human psyche and body [22].

The transhumanistic model of freedom does not at all resemble the moral freedom of humanism, since the anthropological concept and the entire system of values will be completely reformatted today [21]. A. Horyachkovska reveals the key concept and ultimate goal of transhumanism - the creation of a posthuman. This idea also resonates with the theories of post-postmodernism, representing a new stage in the evolution of the human species. Posthuman, according to this concept, is considered as a fundamentally new biogenetic species. In theory, this species would be so modified and improved that it would be able to overcome the limitations of the physical body. It is assumed that the posthuman will have the ability to exist in intangible forms, such as information structures in computer networks. This futuristic concept suggests the possibility of transforming human consciousness into a form of artificial intelligence or metamind. Thus, the posthuman is presented as an entity that goes beyond the traditional understanding of human existence, able to function in the digital space without attachment to the physical body. This idea reflects a radical vision of the future of humanity, where the boundaries between biological and technological, physical and virtual blur, opening new horizons for the development of human consciousness and intelligence [22].

The modern era is characterized by a rapid expansion of the space of human freedom, which goes far beyond the boundaries of traditional social reality. In the context of transhumanist ideas, the freedom of a transhuman covers not only external aspects of life, but also internal ones: mental and neurophysiological states, biochemical and molecular biological processes. This new paradigm of freedom gives a person the status of not only the carrier and potential resource of these processes, but also their full-fledged moderator, which is embodied in the concept of "the right to dispose of one's own body." Thus, a person receives an unprecedented level of control over his biological essence, which opens new horizons for self-determination and self-realization, but at the same time raises complex ethical and legal questions about the limits of such freedom [21]. The modern tendency towards the pragmatic objectification of immaterial phenomena as objects of market relations creates the basis for a radical transformation of ideas about the human body and its potential. In this context, human abilities and attributes are seen as potential means of enrichment, similar to material possessions. This principle of commercialization became the basis for the legitimization of biotechnological manipulation of the human body, including postmortem use, turning it into a kind of medical product and the object of biotechnological experiments. Within the framework of the transhumanist concept of freedom, the human body becomes an attractive resource for economic investment, which opens up new opportunities, but at the same time raises serious ethical questions about the limits of commodification of human nature and the potential consequences of such an approach for society and individual dignity [4]. The concept of the body as a biomaterial and a resource is formed at the intersection of two key trends of modern society. On the one hand, it is the result of the expansion of personal human rights, which gives the individual greater control over his own body. On the other hand, this concept reflects the trend towards all-encompassing commercialization, which permeates all spheres of life, including the sale of biospecimens for banks, the patenting of genes, and the existence of an (often illegal) market for human organs and tissues [5].

According to the definition of S. Ranish, transhumanism acts as a unifying slogan for various cultural, political, philosophical and digital currents that promote techno-futuristic concepts of overcoming the limitations of human biology [23]. V. Vovk notes that transhumanism as a separate movement was finally formed at the end of the 20th century, promoting the idea of overcoming death and aging with the help of advanced technologies. The 20th century became revolutionary for European culture, marked by a number of "turns" - scientific, linguistic, visual, and at the turn of the 20th-21st centuries there was an "anthropological turn", which is characterized by a new attitude of man to his body and an emphasis on physicality. Every historical era and society expresses its essence through ideologies and worldviews, which are reflected in philosophy, science, law, art, religion, rules of behaviour and ideas about bodily beauty. These ideas form a certain ideal, which is considered a standard for its time. Modern transhumanist trends, supported by technological progress, lead to a rethinking of the concept of the body. V. Vovk claims that the traditional understanding of the body as a "soma" - a local autopoietic biosystem - is annulled. A person is no longer considered a "somatic automaton" controlled by the body's instinctive programs. Instead, it appears as an entity that opposes itself to the unity and non-contradiction of the natural world, going beyond biological limitations [24].

Yu. Turyansky rightly notes that the doctrine of transhumanism, which can lead to the restriction of individual freedom for the sake of hypothetical future ideals, does not meet the legal standards of a developed society. He emphasizes the difficulty of finding the "golden mean" between the extremes, emphasizing

the need to define a clear vector for the development of a new generation of somatic rights through law, public institutions and legal policy. The scientist calls for caution in perceiving convergent technologies as a panacea for the future of humanity. He proposes a "third way", which involves strengthening the responsibility of a person for his future, preserving his evolutionary and biological certainty and the maximum realization of somatic rights. This approach requires not only a general strategy for the development of the latest technologies, but also a detailed consideration of each right from the somatic group, clearly determining the acceptability or unacceptability of the opportunities provided by global technological transformations. Such a position emphasizes the need for a balanced approach to the development of technologies and the protection of human rights, avoiding both excessive techno-optimism and unfounded fear of innovation. This requires careful analysis and regulation of every aspect of somatic rights in the context of technological progress [25].

CONCLUSIONS

Based on the above, the following conclusions can be drawn:

1. Transhumanism is a modern philosophical and scientific concept that proposes a radical rethinking of human nature through the use of advanced technologies aimed at overcoming human biological limitations.
2. The development of transhumanist ideas creates new ethical, legal and social challenges, in particular regarding human rights, personal freedom, and the limits of technological intervention in the human body and consciousness.
3. The concept of "cyborgization" of man and the idea of creating a "post-human" raise important questions about the future of humanity, including the potential risks and benefits of radical modification of the human body.
4. Transhumanism is closely related to the development of bioethics, especially in the context of the aging of the population and the search for new approaches to improving the quality of life, which requires a rethinking of traditional ethical norms and legal standards.
5. There is a need to find a balance between technological progress and the preservation of fundamental human rights, which requires the development of new legal mechanisms and ethical principles for the regulation of transhumanist technologies.

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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

RECEIVED: 29.05.2024
ACCEPTED: 28.09.2024

REVIEW ARTICLE

CONTENTS

Novel pharmacologic approaches in resistant hypertension

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ABSTRACT

Resistant hypertension (RH) affects 10% to 18% of all patients with hypertension. It is diagnosed when blood pressure cannot be normalized despite the use of 3 classes of antihypertensive drugs (ACE inhibitors or sartans), calcium antagonists and diuretics in maximum doses. If the above-mentioned drugs are ineffective, spironolactone is most often administered. Recently, valsartan/sacubitrol and flozins have been increasingly used in resistant hypertension, which have been introduced for the treatment of diseases other than hypertension but can be a supplement to previously used drugs in resistant hypertension. Pharmaceutical companies are currently working on a dozen or so antihypertensive drugs. The most advanced studies concern aldosterone synthesis inhibitors, an endothelin receptor antagonist, and a drug that inhibits angiotensinogen synthesis. The results to date allow for the consideration of aprocitentan (a drug that inhibits endothelin receptors) or baxdrostat or another new aldosterone synthesis inhibitor in the treatment of resistant hypertension that does not respond to 4 drugs, including spironolactone. Further studies are needed to confirm the efficacy and safety of these new drugs in the treatment of resistant hypertension.

KEY WORDS: resistant hypertension, baxdrostat, aprocitentan, zilebesiran

Wiad Lek. 2024;77(9):2083-2089. doi: 10.36740/WLek/194090 DOI

INTRODUCTION

Resistant hypertension (RH) is diagnosed when blood pressure cannot be normalized despite the simultaneous use of 3 different antihypertensive drugs, including a diuretic in maximum doses [1]. Diagnosis of true resistant hypertension requires exclusion of non-adherence to an appropriate lifestyle and non-systematic use of drugs. Blood pressure measurements using the ABM method are desirable to exclude white-coat hypertension [1]. Studies indicate that in almost 50% of patients resistant hypertension is diagnosed, which disappears after full-adherence with medical recommendations [2]. Many authors use the term true resistant hypertension (RH) to distinguish it from resistant hypertension caused by non-adherence to medical recommendations (pseudoRH) [3]. So far, the terms resistant and refractory hypertension have been used interchangeably in the literature. Resistant hypertension (RH) is diagnosed when 3 or 4 drugs used simultaneously do not normalize blood pressure. It affects 10-15% of patients with hypertension [1]. On the other hand, refractory hypertension (RfRH) can be diagnosed when treatment is ineffective with the use of five or more antihypertensive drugs listed above, including spironolactone [1]. In recent years, this issue has been discussed more and more often. according to Filippone et al., true resistant hypertension is characterized by volume overload and aldosterone excess, refractory by enhanced sympathetic tone [6]. Refractory hypertension (RfRH) occurs in 0.5% to 4.5% of all patients with hypertension [5,6,7]. Patients with RfRH are usually younger, overweight, and more likely to suffer from type 1 diabetes or obstructive sleep apnea, in contrast to people with resistant hypertension (RH), where usually older people predominate, often black race [6, 7]. RH significantly more often causes heart ischemia (including myocardial infarction), strokes and leads to increased mortality compared to hypertension that responds well to antihypertensive therapy [1]. Even more frequent complications of hypertension occur in RfRH) [7, 8] These patients required more drugs than patients with RH [7]. It is therefore understandable to search for new drugs that would effectively lower blood pressure in patients with resistant or refractory hypertension. Is there any hope that new antihypertensive drugs will be released in the near future that will improve the prognosis in patients with resistant hypertension?

REVIEW AND DISCUSSION

SEARCH FOR NEW ANTIHYPERTENSIVE DRUGS

For many years, the pharmaceutical industry has not produced a new drug dedicated solely to the treatment of hypertension, and the last one was aliskiren introduced to therapy almost 20 years ago. Work is

currently underway on a dozen or so preparations that have the potential to have an effective antihypertensive effect. These include angiotensin 1-7, inhibition of angiotensinogen, aldosterone synthesis, endothelin receptors, mechanisms that increase nitric oxide levels, vaccines, and change intestinal microbiota. Great hopes are currently pinned on a new drug called zilebesiran. It is a new class of siRNA therapeutic that reduces the production of angiotensinogen. The latter compound is synthesized in the adipose tissue in the brain, kidneys, and primarily in the liver [9]. As a result, less angiotensin I and angiotensin II are produced in the body, which translates into a decrease in blood pressure. In subsequent studies, zilebesiran has been shown to prevent the stimulation of the RAS system despite a large increase in renin [9]. Phase I studies of zilebesiran confirmed that a single subcutaneous administration of this drug at a dose of 800 mg reduces the concentration of angiotensinogen by over 90% and this effect is maintained for 6 months, and a drop in blood pressure of over 15 mm Hg lasts for about 8 weeks. These studies also showed that this drug is well tolerated and that adverse effects are rare (local reactions at the site of drug administration in 5 out of 56 patients treated). In particular, no hypotension or liver function damage was observed [10, 11] The next study (KARDIA-1) recruited 107 patients with mild or moderate hypertension. Zilebesiran was administered subcutaneously at doses of 10, 25, 100, 200, 400, and 800 mg or placebo [11]. Finally, some patients were given zilebersan in combination with irbesartan. The reduction in blood pressure correlated with the dose of zilebesiran and was maintained for up to 24 weeks. A single dose of 200 mg or more of zilebesiran caused a reduction in systolic blood pressure of at least 10 mm Hg and diastolic blood pressure of at least 5 mm Hg. The combined administration of zilebesiran with irbesartan caused a greater reduction in blood pressure. The reduction in blood pressure after a single administration of zilebesiran was maintained for 24 weeks [11]. Currently, there are ongoing studies with zilebesiran injections every 3 months or 6 months, as well as studies with the concomitant use of zilebesiran with other antihypertensive drugs. Among others, the Kardia-2 study concerns the addition of zilebesiran to the therapy of patients with uncontrolled hypertension despite the use of standard antihypertensive treatment. Completion of these studies is expected in 2024/2025.

Endothelin is a very strong vasoconstrictor. It could be assumed that endothelin receptor antagonists would be compounds that strongly lower blood pressure. Several types of these antagonists are currently known. These include bosentan and darutensin, but due to numerous serious side effects, they have not found wider therapeutic use. Recently, aprocitentan is a dual ETAR/ETBR receptor antagonist, evaluated in a randomized, double-blind PRESISION study of 730 patients with resistant hypertension [12, 13]. Doses of 12.5 mg or 25 mg of aprocitentan or placebo were administered for over 44 weeks. A significant reduction in systolic blood pressure was observed compared to placebo (P= 0.0042 and P= 0.0046). Blood pressure was measured using unattended automated off-line BP. Aprocitentan caused a greater reduction in blood pressure at night than during the day in the 24-hour ABP study. Fluid retention during aprocitentan treatment was dose-dependent and occurred in 9 to 18% of treated patients and in 2.1% of patients receiving placebo. Mahfooz et al. conducted a meta-analysis of aprocitentan regarding the antihypertensive efficacy of this drug. This meta-analysis shows that this drug, both at a dose of 10 and 25 mg, statistically significantly reduces systolic and diastolic blood pressure [14]. In the third phase of the PRECISION study, the usefulness of aprocitentan in the treatment of resistant hypertension was assessed. The results of aprocitentan treatment allow the use of this drug in elderly patients with renal failure, especially when spironolactone therapy must be discontinued due to its adverse effects. A unique effect of aprocitentan is a significant reduction in proteinuria and nephroprotection properties. There was no difference in the frequency of serious adverse events between the drug and placebo [15, 16]. These beneficial features of this drug resulted in the fact that in March 2024, the United States Food and Drug Administration has been approved under the brand name Tryvio for treatment resistant hypertension in combination with other antihypertensive drugs in adult patients [13].

Hypertension can be caused by excess aldosterone. Spironolactone, which has been used for a long time and effectively reduces resistant blood pressure, is a synthetic analogue of progesterone, which blocks the binding of this drug to the aldosterone receptor [17]. Common side effects of spironolactone include excessive increase in serum potassium concentration. For this reason, it cannot be used in patients with advanced renal failure. Administration of drugs that lower serum potassium concentration may prolong the use of this drug, but does not protect against the development of gynecomastia. Recently, the efficacy and safety of a new aldosterone receptor antagonist called esaxerenone [18,19] was assessed in Japan. The drug proved to be effective and safe, with only a slight decrease in GFR and an increase in serum potassium concentration [19].

Another drug called baxdrostat (inhibiting aldosterone synthesis) has successfully passed the first human

trials. Its half-life is 26-31 hours, which allows it to be administered once daily [20]. In a multicenter placebo-controlled study of 275 patients with therapy-resistant hypertension, baxdrostat was administered at a dose of 0.5 mg, 1 mg or 2 mg for 12 weeks. Patients receiving an aldosterone receptor antagonist, potassium sparing diuretics and patients with GFR <45 ml/min were excluded from the study. At the end of the study, systolic blood pressure decreased by 20.3 mm Hg, 17.7 mm Hg and 12.1 mm Hg in patients receiving 2. mg, 1 mg and 0.5 mg of the study drug/day, respectively. In patients receiving placebo, blood pressure decreased by 9.4 mm Hg. The decrease in blood pressure in patients receiving 1 or 2 mg of the drug was highly statistically significant. No serious side effects were noted during the study; only in two patients the concentration of potassium in the blood exceeded 6 mmol/l.

Sometimes headaches and weakness occurred after using this drug. Hyperkalemia did not cause cardiac arrhythmias. Baxdrostat is therefore well tolerated and does not cause adrenal insufficiency [20]. The HALO study is another evaluation of the efficacy and safety of baxdrostat. The study included 249 patients with hypertension, and the drug was used similarly at a dose of 2 mg, 1 mg and 0.5 mg/day. After 8 weeks of treatment, blood pressure dropped insignificantly compared to placebo. The authors of the study explain the lack of significant decrease in blood pressure by the criteria for qualifying patients for the study. Namely, initial blood pressure was above 180/110 mm Hg, some patients had uncontrolled diabetes and some of the subjects had GFR < 30 ml/min. The study lasted 8 weeks, not 12 weeks as in the previous study [21, 22]. Further evaluations of baxdrostat are currently underway lasting 26 weeks of therapy. The use of baxdrostat in the treatment of hypertension, also resistant, is the subject of further studies [23].

Another drug inhibiting aldosterone synthesis, called lorundrostat, was used at a dose of 50 or 100 mg once daily or at a dose of 12.5 and 25 mg twice daily in 163 patients with uncontrolled hypertension. The decrease in systolic blood pressure was statistically significant. A decrease in serum aldosterone concentration was observed in all patients. In one patient, the treatment was discontinued due to hyponatremia. In 6 patients, the serum potassium concentration exceeded 6 mmol/l. However, the study was not discontinued, only the dose of lorundrostat was reduced [24].

Two drugs (entresto - valsartan/sacubitril and flozyny) recently introduced to the therapy of heart failure and diabetes moderately reduce blood pressure values. The first of these drugs contains valsartan and sacubitril. The latter component inhibits neprilysin, which causes vasodilation and increased natriuresis [25]. According to Rakugi et al., entresto is more effective in lowering blood pressure than olmesartan [26]. Therefore, Wang et al. used entresto alongside the previous drugs in resistant hypertension in hemodialysis patients. The decrease in blood pressure was 20.7/8.3 mm Hg and was statistically significant, the NT-proBNP concentration decreased, and the therapy proved to be safe [27]. Entresto was also successfully used by Jackson AM et al. in the therapy of resistant hypertension and heart failure [28]. Flozins introduced to diabetes therapy have proven effective in the treatment of circulatory failure. It has long been known that these drugs have a diuretic effect [29]. All known flozin preparations slightly but statistically significantly reduce blood pressure. The hypotensive effect of flozins has already been confirmed by meta-analyses [30]. The greatest decrease in blood pressure was observed in the SACRA study. The hypotensive effect of flozins is complex and not fully explained. The diuretic effect of these drugs plays an important role in the hypotensive effect of flozins. Flozins reduce the body weight of patients by an average of 2.2-2.6 kg within 6 months, [31] increase the excretion of uric acid, which leads to a small decrease in this compound in blood serum [32]. Clinical studies also suggest inhibition of sympathetic activity, as well as reduction of vascular stiffness [33]. The first successful trials of using flozins in resistant hypertension have appeared. Ferreira et al. administered empagliflozin or placebo to 7020 patients with hypertension, and among them 22.5% of patients showed hypertension resistant to 3 or 4 antihypertensive drugs, including a diuretic and in 17.2% spronolactone. (34) Adding empagliflozin caused a decrease in blood pressure in the entire study group by 4.5 mm Hg. In 38% of patients with resistant hypertension a decrease in systolic blood pressure below 130 mm Hg was achieved, in the placebo group in 26%. In addition, a decrease in the number of heart attacks and strokes was observed. An interesting report by Obeid et al. is about a 68-year-old man with diabetes and hypertension, despite taking ramipril, bisoprolol, diltiazem, amiloride and furosemide, excluding secondary causes of hypertension and skipping doses of the drug by the patient. Renal artery denervation was also performed. Despite this, the hypertension did not subside. The administration of canagliflozin caused a decrease in blood pressure from 151/87 to 136/83 mm Hg) [35]. The literature reports to date regarding the use of flozins in patients with resistant hypertension encourage the conduct of larger studies assessing the efficacy and safety of such a procedure. It can be assumed that the results of these studies will expand the number of drugs used in resistant hypertension. It can

also be assumed that GLP-1 inhibitors will also be used in the future to treat resistant hypertension. They have a diuretic effect and reduce excess weight more than flozins. However, there are no reports in the literature on this subject so far.

PROPOSALS FOR A NEW TREATMENT REGIMEN FOR RESISTANT HYPERTENSION

According to the current recommendations of experts, in patients with resistant hypertension we use angiotensin-converting enzyme inhibitors or sartans, calcium antagonists and diuretics, usually hydrochlorothiazide or indapamide [1]. If this procedure is ineffective, in light of the studies presented above, now we can optimize the current pharmacological treatment, namely swap sartans for entresto and/or add flozins regardless of the presence of diabetes in the patient with hypertension. The next, fourth drug in the treatment of resistant hypertension depends on the degree of renal function. In patients with glomerular filtration less than 35 ml/min, it is recommended to administer a strong diuretic, e.g. chlorthalidione (but not spironolactone, which will intensify hyperkalemia). In patients with glomerular filtration greater than 35 ml/min, spironolactane (an aldosterone receptor blocker) should be added to the above treatment. Spironolactone lowers blood pressure by a further 8.7 mm Hg after 12 weeks of therapy. Long-term use of this drug can cause dangerous hyperkalemia and gynecomastia in men and menstrual disorders in women. Currently, in the case of contraindications to spironolactone, baxdrostat or aprocitentan can be considered (Table1). Baxdrostat is a drug that inhibits aldosterone synthesis, and at a dose of 2 mg/day it lowers blood pressure by 20 mm Hg. Aprocitentan, a dual endothelin receptor antagonist, lowers blood pressure by about 11 mm Hg and does not increase potassium levels. In patients with GFR lower than 30 ml/min/1.73 m^2 and who do not respond to strong diuretics, aprocitentan should be considered [13]. Many patients with resistant hypertension are overweight and have diabetes. Such patients often have elevated serum endothelin (ET-1) levels. Higher endothelin levels are also often observed in renal failure, as well as in the elderly, hypertensive blacks and especially in women [36-38]. All of the above-mentioned reasons indicate the advisability of administering aprocitentan to the above-mentioned patients with hypertension resistant to therapy with previously used drugs, especially when renin activity is low. It has been shown that there is an inverse relationship between serum endothelin levels and plasma renin activity [39]. Aprocitentan can be used both in patients with spironolactone intolerance and in patients with advanced renal failure and resistant hypertension. In 2021, Sidharta et al conducted a study in patients with severe renal failure (GFR 21 ml/min) who were given aprocitentan at a dose of 50 mg/day. The pharmacokinetics of aprocitentan were similar in patients with severe renal impairment and in healthy subjects, as well as in younger and elderly subjects (the half-life of the drug in patients was slightly longer than in healthy subjects and the maximum drug concentrations in blood were comparable [40].

The next steps in the treatment of resistant hypertension that does not respond to previous treatment are the administration of beta blockers or alpha-1 blockers. Bisoprolol, carvedilol or nebivolol are usually used. These drugs can also be administered to patients with advanced renal failure [1].

Alpha-1 antagonists include Doxazosin (alpha-1 antagonist). In the Treatnent to Prevent Heart Attack study, doxazosin administration was associated with more frequent cardiac complications compared to chlorthalidone therapy, and in the PATHWAY-2 study, the antihypertensive efficacy was lower than that of spironolactone.

After beta and alpha blockers, the next step in treatment is peripheral vasodilators, which strongly lower blood pressure but are burdened with numerous side effects. These include hydralazine (Apresolina). Another drug used in the case of persistent high blood pressure is alpha methyldopa. This drug is used in hypertension during pregnancy but can also be administered in resistant hypertension. In the absence of improvement, some authors recommend the use of clonidine (central sympatholytic agents), which in the ReHOT study significantly lowers systolic and diastolic blood pressure but is burdened with many common side effects.

Moxonidine is an imidazole receptor agonist that works slightly longer than clonidine. The last therapeutic option, according to some authors, is minoxidil. The initial dose of this drug is 5 mg/day and can be gradually increased if no adverse effects occur. This drug causes fluid retention and stimulates the sympathetic system. It often causes hirsutism and for this reason patients discontinue therapy with this drug.

Lack of improvement after the use of the above-mentioned drugs is an indication for renal artery denervation or baroreceptor activation.

The optimal treatment of RfRH is unclear, but sympathetic inhibition (α-β blockade, centrally acting sympathoinhibitors, or both) and aprocitentan seems reasonable. Renal denervation has shown minimal benefit for resistance, but its role in refractory hypertension is probable better [6].

Table 1. Treatment plan of RH

Treatment plan of RH	
ACEi or ARB + CA + Diuretic	
Patients not controlled change	
ARB on valsartan/sacubitrol or/and flozin	
CKD stage 1 to 3	CKD stage 4-5 (not on dialysis)
eGFR> 30 ml/min/1,73 m^2	eGFR < 30 ml/min/1,73 m^2
Patients not controlled	
ADD	ADD
Spironolacton or another MRA	Chlortalidone or anther thiazide-like DD
Patient not controlled	
ADD	ADD
Aprocitentan or baxdrostat	Aprocitentan
Patietnt not controlled	ADD
BB or A1-blocker§BB or A1- blocker	
Patient not controlled	ADD
Centrally acting drug§Centrally acting drug	

Abbreviations:
ACB – angiotensin converting enzyme inhibitor, ARB - sartan, MRA - mineralocorticoid receptor antagonist, BB beta-blockers , CA – calcium, antagonists diuretic (DD) -- ADD. Changes to current recommendations are marked in bold letters.

CONCLUSIONS

True resistant hypertension (RH) affects over 10% of all patients with hypertension. It causes 44% more ischemic heart disease, 57% more strokes. We hope that after the break caused by the Covid-19 pandemic, when almost 80% of pharmaceutical companies were busy searching for a cure for this epidemic, the pharmaceutical industry will now accelerate research on new drugs for hypertension. The most promising trials concern baxdrostat, aprocitentan and zilebesiran. It is already possible to modify the recommendations of the Hypertension Societies from 2023 by adding enresto and flozins to the treatment of resistant hypertension. In the event of lack of effectiveness, it is worth considering the use of endothelin receptor antagonists and aldosterone synthesis blockers, which have not been included in the recommended treatment regimens for resistant hypertension so far. However, they require further studies assessing their effectiveness and safety in these patients.

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CONFLICT OF INTEREST

The Author declare no conflict of interest

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RECEIVED: 30.07.2024
ACCEPTED: 15.09.2024

REVIEW ARTICLE

CONTENTS

For whom the bell tolls: The fear of death and the ways to become less afraid of it

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ABSTRACT

Aim: This paper aims to examine the fear of death and psychotherapeutic techniques to face and confront this fear.

Materials and Methods: The authors used interpretive research paradigm, integrative anthropological approach, and hermeneutic approach.

The data collection was carried out using PubMed, Scopus, Web of Science, Google Scholar databases. Research papers were identified according to search terms: "fear of death", "death anxiety", "death", "mental health", "psychological wellbeing", "culture", "human existence", "philosophy", "existentialism", "cognitive behavioral therapy", "existential psychotherapy", "logotherapy", "acceptance and commitment therapy", "mindfulness", "meaning in life".

Conclusions: According to S. Kierkegaard, a fear, including the fear of death, is the prerogative of a human being, because human beings, unlike animals, have a spirit. It is closely connected with the pinnacle of temporary tension, the moment when we have to act in order to shape or reshape our destiny and our Self. J.-P. Sartre considered anxiety and fear to be integral parts of our being in the world. Being abandoned in the world, an individual does not have a certain future, so he/she is forced to choose his/her own life and Self. None of us can escape the fear of death; each of us, sooner or later, has to face it. But if we cannot avoid something, we must accept it. People have known this simple truth since ancient times.

KEY WORDS: death, fear of death, death anxiety, mental health, psychological wellbeing, culture, human existence, philosophy, existentialism, cognitive behavioral therapy, existential psychotherapy, logotherapy, acceptance and commitment therapy, mindfulness, meaning in life

Wiad Lek. 2024;77(9):2090-2097. doi: 10.36740/WLek/195171 DOI

INTRODUCTION

Death is one of the poles of the fundamental binary opposition "life – death". This opposition is universal and serves as the principle of semantic division of the world into two parts. Within the framework of this binarity, individuals organize and comprehend their experiences, their place in the world seeing "life" as a desirable goal and "death" as something that must be avoided at all costs. Since it is almost impossible to realize and describe one's own death (in this context, the investigations of R.A. Moody, T. Leary, R. Metzner, and R. Alpert are of great interest), it was awareness of the death of the "Other" that determined sociogenesis and cultural genesis.

From ancient times to the present day, the death of an individual, his/her attitude to death, the meaning or meaninglessness of life has always been a relevant, pressing and controversial topic for any human being and humanity as a whole. This incomprehensible and frightening phenomenon seemed mysterious, hidden behind the veil of Isis, the lifting of which revealed the way to the worlds beyond our earthly realm: from the state similar to a dream or transition to another world to the developed concepts of heaven and hell, reincarnation or even the physical immortality of the Taoists. Human culture has used all its wealth to "resist" death. Ideas about death are crucial for worldviews inherent in any given sociocultural community, individual self-awareness, human values, and "maps of meanings". Cultural orientations in relation to death are closely related to the self-awareness of the individual, his/her identity. People tend to perceive death based on the values of their cultures. Changes in the perception of death express shifts in our self-understanding. In fact, the transformation of the image of death in the public consciousness sheds light on the reshaping of human subjectivity.

The institutionalization of death plays an important role in the process of socialization and formation of cultural identity in all societies. In traditional societies, the phenomenon of death shaped culture, religion, and morality. Nowadays, a society (especially the Western society) is trying to "eliminate" death from social and cultural life. Death is no longer a sealed secret. It has come into conflict with scientific rationality and the progress of science and technology. It no longer evokes sacred horror and awe; on the contrary, it should be hidden and transformed. As a result, there is a need for more aesthetic funeral rites. The process of dying should not disturb the harmony of human society. The death of an individual has become the responsibility of hospices, hospitals, and funeral homes. However, the fear of death is constantly present in human souls.

According to YouGov Death Study, 41% of Britons are afraid of their own death that has affected their enjoyment of life [1]. A 2019 survey on respondents' fear of death shows that 11% of respondents in the United States were very afraid of death [2]. According to a survey conducted in Singapore in 2019, 32% of respondents were afraid of death [3]. However, the answers to the surveys and the perception of death in an existential situation can be strikingly different.

Covid-19 pandemic added fuel to the flame becoming an anxiety-provoking global event. Death toll statistics have reminded people of the fragility of their own existence. Tragic situations occurring with relatives, friends, neighbors and even strangers were projected onto themselves causing fear and anxiety for their own lives and the lives of their loved ones. Conducting a systematic review in order to estimate the pooled score of death anxiety during the COVID-19 pandemic, I. Patra et al. revealed that "the standard score of death anxiety in the COVID-19 pandemic was 50% … The findings related to the continent showed that the standard score of death anxiety was 51% in Asia and 42% in Europe and America. In addition, the death anxiety score in studies related to 2020 and 2021 was 51% and 62%, respectively. The results of subgroup analysis revealed that the highest score of death anxiety was related to patients with COVID-19 (59.4%), other patients (58.9%), and the elderly (56.4%). The lowest score of death anxiety was related to the general population (42.9%) and health care workers (48.2%)" [4]. Z. Chalhoub et al. defined death anxiety as the "most significant predictor of fear related to the COVID-19 pandemic" [5]. The COVID-19 pandemic has revealed that the changes are the only constant in life [6]. Changes involve situations of uncertainty, which in most cases are difficult to tolerate, because human beings "seek to understand, predict and control – it helps us learn and it keeps us safe. Uncertainty can feel dangerous because we cannot predict with complete confidence what will happen" [7].

M. Bulut's research suggests that intolerance of uncertainty is a critical variable in the relationship between anxiety provoked by the COVID-19 pandemic and the fear of death [8]. Uncertainty seems dangerous, frightening, preventing us from planning the future, being confident or having a system of expectations that is justified. Death for people is also a huge abyss of uncertainty, since no one has returned from the other world and shared their experiences about the stages of transition to another reality or complete disappearance.

Though human beings, unlike other species, know about their own finiteness, the COVID-19 pandemic increased the awareness of the inevitability of death, aggravated the fear of death, and affected mental health. W. James stated that the awareness of our inevitable death is the "worm at the core" of human existence: "The fact that we CAN die, that we CAN be ill at all, is what perplexes us; the fact that we now for a moment live and are well is irrelevant to that perplexity. We need a life not correlated with death, a health not liable to illness, a kind of good that will not perish, a good in fact that flies beyond the Goods of nature.

It all depends on how sensitive the soul may become to discords… a little cooling down of animal excitability and instinct, a little loss of animal toughness, a little irritable weakness and descent of the pain-threshold, will bring the worm at the core of all our usual springs of delight into full view, and turn us into melancholy metaphysicians. The pride of life and glory of the world will shrivel. It is after all but the standing quarrel of hot youth and hoary eld. Old age has the last word: the purely naturalistic look at life, however enthusiastically it may begin, is sure to end in sadness" [9]. This "worm at the core" may dramatically undermine human experience of happiness; cause mental health disorders; lead to a loss of meaning in life which will end anyway. L. Iverach, R.G. Menzies and R.E. Menzies argue that death anxiety is a transdiagnostic construct that underlies the "development and maintenance of numerous psychological conditions" [10]. The fear of death is related to illness anxiety, somatoform disorders, panic disorder, common specific phobias, obsessive compulsive disorder, agoraphobia, post-traumatic stress disorder, depressive disorders. Death anxiety is related to social anxiety disorder and eating disorders [11]. Hence, dealing with only one disorder does not address the underlying death-related fears, which ultimately results in the new mental health issues because the core problem remains untouched [11].

AIM

This paper aims to examine the fear of death and psychotherapeutic techniques to face and confront this fear.

MATERIALS AND METHODS

The authors used interpretive research paradigm, integrative anthropological approach, and hermeneutical approach.

The data collection was carried out using PubMed, Scopus, Web of Science, Google Scholar databases. Research papers were identified according to search terms: "fear of death", "death anxiety", "death", "mental health", "psychological wellbeing", "culture", "human existence", "philosophy", "existentialism", "cognitive behavioral therapy", "existential psychotherapy", "logotherapy", "acceptance and commitment therapy", "mindfulness", "meaning in life".

It should be noted that the concepts of "fear" and "anxiety" are not identical and may carry different meanings. M. Heidegger explained anxiety as the state in which the threat is nowhere: "Anxiety 'does not know' what that in the face of which it is anxious is" [12]. It is something more extensive and diffuse than the immediate manifestation of fear, which is specific [13]. However, as M. Heidegger emphasizes, there is an essential connection between fear and anxiety. Fear and anxiety are distinct but inseparable. They are immanent to each other and to life itself. These ontological phenomena are modes of human existence. Therefore, in this article we will use the concepts of fear and anxiety interchangeably.

REVIEW AND DISCUSSION

The fear of death is as old as the world and human life itself. G. Zilboorg states that no one is free of the fear of death which underlies many psychopathological conditions: "behind the sense of insecurity in the face of danger, behind the sense of discouragement and depression, there always lurks the basic fear of death, a fear which undergoes most complex elaborations and manifests itself in many indirect ways" [14]. As C. Wahl put it, "death is itself not only a state, but a complex symbol, the significance of which will vary from one person to another and from one culture to another…" [15]. According to E. Becker, the fear of death haunts the human race like nothing else. This fear is a driving force of any life activity. Given that a human being is at the intersection of the natural and symbolic worlds and it is the symbolic identity that distinguishes him/her from the natural world, each of us is in the state of existential paradox: we are unique beings towering above the world and at the same time the flesh that must inevitably perish. This unbearable situation must be overcome, at least in the symbolic world [16]. E. Becker argued that "man's innate and all-encompassing fear of death drives him to attempt to transcend death through culturally standardized hero systems and symbols" [17].

Thus, the terror of death governs human life, even if we are not consciously death aware. Awareness of our finite existence awakens thoughts about the meaninglessness of everything around including ourselves. In an attempt to give meaning to this elusive moment, people strive to achieve symbolic immortality, whatever that means. At different times, in different cultural-civilizational communities, this issue was addressed in its own way. Trying to keep death at bay, individuals elaborate social constructs, create own worldviews in which the fear of death becomes more controllable and bearable. Funeral rites, the cult of ancestors, rituals, various forms of religions, ideas ranging from non-being to heaven and hell, architectural monuments, works of art, awards, statuses, projects of the future, immortality projects, human superiority and everything that boosts self-esteem have performed a psychotherapeutic function in the fight against the fear of death. i.e., the human self should become meaningful again: I am, I am significant.

Developing E. Becker's ideas, S. Solomon, J. Greenberg and T. Pyszczynski introduced Terror Management Theory (TMT), according to which "the uniquely human awareness of death gives rise to potentially paralyzing terror that is assuaged by embracing cultural worldviews and meeting or exceeding the standards of value associated with them (i.e., self-esteem) in pursuit of literal and/or symbolic immortality. Convergent empirical support for TMT was originally obtained by studies demonstrating that: momentarily elevated or disproportionately high self-esteem reduces anxiety, autonomic arousal, and defensive cognitive distortions produced by psychological and physical threats; making MS (*mortality salience*) increases defense of the cultural worldview and self-esteem striving; and threats to cherished cultural beliefs or self-esteem increase the accessibility of implicit death thoughts (DTA)" [18].

Trying to insulate themselves from the deep fear of death, people use proximal and distal defenses based on whether their fears are conscious or unconscious. If they are conscious, people combat them through proximal defenses exaggerating their health, denying their vulnerability, or just suppressing thoughts about death. Distal defenses deal with unconscious fears – people strive to see themselves as valuable contributors to the world, develop cultural worldviews, create meanings, purposes, and values that offer literal or symbolic immortality [19]. Although Terror Management Theory as any complex multidimensional theory, has attracted its share of criticism, it is an effective tool

that can help in understanding our existential world, the fear of death and develop strategies to address these issues [20].

The power of death is so strong to resist, and there is still no one "always working" approach to solving this problem. As Shakespeare noted, "death, a necessary end, will come when it will come", so it seems reasonable to come to terms with death, that is, to accept this truth. G. Gesser, P.T.P Wong and G.T. Reker defined three types of death acceptance:

- Approach-Oriented Death Acceptance – death is viewed as a passageway to a happy afterlife; religious beliefs play a main role in this type of acceptance;
- Escape-Oriented Death Acceptance – death is viewed as an escape from our painful life; health conditions, suffering, loneliness, hopelessness play a main role in this type of acceptance;
- Neutral Acceptance – death is neither welcomed nor feared; this type of acceptance is based on the awareness of the mortality of all living things [21].

Approach-Oriented Death Acceptance ought to be positively related to happiness, optimism and hopefulness, while Escape-Oriented Death Acceptance indicates a low level of wellbeing and hopelessness. As for Neutral Acceptance, it, on the one hand, may motivate individuals to make their lives meaningful, to be "here and now", on the other hand, perception of ourselves as creatures who come to this world for a moment and are doomed to die may lead to doubt about the meaning of life, which is not conducive to psychological wellbeing [21].

Although all types of death acceptance help reduce the level of death anxiety, R.E. Menzies and R.G. Menzies emphasized that "neutral acceptance appears to produce the lowest levels of death anxiety" [11] and compared it to the Stoic approach. And indeed, when it comes to such deep existential problems, it is philosophy that acts as a guiding star in the ocean of chaos, darkness, and fear. The Stoics taught that some things were in our control and others not. Death, like birth, is beyond our control, thus, we have to accept it. Epictetus wrote: "where I can escape death: discover for me the country, show me the men to whom I must go, whom death does not visit. Discover to me a charm against death. If I have not one, what do you wish me to do? I cannot escape from death" [22].

Neutral Death Acceptance is also represented by Epicureanism – another Hellenistic philosophical school. Being aware that death is a part of human existence and the basic human fear, Epicurus addressed this issue: "Become accustomed to the belief that death is nothing to us. For all good and evil consists in sensation, but death is deprivation of sensation. And therefore, a right understanding that death is nothing to us makes the mortality of life enjoyable, not because it adds to it an infinite span of time, but because it takes away the craving for immortality. For there is nothing terrible in life for the man who has truly comprehended that there is nothing terrible in not living. So that the man speaks but idly who says that he fears death not because it will be painful when it comes, but because it is painful in anticipation. For that which gives no trouble when it comes, is but an empty pain in anticipation. So, death, the most terrifying of ills, is nothing to us, since so long as we exist, death is not with us; but when death comes, then we do not exist. It does not then concern either the living or the dead, since for the former it is not, and the latter are no more" [23].

Can we cultivate death acceptance in our complex, contradictory, ever-hurrying world, in which, on the one hand, the topic of death is most often avoided in interpersonal communication and, on the other hand, death appears everywhere in popular culture and becomes something ordinary? Is it possible to loosen the grip of the suffocating fear of death? Today it is believed that the leading place in correcting the fear of death is occupied by cognitive behavioral therapy (CBT). It helps people reinterpret situations allowing them to find ways to deal with their fears more constructively. Cognitive behavioral therapists encourage clients to explore some of the complex reasons behind their fear to help them better understand it and discover their maladaptive unrealistic beliefs about dying and death [11]. Since avoidance is a common way of dealing with the fear of death, CBT focuses a lot on exposure (in vivo or imaginal) allowing clients to face their fears. Entering the frightening situation, clients "learn" to calm themselves gradually approaching their worst fear and learning to accept it. In vivo exposure includes reading obituaries, preparing a will, visiting cemeteries, hospices, funeral homes, etc., i.e., visiting places and engaging in activities directly related to death. Imaginal exposure involves writing a story about different aspects of client's own death or deaths of their loved ones, about fears concerning death, pain, suffering. If certain unpleasant sensations are signals of fear of death, interoceptive exposure is used [24].

Definitely, CBT effectively copes with certain symptoms by changing conscious thoughts and behavior, but this approach does not consider the personality in its entirety. As F. Faranda put it, CBT and the general shift in the orientation of psychotherapy are associated not so much with the advantages of one approach over another, but with our fear of something inside us [25], with the abyss and darkness of our natural (vs

symbolic) part at which sooner or later we have to look. As R.E. Menzies and R.G. Menzies rightly stated, the "failure to address death fears may in fact be due the death anxiety of clinicians and researchers themselves. How can we begin to work with clients' concerns about death and dying if we ourselves avoid the subject in our own life?" [11].

Existential psychology and, accordingly, psychotherapy try to cope with this existential abyss. Psychology is deeply rooted in a certain type of philosophy. If scientific psychology adheres to theories and techniques that have been developed and validated through the use of the scientific method and depends on the objective measurement and the replication of results under controlled or known conditions [26] by and large following the path of R. Descartes, then existential psychology is based on the philosophy of existentialism. Focusing on naturalism and empiricism and searching for general laws, scientific psychology to some extent absolutizes and universalizes the reality, in which the individuals have to act according to its laws. Moreover, cartesian "mind – body" dualism has led to the collapse of the holistic phenomenon of a human being and accordingly to the deep contradictions [27]. At the same time, existential psychology interprets a human being as an indivisible whole: there are no separate mental and physical phenomena, and we have to deal with human wholeness and indivisibility approaching his/her core (where this deep and inescapable fear, the fear of death, resides).

Within the framework of existential psychodynamics, the basic conflict of an individual is determined by his/her confrontation with the givens of existence. I.D. Yalom defines these givens of existence as "certain ultimate concerns, certain intrinsic properties that are a part, and an inescapable part, of the human being's existence in the world" [28]. He identified four ultimate givens: death, freedom, isolation, and meaninglessness. Existential conflicts are generated by the individual's confrontation with any of these life facts. Of these, death is the primary source of anxiety and the primary fount of psychopathology [28]. One of the classic boundary situations, in which a person can really answer the question of "who" he/she is and "why" he/she is, is a confrontation with personal death, which can cause a significant change in a style and character of an individual's life. Indeed, as I. Yalom emphasized, "death is of such momentous importance that it can, if properly confronted, alter one's life perspective and promote a truly authentic immersion in life" [28].

A confrontation with own death pulls an individual out of the trap of oblivion, transfers him/her to another mode of existence, in which people take personal responsibility for their lives. Existential psychotherapists encourage clients to visualize their death; to write their own obituaries or epitaphs; to draw a line on paper, with one representing birth and other – death, and to draw a cross where they believe they currently are; to disidentify from what they are used to consider themselves; to use a guided fantasy technique to increase death awareness; to interact with the dying, etc. [28].

Philosophy plays an important role in existential psychotherapy. For individuals who express concern about their nonexistence after their death, I. Yalom recommended to find comfort in Epicurus's symmetry argument: "after death I will be in the same state of nonbeing as before birth" [29]. Confrontation with death becomes a source of personal change – an individual realizes that life cannot be postponed; disidentifies with everything insignificant to which he/she holds on within his/her illusion of life; begins to appreciate what he/she has. Despite its apparent depressive overtones, existential psychotherapy helps to overcome existential vacuum; brings people back to life in a broad sense of the word; reveals the world in a new light, the world, in which they can live to the fullest. Choosing to live independently and fully, an individual creates his/her own meanings that are really important to him/her, feels more joy in life, and is more capable of healthy interactions with other people.

It is a great challenge to find (or create) meanings in life. People form their values, goals, attitudes, preferences under the influence of the agents of primary and secondary socialization, i.e., their meanings are conditioned by others and are often not critically analyzed. As A. Maslow put it, people do not listen to themselves but to the voices of their parents, government, authorities, traditions, etc. However, true existence in the face of inevitable death and other givens is possible only if we shape our life and create our own meanings. Then, even in the most difficult situations, we can overcome all hardships. As F. Nietzsche said: "If you have your why for life, you can get by with almost any how" [30]. The meaning of life has become the cornerstone of logotherapy – a therapeutic approach developed by V. Frankl. Believing that it was possible to turn suffering into self-development, V. Frankl helped clients to struggle with existential vacuum and uncover their true meanings. According to V. Frankl, the purpose of logotherapy is to cope with the suffering caused by the philosophical problems posed by life [31]. The task of a logotherapist is to help his/her clients in finding their meanings, since meanings cannot be given. By finding meanings, people are able to rise above their difficulties, fears, and doubts and outgrow their own limits.

Although it seems to us that death deprives life of meanings, V. Frankl stated that "the transitoriness of our existence in no way makes it meaningless. But it does constitute our responsibleness; for everything hinges upon our realizing the essentially transitory possibilities. Man constantly makes his choice concerning the mass of present potentialities; which of these will be condemned to nonbeing and which will be actualized? Which choice will be made an actuality once and forever, an immortal "footprint in the sands of time"? At any moment, man must decide, for better or for worse, what will be the monument of his existence" [31]. The studies conducted indicate that logotherapy significantly reduces death anxiety in the elderly; in mothers of children with cancer; in the women with breast cancer; in diabetic patients with depression; in multiple sclerosis patients [32-34]. M. Ameli and F.M. Dattilio suggested enhancing cognitive behavioral therapy with logotherapy providing examples of how logotherapy techniques may be incorporated into a cognitive behavioral framework [35].

Acceptance and Commitment Therapy (ACT) is no less effective in combating the fear of death. ACT is an action-oriented approach to psychotherapy developed by S.C. Hayes. It is focused on values, compassion, living "here and now", forgiveness, striving for true self and contributes to the acceptance of our hardships, negative thoughts and feelings since they are a part of our life. ACT aims to develop our psychological flexibility through acceptance; cognitive defusion; being mindful in the present moment; expansion of the self; values; and committed actions. It helps to "create a rich and meaningful life, while accepting the pain that inevitably goes with it" [36].

Mindfulness plays an important role in ACT. Mindfulness is a certain mental state and a therapeutic technique that rely on the ability to be focused on the present moment, "here and now". Mindfulness can be practiced anytime, anywhere and can be applied to any situation. It effectively supports ACT by developing nonjudgmental acceptance of clients' thoughts and feelings. Research shows that ACT decreases death anxiety in clients with OCD, in patients with multiple sclerosis, in palliative care, in cancer patients, in the elderly [37-39].

Examining the effects of different psychosocial interventions on death anxiety, J. Lu et al. came to the conclusion that rational-emotive hospice care therapy "exhibited superior efficacy as a psychological treatment for reducing the death anxiety of patients" [40]. Rational emotive behavioral therapy was formulated by A. Ellis and was inspired by the Stoic belief that the ideas of things make us happy or unhappy, but not things themselves. In addition, J. Lu et al. attached special importance to logotherapy (including group therapy), spirituality therapy training, and acceptance and commitment therapy hypothesizing that "combining logotherapy and spirituality therapy training or acceptance and commitment therapy would yield a more favorable effect on DA (*death anxiety*) than employing rational emotive hospital care therapy alone" [40; emphasis added].

CONCLUSIONS

According to S. Kierkegaard, a fear, including the fear of death, is the prerogative of a human being, because human beings, unlike animals, have a spirit. It is closely connected with the pinnacle of temporary tension, the moment when we have to act in order to shape or reshape our destiny and our Self. J.-P. Sartre considered anxiety and fear to be integral parts of our being in the world. Being abandoned in the world, an individual does not have a certain future, so he/she is forced to choose his/her own life and Self. None of us can escape the fear of death; each of us, sooner or later, has to face it. But if we cannot avoid something, we must accept it. People have known this simple truth since ancient times. Various cultures throughout history have developed their own ways of dealing with the fear of death that found expression in the religious and philosophical teachings of humankind, to which many people still resort today. However, in the contemporary world, in which the exacerbation of existential anxiety has reached monstrous proportions, more and more people terrorized by wars, disasters, impending environmental, sociopolitical and cultural problems abandon traditional ideas and feel cut off from the integral fabric of being.

Modern psychotherapy offers a range of psychotherapeutic techniques that can help confront the fear of death, such as cognitive behavioral therapy, existential psychotherapy, logotherapy, acceptance and commitment therapy, rational-emotive hospice care therapy and some others. These techniques are not always suitable for everyone. One glove does not fit all. Probably, that is why it is very difficult to work within the framework of one protocol in such non-protocol existential situations as the fear of death, loss of meaning of one's existence, horror in the face of nonexistence. Therefore, it seems appropriate to direct joint efforts towards elaboration and development of effective strategies to confront and overcome the fear of death – strategies that would combine the strengths of various techniques, could be tailored to fit personal needs of a client, and in the spirit of a holistic paradigm would be integrated and implemented by a transdisciplinary team of specialists.

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Tetiana Danylova acknowledges the support provided by the Institute of International Education's Scholar Rescue Fund (IIE-SRF) and the Graduate School for Social Research, Institute of Philosophy and Sociology of the Polish Academy of Sciences.

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

RECEIVED: 18.05.2024
ACCEPTED: 27.09.2024

REVIEW ARTICLE

CONTENTS

Psychosocial aspects of rehabilitation of the National guard of Ukraine soldiers injured in combat

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ABSTRACT

Aim: To identify the main problems and analyse the psychosocial aspects of the rehabilitation of servicemen of the National Guard of Ukraine and to develop a technology for the formation of psychological skills for psychosocial recovery in order to use it in the practice of psychosocial assistance to servicemen of the National Guard of Ukraine who were injured during the russian-ukrainian war.

Materials and Methods: The following research methods were used to study the psychosocial aspects of the rehabilitation of soldiers of the National Guard of Ukraine who were wounded during hostilities: comparative, content analysis, analysis of activity products, synthesis, specification.

Conclusions: on the basis of multidisciplinary approaches, the main problems were identified and the psychosocial aspects of the rehabilitation of soldiers of the National Guard of Ukraine were analysed, and the technology of forming psychological skills for psychosocial recovery was proposed with the aim of using it in the practice of psychosocial assistance to soldiers of the National Guard of Ukraine who were injured during the russian-ukrainian conflict war.

KEY WORDS: wounded servicemen, psychosocial rehabilitation, families of military combatants, psychological problems of families of combatants, social and psychological assistance to families of military personnel, technology of formation of psychological skills

Wiad Lek. 2024;77(9):2098-2103. doi: 10.36740/WLek/194770 DOI

INTRODUCTION

Rrussia's full-scale military aggression against independent Ukraine is causing large human, material, economic, demographic, psychological, and environmental losses and is causing psychological problems for the population and military soldiers directly involved in the hostilities.

When returning to civilian life, combatants experience new stresses related to social adaptation, psychological difficulties in communication, problems of professional self-determination, lack of understanding from their loved ones, etc. Injuries, traumatic experience gained during combat operations, problems with treatment negatively affect the health of the National Guard of Ukraine (NGU) and at the same time exacerbate the above difficulties [1, 2].

The current psychosocial situation in Ukraine in the context of russia's large-scale aggression and the annexation of part of Ukrainian territories is discussed and understood by representatives of various scientific communities, including psychotherapy [3]. The Ministry of Health of Ukraine predicts that more than 15 million Ukrainians will need psychological support because of this war [4]. At the same time, about 3 million people will need to be prescribed medication, and after the war, at least 20% of people will have negative mental health consequences.

Researchers have found that between 20 % and 40 % of combatants need psychological assistance [5]. At the same time, symptoms of acute trauma are detected in 60-80% of combatants who witnessed the deaths of their comrades or civilians or saw the bodies of the dead. The risk of mental health symptoms is much higher in younger soldiers, aged 18-24, who have symptoms of depression or have had problems with alcohol. Symptoms of post-traumatic stress disorder (PTSD) develop in about 12-20% of soldiers who have suffered combat trauma but have not sought psychological help [5].

Thus, the relevance of the problem we have identified lies in the fact that today there is an urgent need to identify and improve a set of measures related to the psychosocial aspects of rehabilitation of the National Guard of Ukraine soldiers who were injured during hostilities.

AIM

The aim is on the basis of multidisciplinary approaches of studying foreign and domestic experience of integration of rehabilitation with psychosocial work, identify the main problems, analyze the psychosocial aspects of rehabilitation, and develop the author's technology for the formation of psychological skills for psychosocial recovery with the aim of using it in the practice of psychosocial assistance to servicemen of the National Guard of Ukraine, who were injured under during the Russian-Ukrainian war.

MATERIALS AND METHODS

The research was based on scientific literature and regulatory documents. The following research methods were used to study the psychosocial aspects of rehabilitation of the National Guard of Ukraine soldiers who were injured during combat operations and to achieve results in improving the technology of this process: comparative, generalization, multidisciplinarity, conceptual and comparative analysis, system-structural analysis of regulatory documents, synthesis, specification, etc.

REVIEW AND DISCUSSION

Research conducted over the past 15 years is based on the experience of rehabilitation of victims in modern wars from Operation Desert Storm to the current russian-Ukrainian war, they confirm that it is very important to provide adequate and prompt medical, social and psychological assistance to combatants, especially those who have been wounded [6-8]. After all, a sharp change in social status and financial situation, domestic dislocation, which is inherent in many families of Ukrainian soldiers today, medical problems and the psychological state of the wounded, significantly affect all aspects of the life of combatants.

Researchers Y. Kuchyn and V. Horoshko stated that during the hostilities in eastern Ukraine in 2016, the number of patients with gunshot wounds to the extremities was about 64% [9, 10]. Treatment of PTSD in these patients does not result in a positive outcome in 82.1% of cases. The reasons for this are the high incidence of chronic pain syndrome and the persistence of pathological functioning in the treatment of PTSD. In 30-40% of cases, the treatment of pain in patients with gunshot wounds and PTSD does not have a positive effect. In our opinion, only systematic, targeted, comprehensive, integrative psychosocial support at the level of all social and professional groups can be a key factor in preventing and overcoming the negative effects of combat stressful situations and recovery from injury. It is also necessary to take into account that a significant number of veterans create a kind of barrier around themselves in order to maintain control over their own intimate experiences related to important people and events. This leads to withdrawal, isolation from family and friends, colleagues, etc.

On 15 March 2024, the Cabinet of Ministers of Ukraine adopted Resolution No. 296 «On Approval of the Procedure for Conducting Restorative (Post-Isolation, Reintegration) Measures, Adaptation, Support (Accompaniment) of Persons in Respect of Whom the Deprivation of Personal Liberty as a Result of Armed Aggression against Ukraine has been Established, after Their Release». And on 18 March 2024, the Cabinet of Ministers of Ukraine adopted Resolution No. 307 «Some issues of mental, sports, physical, psychological rehabilitation and professional adaptation of war veterans, their family members and some other categories of persons». It also provides for the mechanism of using funds allocated in the state budget under the budget programme «Measures for Mental, Sports, Physical, Psychological Rehabilitation and Professional Adaptation «.

The analysis of the goals, functions and tasks laid down in the above-mentioned programme documents of the state of Ukraine and taking into account the main directions of psychological assistance to combatants, allows us to emphasise that the purpose of work with soldiers who have returned from the front should be to overcome the negative psychological consequences of performing military service duties; restore the proper level of their mental health; correct the psychological functions, qualities, properties of the serviceman; increase psychological competence; carrying out the selection of specialists who will be able to return to the performance of their professional duties in the future [11, 12].

At the same time, statistics from wars and local military conflicts indicate that gunshot wounds to the extremities account for the highest frequency of injuries among soldiers, at 54-70%. According to the Medical Forces Command of the Armed Forces of Ukraine, gunshot wounds of the extremities account for approximately 64 %, of which 74.8 % are soft tissue wounds and 25.2 % are gunshot fractures [8]. Therefore, in the further disclosure of the scientific material in the article, we will not refer to psychosocial work with soldiers who have sustained gunshot wounds to the extremities.

The All-Ukrainian Mental Health Programme «Are You OK?» is currently being implemented in the country on the initiative of Olena Zelenska, and a link to it is available on the home page of the National Guard of Ukraine website. The main mission of the programme is to make mental health care a daily habit for Ukraini-

ans. With regard to psychosocial work with wounded soldiers, the Ministry of Defence of Ukraine's website now has a handbook entitled «Help after injury» in the section «Caring for a serviceman» (https://turbota.mil.gov.ua), which was developed for soldiers who were injured, traumatised or ill during their military service.

Let's consider the main stages of psychological recovery and psychosocial aspects of rehabilitation of wounded soldiers of the NGU. This activity consists of two stages that the wounded go through. The first stage of psychological recovery after an injury is acceptance. The shock stage is followed by emotions of pain, anger, fear, apathy, sadness or disappointment. At this time, psychologists advise family and friends to support the wounded, not to feel sorry for the wounded and not to grieve more than the wounded at such moments. It is recommended to gradually restore the person's taste for life through care and support, strengthen independence, give choices and communicate as an equal.

The second stage of psychological recovery after an injury is to get over the pain, depression, anger, resentment, etc. The duration of this stage is individual. At this stage, it is recommended to make plans with the person for the future, encourage them to act, return to a normal lifestyle, form new habits, discover new resources and abilities, get a different education, etc. When organising psychological counselling, individual and group forms of work with the wounded can be used (conversations, training exercises, lectures, creative exercises, role-playing games, exercises for personal growth, etc.) According to scholar Yurii Bryndykov, the effect of such work will be much higher if close cooperation with public administration and social services is established [13]. The principles of patient-centredness, timeliness, systematicity, comprehensiveness and others become the main ones in such rehabilitation work [14].

Based on our experience, developing specialized skills such as emergency and medical first aid can be effective in the psychosocial rehabilitation of injured soldiers, helping them to overcome current and future challenges [15]. One of these options for developing psychological skills for psychosocial recovery was developed by the US Department of Health and Human Services for survivors of traumatic events [16]. The combination of the provisions of this option with the materials of the study of the peculiarities of the modern rehabilitation of combatants conducted by the authors of the article allowed us to propose the following technological sequence of forming psychological skills of soldiers who suffered limb injuries for use in the practice of psychosocial assistance.

The first block is the formulation of the problem, the goals of the work and the development of problem-solving skills. Soldiers who have experienced a traumatic event due to injury often have difficulty performing everyday, ordinary tasks. They are hampered by stress, anxiety, maladjustment, and loss of muscle strength, which are consequences of the injury and are also related to the treatment process in hospitals. Problem-solving skills can help to restore a sense of control, increase self-efficacy, search for resources, get rid of helplessness, expand the understanding of the situation, etc. Several sessions of individual work (up to 40 minutes) are enough to master the skills, which consist of the following main blocks of work: clearly defining and clarifying the client's problems, formulating goals; searching for directions and methods through brainstorming or generating ideas; choosing the best solution.

The next step is to determine the place of the problem in a person's life, why it interferes, what it affects, what feelings and experiences it causes. After that, ideas are generated or brainstormed, i.e., any spontaneous options that may be useful for solving the problem are voiced and written down. This includes identifying a circle of people, relatives who can help; learning ways to relieve tension; learning methods of rational thinking and calming, unloading; identifying types of psychological protection and rethinking them; updating useful experiences from the past; developing new skills; surfing useful websites and contacting social services or psychologists, etc.

The last step in this block is to evaluate each option and choose the best one, when all the «strong and weak» options are discussed. This can take the form of a follow-up meeting with a psychologist (supervisor, social worker), self-analysis of the work done, discussion with and help from close people.

The second block is positive activity. After a traumatic event, people often stop doing things that used to bring pleasure or were personally meaningful, which often leads to a decrease in mood, lack of energy in life or feelings of loneliness, hypochondria, etc. However, it can be helpful to draw up a special activity plan that will increase concentration and focus on the positive.

Assistance in identifying, planning and engaging in positive, enjoyable activities that are practically and emotionally meaningful for wounded soldiers, helping to restore a normal daily routine, improve mood and restore a sense of control, etc. A session aimed at developing these skills usually takes 30 minutes and consists of two main steps: identifying activities and planning them using a calendar. To improve the mood of a wounded soldier, it is important to increase positive experiences, i.e. to engage him in positive activities. It is worth explaining that there is a clear relationship between thoughts, feelings and behaviour. Therefore,

it is suggested that in situations where it is not possible to change the emotional state, it is possible to work on changing thoughts or behaviour. The following forms are offered to facilitate the choice of activity: indoor activity, outdoor activity, social activity. It is important to record the types of activity in a calendar that is placed at home or in the hospital, in a place that is easy to see.

The third block is work with reactions. Doctors note that after a traumatic event, the human body usually has a lower threshold to danger [16]. Therefore, even in a relaxed state, a soldier in a hospital after being wounded may feel vulnerable to imaginary dangers. In the absence of real threats, these reactions interfere with recovery and can damage physical and mental health, as well as relationships with other people.

After a traumatic event, soldiers experience unpleasant physical and emotional reactions caused by negative experiences and triggers. These reactions can lower mood, impair communication with others, decision-making, physical health, etc. Therefore, it becomes important to learn special techniques to manage these reactions and use them in everyday life to reduce the level of stress and anxiety of the wounded.

The next step is to identify the types of distress reactions in a soldier: which reactions are most disturbing or interfere with the usual way of life; which reactions are most difficult to master, etc. The triggers of such reactions can be various external (events, people, situations, things) and internal (thoughts, roles, status) factors. Knowledge of these indicators helps to avoid undesirable chain reactions in the future.

Noteworthy are such methods of overcoming unpleasant physical and emotional reactions as methods of emotional and volitional self-regulation of mental states, with the help of which it is possible to learn techniques of relaxation or detachment when such reactions arise. This can be autogenic training according to I. Schultz, progressive muscle relaxation according to Jacobson, breathing regulation according to G. Benson's method, «belly breathing» according to the Qigong system.

For soldiers who have experienced traumatic events with some manifestations of post-traumatic stress disorder, it is advisable to provide additional information about the stress reactions of re-experiencing, avoidance, numbness and hyperactivation, with the emphasis on the fact that these reactions can be gradually controlled. The goal here is to learn to control distressing emotions. Methods that can be used: reminding yourself that post-stress reactions are common; positive activity; spending time with loved ones; using any relaxation techniques (meditation, autogenic exercises, yoga, listening to relaxing music, etc.); rationalising thoughts; attending individual or group psychotherapy sessions. It is advisable to avoid the use of alcohol or psychoactive substances. It is also advisable to draw up a detailed action plan for a warrior to cope with unpleasant reactions.

A session for this purpose can last up to 45 minutes and consists of defining and identifying reactions that cause distress and their triggers, learning coping skills, replacing them and developing an appropriate plan.

The fourth block is the rationalisation of thoughts. Scientists have stated that after a traumatic event, people's opinions about the world and themselves in it often change [16]. Today, it is a common phenomenon when soldiers, after the horrors of war, look at the world as a hostile environment and have difficulty trusting other people, even their loved ones. In order to change such behaviour and emotions, it is advisable to replace negative or irrational thoughts with positive or rational ones.

A session aimed at rationalising thoughts according to the Albert Ellis methodology can last up to 45 minutes and consist of the following stages [17]: joint identification of irrational thoughts, their discussion and replacement with rational ones; assessment of the strength of arguments (sufficient or not); repeating the procedure when irrational thoughts reappear.

The fifth block is healthy social connections. Social support is considered to be one of the most important protective factors that helps to survive a traumatic event. Social support from family, relatives, and friends can significantly accelerate recovery from a traumatic event by meeting the emotional and practical needs of a soldier. Therefore, according to our practical experience, the best way to overcome it is to discuss it with other people, as it becomes important for a wounded soldier to seek support more effectively and provide it to others.

Building skills to establish healthy social connections will be especially useful for soldiers who feel isolated, lonely, living in a new environment, insecure and do not have family or friends with whom to discuss their feelings and experiences of war. This is especially true for severely wounded soldiers who are limited in movement and communication.

It is important to explain to such soldiers that negative thoughts, emotions and related unpleasant reactions can affect relationships with other people, lead to distancing, and form attitudes of distrust and alienation. It is human communication that can help to feel cared for by others, to be needed, to be part of society, to overcome the problems that arise in war-wounded soldiers.

A drawing of a social grid, for example, in the form of a solar system, in which the soldier is centrally located, is quite effective and visual. The planets, as in the case of

the solar system, can be a father and mother, husband or wife, friends, children, comrades-in-arms, doctors and psychologists, representatives of social services, a chaplain, teacher or trainer, commander, subordinates, members of various groups, etc.

The next step is to review these relationships with answers to the following questions: who is currently the most important for the soldier in the scheme of social connections and the hierarchy of empathy; with whom he can share his experiences and get useful advice; who can help and who needs help; with whom it is necessary to restore connections or improve relationships; with whom it is necessary to spend more or less time, why; whether there is a need to increase social activity and the number of social connections.

The last step of the proposed technology, which we have adapted to the conditions of today's russian-ukrainian war, is for the soldier to create a specific action plan for the next few weeks. For example, «tomorrow I will call the commander and thank him for his help, offer my experience to other soldiers in the unit», «tomorrow I will offer my services to young soldiers as an advisor or weapons consultant», «I will call my friends and offer to meet them near the hospital», «I plan to join a group where soldiers share their impressions of prostheses and how to adapt to them quickly», «I will join a community of veterans involved in veteran sports», «tomorrow I will start helping more soldiers in the hospital who are seriously injured and need support», etc.

The session is recommended to last for 30 minutes. Its goal is to create and maintain healthy social connections, and the main stages can be: building a visual map of social connections, reviewing it, implementing a social support plan, etc. The programme of such work should be based on observance of important points in working with wounded soldiers: First, it is the specificity of the individual programme with its maximum approximation to the real life of the soldier (hospital, rehabilitation centre, family); second, this programme should be flexible and variable in accordance with the requests and achievements of the soldier in working on himself (regardless of the personality of the psychologist or supervisor, circumstances of life, etc;) thirdly, it should allow for reflection on the results of each stage or steps taken by the psychologist or counsellor and the wounded soldier himself to get closer to the goal, taking into account the skills already developed, as mentioned above; Fourth, it is important to follow the protocols recommended by the Ministry of Veterans Affairs of Ukraine during the psychosocial rehabilitation of wounded soldiers, but to assess the individual course of such work and make timely adjustments during practical work with them; fifth, to use supervision as a form of professional support and training for psychologists who want to improve their work with clients, develop their competencies and prevent professional burnout.

CONCLUSIONS

The study of foreign and domestic experience in the rehabilitation of wounded soldiers shows that today the need for its integration with such an important area as psychosocial work is not fully taken into account. Therefore, on the basis of multidisciplinary approaches, the main problems are identified and the psychosocial aspects of the rehabilitation of the National Guard of Ukraine are analysed, and the technology of forming psychological skills for psychosocial recovery is proposed for its use in the practice of psychosocial assistance to the National Guard of Ukraine, who were injured during the russian-ukrainian war.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 16.06.2024
ACCEPTED: 15.09.2024

CASE STUDY

CONTENTS

Mine-explosive trauma of the maxillofacial region: current state of the problem and description of a case from practice

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ABSTRACT

Military conflicts, terrorist attacks and wars around the world pose a wide range of questions to the medical community about providing medical care to military personnel and civilians with mine-explosive trauma, which is characterized by simultaneous damage of various anatomical areas, including the maxillofacial region. The purpose of this work was to describe a case from practice of treating a patient with a mine-explosive trauma, which manifested by a fracture of the right zygomatic-orbital complex and the upper jaw on the right. Using own case from practice, the authors showed that the treatment of patients with mine-explosive trauma of the maxillofacial region is long-term, multi-stage and should take place in a specialized hospital with the involvement of a team of multidisciplinary specialists.

KEY WORDS: mine-explosive trauma, maxillofacial region, case from practice

Wiad Lek. 2024;77(9):2104-2110. doi: 10.36740/WLek/196165 DOI

INTRODUCTION

The high intensity of military conflicts and terrorist attacks in the world, the full-scale war in Ukraine have led to a significant increase in the frequency of mine-explosive traumas among military personnel and civilians [1]. Mine-explosive traumas are a common cause of loss of work ability and disability, characterized by high mortality. The frequency of losses during combat operations as a result of mine-explosive traumas reaches 25% [2].

Mine-explosive traumas occur as a result of a one-time action on the human body of damaging factors of heterogeneous characteristics caused by a mine-explosive device (shock wave, gas flame jet, ammunition fragments, toxic products of explosion and combustion, etc.) [3]. Damage caused by a mine-explosive trauma depends on the person location at the time of the explosion, the type of explosive device and its strength, the presence or absence of protective equipment [4]. Mine-explosive traumas are characterized by simultaneous injury of several organs and systems (two or three anatomical areas or more) [5]. The most common locations of injuries in mine-explosive traumas, according to various scientists, are the extremities, head and neck, chest, abdomen, etc. [6, 7]. This category of patients is characterized by the frequent development of traumatic shock and multiple organ failure [2]. Victims of mine-explosive traumas require immediate and highly qualified medical care followed by a range of rehabilitation measures.

AIM

The purpose of this work was to describe a case from practice of treating a patient with a mine-explosive trauma, which manifested by a fracture of the right zygomatic-orbital complex and the upper jaw on the right.

CASE REPORT

Patient M., 35 years old, was admitted to the hospital on June 14, 2023. After a comprehensive examination, a comminuted fracture of the right zygomatic-orbital complex and the upper jaw on the right was diagnosed (Fig. 1). During hospitalization, the patient signed a written consent that his photographs and medical

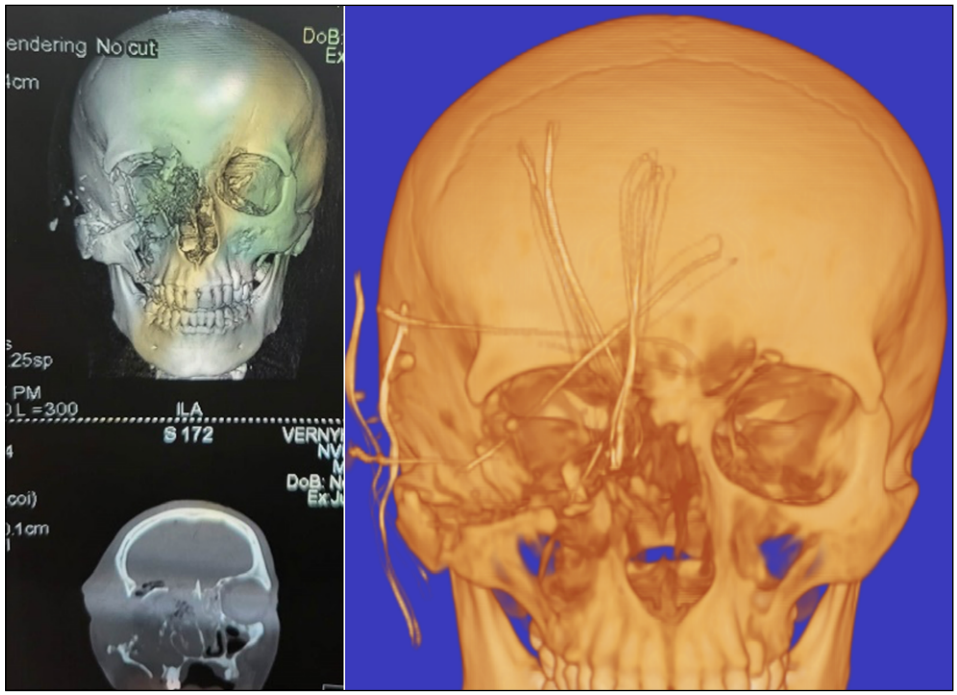

Fig. 1. 3D computed tomography of the skull of patient during hospitalization on June 14, 2023.

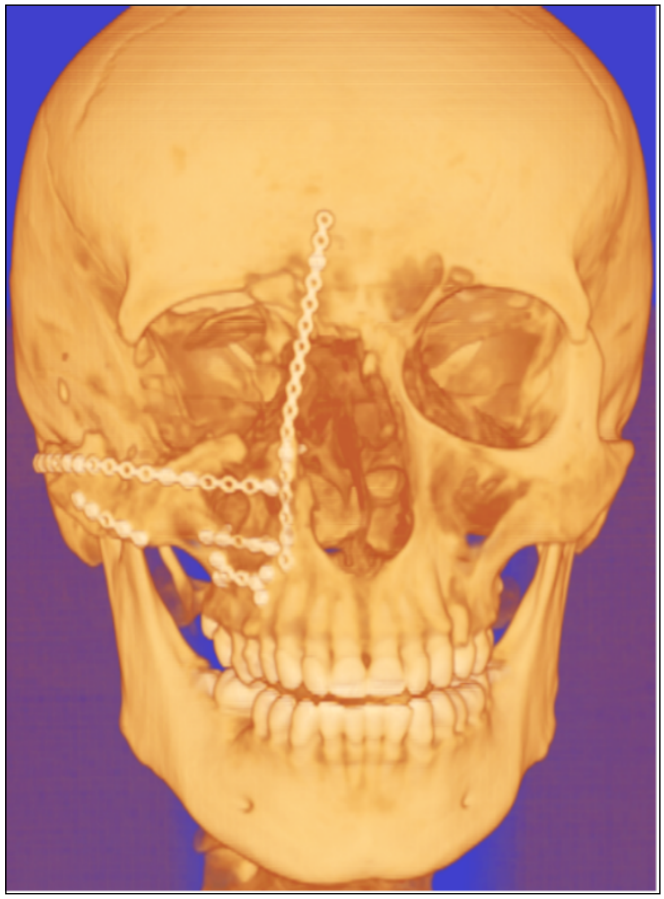

Fig. 2. 3D computed tomography of the skull of patient after surgery on June 16, 2023.

records would be analyzed and published as an article in a journal. It is known from the anamnesis that the patient received a mine-explosive trauma on June 6, 2023. At the previous stages of providing medical care to the patient, primary surgical treatment of the wound and removal of the right eyeball were performed. On June 15, 2023, a conference of doctors was held with the participation of a neurosurgeon, a neuropathologist, an ophthalmologist, an otorhinolaryngologist, and a maxillofacial surgeon and the tactics of treatment were decided.

On June 16, 2023, the patient underwent surgery. Wound revision and reposition of bone fragments were made using intraoral and external approaches through the wound. Using microplates, metal osteosynthesis of the right zygomatic-orbital complex and the upper jaw on the right was performed in the areas of the zygomatic arch, nasolabial buttress, and anterior wall of the maxillary sinus (Fig. 2). During the revision of the wound, tissue fragments were obtained, which were subsequently subjected to morphological examination. During the survey microscopy of the latter, fragments of connective, muscle and bone tissues with pronounced dystrophic-necrotic changes, signs of circulatory disorders, and focal moderately pronounced polymorphic cellular infiltration were determined (Fig. 3). The latter was mainly represented by neutrophilic leukocytes, macrophages and lymphocytes.

Taking into account the presence of massive bone defects in patient, the method of electrical stimulation was used to activate the processes of reparative osteogenesis, the effectiveness of which was proven by us in the previously conducted morphological study of the experimental material [8, 9]. It was used an electrode for electrical stimulation, connected to the negative pole of a battery with a diameter of 8×2.5 mm (Fig. 4). The electrode was attached to the microplate, which fixed the fracture

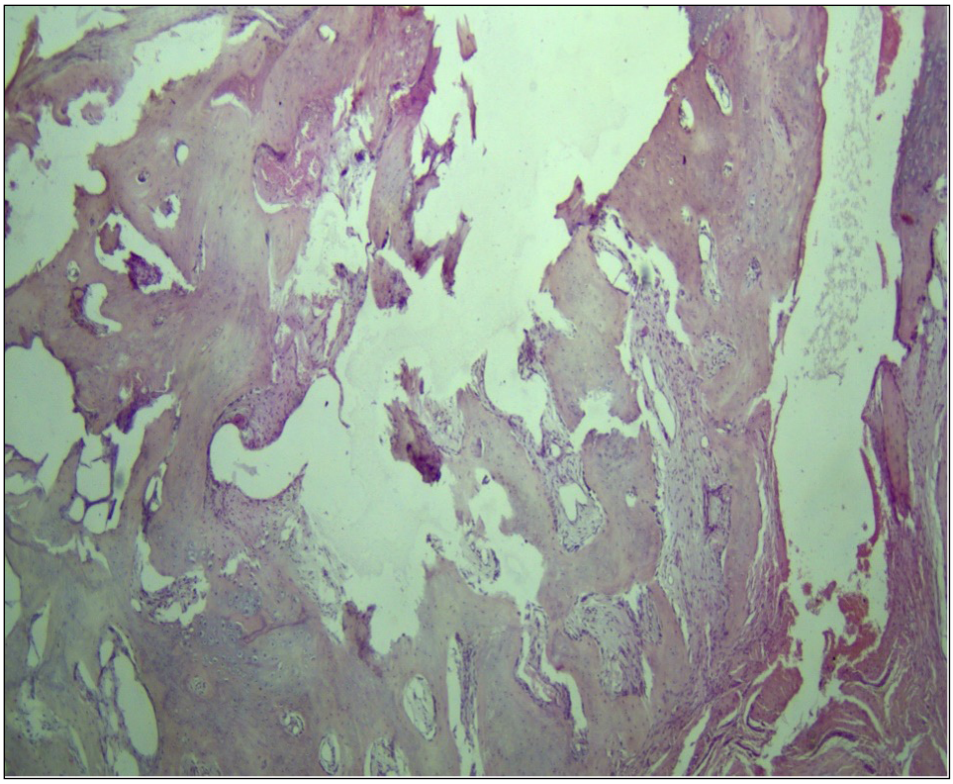

Fig. 3. Bone, muscle and connective tissue fragments with alterative, hemodynamic and inflammatory changes. Hematoxylin and eosin staining, ×40.

Fig. 4. Appearance of the electrode that was used for electrical stimulation.

fragments. The battery was introduced into the oral cavity and fixed with a vicryl to the mucous membrane of the transitional fold of the upper jaw. The duration of the electrical stimulation procedure was 30 days, after which the device was removed under local anesthesia.

On June 28, 2023, the patient was discharged from the hospital for further conservative treatment in an outpatient setting. On September 5, 2023, patient M. was re-hospitalized to the hospital with the diagnosis "Facial deformation in the area of the right zygomatic-orbital complex, bone defect of the outer edge and bottom of the orbit on the right". The patient underwent reconstruction of the orbit with the help of an individually made implant (Fig. 5-7), which was developed by

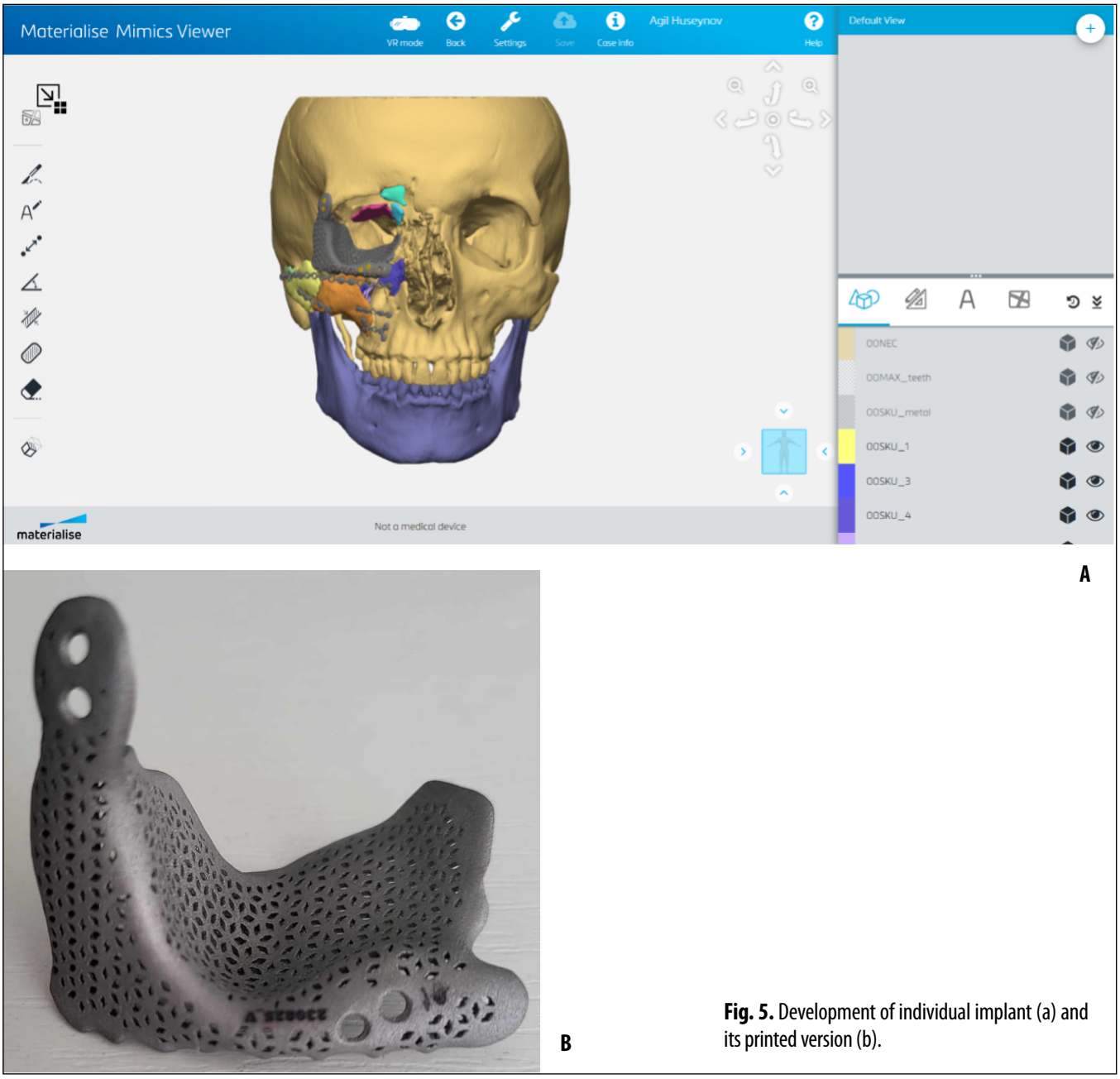

Fig. 5. Development of individual implant (a) and its printed version (b).

the engineers of the MATERIALISE company (Belgium) using the Materialise Mimics Viewer program and printed by the 3D MetalTech company (Ukraine) (Fig. 5). The microplate was removed from the nasolabial suture area before installing the implant, during which small tissue fragments were obtained and sent to the morphological study. During the morphological study of the latter, connective and lamellar bone tissue, which are the formed regenerate components, were determined (Fig. 8). The results of the morphological study of clinical material proved the effectiveness of the electrical stimulation method of the reparative osteogenesis processes, which coincides with the results of other scientists and the morphological study of the experimental material conducted by us earlier [8-11].

On December 11, 2023, patient underwent the next stage of surgical treatment, during which a bed for an eye prosthesis was formed. On January 10, 2024, ophthalmologists performed a prosthetic procedure on patient's right eye, after which he was discharged from the hospital.

After mine-explosive trauma, physical therapy is extremely important, which contributes to the physical and psychological recovery of the person and the return to a full-fledged life [12].

CONCLUSIONS

Military conflicts, terrorist attacks and wars around the world pose a wide range of questions to the medical

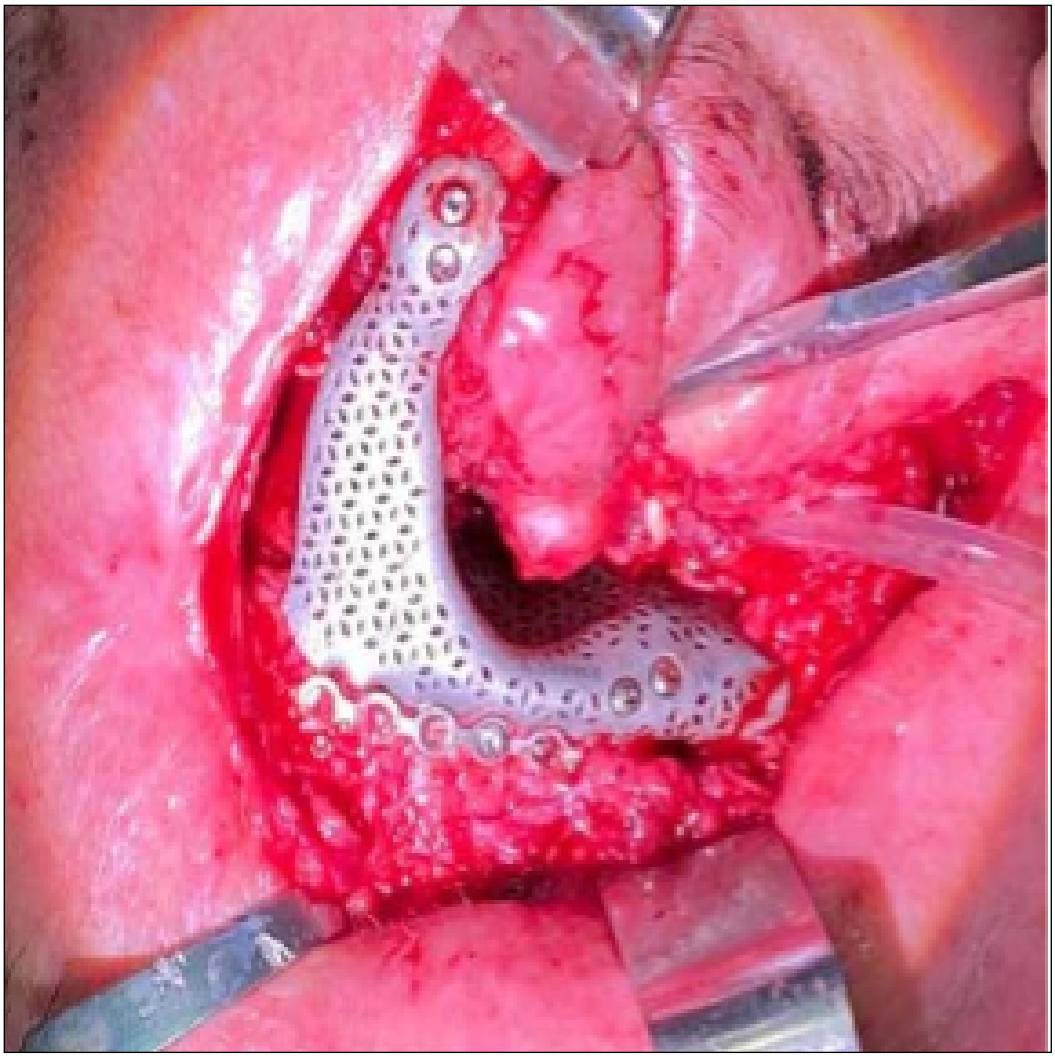

Fig. 6. View of the installed implant during the operation.

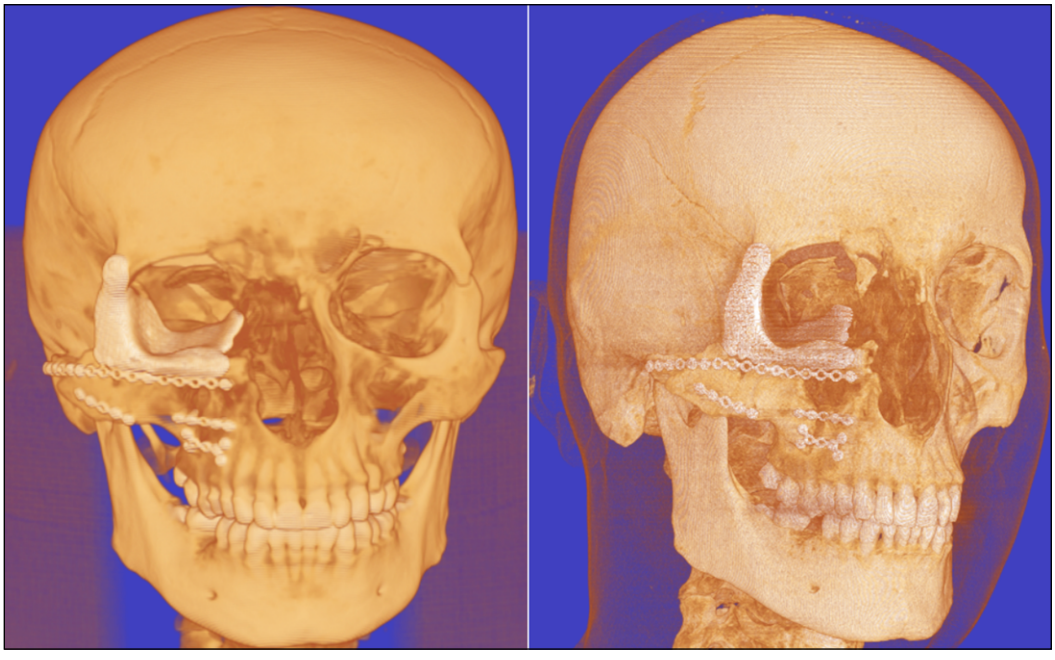

Fig. 7. 3D computered tomography of the skull of patient after the reconstruction of the orbit bottom using an individual implant.

community about providing medical care to military personnel and civilians with mine-explosive trauma, which is characterized by simultaneous damage of various anatomical areas, including the maxillofacial region. Using own case from practice, the authors showed that the treatment of patients with mine-ex-

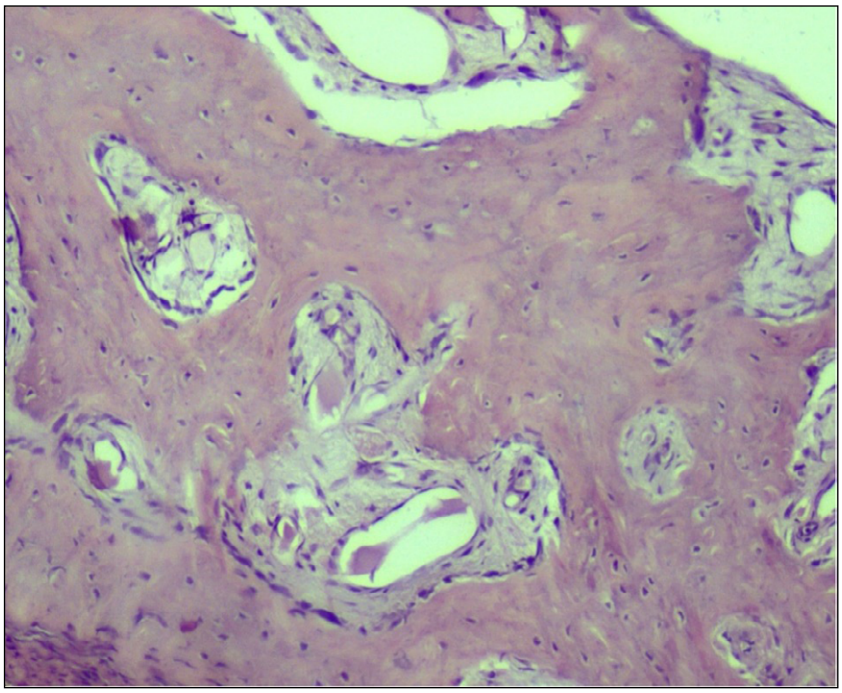

Fig. 8. Lamellar bone tissue from the area of the formed regenerate. Hematoxylin and eosin staining, ×400.

plosive trauma of the maxillofacial region is long-term, multi-stage and should take place in a specialized hospital with the involvement of a team of multidisciplinary specialists.

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CONFLICT OF INTEREST
The Author declare no conflict of interest

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RECEIVED: 05.06.2024
ACCEPTED: 03.10.2024