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Prevalence and structure of pain syndrome in patients with stroke and Covid-19

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ABSTRACT

Aim: To investigate the prevalence and characteristics of pain syndrome in patients with ischemic stroke against the background of COVID-19 over a 3-month period.

Materials and Methods: A total of 34 patients with laboratory-confirmed COVID-19 and ischemic stroke were examined during the acute phase. Pain syndrome onset was studied within 3 months of stroke development, focusing on musculoskeletal pain, central post-stroke neuropathic pain, shoulder pain, and headache. Pain intensity was measured using the Visual Analog Scale and the DN4 questionnaire was applied to determine neuropathic pain.

Results: Pain syndrome developed in 75% of patients. Musculoskeletal pain (58.38%) and headache (54.2%) were the most common. More than half of patients experienced a combination of pain types, with musculoskeletal pain and headache frequently co-occurring in one-third of cases. Moderate pain syndrome was the most common (48.7%). Headache positively correlated with COVID-19 severity ($r = 0.486$, $p = 0.005$), and shoulder pain positively correlated with stroke severity ($r = 0.517$, $p = 0.002$).

Conclusions: Identifying the prevalence, structure, and severity of pain syndrome in combined ischemic stroke and COVID-19 cases highlights the importance of timely recognition and management. Effective intervention can prevent pain syndrome chronification, improve functional recovery, and enhance patients' quality of life.

KEY WORDS: stroke, COVID-19, headache, central post-stroke pain, shoulder pain

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INTRODUCTION

Stroke is a leading cause of disability and mortality worldwide. Pain syndrome (PS) after stroke significantly contributes to disability and reduced quality of life, alongside symptoms such as paresis-paralysis, speech impairment, and sensory disturbances [1]. The prevalence of post-stroke pain ranges from 19% to 74% [2, 3], with chronic pain observed in 45–65% of patients, 70% of whom experience it daily. PS is one of the most common complications post-stroke, along with depression, falls, and urinary tract infections [2, 4–6]. Risk factors for PS include female gender, advanced age, depression, spastic paresis, and lesion localization in the brainstem or thalamus [7].

The COVID-19 pandemic has added new challenges, infecting over 676 million people and causing more than 6.8 million deaths globally [8]. Neurological manifestations in COVID-19, including myalgia, joint pain, headaches (HA), and neuropathic pain, are reported both in the acute phase and post-acute period [9]. These symptoms result from systemic inflammation, direct neuropathic mechanisms, or complications of infection and treatment [10].

COVID-19 also increases the risk of stroke, with an incidence twice as high compared to non-COVID-19 patients and an approximate rate of 5% [12, 13]. Stroke in the context of COVID-19 is associated with greater severity, higher mortality [14], younger age, and more comorbidities such as diabetes and obesity [15],], as well as a higher prevalence of undetermined pathogenic subtypes [16].

However, data on the prevalence and characteristics of PS in combined stroke and symptomatic SARS-CoV-2 infection remain scarce. The overlap of these conditions may enhance pain intensity, increase neuropathic pain prevalence, and create unique variants of requiring further study. Understanding these aspects is critical for improving diagnostics, treatment, and rehabilitation, thereby enhancing quality of life and reducing societal burdens.

AIM

To investigate the prevalence and characteristics of pain syndrome (PS) in patients with ischemic stroke against the background of COVID-19 over a 3-month period.

MATERIALS AND METHODS

We examined 32 patients with ischemic stroke (IS) and laboratory-confirmed COVID-19 (PCR test), who were hospitalized at Kyiv City Clinical Hospitals No.1 from April 2020 to November 2021.The clinical severity of COVID-19 was assessed based on symptoms, physical examination, laboratory test results, and findings from instrumental diagnostic methods [17]. Inclusion criteria were:mild to moderate COVID-19 severity, ability to establish contact with the patient to assess and characterize PS. The diagnosis of IS was based on clinical neurological examination and neuroimaging results, including spiral computed tomography (sCT) or magnetic resonance imaging (MRI). The National Institutes of Health Stroke Scale (NIHSS) was used to assess stroke severity, spasticity was evaluated using the Modified Ashworth Scale (MAS) [18]. Assessment of spasticity involved flexion and extension movements around the joints of the upper limb (shoulder, elbow, wrist, and fingers) and lower limb (hip, knee, and ankle) in a resting state. The study was conducted in accordance with the Helsinki Declaration of Ethics [19]. We assessed the development of PS following a stroke, starting from hospitalization and up to 12 weeks after the acute infection. The following variants of PS were distinguished: Shoulder pain (SP) on the paretic side, headache (HA), musculoskeletal pain (MSP), central (neuropathic) post-stroke pain (CPSP).

The presence and characteristics of PS (type and intensity) were determined through interviews and questionnaires conducted during hospitalization or via telephone interviews. To diagnose CPSP, the classification of central neuropathic pain and the DN4 questionnaire (Douleur Neuropathique 4) were applied [20]. A positive response to 4 out of 10 items on the DN4 questionnaire indicated the presence of central post-stroke neuropathic pain.

The pain was evaluated quantitatively using the Visual Analog Scale (VAS, 0–10 cm) and categorized using the Numeric Rating Scale (NRS): 1–3 points: mild pain, 4–6 points: moderate pain, 7–10 points: severe pain. In cases of combined pain types, VAS scores were assessed separately for each type of pain.

Statistical analysis was performed using IBM SPSS Statistics 22. Descriptive statistics were calculated, and normality was tested with the Chi-square test. Data with normal distribution were presented as the mean (M) ± standard deviation (SD) and analyzed using the paired or independent Student's t-test. Non-normally distributed data were analyzed with the Wilcoxon signed-rank test or the Mann-Whitney U test. Relative values were compared using Pearson's χ^2 test or the one-sample χ^2 test. Correlation analysis employed Pearson or Spearman coefficients, with significance set at $p < 0.05$.

RESULTS

The mean age was 71.6 years, with a male-to-female ratio of 53.3% to 43.8%. The mean NIHSS score for stroke severity was 11.5, corresponding to a moderate severity level. The most common stroke subtype was atherothrombotic (40.6%). Vascular lesions were predominantly localized in the carotid arterial system (50.0%). The majority of patients experienced a mild course of COVID-19 (78.1%), while 21.9% had a moderate course (Table 1).

Among the 32 examined patients with stroke and COVID-19, PS was detected in 24 patients (75.0%). In 7 patients PS was observed during hospitalization, in 17 patients PS developed within 3 months from disease onset during ongoing symptomatic COVID-19.

In more than half of patients with PS (46.9 %) large artery involvement was identified.

Among patients with PS in 11 patients (45.8%) the stroke occurred in the carotid arterial system, in 10 patients (41.7%) the infarction focus was localized in structures supplied by the vertebrobasilar arterial system. In 3 patients (12.5%) both vascular territories were affected (Table 2).

There were no significant differences in the distribution of pathogenetic subtypes between patients with and without PS. Additionally, no differences were found in the prevalence of different subtypes among patients with PS in cases of combined stroke and COVID-19. There was also no difference in the prevalence of PS depending on the clinical severity of COVID-19: among patients with a mild course, PS was observed in 18 (72.0%), and with a moderate course – in 6 (85.7%), $p = 0.459$. The analysis of patients with ischemic stroke by the structure of PS is presented in (Table 3).

The data indicate that the most common type of pain disorder was MSP, observed in 14 patients, which accounted for 58.3% of patients with PS and 43.8% of all examined patients. Patients reported pain in the neck and shoulder girdle (6 patients), lower back and sacral region pain was noted by 3 patients,1 patient reported pain in the lower legs. In 4 patients, the PS had a multiple nature, simultaneously affecting several anatomical regions.

HA was the second most common cause of PS, occurring in more than half of patients with PS (54.2%) and in 40.6% of all examined patients. In 2 patients, the HA was described as bilateral, localized in the temporo-parietal and occipital regions, resembling vascular pain associated with elevated blood pressure. In 3 patients, the HA was migraine-like. For the remaining patients, the HA resembled daily tension-type HA and developed on days 4–6 after the stroke, in the absence of any preexisting HA complaints.

Shoulder pain (SP), observed in a quarter of patients, was associated with various factors: subluxation of the shoulder joint (due to spasticity and restricted range of motion),tissue contracture around the shoulder caused by prolonged immobilized positioning, features of adhesive capsulitis. SP in 4 patients was combined with spastic hemiparesis in the right limbs (3–4 on the MAS scale). In another 3 cases, it was associated with central monoparesis without significant spasticity (2 points on the MAS scale). At the same time, the frequency of pain types such as HA, SP, and MSP did not show any statistical differences (all $p > 0.05$).

CPSP was found in 2 patients associated with lesions in the lenticulo-capsular region, in 1 patient - with a lesion in the lateral medulla, in 1 patient with a lesion in the pons, in 1 patient, with a lesion in the parietal cortex. CPSP occurred significantly less frequently than HA, $p = 0.026$ and MSP, $p = 0.014$ (Table 4).

It was shown that in more than half of cases (58.3%), patients reported a combination of two types of pain that developed after a stroke. In one patient, three pain syndromes occurred simultaneously (Table 5).

It was determined that the most common combination of two pain variants was MSP and HA, observed in 8 patients (33.3%). The mean pain score on the VAS was 4.7 ± 1.7 points (range: 2–8 points), with no significant differences depending on the type of pain, all $p > 0.05$ (Table 6).

Among all presentations of pain syndrome and its combinations (40 variants), moderate PS was the most common, occurring in 47,5% of cases.

An assessment of pain intensity depending on the type of PS revealed certain features (Table 7).

Thus, MSP belonged to the «mild and moderate» categories with the number of mild cases exceeding moderate ones by more than 1.5 times. HA of moderate intensity occurred more frequently than other gradations – 2.5 times more often than mild pain and 4 times more often than severe pain. Among patients with CPSP 80% reported moderate and severe pain. Shoulder pain, associated with spasticity, was characterized as moderate in 50% of cases.

Correlation analysis revealed significant associations: between HA and COVID-19 severity ($r = 0.486$, $p = 0.005$), between stroke severity (NIHSS score) and SP($r = 0.517$, $p = 0.002$).

Table 1. General characteristics of patients (n=32)

Characteristic	Indicator
Age (years), M±SD	71.6 ± 4.5
- Male n (%)	18 (53.3)
- Female n (%)	14 (43.8)
Stroke severity (NIHSS), M±SD	11.5 ± 2.7
Stroke subtype, n (%)	
- Atherothrombotic	13 (40.6)
- Atrial fibrillation	9 (28.1)
- Lacunar infarcts	7 (21.9)
- Undetermined mechanism	3 (9.4)
Vascular localization	
- Carotid arterial system	16 (50.0)
- Vertebrobasilar arterial system	13 (40.6)
- Both localization	3 (9.4)
COVID-19 course; n (%):	
Mild:	25 (78.1)
Moderate:	7 (21.9)
Pain syndrome; n (%):	24 (75)

DISCUSSION

Among hospitalized patients with COVID-19, ischemic stroke occurred in 1.1–4.7% of cases with a cumulative prevalence of 2% [21].

This urges researchers to devote serious attention to studying the pathophysiological and clinical aspects of

Table 2. Prevalence of pain syndrome depending on stroke subtype and vascular localization

Indicator	Proportion of subtype and vascular localization in PS, n (%)		Prevalence of PS within each subtype/vascular localization		
	n=24	P	n=32	n (%)	P
Stroke subtype					
Atherothrombotic	9 (37.5)	0.228	13	9 (69.2)	0.848
Cardioembolic	7 (29.2)	9	7 (77.8)		
Lacunar	6 (25.0)	7	6 (85.7)		
Undetermined	2 (8.3)	3	2 (66.7)		
Vascular localization					
Carotid	11 (45.8)	0.093	16	11 (68.8)	0.507
Vertebrobasilar	10 (41.7)		13	10 (76.9)	
Both localization	3 (12.5)		3	3 (100.0)	

Table 3. Distribution of patients with IS and COVID-19 by type of PS

Type of pain syndrome	Absolute (n)	% of Total patients (n=32)	% of Patients with PS (n=24)
CPSP	5	15.6	20.8
Headache	13	40.6	54.2
Shoulder Pain	8	25.0	33.3
Musculoskeletal Pain	14	43.8	58.3

Table 4. Pain syndrome comparison: statistical significance (p-values)

Pain syndrome comparison	p-value
CPSP vs Headache	0.026
CPSP vs MSP	0.014
Headache vs SP	>0.05
Headache vs MSP	>0.05
Shoulder Pain vs MSP	>0.05

this comorbidity. Our study focuses on the prevalence and features of PS in stroke patients with COVID-19 and, to our knowledge, is the first to address this issue in such a direction. Our interest in this practically unexplored topic can be explained by the fact that PS in COVID-19 alone is a prominent and quite persistent element. The overall prevalence of pain reported after COVID-19 was 34.2%, which is higher than for any other symptoms and exceeds post-influenza pain (24.0%) [26]. According to FAIR Health data, which includes more than 34 billion private medical records, persistent pain was reported in 5.1% of COVID-19 patients and was identified as one of the five most common symptoms lasting 30 days or more after the initial diagnosis [23].

Among the patients we examined with combined IS and COVID-19, PS developed in 75% of cases, which exceeds the rates reported in the literature for the stroke population before the pandemic [4, 6, 24]. One of the most common types of pain in the structure of post-stroke pain is HA, and this is also true for the neurological manifestations of COVID-19 in the absence of stroke. The overall prevalence of HA in hospitalized COVID-19 patients ranges from 11% to 34% [25], while data from Carvalho LCLS et al. [26] showed that it persisted after the acute phase of COVID-19 in 52% of patients.

Table 5. Distribution of patients with IS and COVID-19 by combination of pain types (n=24)

Pain syndrome	CPSP	Headache	Shoulder Pain	Musculoskeletal Pain
CPSP	-	0 (0.0%)	3 (12.5%)	1 (2.4%)
Headache	0 (0.0%)	-	2 (8.3%)	8 (33.3%)
Shoulder Pain	3 (12.5%)	2 (8.3%)	-	3 (12.5%)
Musculoskeletal Pain	1 (2.4%)	8 (33.3%)	3 (12.5%)	-

Table 6. Pain score on VAS by pain syndrome type

Pavin syndrome	N	M ± SD	Min–Max
CPSP	5	5.4 ± 2.1	3 – 8
Headache	13	4.9 ± 1.5	3 – 8
Shoulder Pain	8	5.3 ± 1.8	3 – 8
Musculoskeletal Pain	14	3.9 ± 1.5	2 – 6

Table 7. Distribution of patients by pain intensity gradations on the VAS Scale depending on PS type

Pain syndrome	Pain intensity						Total	
	Mild		Moderate		Severe			
	Abs.	%	Abs.	%	Abs.	%	Abs.	%
CPSP	1	20.0	2	40.0	2	40.0	5	100
Headache	3	23.1	8	61.5	2	15.4	13	100
Shoulder Pain	2	25.0	4	50.0	2	25.0	8	100
Musculoskeletal Pain	9	64.3	5	35.7	0	0.0	14	100

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According to the latest meta-analysis, HA after stroke in patients without signs of COVID-19 occurs with a prevalence of 6–44%, is predominantly characterized as tension-type HA, and is associated with stroke in the posterior circulatory territory and with the female gender [30]. Our results showed the presence of HA in more than half of patients with PS (54.2%), which was higher than in cases of isolated COVID-19 infection during the same period and exceeded the prevalence of this symptom in the "stroke without COVID-19" group. Unlike Harriott A.M. et al. [27], we did not find a difference in the prevalence of HA depending on the affected vascular territory. At the same time, we identified a moderate, positive, and statistically significant correlation between the prevalence of HA and the severity of COVID-19, which aligns with the findings of these researchers.

SP after stroke is a common phenomenon, with a reported prevalence ranging from 22% to 47% [28]. In cases of stroke combined with COVID-19, our data revealed SP in 25% of all patients examined and in one-third of those with pain syndrome. Thus, the prevalence of this syndrome did not differ significantly from that in the "non-COVID" stroke group. However, we observed a correlation with stroke severity, rather than with the clinical severity of COVID-19.

MSP are frequently observed in COVID-19 during both the acute and subacute periods. According to a systematic review and meta-analysis [21], their prevalence was 92.3% during hospitalization, 72.7% after 2 weeks, and 56.3% after 1 month.

In patients with IS and COVID-19, our data showed MSP in 58.3% of patients. This frequency was more than 4 times higher than the frequency of MSP reported in stroke patients without COVID-19 by Cherenko T.M. [6]. However, other studies [29] report a significantly higher frequency of MSP, though 30.9% of cases were identified as having a local nociceptive nature.

In isolated studies [30], it has been reported that stroke can potentially cause prolonged neuropathic pain in 7–8% of patients after COVID-19 within 1 year (without specifying central or peripheral neuropathic pain). In our study, the prevalence of central post-stroke neuropathic pain (CPSP) in the "COVID-19 + stroke" group was over 20% within 12 weeks. This higher rate, compared to stroke without COVID-19, likely reflects the synergistic effect of the underlying pathophysiological mechanisms.

The study has certain limitations, as our conclusions do not cover cases of combined stroke and COVID-19 with a severe clinical course, nor was the prevalence of pain syndromes during the long COVID investigated. At the same time, these aspects require further research to optimize approaches to pain syndrome management.

CONCLUSIONS

Thus, acute and subacute PS in patients with COVID-19 and stroke represents a significant medical issue, affecting 75% of such patients within 12 weeks of the disease. In the spectrum of pain disorders, the most common were MSP (58.3%) and HA (54.2%), while SP was observed in 33% of cases. Notably, CPSP was recorded with a high prevalence of 20.8%. In more than half of the cases, PS involved a combination of two pain types: MSP and HA were observed in one-third of patients. Moderate pain was the most frequent, occurring in 48.7% of cases, while severe pain was recorded in 15.4%.

Thus, PS links two global medical challenges through common clinical intersections. Identifying the specific prevalence , structure, and severity of PS in the combination of ischemic stroke and COVID-19 will help focus attention on its timely recognition and management. This will enable the prevention of pain chronicity, improve functional recovery after stroke, and enhance patients' quality of life.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Epidemiology of endometriosis in female evacuated from the Eastern Ukrainian military conflict regions: results a multicenter study (2022-2024)

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ABSTRACT

Aim: To estimate the prevalence and risk factors associated with endometriosis in female evacuated from the Eastern Ukrainian military conflict regions.

Materials and Methods: A prospective multicenter cohort study was based on surveillance data for endometriosis. 982 women undergoing pelvic surgery were compared to 264 patients unexposed to surgery. The study cohort included women, who underwent a diagnostic and/or therapeutic laparoscopy or laparotomy in 2022-2024. Endometriosis among women was diagnosed visually at laparoscopy/laparotomy or by pelvic magnetic resonance imaging.

Results: The prospective multicenter study included 1,246 women. The overall prevalence of endometriosis was 31.5%. Prevalence of the three types of endometrioses included peritoneal/superficial endometriosis, ovarian endometriotic cyst/endometrioma and deep infiltrating endometriosis was 13.8%, 9.8%, and 4.8%, respectively. Three hundred sixty-one women 36,8% in the surgical group and thirty-one women 13.3% in the community treatment group (unexposed to surgery, population cohort) were diagnosed with incident endometriosis. The pelvic pain, infertility and early age at menarche to be a consistent risk factor for endometriosis in both the operative and population cohorts. Data analysis showed that odds were decreased for gravidity, parity, and BMI. Factors that increased the odds of endometriosis diagnosis included dysmenorrhea, older age at first sex, pelvic pain as a surgical indication for laparoscopy, and higher education.

Conclusions: Our study findings demonstrate the high prevalence of endometriosis among women in Ukraine. The pelvic pain, infertility and early age at menarche to be a consistent risk factor for endometriosis in both the operative and population cohorts.

KEY WORDS: women evacuated from the military conflict regions, endometriosis, prevalence, risk factors, Ukraine

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INTRODUCTION

Endometriosis is a common benign gynecological disease in female of reproductive age, but the prevalence of these condition is not exactly known. According to the literature, prevalence of endometriosis affects up to 10% of females worldwide [1] and it most often occurs on or around reproductive organs in the pelvis, including Fallopian tubes, Ligaments around the uterus, Lining of the pelvic cavity, Ovaries, Outside surface of the uterus, Cervix, Vagina or vulva, Space between the uterus and the rectum or bladder.

Endometriosis significantly impacts women's reproductive health. This disease not only leads to reduced female fertility but may also be a risk factor for adverse pregnancy outcomes [2-5]. Moreover, endometriosis associated with depression, fatigue, and a reduction in work productivity, leading to a substantial economic burden [6]. According to the literature, endometriosis

imposes a yearly cost of ~$78 billion in the United States [7]. The health care costs for endometriosis were approximately $4,000 per affected woman [8]. In Belgium annual total cost per woman with endometriosis was €9579 [7].

Endometriosis predominantly affects women in their reproductive years and characterized by lesions resembling the endometrium found outside the uterus (ectopic endometrium), primarily in the pelvic tissues and organs [9, 10]. In addition, ectopic endometrium can invade almost any part of the body, including the lungs and pleura, but most commonly the pelvic organs and parietal peritoneum [11]. The most common signs of endometriosis in female are painful periods, painful intercourse, chronic pelvic pain, ovarian cysts, and infertility [12]. However, these symptoms aren't exclusive to endometriosis, there may be other conditions. The varied symptomatology can also be attributed to other conditions [13]. The complex diagnostic challenge of endometriosis drives the delay in diagnosis. In addition, there are no effective biomarkers for the detection of endometriosis.

The pathogenesis of endometriosis is multifactorial and include retrograde menstruation, celomic metaplasia, embryologic rests, and lymphovascular spread [14]. Etiology and the association between the severity and location of endometriosis remains unclear [15]. Current surgical and medical approaches (including hormonal treatments) to endometriosis are ineffective for a sizable proportion of women. According to the literature, approximately 50% of women with endometriosis have recurrent symptoms over a period of 5 years, irrespective of the treatment approach [16]. An improved understanding of the pathogenesis of endometriosis is needed for the development of targeted medical approaches. However, no studies have been conducted on endometriosis in women living in the zone of the Ukrainian-Russian military conflict. Knowledge of endometriosis and risk factors in these regions are unknown.

AIM

The aim this study to estimate the prevalence and risk factors associated with endometriosis in female evacuated from the Eastern Ukrainian military conflict regions.

MATERIALS AND METHODS

STUDY DESIGN AND POPULATIONS

A prospective multicenter cohort study was based on surveillance data for endometriosis. The study cohort comprised 1,246 women, aged 18–49 years, who were evacuated from the Eastern Ukrainian military conflict regions. Participants in this study were admitted to gynecologic departments at hospitals located in the Kyiv (4 hospitals), Vinnytsia (2 hospitals), Lviv (2 hospitals), Kharkiv (2 hospitals) and Odesa (2 hospitals) sites. In this study, 982 women undergoing pelvic surgery (exposed to surgery, operative cohort) were compared to population cohort of 264 women (unexposed to surgery, population cohort). The cohort included currently menstruating women, who underwent a diagnostic and/or therapeutic laparoscopy or laparotomy at 1 of 4 participating hospitals in June 1st, 2022 through December 31st, 2024. In this study, any surgical indication was acceptable and included pelvic pain, pelvic mass, menstrual irregularities, fibroids, tubal ligation, and infertility. Endometriosis among women was diagnosed visually at laparoscopy/laparotomy or by pelvic magnetic resonance imaging (MRI) in the operative and population cohorts, respectively.

Exclusion criteria: previous laparoscopic diagnosis of endometriosis; currently breast-feeding; history of cancer other than nonmelanoma skin cancer; currently hormonal therapy; women with urinary tract infection and gastrointestinal infections; women who refused to sign the informed consent form.

DEFINITION

In the present study, endometriosis is defined as a disease characterized by the presence of endometrium-like epithelium and/or stroma outside the endometrium and myometrium, usually with an associated inflammatory process [16]

DATA COLLECTION

In this study, we analyzed the inpatient data medical records to identify and describe the epidemiology of endometriosis. Medical records and epidemiological data were used to find risk factors for endometriosis. A standard data collection form was created to extract demographic and clinical data. The standardized data collection protocol included interview administered at baseline, an anthropometric assessment including body mass index (BMI). All participants were queried regarding sociodemographic characteristics, medical and reproductive history, pain, and life-style. The protocol was administered for the operative cohort and population cohort (patients unexposed to surgery). In addition, for operative cohort completed a standardized report immediately following surgery to capture gynecologic pathology and endometriosis

Table 1. Characteristics by female evacuated from the Eastern Ukrainian military conflict regions and endometriosis diagnosis, 2022-2024 (n=1,246)

	Endometriosis							
	Operative cohort (n = 982)				Population cohort (n = 264)			
	Endometriosis (n = 361)		No endometriosis (n = 621)		Endometriosis (n = 31)		No endometriosis (n = 233)	
	n	%	n	%	n	%	n	%
Age, y								
<20	9	2.6	15	2.4	0	0	9	3.9
20–24	42	11.6	57	9.2	8	25.8	43	18.5
25–29	91	25.2	121	19.5	2	6.5	46	19.7
30–34	84	23.3	129	20.8	6	19.4	36	15.5
≥35	135	37.4	299	48.1	15	48.4	99	42.5
Marital status								
Married	304	84.2	481	77.5	28	90.3	185	79.4
Other	57	15.8	140	22.5	3	9.7	48	20.6
Education								
Less than College grad	114	31.6	201	32.4	17	54.8	104	44.6
College grad or higher	247	68.4	420	67.6	14	45.2	129	55.4
Alcohol consumer								
Never	84	23.3	138	22.2	7	22.6	66	28.3
Past	116	32.1	207	33.3	13	41.9	82	35.2
Present	161	44.6	276	44.4	11	35.5	85	36.5
Age at first consenting sex								
≤17	174	48.2	345	55.6	8	25.8	114	48.9
18–20	95	26.3	147	23.7	14	45.2	78	33.5
≥21	92	25.5	129	20.8	9	29.0	41	17.6
Ever use oral contraceptives								
No	40	11.1	98	15.8	3	9.7	36	15.5
Yes	321	88.9	523	84.2	28	90.3	197	84.5
Gravidity								
Nulligravid (0)	154	42.7	163	26.2	11	35.5	95	40.8
Gravid (≥1)	207	57.3	458	73.8	20	64.4	138	59.2
Parity (no. of live births)								
Nulliparous	71	19.7	76	12.2	4	12.9	36	15.5
Parous	290	80.3	545	87.8	27	87.1	197	84.5
Age at first pregnancy, y								
<20	143	39.6	221	35.6	1	3.2	48	20.6
20–24	134	37.1	202	32.5	12	38.7	94	40.3
25–29	66	18.3	109	17.6	9	29.0	68	29.2
30–34	13	3.6	71	11.4	5	16.1	15	6.4
35–39	5	1.4	14	2.3	1	3.2	4	1.7
≥40	0	0	4	0.6	3	9.7	4	1.7
History STIs								
No	304	84.2	481	77.5	28	90.3	185	79.4
Yes	57	15.8	140	22.5	3	9.7	48	20.6
History of abnormal pap smear								
No	281	77.8	461	74.2	26	83.9	165	70.8
Yes	80	22.2	160	25.8	5	16.1	68	29.2

Table 1. Cont.

Ever seek infertility treatment								
No	239	66.2	514	82.8	23	74.2	211	90.6
Yes	122	33.8	107	17.2	8	25.8	22	9.4
Surgical indication								
Pelvic pain	228	63.2	188	30.3				
Pelvic mass	49	13.6	107	17.2				
Menstrual irregularity	38	10.5	97	15.6				
Fibroids	17	4.7	95	15.3				
Tubal ligation	15	4.2	89	14.3				
Infertility	14	3.9	45	7.2				
Menarche, y								
≤11	168	46.5	325	52.3	15	48.4	64	27.5
12–13	121	33.5	189	30.4	11	35.5	131	56.2
≥14	72	19.9	107	17.2	5	16.1	38	6.3
BMI (mean±SD)	27.6 ± 7.7		29.4 ± 8.2		27.2 ± 5.5		27.8 ± 6.4	

SD, standard deviation; STIs, sexually transmitted disease; BMI, Body Mass Index.

diagnosis and staging using the revised criteria from the American Society for Reproductive Medicine (rASRM) [17]. rASRM staging was categorized as: stage I, minimal (scores 1–5); stage II, mild (scores 6–15); stage III, moderate (scores 16–40); or stage IV, severe (scores >40) [17]. All women in the population cohort (patients without prior surgery) underwent a pelvic MRI to assess any gynecologic pathology including endometriosis. Using protocol for pelvic imaging, 1 radiologist supervised and evaluated all MRI. These results were confirmed by a second radiologist with expertise in gynecologic imaging. In present study endometriosis among women diagnoses were derived from visualization by the surgeon in the operative cohort and from MRI in the population cohort (patients unexposed to surgery). Histologically confirmed endometriosis required the presence of endometrial glands and/or stroma.

ETHICS

The study was approved by the Institutional Ethics Committee of the Ukrainian center of maternity and childhood of the National Academy of Medical sciences of Ukraine. All participants provided informed consent before any data collection.

STATISTICAL ANALYSIS

In this study was performed by using SPSS program (Version 18, Chicago, IL, USA). Descriptive statistics were reported in numbers and percentages. In this study the associations between categorical variables were assessed by $\chi 2$ statistic or the Student t test. Odds ratio (OR) and 95% confidence interval were also calculated for all factor. Factors associated with endometriosis were investigated using logistic regression analysis model. We used stepwise logistic regression in a backward manner. In ordinal regression analysis, predictors which probably reflect symptoms of endometriosis (such as dyspareunia, dysmenorrhea and pelvic pain) were examined. Logistic regression estimated the adjusted odds ratios (AORs) and 95% confidence intervals for each cohort. We in each category compared the mean predicted probability in that particular category with the observed probability. In this study *P* values less than 0.05 (< 0.05) were considered to be statistically significant.

RESULTS

PREVALENCE OF ENDOMETRIOSIS

A total the study cohort comprised 1,246 women, who were evacuated from the Eastern Ukrainian military conflict regions. The overall prevalence of endometriosis among these women was 31.5% [95% confidence interval (CI), 30.2-32.8]. The number of surgically visualized endometriosis among women in the operative cohort was 361/982 and 31/233 in the population cohort based on MRI. MRI-visualized endometriosis in the population cohort consisted of primarily ovarian

Table 2. Risk factors for endometriosis by female evacuated from the Eastern Ukrainian military conflict regions, 2022-2024 (n = 1,246)

Risk factor	Operative cohort (n = 982)		Population cohort (n = 264)	
	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Sociodemographic				
Age, y	0.97 (0.94–0.99		1.02 (0.95–1.09)	
Poverty level	1.54 (0.83–2.81)	1.89 (1.00–3.53)	0.87 (0.18–4.25)	0.87 (0.18–4.54)
Educated level	1.63 (1.00–2.64)	1.83 (1.12–3.00)	0.58 (0.11–2.98)	0.58 (0.11–3.13)
Reproductive history				
Gravid vs nulligravid	0.48 (0.34–0.72)	0.49 (0.33–0.76)	1.25 (0.41–3.95)	1.03 (0.28–3.79)
Parous vs nulliparous	0.47 (0.33–0.69)	0.42 (0.27–0.65)	1.31 (0.43–4.03)	1.06 (0.27–3.97)
Infertility history	2.51 (1.62–3.86)	2.44 (1.56–3.78)	7.14 (1.73–29.8)	7.92 (1.67–37.4)
Age at first consenting sex	1.06 (1.01–1.13)	1.06 (1.02–1.14)	1.08 (0.87–1.31)	1.06 (0.88–1.29)
Surgical indication for laparoscopy	3.91 (2.64–5.77)	3.66 (2.45–5.51)		
Menstruation				
Early age at menarche, y	2.79 (1.47–5.31)	2.47 (1.29–4.74)	3.97 (2.64–5.73)	3.68 (2.45–5.57)
Dysmenorrhea (yes/no)	1.07 (0.95–1.19)	1.05 (0.93–1.18)	1.38 (0.28–6.59)	1.42 (0.28–7.16)
Pelvic pain	3.91 (2.64–5.77)	3.66 (2.45–5.51)	5.48 (2.66–9.88)	5.41 (2.61–7.72)
Body mass index, kg/m^2	0.95 (0.94–0.98)	0.95 (0.94–0.98)	1.01 (0.93–1.09)	1.01 (0.93–1.09)

endometriomas, and included nodular implants (stage 3–4 by rASRM). The prevalence of endometriosis by rASRM stage ranged from 4.6% for stage 4 to 23.8% for stage 1. Of all endometriosis cases, 36.8% were peritoneal/superficial endometriosis, 48.4% were ovarian endometriotic cyst/endometrioma and 14.8% were deep infiltrating endometriosis. Prevalence of the three types of endometrioses included peritoneal/superficial endometriosis, ovarian endometriotic cyst/endometrioma and deep infiltrating endometriosis was 13.8%, 9.8%, and 4.8%, respectively. Three hundred sixty-one women 36,8% [95% (CI), 35,4-38,0) in the surgical group and thirty-one women 13.3% [95% (CI), 12.3-14.3) in the community treatment group (unexposed to surgery, population cohort) were diagnosed with incident endometriosis. Detailed of socio-demographic, gynecological and clinical characteristics of study participants who were evacuated from the Eastern Ukrainian military conflict regions are presented in Table 1.

RISK FACTORS FOR ENDOMETRIOSIS

In this study, logistic regression identified only three consistent risk factors across both cohorts—history of infertility, early age at menarche and pelvic pain. A history of infertility increased the odds of endometriosis diagnosis in the surgical cohort (adjusted odds ratio [AOR], 2.44; 95% CI, 1.56–3.78) and in the population-based cohort (AOR, 7.14; 95% CI, 1.73–29.8), even after adjusting for age. A pelvic pain history increased the odds of an endometriosis diagnosis in the operative cohort (adjusted odds ratio [AOR], 3.66; 95% CI, 2.45–5.51), and in the population cohort (AOR, 5.51; 95% CI, 2.61–7.72). Also, in our study, early menarche history increased the odds of an endometriosis diagnosis in the operative cohort (adjusted odds ratio [AOR], 2.47; 95% CI, 1.47–5.31), and in the population cohort (AOR, 3.97; 95% CI, 2.64–5.73), even after adjusting for age and study site. Data analysis showed that odds were decreased for gravidity, parity, and BMI. Factors that increased the odds of endometriosis diagnosis included dysmenorrhea, older age at first sex, pelvic pain as a surgical indication for laparoscopy, and higher education. In this study, we found no association with endometriosis for any aspect of menstrual history (Table 2).

DISCUSSION

This study was performed to estimate the prevalence and risk factors associated with endometriosis in female evacuated from the Eastern Ukrainian military conflict regions, who admitted to gynecologic departments at regional hospitals located in the Kyiv, Vinnitsa, L'viv and Odesa sites. In this study, women undergoing pelvic surgery were compared to population cohort (unexposed to surgery). The incidence of surgically visualized

endometriosis in the operative cohort was 36.8% and 13.3% in the population cohort. The prevalence of endometriosis by rASRM stage ranged from 4.6% for stage 4 to 23.8% for stage 1. Of all endometriosis cases, 36.8% were peritoneal/superficial endometriosis, 48.4% were ovarian endometriotic cyst/endometrioma and 14.8% were deep infiltrating endometriosis. Prevalence of the three types of endometrioses included peritoneal/superficial endometriosis, ovarian endometriotic cyst/endometrioma and deep infiltrating endometriosis was 13.8%, 9.8%, and 4.8%, respectively. In this study we found infertility, pelvic pain, and early age at menarche to be a consistent risk factor for endometriosis in both the operative and population cohorts. Other risk factors either decreased or increased the odds of an endometriosis diagnosis.

Endometriosis is a common, chronic, gynecological condition that affects approximately 5–10 % of women of reproductive age worldwide [18]. According to the literature, endometriosis is estimated to affect approximately from 176 million [8] to 190 million of reproductive-age women worldwide [19]. The true prevalence of these disease in women is unknown. Literature data on the prevalence of endometriosis vary widely among population samples and diagnostic approaches, and on characteristics of the study population. In addition, differences in prevalence and diagnosis of female endometriosis patients are caused by differential access to healthcare, views, society, economy, education, medical conditions, and security systems and other factors. Moradi Y, et al. reported that the prevalence of endometriosis by the continent ranged from 17% for Europe to 36% for Asia [1].

Despite the high prevalence of endometriosis, diagnosing the condition remains challenging. In USA during the 10-year study period (2006-2016), among 332,0562863 women incident endometriosis cases were identified for an average incidence 24.3%. The distribution of the 2863 incident cases by the diagnosis modality was as follows: 45.5% surgical, 5.7% imaging, and 48.8% clinical [20]. The results of our study showed that the prevalence of endometriosis diagnosed with laparoscopy, ultrasound and magnetic resonance imaging (MRI) methods was higher than endometriosis diagnosed with other diagnostic methods. Other studies have shown the same results regarding the prevalence of endometriosis. Another study showed the same results prevalence of endometriosis [1, 21].

According to the literature, many women with this pathology are asymptomatic, while others may report non-specific symptoms. Shafrir AL, et al. reported that the prevalence ranged from 2 to 11% among asymptomatic women, 5 to 50% among infertile women, and 5 to 21% among women hospitalized with pelvic pain [19]. In our study, prevalence of asymptomatic women with endometriosis was 13.3%. A previous study, the prevalence of endometriosis among Ukrainian women was 28.4% [22]. In present study the overall prevalence of endometriosis among women in present study was 31.5%.

Currently, the etiology of endometriosis remains unknown. Prior studies identified a variety of endometriosis risk factors including abnormal or heavy bleeding, dysmenorrhea, dyspareunia, dysuria, and pelvic pain, age, alcohol use, early menarche, family history of endometriosis, infertility, intercourse during menses, low body weight, prolonged menstrual flow [14, 19, 22-26]. In present study, infertility, pelvic pain, and early age at menarche to be a consistent risk factor for endometriosis in both the operative and population cohorts. Other risk factors either decreased or increased the odds of an endometriosis diagnosis.

STRENGTH AND LIMITATIONS

Strength: The main strength of this study is the utilization of surveillance data for prevalence and causal inference of endometriosis. This work may be considered the first of epidemiological studies in Ukraine in order to estimate the prevalence and risk factors associated with endometriosis in female evacuated from the Eastern Ukrainian military conflict regions. Our results provide new data for a comprehensive assessment of the prevalence and risk factors of endometriosis in Ukraine.

Limitations: (1) The prevalence and risk factor data are derived exclusively in female evacuated from the Eastern Ukrainian military conflict regions. Therefore, the findings may not be extrapolated to other female groups and regions. More comprehensive studies across different regional groups of female are needed; (2) Although the available large female evacuated from the military conflict regions data, follow-up research efforts should focus on further expanding the sample size to produce more precise assessments on endometriosis.

CONCLUSIONS

Our study findings demonstrate the high prevalence of endometriosis among women in Ukraine. The pelvic pain, infertility and early age at menarche to be a consistent risk factor for endometriosis in both the operative and population cohorts. However, whether these factors are causal or merely represent a feature of the pathophysiological process remains uncertain. The lack of an effective diagnosis of endometriosis in female leads to delayed or missed diagnosis are associated with reduced quality of life and high financial costs to

the patient and the healthcare system. An improved understanding of the pathogenesis of endometriosis is needed for the development of targeted medical approaches. Given the high prevalence of endometriosis, the negative effect of the disease on female health and the high associated economic costs, biomarkers are urgently needed, as are new therapeutics that target the varied physiological pathways related to the development and progression of endometriosis. These can be achieved through collaborative, multidisciplinary research and the prioritization of endometriosis as an important public health issue.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Comparative analysis of treatment options for impacted mandibular canines in different age groups of patients. A retrospective study

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ABSTRACT

Aim: To analyze the effectiveness of traditional treatment options for impacted mandibular canines in adolescents and young people while considering age, the number of impacted teeth, type of treatment, and orthodontic appliances.

Materials and Methods: The retrospective investigation of the data of the case histories of patients with impacted mandibular canines was done: 53 cases in children and adolescents (Q1-Q3 = 13 to 15 years) (Group I) and 19 in young adults (Q1-Q3 = 21.25 to 33.5 years) (Group II). The anamnesis data and clinical examination, x-ray findings, jaw model findings, treatment methods, and results were studied.

Results: In Gr. I, unilateral impaction prevailed (71.7%), whereas 20.8% of patients had bilateral impaction of mandibular canines. In Gr. II, 57.9% of patients had unilateral impaction, whereas 26.3% had bilateral impaction of mandibular canines. There is a strong correlation between the treatment outcomes and the patient's age, the number of impacted teeth, and the type of orthodontic appliance.

Conclusions: The findings indicate the risk of poor treatment effect increases with the patient's age and the kind of appliances. the number of retained teeth, and their location in the jaw.

KEY WORDS: tooth impaction, orthodontic traction, lower canines, custom-made fixed appliance

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INTRODUCTION

Numerous studies indicate that between 1–3.5% of individuals in the white population have impacted upper canines, while 0.92–1.35% have impacted lower canines, making these conditions the second most prevalent after the impaction of third molars . Although there are numerous theories explaining tooth impaction, the specific causes of this condition remain unknown [1,2].

Clinical tests play a crucial role in diagnosing impaction, and panoramic radiographs are frequently used to confirm the clinical diagnosis of canine impaction. Cone beam computed tomography (CBCT) is the most effective procedure for identifying root resorption in adjacent teeth, examining the position of the root of the impacted tooth in the jawbone. In orthodontics, the degree of canine angulation with the presence of space in the dentition at the diagnostic stage are considered a risk factors of successful eruption [3,4].

The management of tooth impaction involves a combination of surgical, orthodontic, and periodontal approaches, each with a specific sequence and level of intervention. Treatment options for ectopically positioned canines include observation, interceptive treatment, orthodontic extrusion, transalveolar transplantation, and canine extraction [5-7]. A significant number of scientific sources indicate the predominant use of fixed appliances with additional anchorage during the orthodontic stage of treatment.

Current scientific advances, including the rationale for various treatment options for tooth impaction and the ability to manage the treatment process, require the identification of factors that influence the treatment of this anomaly.

AIM

The study aimed to analyze the effectiveness of traditional treatment options for impacted lower canines

Table 1. Prevalence of lower canine impaction in groups, types, and effectiveness of treatment

№	Background and parameters	Characteristics	Group I			Group II		
			N 53, abs. (%)	girls	boys	N 19, abs. (%)	females	males
				N 30, abs. (%)	N 23, abs. (%)		N 13, abs. (%)	N 6, abs. (%)
1	Type of impaction	Unilateral	38(71.7)	21(70.0)	17(73.9)	11(57,9)	9(69.2)	2(33.3)
		Bilateral	11(20.8)	6(20.0)	5(21.7)	5(26,3)	2(15.4)	3(50.0)
		3 or more teeth	1(1.9)	1(3.3)	0	1(5,3)	0	1(16.7)
		Transmigration	3(5.7)	2(6.7)	1(4.3)	2 (10,5)	1(7.7)	1(16.7)
2	Consent	Yes	42(79.2)	27(90.0)	15(65.2)	11(57.9)	7(53.8)	4(66.7)
		No	11(20.8)	3(10.0)	8(34.8)	8(42.1)	6(46.2)	2(33.3)
3	Type of treatment	Interception	18(34.0)	11(36.7)	7(30.4)	1(5.3)	1(7.7)	0
		Orthodontic appliances and surgical exposure	23(43.4)	15(50.0)	8(34.8)	10(52.6)	6(46.2)	4(66.7)
		Refusal	12(22.6)	4(13.3)	8(34.8)	8(42.1)	6(46.2)	2(33.3)
4	Type of orthodontic appliances	Removable	7(13.2)	6(20.0)	1(4.3)	0	0	0
		Fixed	21(39.6)	11(36.7)	10(43.5)	6(31.6)	5(38.5)	1(16.7)
		Custom fixed	4(7.5)	2(6.7)	2(8.7)	0	0	0
		Removable and fixed	1(1.9)	0	1(4.3)	0	0	0
		Fixed and custom fixed	9(17.0)	8(26.7)	1(4.3)	4(21.1)	2(15.4)	2(33.3)
		Refusal	11(20.8)	3(10.0)	8(34.8)	9(47.4)	9(69.2)	0
5	Treatment	Effective	25(47.2)	15(50.0)	10(43.5)	2(10.5)	2(15.4)	0
		Satisfactory	14(26.4)	10(33.3)	4(17.4)	6(31.6)	3(23.1)	3(50.0)
		Unsatisfactory	2(3.8)	1(3.3)	1(4.3)	0	0	0
		Extraction	3(5.7)	1(3.3)	2(8.7)	6(31.6)	4(30.8)	2(33.3)
		Observation	7(13.2)	3(10.0)	4(17.4)	5(26.3)	4(30.8)	1(16.7)
		Didn't receive treatment	2(3.8)	0	2(8.7)	0	0	0

in adolescents and young people while taking into account age, the number of impacted teeth, type of treatment, and orthodontic appliances

MATERIALS AND METHODS

After analyzing medical records of individuals, who sought consultation and treatment for "positional anomalies of teeth" at the orthodontic department of the Dental Medical Center of Bogomolets National Medical University (NMU) between 2018 and 2023 years, a total of 812 clinical cases were identified in the "impacted teeth" category. During our subsequent examination of the medical records, we specifically selected the patients with mandibular canine impaction. Among these records, there were 53 (6,5%) cases involving children and adolescents and 19 (2,3%) cases involving young adults. The participants were divided into two groups. Group I (hereinafter referred to as Gr. I) included 53 children and adolescents (30 females and 23 males). The average age of these patients (median value and interquartile range) was Mel=14 years (Q1-Q3 = 13 to 15 years). Group II (hereinafter referred to as Gr. II) included 19 young adults (13 women and 6 men). The average age was MeII=25 years (Q1-Q3 = 21.25 to 33.5 years). The study received approval from the Bioethics Committee of Bogomolets NMU. Every participant, or their parents or guardians, provided informed consent for diagnostic and treatment procedures, as well as observation. This consent was obtained following the Declaration of Helsinki of the World Medical Association, which outlines ethical principles for medical research involving human subjects.

The collected data were analyzed using EZR v. 1.66 (a graphical user interface for R statistical software version 4.3.1 developed by the R Foundation for Statistical Computing in Vienna, Austria) [8]. To predict the risks of reduced effectiveness in treating retained

Table 2. Analysis of univariate logistic regression models for predicting the risk of not achieving the full treatment effect

Factor variables		Coefficient of the model, b±m	The level of significance of the difference OR from 1, p	Model odds ratio indicator, OR (95% CI)	Area under the operating characteristics curve, AUC (95% CI)
Gender	F		Reference		–
M	-0.22±0.59	0.703	–		
Age, years		0.40±0.16	**0.014**	**1.49 (1.08 – 2.06)**	**0.79 (0.65 – 0.89)**
Impaction	1		Reference		**0.66 (0.52 – 0.79)**
	2 or 3	1.83±0.73	**0.012**	**6.29 (1.49 – 26.4)**	
Type of treatment	1		Reference		**0.70 (0.56 – 0.82)**
	2	1.88±0.67	**0.005**	**6.56 (1.77 – 24.4)**	
Appliances	2		Reference		**0.71 (0.57 – 0.83)**
	1 or 3	0.13±0.75	0.858	–	
	4 or 5	2.48±0.87	**0.004**	**12.0 (2.20 – 65.5)**	

Note. Factor variables (Table 1): Impaction: 1 - Unilateral, 2 - Bilateral, 3 - 3 or more teeth.
Type of treatment: 1 - interception, 2 - orthodontic appliance with surgical exposure.
Appliances: 1 - removable, 2 - fixed, 3 - custom fixed, 4 - removable+fixed, 5 - fixed+custom-made fixed.
Reference vs. others (2 or 3 combined).

Table 3. Analysis of the three-factor logistic regression model for predicting the risk of not achieving the full therapeutic effect

Independent factor variable		Coefficient of the model, b±m	The level of significance of the difference OR from 1, p	Model odds ratio indicator, OR (95% CI)
Age, years		0.49±0.20	**0.016**	**1.63 (1.10 – 2.43)**
Impaction	1		Reference	
	2 or 3	1.76±0.94	0.062	5.80 (0.92 – 36.7)
Appliances	2		Reference	
	1 or 3	0.94±0.88	0.289	–
	4 or 5	2.33±0.98	**0.007**	**10.3 (1.50 – 71.3)**

Note. Factor variables (Table 1): Impaction: 1 - Unilateral, 2 - Bilateral, 3 - 3 or more teeth.
Appliances: 1 - removable, 2 - fixed, 3 - custom fixed, 4 -removable+fixed, 5 - fixed+custom-made fixed.
Reference vs. others (2 or 3 combined).

mandibular canines, the method of building logistic regression models was used. The degree of correlation of factor signs with the probability of decreased treatment effectiveness was assessed using odds ratio (OR) indicators along with a corresponding confidence interval (CI). The model was evaluated using receiver operating characteristic (ROC) analysis to determine the optimal cut-off point (OCP). The results of the ROC analysis are presented as the average area under the ROC curve (AUC) with its 95% confidence interval (95% CI), sensitivity (Se), and specificity (Sp) corresponding to the discriminating point. The critical level of significance in the analysis is α=0.05. The interpretation of the area under the ROC curve concerning diagnostic accuracy is as follows: 0.9-1.0 - excellent, 0.8-0.9 - very good, 0.7-0.8 - good, 0.6-0.7 - satisfactory, 0.5-0.6 - unsatisfactory; a value of 0.5 indicates the marker is not suitable for prognosis [9]. The ROC analysis yields the average value of the area under the ROC curve (AUC), with its 95% confidence interval (95% CI), sensitivity, and specificity at the discriminating point.

RESULTS

The distribution of impacted canines was as follows in number and location. In Gr.I (Table 1), the prevalence of unilateral impaction of the mandibular canine was higher in girls and boys, with rates of 70.0% and 73.9%, respectively. A total of 20.8% of individuals had bilateral impaction of mandibular canines, whereas just one person had retention of both maxillary and mandibular canines. Three patients demonstrated transmigration of the mandibular canines. They intersected the mandible's symphysis at various angles relative to the midline. In Gr. II, over 50% of the patients had unilateral impaction of the mandibular canine, whereas a quarter had bilateral

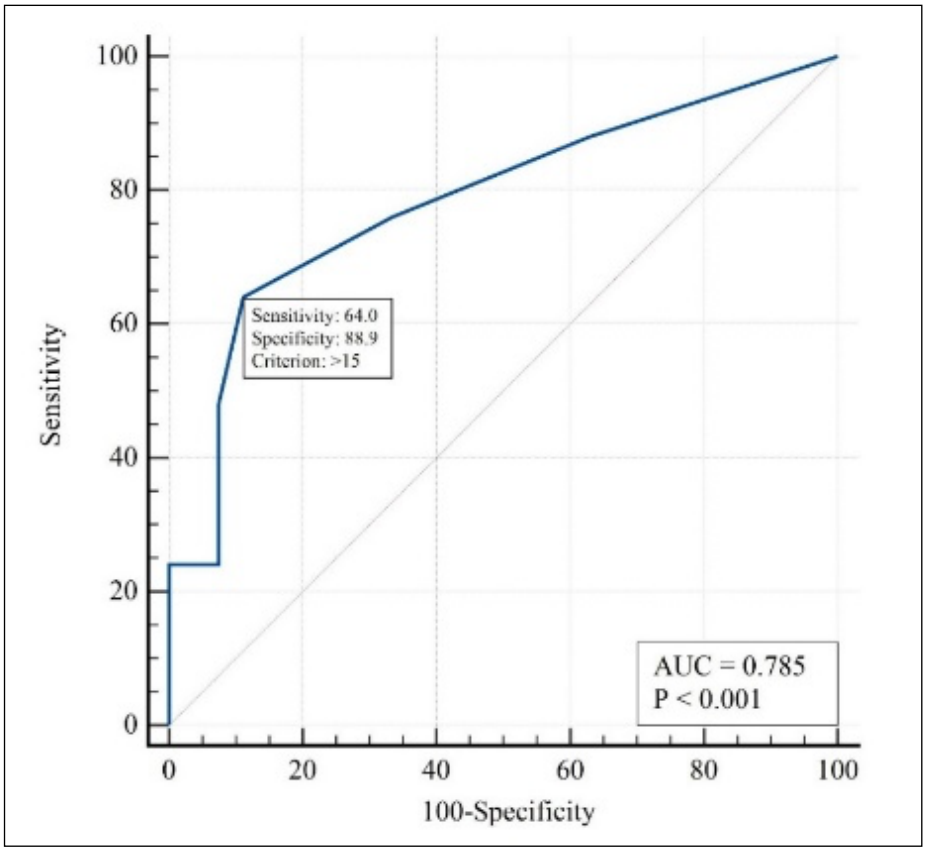

Fig. 1. Receiver operating characteristic (ROC) curve for predicting the risk of not achieving the full treatment effect by patient age.

impaction of the mandibular canine (Table 1). In one case, three canines were impacted. Mandibular canine transmigration was detected in two adults.

Following a thorough assessment, 79.2% of patients, mostly girls, agreed to treatment in Gr. I. One-third of the boys refused treatment. In Gr. II, a total of 57.9% of individuals provided their consent for treatment. Nevertheless, in this particular group, a significant proportion of women (46.2%) refused treatment (Table 1). In Gr. I, the impaction of mandibular canines was managed by a combination of interception (34%) and by consistent planned use of orthodontic appliances and surgical exposure (43.4%). Gr. II exhibited a significantly higher proportion of patients who declined therapy (42.1%) in comparison to Gr. I (20.8%). Orthodontic appliances and surgical exposure were used for canine eruption, mainly, in Gr.II (52.6%) (Table 1).

It has been established that orthodontic appliances were used more often simultaneously and sequentially: removable and standard fixed appliances, as well as standard fixed appliances and custom-made fixed appliances. In Gr. I, a minority of patients (13.2%) were treated with removable appliances, while the majority (39.6%) were treated with standard fixed appliances. In complicated cases, standard fixed appliances were combined with auxiliary anchorage and custom-made fixed appliances (17.0%) (Table 1). In Gr. II, 31.6% of patients underwent treatment using standard fixed appliances with auxiliary anchorage, while 21.1% of patients were treated using standard fixed and custom-made fixed appliances, either simultaneously or sequentially. In Gr. I, one adolescent willingly consented to undergo treatment but refused to use any orthodontic appliances after a temporary canine tooth extraction. In Gr. I, dental extractions were performed on 5.7% of patients (two teeth were in transmigration). One patient refused to have the extraction procedure, opting instead for observation. One tooth of the teenager was removed due to the formation of a cyst. In Gr. II, extraction was used as a treatment strategy for impaction in 31.6% of cases. Patients who refused treatment and did not have their teeth removed were periodically observed. The proportion of such patients was 13.2% in Gr. I and 26.3% in Gr. II (Table 1).

Throughout treatment, one adolescent refused to continue treatment, so further analysis was for 52 participants. Effective treatment were observed in 27 individuals, corresponding to the outcome variable Y=0 (effect achieved), which determined the treatment as effective. Out of the total number of patients, 25 cases were classified as Y=1 (no effect), where the treatment was assessed as satisfactory, unsatisfactory, or tooth extraction. The analysis was conducted for 5 variables:

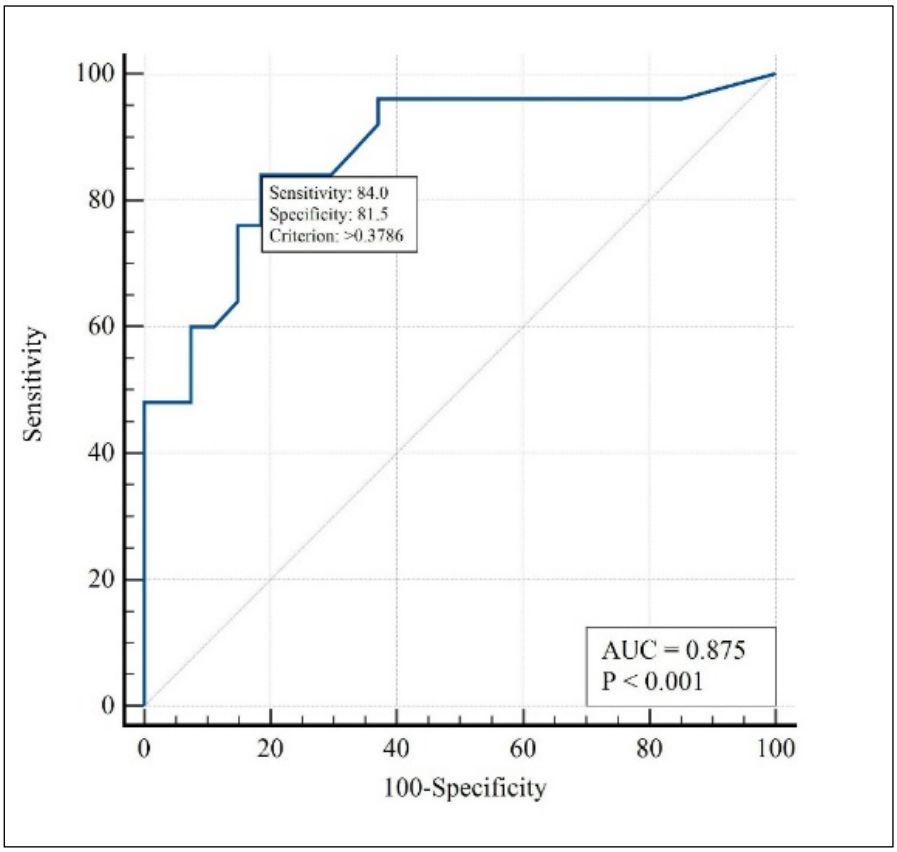

Fig. 2. Receiver operating characteristic (ROC) curve of the three-factor logistic regression model for predicting the risk of not achieving the full treatment effect.

gender, age, retention, orthodontic appliances, and type of treatment (Table 2).

A univariate analysis revealed no significant correlation between the risk of not achieving the full treatment effect and the gender of the individuals being treated (p=0.703). There was a statistically significant increase (p=0.014) in the risk of not achieving the full treatment effect with increasing patient age, OR = 1.49 (95% CI 1.08 - 2.06) for each year. The study established a significantly increased risk (p=0.012) of not achieving the full treatment effect for those with bilateral impaction and the presence of three or more impacted teeth in both jaws OR = 6.29 (95% CI 1.49 - 26.4) compared to those with unilateral impaction. When using orthodontic appliances and surgical intervention, there is a greater risk of not achieving the full treatment effect (p=0.005), OR= 6.56 (95% CI 1.77 - 24.4) compared to interceptive treatment. There was also a significantly higher risk (p=0.004) of not achieving the full treatment effect: OR = 12.0 (95% CI 2.20 - 65.5), when comparing the combination of removable and standard fixed appliances to the combination of standard fixed appliances and custom-made fixed appliances. For such approaches the correlation between patient age and the risk of not achieving the full treatment effect is pronounced - AUC = 0.79 (95% CI 0.65 - 0.89). Figure 1 shows the operating characteristics curve of this model.

When selecting the optimal threshold (age>15 years) based on the Youden Index, the sensitivity of this model is 64.0% (95% CI 42.5% - 82.0%), and the specificity is 88.9% (95% CI 70.8% - 97.6%). The sensitivity and specificity of a univariate logistic regression model for predicting the risk of not achieving the full treatment effect depending on patient age (64% and 88.9%, respectively) indicate its favorable prognostic value in predicting the treatment outcomes for mandibular canine impaction provided that the patient's age is > 15.

The method of constructing multivariate logistic regression models was used to identify a group of independent factor variables that are linked to the risk of not achieving the full treatment effect... We identified three main risk factors: age, impaction, and orthodontic appliances. The model constructed using these variables is adequate (chi-square= 27.7 with 4 degrees of freedom, p<0.001). The findings of the multivariate analysis are displayed in Table 3.

In the multivariate logistic regression model, after considering other factors, it was found that the patient's age was associated with an increased risk (p=0.016) of not achieving the full treatment effect, OR= 1.63 (95% CI 1.10 - 2.43) for each year (taking into account the effect of impaction and orthodontic appliances). Additionally, there was a significantly higher risk (p=0.007) of not achieving the full treatment effect when using a combination of appliances, specifically removable appliances with standard fixed appliances,

OR= 10.3 (95% CI 1.50 - 71.3) compared to treatment with a combination of standard fixed appliances and custom-made fixed appliances. This analysis took into consideration the impact of patient age as well as the number and location of impacted teeth in the jaw. Figure 2 shows the operating characteristics curve of this model.

When selecting the optimal threshold based on the Youden Index, this model has a sensitivity of 84.0% (95% CI 63.9% - 95.5%), and a specificity of 81.5% (95% CI 61.9% - 93.7%), which, according to the generally accepted classification, indicates their significant prognostic value in predicting the treatment outcomes for mandibular canine impaction, taking into account the patient's age, the number and location of impacted canines, as well as the choice of orthodontic appliances.

Removable orthodontic appliances and standard fixed appliances were used for creating interdental space in the dentition for traction of impacted canines in Gr.1 (13.2% and 39.6%). In difficult cases (significant canine displacement, horizontal location), 17.0% of patients in Gr.1 and 21.1% in Gr.II used a combination of standard fixed orthodontic appliances with custom-made fixed appliances.

DISCUSSION

Mandibular canine impaction, translocation, and transmigration are infrequent phenomena. Multiple scientific reviews revealed the occurrence of mandibular canine impaction ranges between 0.008% and 1.7%. This is a big range of results that can be explained by different focusing on populations ranging from orthodontic patients to the general population, different ethnic groups, and different sample sizes [1,10,11]. In compartment to those data our results of mandibular canine impaction manifestation in persons of the "impacted teeth" sample proved 6,5% in children and adolescents and 2,3% in adults with the prevalence of unilateral impaction (71,7% to 57,9%). In our study, most mandibular-impacted canines occurred unilaterally without significant differences between the right and left sides which was also confirmed by the Chowdhary S. et al., review [12].

Experienced orthodontists believe that the extraction of a deciduous canine should occur between the ages of 10 and 13, ensuring the natural eruption of the permanent canine. The timing of diagnosis is critical in terms of treatment options and therapeutic prognosis [1]. In our study, we found mandibular canine impaction in 22.6% of children aged 13 years, and, after data analysis, we can conclude that the risk of not achieving the full treatment effect after the patient's age increased (OR= 1.63 (95% CI 1.10 - 2.43) for each year. This emphasizes the importance of early identification of impaction to ensure a safer course of treatment- interception.

Stabryła J, et al. [2] proved that changes in canine angulation from a vertical to a horizontal location in the dental arch can complicate treatment. According to our investigation, a strong correlation has been established between the number of impacted teeth, their location, and the effectiveness of treatment results.

The most commonly employed treatment strategy for impacted mandibular canine surgical removal, because it was considered easier and faster than bringing the canine to its actual position[1]. Another approach is supported by the study of Stabryła J, et al [2], according to which orthodontic extrusion was most often performed to impacted mandibular canine eruption (33%), and, such treatment was successful in 95% of cases. Our research results support this data and show that for children, adolescents, and adults the most common treatment options were orthodontic traction with surgical exposure (Table 1). An univariate logistic regression model for predicting the risk of not achieving the full treatment effect depending on patient age indicates its favorable prognostic value in predicting the treatment outcomes for mandibular canine impaction provided that the patient's age is > 15.

The traction of impacted mandibular canines is always a challenge for the orthodontist. Each case of an impacted canine should be studied individually, concerning the location of the impacted tooth in the alveolar bone, occlusion, and the patient's profile [2, 12]. Agastra E. et al. review showed that the percentage of favorable to unfavorable impaction was 28.6% and 71.4% respectively [4] In compartment to this data our results show 47, 2% effective treatment for children and adolescents and 10,5% for adults.

The development of custom-made fixed appliances was necessary to achieve more effective results for challenging cases and adult patients. According to the findings of Germano F. et al. [13] and Topka A. et al. [14], these appliances allow forces to be controlled in specific directions and durations, minimizing the negative effects on abutment teeth. The findings of Inchingolo A. et al. [6] confirm that ensuring the correct aligning of the teeth in the dental arch and preserving the health of the surrounding gingival tissue is possible with the help of traction of the impacted tooth, which should imitate the natural process of tooth eruption.

Although treatment can be lengthy, successful eruption can be achieved with appropriate biomechanics, aided by CAD/CAM technologies, as well as Artificial Intelligence to minimize adverse effects and achieve the best results in rehabilitating patients with various anomalies, including teeth impaction [3,13,15].

CONCLUSIONS

The findings after our research indicate the increase in the risk of poor treatment results with the increasing patient age, with the type of appliances. the number of impacted teeth, and their location in the jaw.

Artificial Intelligence and digital diagnostics of pathology, digital design, and manufacturing of orthodontic appliances should become a promising direction for increasing the effectiveness of teeth impaction treatment, so it needs active development.

Further research is necessary to establish clinical guidelines for more active detection and early treatment of dental impactions in children and to develop algorithms for using orthodontic appliances in adults as an alternative to tooth extraction.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CONTENTS

Changes in morphological and immunohistochemical parameters of tumors during neoadjuvant hormone therapy in postmenopausal patients with luminal type of breast cancer

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ABSTRACT

Aim: Breast cancer (BC) remains the most common malignant disease among women in Ukraine and worldwide. The indication for neoadjuvant hormone therapy (NAHT) is HR+/HER2- breast cancer of stage II-III. However, there are currently insufficient data on its impact on the IHC biological characteristics of tumors and clinical outcomes.

Materials and Methods: The study was conducted at the Uzhhorod Treatment and Diagnostic Oncology Center during 2015–2023. At the diagnostic stage, morphological verification and IHC profile were determined based on trephine biopsy material. The study included 28 patients with HR+/HER2- BC, aged from 38 to 75 years old. All patients were prescribed NAHT with the use of aromatase inhibitors for 6.10±2.23 months. The expression levels of ER, PR, Ki-67, category G were assessed before and after NAHT using histological and IHC analyses.

Results: After NAHT, we observed a decrease in Ki-67 levels from 25.31% to 10.25%, ER expression levels from 97% to 84%, and PR expression levels from 87% to 11%. There were changes in category G: G1 was observed in 45.83% of patients, G2 in 54.17%. The staging of BC also decreased. A moderate degree of curative tumor pathomorphosis was achieved in 76.47% of patients, and complete regression (pCR) in 5.88%.

Conclusions: Neoadjuvant hormone therapy demonstrated high efficacy in the treatment of patients with luminal HR+/HER2- BC of stage II-III. In most cases, it was possible to reduce the proliferative activity of the tumor, which was accompanied by a decrease in tumor staging. This made it possible to perform organ-preserving surgeries in 60.71%, which confirms the feasibility of using NAHT as an alternative to chemotherapy in certain groups of patients.

KEY WORDS: breast cancer, neoadjuvant hormone therapy, estrogen and progesterone receptors, Ki-67 tumor proliferation index

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LIST OF ABBREVIATIONS
BC - breast cancer
IHC - immunohistochemical study
NAHT - neoadjuvant hormone therapy
HR+ - positive hormone receptors
HER2- human epidermal growth factor receptor 2
ER - estradiol receptors
PR - progesterone receptors
Ki-67 - tumor proliferation index
NACT - neoadjuvant chemotherapy
pCR - pathological complete regression

INTRODUCTION

Breast cancer (BC) is the most common malignant disease and the leading cause of death among women worldwide [1]. In 2020, 2,261,419 cases of BC were diagnosed in the female population worldwide, of which 531,086 were diagnosed in Europe [2]. In 2021, the standardized incidence rate per 100,000 population for women in Ukraine was 62.4 [3]. The indication for prescribing neoadjuvant hormone therapy (NAHT) is HR+/HER2- BC of stage II-III [4]. Despite effective changes after NAHT, it is used quite rarely [5]. Approximately 70-75% of breast malignant tumors are hormone receptor positive (HR+), so the use of NAHT should be considered as a priority for the treatment of patients with such tumors [6]. The low cost and availability of drugs are also a significant advantage of NAHT, since 70% of deaths from breast cancer occur in patients with low or middle incomes [1]. The expression levels of ER, PR and Ki-67 are considered to be prognostic indicators of tumor response to NAHT. As described in studies, expression levels tend to decrease with NAHT [7]. There is no consensus on the duration of NAHT [8,9]. Some believe that 3-6 months before surgery is the gold standard [9], while others believe that longer use is necessary [8]. At the conference "Optimization of Treatment for Patients with Primary Breast Cancer - a Brief Summary

Table 1. ER, PR, Ki67 and Grade data before and after NAHT

	count		mean		median		Std		q25		q75		min		max	
Surgery	After	before	after	before	after	before	after	before	after	before	after	before	after	before	after	before
ER	15	15	84,66667	97,33333	90	100	20,91365	7,98809	80	100	97,5	100	20	70	100	100
PR	15	15	11,66667	74,73333	5	90	15,43033	35,07638	0	65	22,5	100	0	0	40	100
Ki67	16	16	10,25000	25,31250	10	20	9,16151	16,87886	2	15	20	40	1	5	30	60
Grade	24	24	1,54167	1,87500	2	2	0,50898	0,53670	1	2	2	2	1	1	2	3

of the Consensus Discussion" (St. Gallen, 2023), up to 70% of the discussion participants reported that the use of NAHT 2-4 weeks before surgery and monitoring of Ki-67 dynamics can provide valuable information for deciding to administer chemotherapy [10]. In a prospective cohort study of 146 patients with ER+/ HER2- BC, NAHT led to effective changes [11]. It has been noted that for patients with HR+ BC, it is preferable to use NAHT rather than NACT, as the pCR rate was lower with NACT [12]. However, other researchers have reported that the frequency of organ- preserving surgeries and clinical response HR+ BC with NAHT are similar to those with NACT [4]. NAHT is also the method of choice for elderly women who have contradictions to or refuse neoadjuvant chemotherapy, as well as in cases of inoperable tumors [6]. It has been reported that 20–30% may have primary resistance to NAHT. Treatment of BC with hormonal drugs, even in cases of resistance, is less toxic [6]. There are opinions that Ki-67 cannot reflect the regression of the primary tumor after NAHT, but instead it is a predictor for assessing the progression-free survival of patients with BC [13]. Approximately 60-80% of patients can undergo organ-preserving surgery after NAHT [6]. Organ-preserving surgeries have a positive effect on the psychosocial and cosmetic outcomes for patients with BC, and they are the main advantage of NAHT [14].

AIM

To study the effect of NAHT on the morphological and IHC parameters of the tumor in luminal types of breast cancer.

MATERIALS AND METHODS

Examination and treatment of 28 patients with HR+ / HER2- BC of stage II-III was carried out at the Uzhhorod Treatment and Diagnostic Oncology Center in 2015-2023. The age group of patients was from 38 to 75 years, with an average age of 56.52 ± 9.83 years. All women had postmenopausal status at the time of NAHT. Those patients who were premenopausal at the time of diagnosis of BC and before the start of NAHT were transferred to artificial menopause by surgery or medication. Confirmation of the presence of luminal type malignant tumors was performed on trephine biopsy material with histological and IHC studies, which obtained data on the expression levels of ER, PR, Ki-67 and category G. IHC studies were performed using the electrochemiluminescent method in the certified laboratory CSD (Kyiv). After completion of NAHT and surgeries, a postoperative histological and IHC study was performed with the determination of the degree of therapeutic pathomorphosis using the RCB System, which allowed to detect changes in the morphological parameters of tumors and assess their sensitivity to NAHT. 28 patients were prescribed aromatase inhibitors (letrozole), the average duration of administration was 6.10 ± 2.23 months. NAHT was discontinued for patients on the day of surgical interventions for BC or one day before surgery.

STATISTICAL METHODS

The study results were statistically processed in the Google Colab environment using the TableOne, scipy and DescTools libraries. Comparison of results before and after surgery was performed using the Wilcoxon paired test (for ER, PR, Ki-67) and the Stewart-Maxwell test (for comparing the G category and cancer stage before and after treatment). Paired comparisons were used to analyze the pre- and post-surgery indicators. In this case, only patients for whom both indicators were present were taken into account. The expression levels of ER, PR were compared in 15 patients, the Ki-67 level in 16, and category G in 24. Descriptive statistics are given in Table 1.

RESULTS

Expression levels of ER. Before the start of NAHT administration in patients with HR+ BC of stage II-III, the average ER value was within 97.33%. Since the distribution of differences between ER levels before and after surgery was not normal according to statistical methods, as determined by the Shapiro-Wilk test, p=0.0176, the nonparametric Wilcoxon test was used

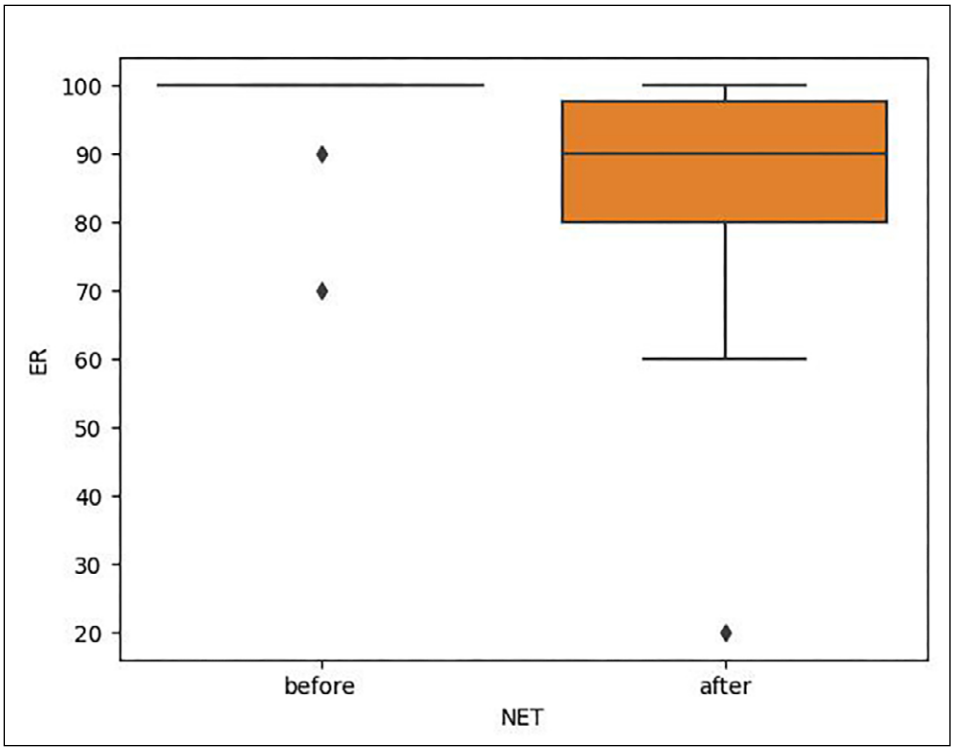

Fig. 1. Comparison of ER expression levels before and after NAHT.

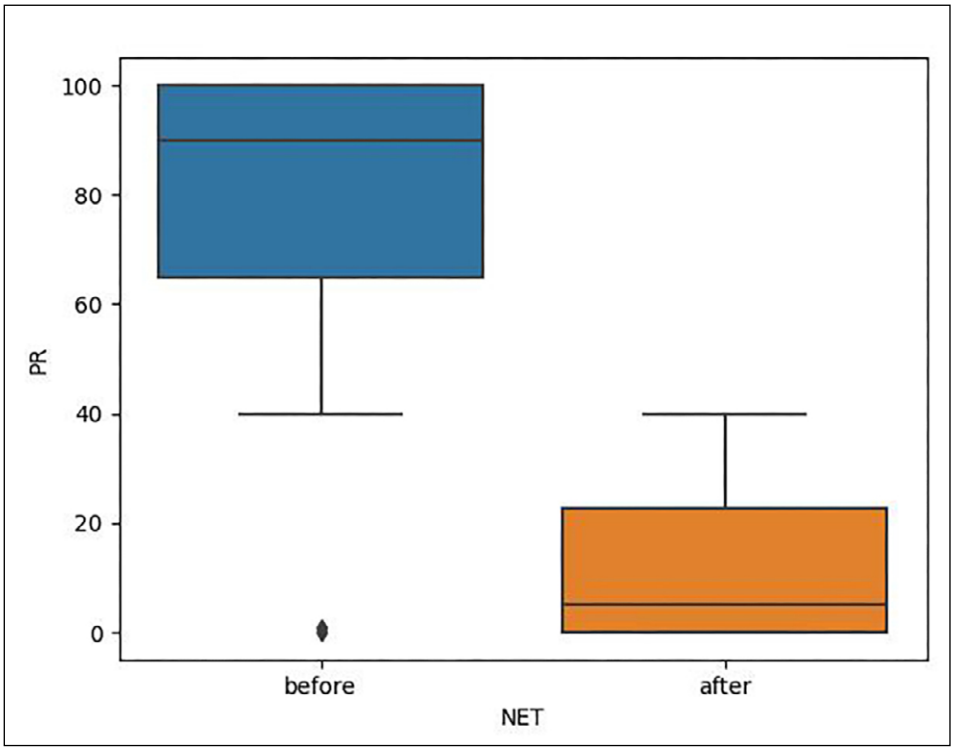

Fig. 2. Comparison of PR expression levels before and after NAHT.

to determine the difference between the groups. The value of p=0.02236 was obtained, so we reject the hypothesis of equality of medians before and after surgery. After completion of NAHT, based on the results of the postoperative IHC study, the levels of ER expression were reassessed. The average value after NAHT was 84.66%, therefore, we can conclude that the expression levels of ER decreased. Fig. 1. presents changes in the expression levels of ER.

Expression levels of PR. Before the start of NAHT, the average value of PR expression was 74.73% (Table 1, Fig. 2). In this case, the distribution of differences between the level of PR expression before and after surgery is not normal, as determined by the Shapiro-Wilk test (p=0.00596), therefore, the non-parametric Wilcoxon test was used to determine the difference between the groups. The value of p=0.00182 was obtained, so we reject the hypothesis of equality of medians before and

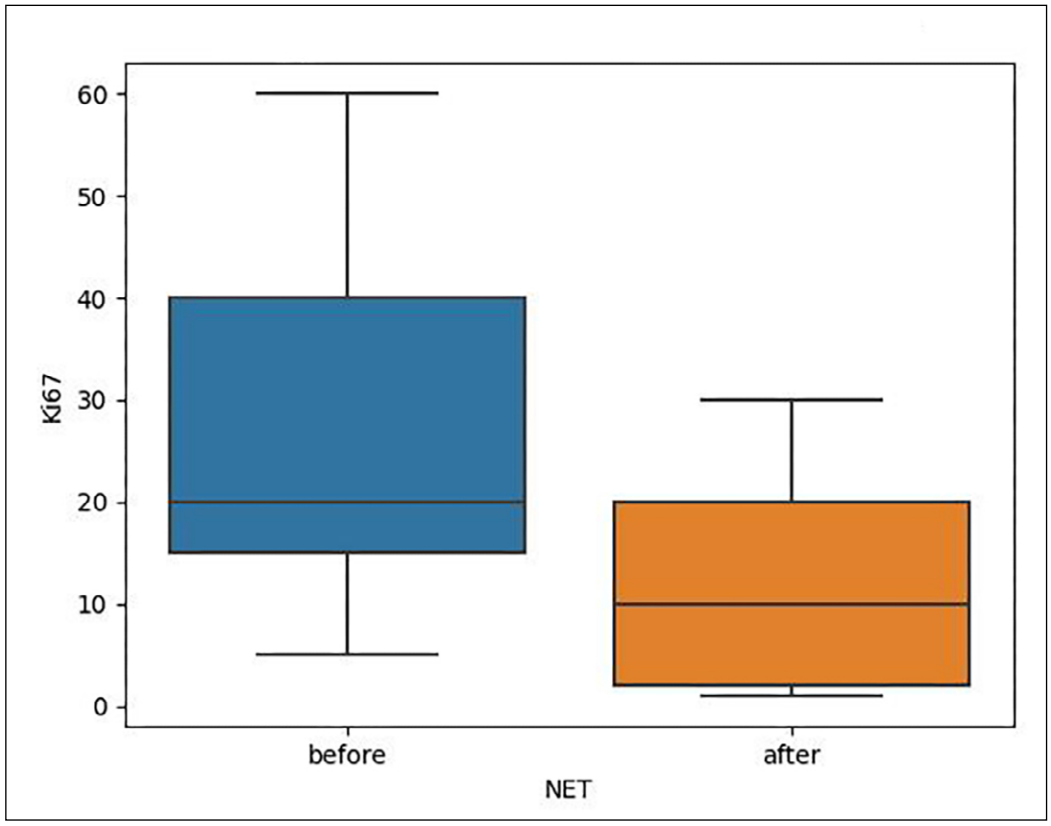

Fig. 3. Comparison of Ki-67 levels before and after NAHT.

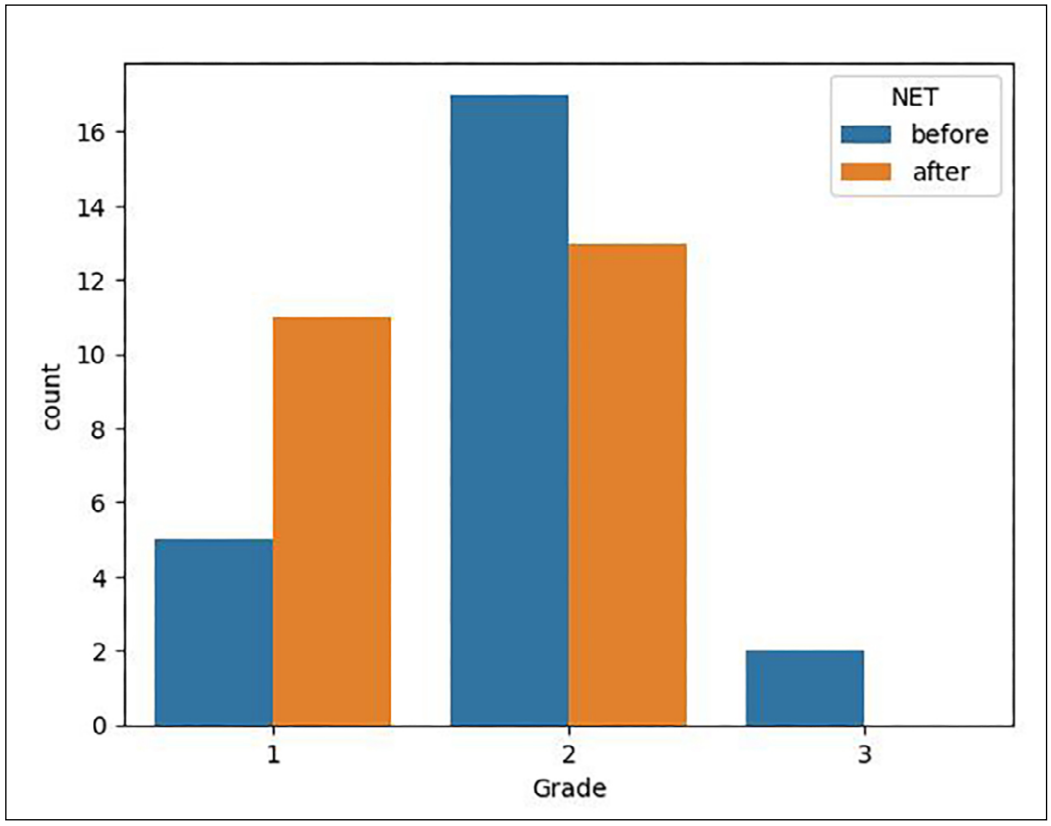

Fig. 4. Distribution by category G before and after NAHT.

after surgery. After the completion of the NAHT course, the average value of PR expression levels was 11.66%.

Ki-67 level. Before the start of the NAHT course, the Ki-67 level averaged 25.31%, and after NAHT this indicator decreased to an average value of 10.25% (Table 1, Fig. 3). The Shapiro-Wilk test revealed a normal distribution of the differences in Ki-67 before and after NAHT (p=0.62454), but the IQR method revealed an outlier (a difference of 40 units). Therefore, the non-parametric Wilcoxon test was used to assess the differ-

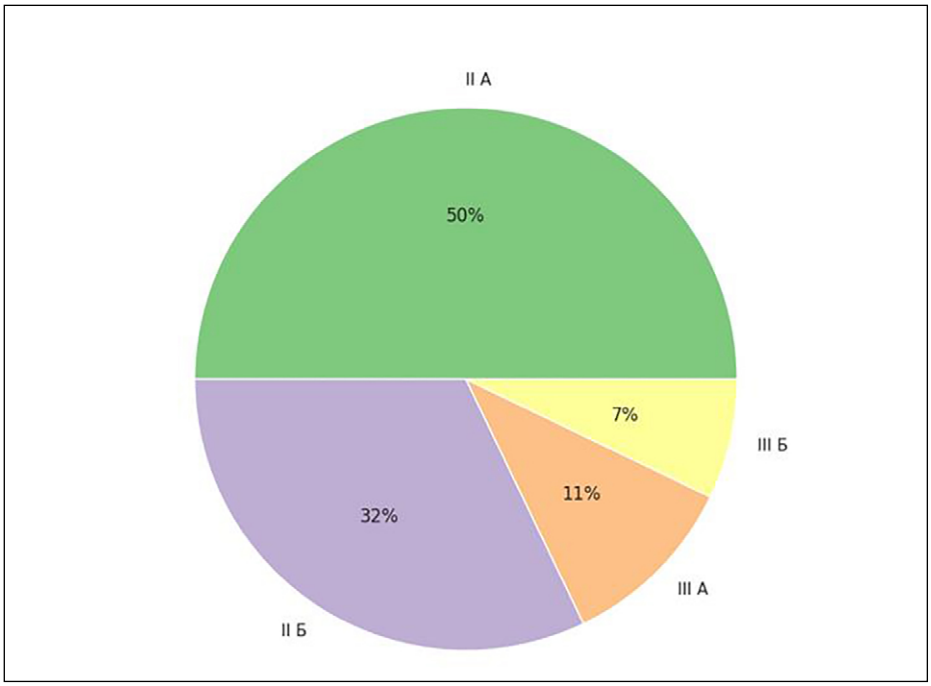

Fig. 5. Distribution by cancer stage before NAHT.

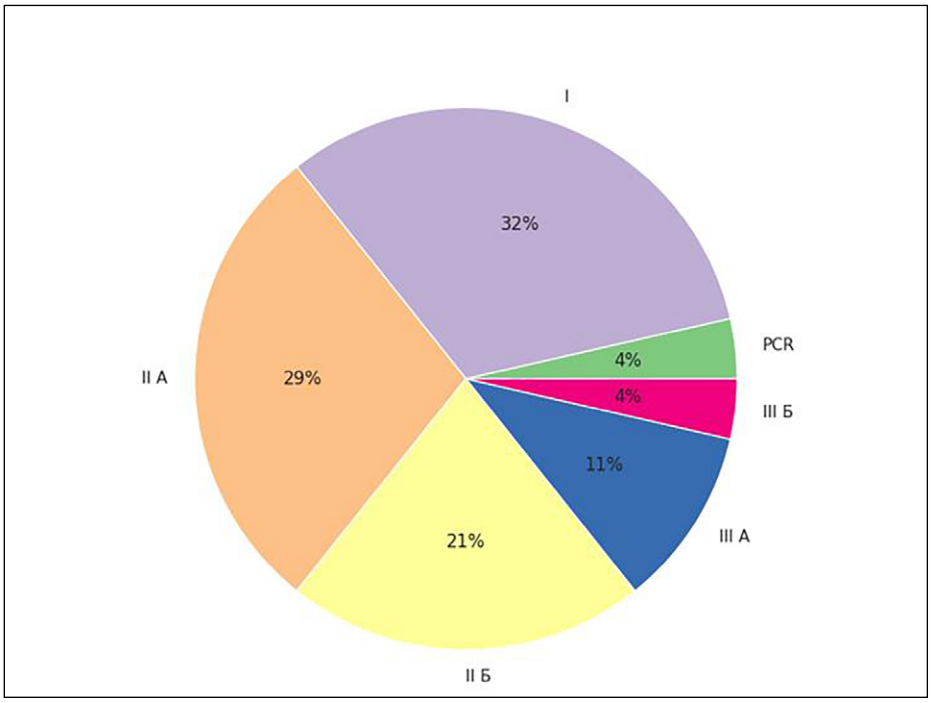

Fig. 6. Distribution by cancer stage after NAHT.

ence between the groups. The value of p=0.00021 was obtained, and we rejected the hypothesis of equality of medians before and after surgery. We can conclude that the Ki-67 level decreased after NAHT.

Category G. The Stewart-Maxwell test was used to compare the distribution of category G before and after surgery, and at p=0.01832 the hypothesis that the distribution of categories G was in the same proportion in both groups was rejected. The average value for category G before NAHT was 1.87, after NAHT 1.54 (Table 1). Changes in category G are shown in more detail in Fig. 4. When compared in percentage terms, before NAHT 7 of 28 patients had G1 (25%), 19 had G2 (67.86%) and 2 had G3 (7.14%). After NAHT, category G was compared in 24 patients, of which 11 had G1 (45.83%), and 13 had G2 (54.17%), and G3 was absent.

Staging of the malignant process. The Stewart-Maxwell test was used to compare the distribution of cancer stages before and after surgery, and at p = 0.04038, the hypothesis that the distribution of cancer stages was in the same proportion in both groups was rejected. At the time of diagnosis and before the start of NAHT, none of the 28 patients had stage I, 50% had IIA, 32% had IIB, 11% had stage III A, and 7% had stage III B (Fig. 5). After

the completion of the NAHT course, restaging revealed significant changes. A complete pathological tumor response (pCR) was achieved in one of the 28 women, stage I was observed in 32% of women, stage II A in 29%, stage II B in 21%, stage III A in 11%, and stage III B in 4% (Fig. 6).

The degree of therapeutic pathomorphosis-RCB. RCB was determined in 17 patients. Complete tumor RCB-0 (5.88%) was achieved in one patient, 13 patients had regressed malignant moderate residual tumors - RCB II (76.47%), and 3 patients had severe residual tumors - RCB III (17.65%).

Types of surgeries performed. After NAHT, 17 patients out of 28 underwent quadrantectomy with axillary lymphadenectomy (60.71%), and 11 patients underwent radical mastectomy with preservation of both pectoral muscles and axillary lymphadenectomy (39.29%).

DISCUSSION

Thus, after NAHT in postmenopausal patients with luminal type BC of stage II-III, significant changes were achieved. Aromatase inhibitors were used for NAHT, which inhibit estrogen biosynthesis and thus block the proliferative effect on hormone-sensitive tumor cells [6]. When comparing the results of IHC studies before and after the completion of NAHT, a decrease in the expression levels of ER, PR, Ki-67 was observed. For ER, the average value before the start of NAHT was 97.33%, and after NAHT 84.66% (p=0.02236). For PR, this indicator before NAHT was 74.73%, and after 11.66% (p=0.00596). The levels of the Ki-67 proliferation index before NAHT had an average value of 25.31%, after the completion of NAHT 10.25% (p=0.62454). Category G also underwent changes, since before the start of therapy the average value was 1.87, after completion of the NAHT course 1.54 (p=0.01832). According to large randomized studies, the use of NAHT before surgery in postmenopausal women reduces tumor size, lowers Ki-67 levels and prevents complete removal of the breast [1, 7, 11]. In our study, at the time of diagnosis, the patients had stage II-III BC. As a result of NAHT, it was possible to achieve changes in staging. Stage I was observed in 32% of women after NAHT, pCR was achieved in one patient (p=0.04038). The average duration of NAHT was 6.10 ± 2.23 months. As a result of the use of NAHT in a group of 28 women, in 60.71% of cases we performed organ-preserving interventions. According to some publications, after 3 months of NAHT, organ-preserving surgeries can be performed in 69.8% of cases, and after 2 years, in 83.5% of cases [15]. Therefore, HR+ tumors are sensitive to NAHT, which has improved the immediate treatment results of patients.

CONCLUSIONS

1. After NAHT, there is a decrease in the expression levels of ER and PR. The expression levels of ER and PR decreased from 97% to 84%, and from 87% to 11%, respectively.
2. The use of NAHT leads to a decrease in the level of Ki-67 from 25.31% to 10.25%.
3. As a result of NAHT, changes occur in the degree of tumor differentiation (category G). After the completion of NAHT, its increase was observed in 24 patients - in 11 to G1 (45.83%), and in 13 to G2 (54.17%).
4. The stage of the BC also decreases - at restaging after NAHT, 32% of women had stage I, in 50% stages II A and II B, and only in 15% stages III A and III B.
5. As a result of NAHT, 13 patients out of 17 (76.47%) achieved a moderate degree of therapeutic tumor pathomorphosis, and one (5.88%) had complete tumor regression.
6. The use of NAHT made it possible to perform organ-preserving surgeries in 60.71% of cases.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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

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ORIGINAL ARTICLE

CONTENTS

Morphological studies of changes in dental hard tissues following different methods of dental deposit removal

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ABSTRACT

Aim: To study structural changes in hard tooth tissues after manual, ultrasonic, and sonic methods of removing dental deposits and identifying the most rational method for various supragingival deposits.

Materials and Methods: Eight patients with supragingival deposits were examined, and eight teeth planned for extraction due to orthodontic or surgical indications: three teeth with dental plaque, three with mineralized deposits, and two with smoker's plaque. Of the three patients with dental plaque, one underwent manual scaling, another ultrasonic scaling, and the third sonic scaling. Similarly, among the three patients with dental calculus, manual, ultrasonic, and sonic scaling were performed. In the two patients with smoker's plaque, ultrasonic and sonic scaling were applied. After tooth extraction, morphological studies were conducted.

Results: Manual scaling of dental plaque left residual deposits and damaged enamel surfaces. For dental calculus, minimal residual deposits and relatively intact enamel were observed. Ultrasonic scaling caused microscopic changes: destruction of enamel prisms, partial fragmentation of the reticular layer in teeth with plaque, partial destruction of superficial enamel layers, thickening of the reticular layer with PAS-positive vegetations in teeth with calculus. In teeth with smoker's plaque, enamel prism destruction, hyperplasia, and reticular layer fragmentation were observed. Sonic scaling caused more pronounced destructive changes.

Conclusions: Manual scaling is the most rational method for supragingival mineralized deposits, while the air-abrasive method is preferred for non-mineralized deposits and smoker's plaque. The destructive changes caused by ultrasonic scaling limit its clinical applicability. Sonic scaling is unsuitable for removing dental debris due to its aggressiveness.

KEY WORDS: dental plaque, dental calculus, manual scaling, ultrasonic scaling, sonic scaling, microscopic study

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INTRODUCTION

It is well known that professional oral hygiene plays a significant role in the comprehensive treatment and prevention of dental caries and periodontal diseases [1, 2]. One of its important components is the removal of non-mineralized and mineralized dental deposits (soft dental plaque, dental biofilm (plaque), dental calculus) as well as smoker's plaque [2, 3].

It should be noted that the majority of studies by national and foreign authors are devoted to examining the structure and characteristics of subgingival dental deposits [4, 5], their impact on tooth and periodontal tissues [6, 7], and the methods for their removal [8]. However, studies on supragingival dental deposits are rare [9]. In this regard, we have previously carried out morphological studies of extracted teeth with various types of supragingival dental deposits [10]. The identified different nature in the structural changes of enamel and its covering tissues, the amelo-dentinal junction, and dentin during the formation of non-mineralized and mineralized dental deposits, as well as smoker's plaque on the tooth surface was an important argument in the search for the most rational methods of dental plaque removal.

Currently, dentists have access to a huge arsenal of various types of instruments, allowing for effective removal of dental deposits with minimal discomfort to patients and without damaging the soft tissues surrounding the teeth. At the same time, the choice of a particular method of dental plaque removal (chemical, abrasive, sonic, ultrasonic, or manual scaling) is often made arbitrarily, based on the practitioner's experience, without considering the nature

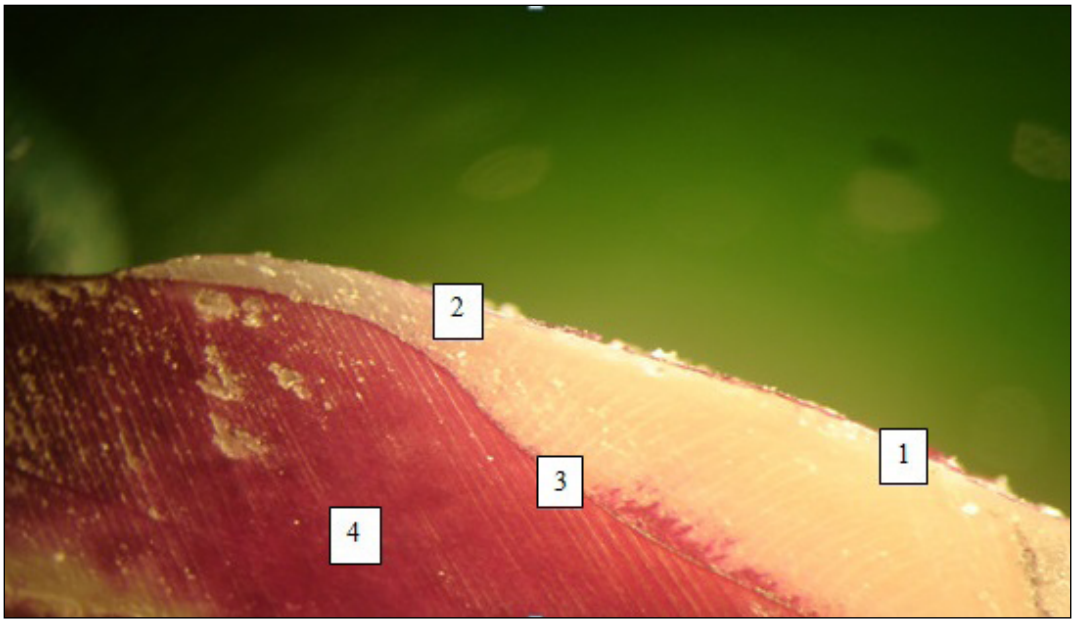

Fig. 1. Changes in tooth enamel after manual scaling of dental plaque: 1 – "eroded" enamel surface; 2 – remnants of dental plaque; 3 – amelo-dentinal junction; 4 – dentin. Staining: PAS-Alcian blue. Magnification: x16.

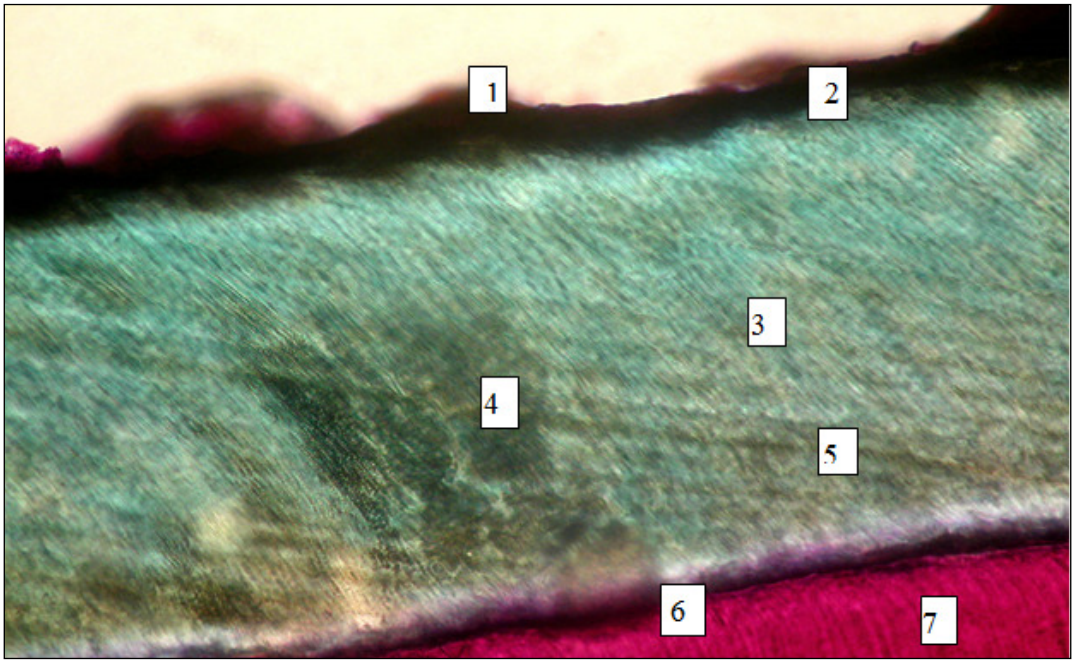

Fig. 2. Remnants of soft dental plaque after manual scaling: 1 – remnants of dental plaque; 2 – cuticle; 3 – preserved bundles of enamel prisms; 4 – partially destroyed bundles of enamel prisms; 5 – Retzius lines; 6 – amelo-dentinal junction with structural disruptions; 7 – dentin. Staining: PAS-Alcian blue. Magnification: x400.

of the dental deposits [8]. However, everyone is convinced of the need to achieve an ideally smooth tooth surface to prevent the recurrence of plaque formation [11]. Most authors believe that achieving a perfectly smooth tooth surface after removing dental deposits is impossible [12]. There is no unanimous opinion regarding the effects of the most commonly used manual, ultrasonic (piezoelectric and magnetostrictive), and sonic instruments on the tooth surface. There are conflicting opinions in the literature about the surface roughness and damaging properties of ultrasonic, sonic and manual scaling. Some researchers believe that ultrasonic scaling is the most sparing method [13], while others consider manual scaling is preferable [14], and the use of sonic scalers is recommended only for long-standing hard dental deposits [13].

Therefore, to date, there is no study in the literature that would allow creating a differentiated approach to selecting a method for removing supragingival dental deposits depending on the nature of dental deposits and structural changes in adjacent tissues, as well as the

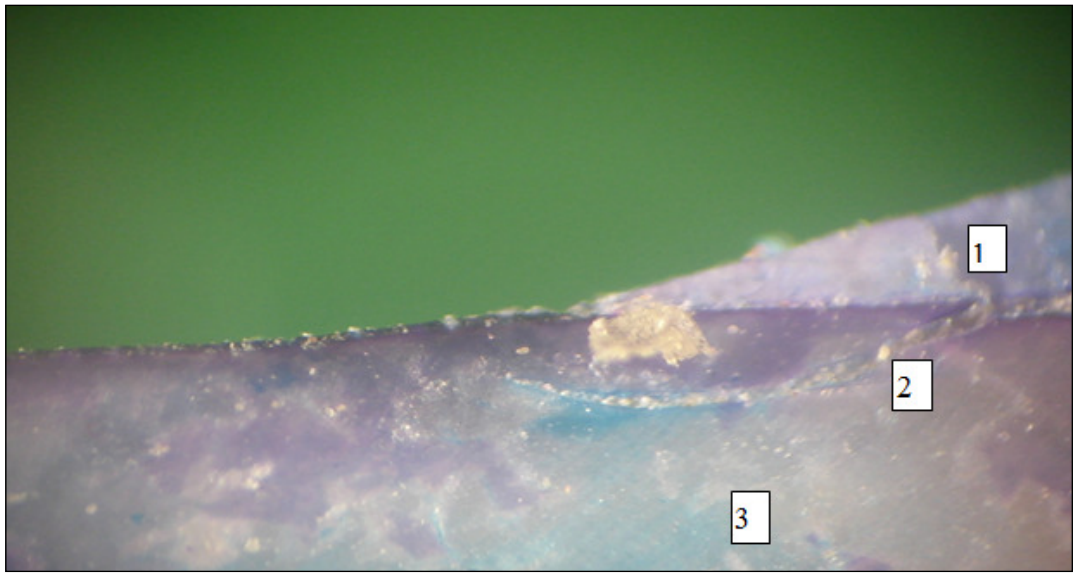

Fig. 3. Remnants of dental calculus in the cervical region of the tooth after manual scaling: 1 – preserved enamel; 2 – enamel invagination into root dentin; 3 – interglobular dentin. Staining: PAS-Alcian blue. Magnification: x16.

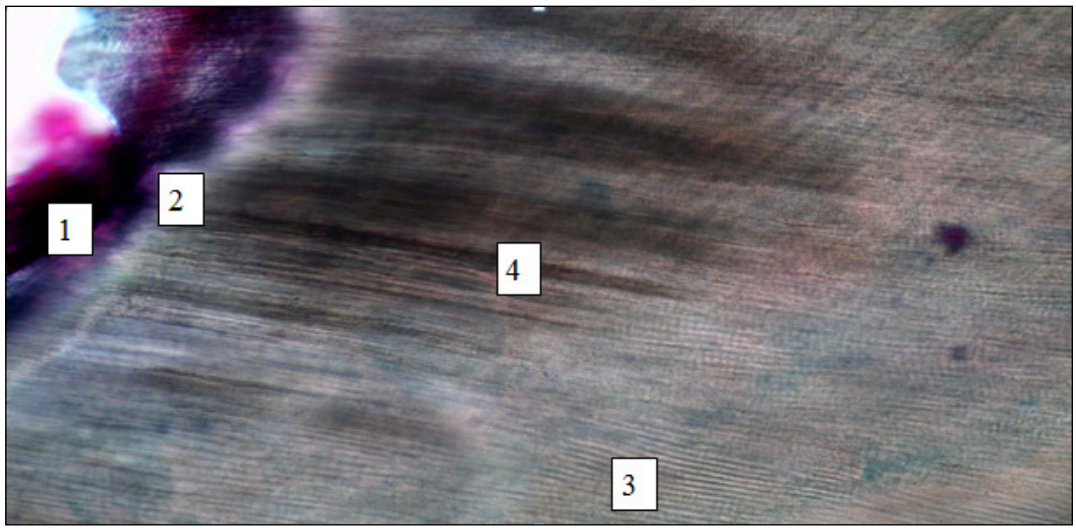

Fig. 4. Microscopic changes in enamel adjacent to dental calculus after manual scaling (equatorial region): 1 – remnants of dental calculus; 2 – the boundary between dental calculus and adjacent enamel; 3 – preserved enamel prisms; 4 – partially or completely destroyed enamel prisms. Staining: PAS-Alcian blue. Magnification: ×400.

effect on the tooth surface of mechanical and physical methods of their removal.

AIM

The aim of this work was to study the structural changes that occur in the hard tissues of the tooth after performing manual, ultrasonic, and sonic methods of removing dental deposits and to determine the most rational method for various supragingival deposits.

MATERIALS AND METHODS

To achieve the study's objective, an examination of 8 patients with various types of supragingival dental deposits was carried out. These patients were scheduled for the removal of 8 teeth due to orthodontic and surgical indications: 3 teeth with dental plaque, 3 with mineralized dental deposits, and 2 with smoker's plaque. In one of three patients with dental plaque, dental deposits were removed using the instrumental method (manual scaling) [15], in the second – with the ultrasonic scaler Cavitron Select SPS (Dentsply, USA), and in the third – with the sonic scaler TopMed TM-AS2000, M4 (China). Similarly, scaling of teeth was performed in patients with mineralized dental deposits. In two patients with smoker's plaque, the removal of supragingival deposits was performed using the ultrasonic and sonic scalers, respectively. Manual scaling of smoker's plaque in patients was

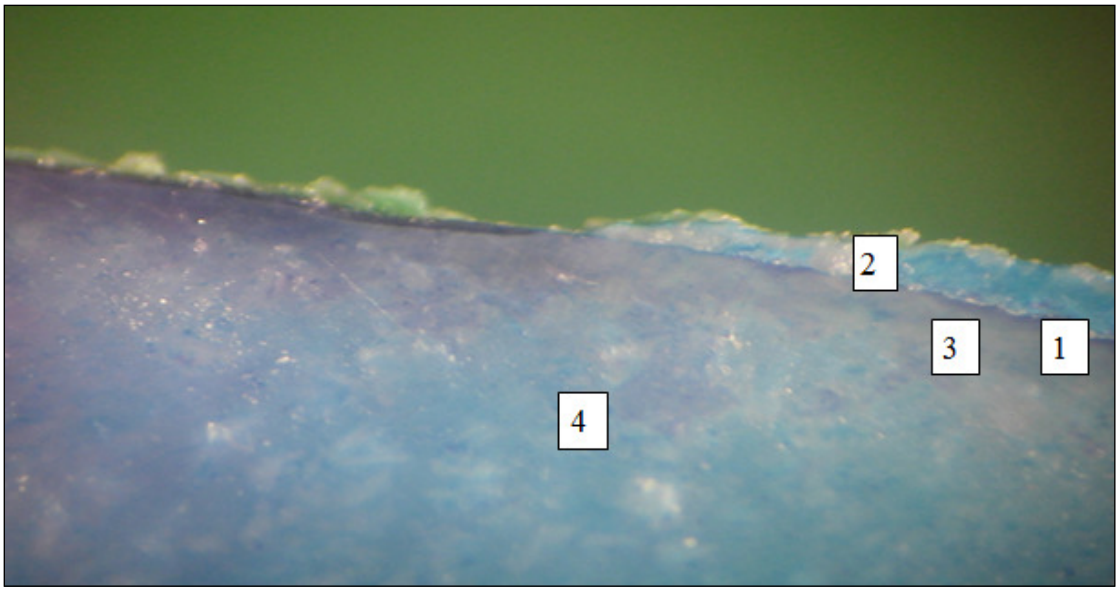

Fig. 5. Condition of hard dental tissues in the cervical area after ultrasonic scaling of soft dental plaque: 1 – amelo-dentinal junction; 2 – "eroded" enamel; 3 – dentin; 4 – interglobular dentin. Staining: PAS-Alcian blue. Magnification: ×16.

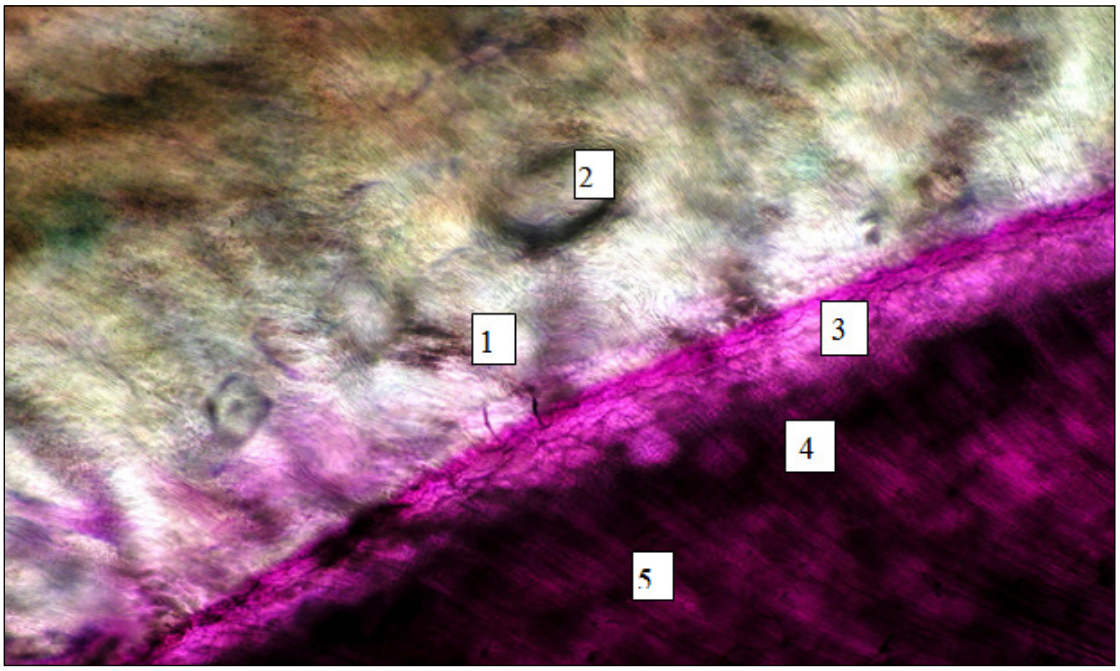

Fig. 6. Condition of hard dental tissues after ultrasonic scaling of supragingival dental calculus: 1 – partial or complete destruction of enamel prism bundles; 2 – granular structures of destroyed enamel; 3 – thickening of the reticular layer; 4 – destroyed layer of odontoblastic terminal processes; 5 – "dead tracts" near the amelo-dentinal junction. Staining: PAS-Alcian blue. Magnification: ×400.

not performed due to the impracticality of using this method in clinical practice. The tooth extraction procedure was carried out the day after the removal of dental deposits.

The extracted teeth were stored in a 10% neutral formalin solution. Morphological studies were carried out according to the previously described methodology [10].

The research was conducted at the Department of Pathological Anatomy and Forensic Medicine of Poltava State Medical University. All research was performed with the voluntary consent of the participants, in compliance with contemporary requirements for scientific research, as outlined in the "Declaration of Helsinki of the World Medical Association" (ethical principles for medical research involving human subjects) – 6th revision (Seoul, 2008). The study was approved by the Ethics and Academic Integrity Commission (Protocol No. 6/27 dated June 28, 2024).

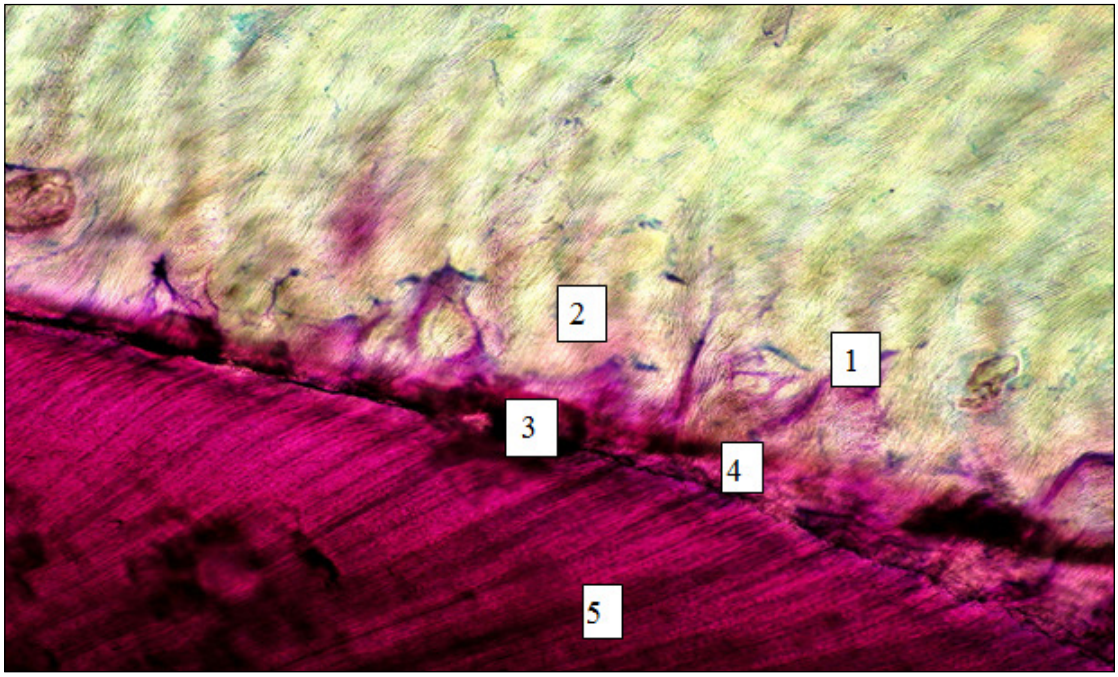

Fig. 7. Condition of hard dental tissues during ultrasonic scaling of smoker's plaque: 1 – destroyed bundles of enamel prisms; 2 – penetration of nicotine pigment into the interprismatic spaces of Hunter-Schreger lines; 3 – fragmentation of the reticular layer; 4 – destruction of the reticular layer with the formation of PAS-positive vegetations; 5 – "dead tracks" in the dentin. Staining: PAS-Alcian blue. Magnification: x400.

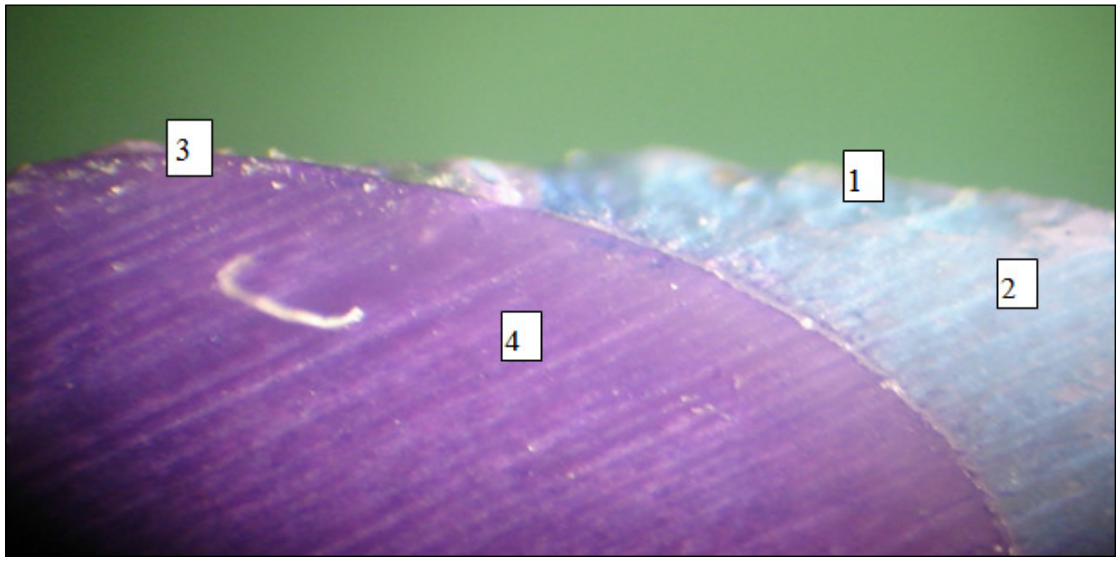

Fig. 8. Condition of hard dental tissues in the cervical area after sonic scaling of dental plaque: 1 – "eroded enamel"; 2 – preserved enamel; 3 – destroyed cementum; 4 – preserved regular dentin. Staining: PAS-Alcian blue. Magnification: x16.

RESULTS

After the removal of dental plaque using a manual method, thick histochemically stained PAS-Alcian blue sections revealed that the enamel surface displays an "eroded" appearance due to the absence of the cuticle. In certain areas near Nasmyth's membrane, represented as a light band, a small amount of PAS-Alcian-positive soft deposits is preserved, which do not penetrate into the enamel's thickness (Fig. 1). In these cases, the enamel is stained in various shades of yellow. The amelo-dentinal junction exhibits a winding course, with single enamel tufts extending from it.

The results of thin-section studies demonstrate the presence of dental plaque remnants across the entire enamel surface as dark pink homogeneous masses (Fig. 2). Alongside preserved bundles of enamel prisms stained blue, well-defined Retzius lines of biomineralization are distinctly visible. In certain areas of the enamel, partial destruction of enamel prism bundles is observed. These prisms are stained a darker shade and are distinctly bordered by lighter lamellae. Near

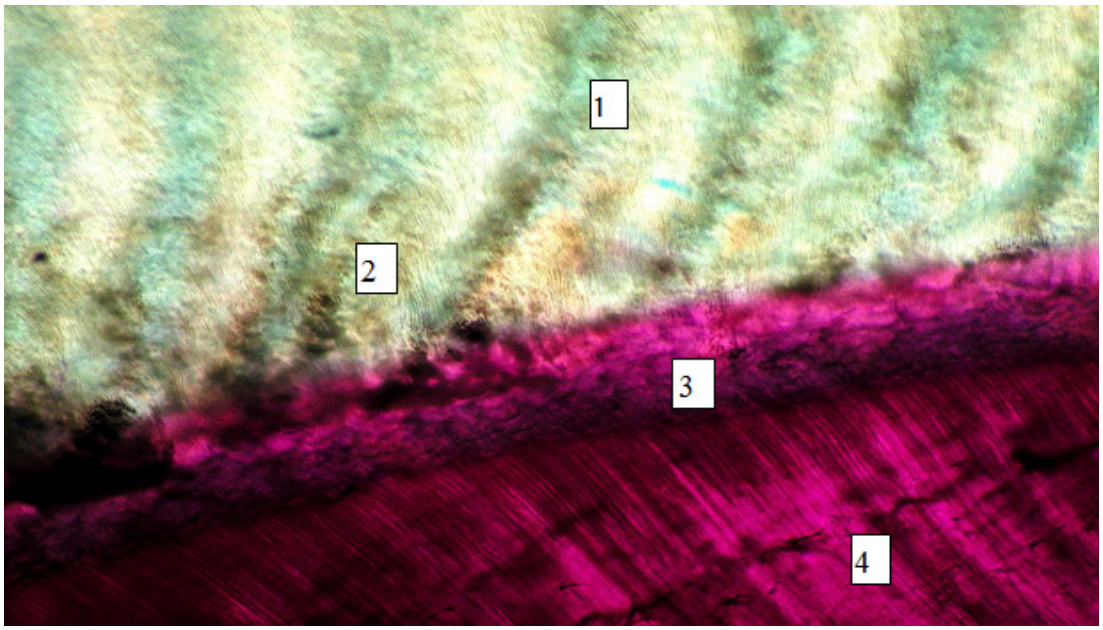

Fig. 9. Condition of hard dental tissues after sonic scaling of mineralized dental deposits: 1 – greenish-blue bands of vertical Hunter-Schreger lines; 2 – yellow-brown bands of vertical Hunter-Schreger lines; 3 – hyperplasia of the reticular layer; 4 – interglobular dentin. Staining: PAS-Alcian blue. Magnification: x400.

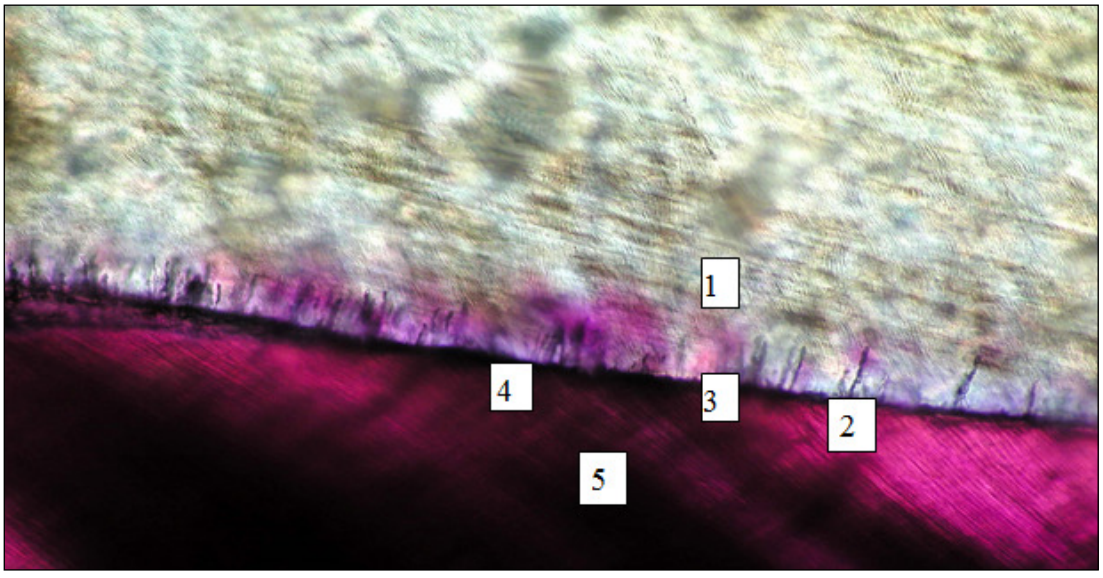

Fig. 10. Condition of hard dental tissues after sonic scaling of smoker's plaque (equatorial part): 1 – enhanced pattern of Retzius lines; 2 – hyperplasia of enamel spindles; 3 – preserved enamel tufts; 4 – reticular layer; 5 – "dead tracks" in the dentin near the amelo-dentinal junction. Staining: PAS-Alcian blue. Magnification: x400.

the damaged enamel prisms, fragmentation of the amelo-dentinal junction and disruption of its structure are noted.

It was established that after manual scaling of supragingival dental calculus in the cervical area, remnants of calculus partially persist (Fig. 3). These remnants exhibit a whitish color due to localized calcification. Preserved areas of enamel invaginate into the dentin and are stained dark purple. The enamel adjacent to the calculus has a triangular shape and is stained light blue. In areas adjoining the calculus, interglobular dentin is present, appearing light lilac against the background of destroyed dentinal tubules.

When examining histochemically stained thin sections, it was found that the calculus remnants exhibit various shades of dark purple (Fig. 4). At its boundary with prismatic enamel, a light homogeneous band can be observed, separating it from the bundles of enamel prisms. The enamel prism bundles display varying colors, ranging from blue to dark gray. The blue color corresponds to intact bundles of enamel prisms, while the dark gray indicates fragmented or entirely destroyed ones.

After ultrasonic scaling of tooth surfaces with non-mineralized dental plaque (dental biofilm), complete removal of deposits is observed. At the same time, the enamel exhibits an "eroded" surface, particularly in the cervical region (Fig. 5). It is noteworthy that two types of altered enamel prism bundles are identified in this area. In the first case, the enamel prism bundles stain blue, with a partially fragmented reticular layer observed. In the second case, the enamel stains light gray, which evidently corresponds to completely destroyed enamel prism bundles. Furthermore, the reticular layer at the amelo-dentinal junction is almost entirely destroyed. The dentin layer adjacent to the enamel stains gray-blue and contains individual layers of interlobular dentin.

After the removal of mineralized debris with an ultrasonic scaler, areas of enamel adjacent to it demonstrated complete removal of supragingival dental deposits. In certain areas, partial or complete destruction of enamel prism bundles was observed, along with small foci of granular structures. In the zones adjacent to the enamel, the reticular layer was thickened and represented by fibrous PAS-positive structures, which separated the enamel from the zone of completely destroyed terminal odontoblast processes. These processes appeared as dark formations extending into the so-called "dead tracts", which, according to Hasiuk A. P. et al. [16], result from fragmentation or complete destruction of odontoblast processes (Fig. 6).

After ultrasonic scaling of smoker's plaque, the enamel exhibits an "eroded" appearance. Significant destructive changes are observed within the enamel structure. Thus, bundles of enamel prisms are stained in various shades ranging from yellow to brown. Individual bundles are demarcated by darker brown stripes and are arranged along the vertical lines of Hunter-Schreger bands, apparently caused by nicotine pigment. The amelo-dentinal junction after ultrasonic scaling shows a wavy course with areas of partial hyperplasia of the reticular layer at the boundary with dentin. At the same time, fragmentation of the reticular layer is noted in the deeper enamel, accompanied by the formation of PAS-positive structures in the thickness of this formation and their vegetation (Fig. 7).

It has been established that when sonic scaling is used to remove dental plaque, the enamel in the cervical area has a pronounced "eroded" surface. Bundles of enamel prisms are partially destroyed and stain in shades ranging from blue to light blue, while the pattern of the amelo-dentinal junction remains intact. However, the layer of cementum adjacent to the enamel is partially or completely destroyed. Partial destruction of the adjacent dentin is also noted, especially in the cervical region of the tooth (Fig. 8).

During microscopic examination of histochemical tooth sections stained with PAS-Alcian blue, after the removal of mineralized dental deposits via ultrasonic scaling, partial destruction of the superficial enamel layers was observed. In areas with preserved enamel, distinct bundles of enamel prisms were noted, appearing as vertical Hunter-Schreger bands. These exhibited either a blue-green or yellow-brown coloration. Ultrasonic scaling revealed thickening of the reticular layer and its hyperplasia due to an increase in PAS-positive fibrous structures within it. Additionally, directly in the zone of regular dentin, which stains red, interglobular dentin bands with a dark cherry color were observed (Fig. 9).

Morphological examination of hard tooth tissues after removal of smoker's plaque using a sonic scaler revealed its absence and a "eroded" enamel surface. The terminal ends of enamel prisms appeared light yellow and brown. In certain areas, the Retzius lines showed intensified patterns, stained in dark color. Preserved enamel tufts and hyperplastic enamel spindles at the interface with the reticular layer also exhibited a dark color, indicating the accumulation of nicotine pigment. In the dentin near the amelo-dentinal junction, "dead tracts" were revealed (Fig. 10).

DISCUSSION

Thus, the analysis of the morphological study results established that after the removal of all types of dental deposits through various scaling methods, the structural changes in enamel, amelo-dentinal junction, and dentin, previously identified by us [10], persist. However, ambiguous changes in hard dental tissues were revealed with different types of scaling of non-mineralized and mineralized dental deposits.

The results of microscopic studies indicate that after manual scaling, dental deposits partially remain on the tooth surface – less so in cases of supragingival calculus and more significantly with dental plaque. This confirms the authors' data [13, 14] regarding the ineffectiveness of manual scaling in patients with soft dental deposits and its advantage in the presence of mineralized deposits.

The established changes in enamel after ultrasonic scaling of different types of dental deposits demonstrate partial or complete destruction of enamel prism bundles. Similar destructive processes were observed at the amelo-dentinal junction and in the superficial layers of dentin. Based on the mechanism of ultrasonic cavitation in biological media [17], it can be argued that the cause of enamel surface damage and destruction of the reticular layer may lie in the differences in micro-

hardness among dental plaque, enamel, and dentin. At the same time, high-frequency microwaves passing through tissues with varying hardness levels may, due to changes in oscillation frequency, cause the rupture of chemical bonds and contribute to defect formation in enamel and degenerative alterations at the amelo-dentinal junction. The ineffectiveness of this scaling method has been confirmed in a few clinical studies [14, 10] and aligns with the results of our microscopic investigations.

Pronounced destructive processes in enamel (damaged surface layers, hyperplasia of enamel spindles) and at the amelo-dentinal junction (thickening of the reticular layer) observed during sonic scaling of non-mineralized and mineralized dental plaque confirmed the clinical observations of the authors [13] regarding the aggressiveness and impracticality of using this method in clinical practice.

Thus, the results of morphological studies suggest that the most rational method for removing mineralized dental deposits is manual scaling, while the removal of non-mineralized plaque and smoker's plaque is best achieved through air-abrasive techniques. The latter method, in our opinion and according to some authors [8, 10, 11], may be the most rational approach; however, this requires further confirmation. Given that destructive changes in dental tissues and deep penetration of nicotine pigment into enamel persist regardless of the type of scaling, there is a need for additional remineralization therapy and teeth whitening using known methods [18].

CONCLUSIONS

1. The conducted morphological studies made it possible to objectively assess the condition of enamel and adjacent tissues, occurring after different methods of supragingival dental calculus removal.
2. It was established that removing non-mineralized dental deposits (dental plaque) and smoker's plaque using sonic, ultrasonic, and manual scaling methods is inefficient. An air-abrasive system may be the method of choice for these types of deposits.
3. Pronounced destructive processes in the enamel (damaged surface layers, hyperplasia of enamel spindles) and the amelo-dentinal junction (thickening of the reticular layer), observed during sonic scaling of non-mineralized and mineralized dental deposits, indicate significant aggressiveness and underscore the impracticality of its use in clinical practice.
4. It has been proven that the most rational method for removing supragingival mineralized dental deposits is manual scaling. Destructive changes in the hard tissues of teeth (partial destruction of surface enamel layers, hyperplasia of the reticular layer with the formation of PAS-positive vegetations) observed (identified) during ultrasonic scaling limit its application in clinical practice.
5. Considering that, regardless of the type of scaling, the enamel surface remains uneven, with destructive changes in the tooth tissues and deep penetration of nicotine pigment, there is a need for mandatory polishing of tooth surfaces as well as additional remineralization therapy and tooth whitening procedures.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Quality of life in patients with chronic slow-transit constipation according to the PAC-QOL scale one year after surgical treatment: comparison with preoperative data and reference values

Ivan M. Leshchyshyn, Leonid Yu. Markulan, Olga I. Okhotska, Yaroslav M. Susak, Pavlo L. Byk
O.O. BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

ABSTRACT

Aim: To assess the impact of surgical treatment on quality of life in patients with chronic slow transit constipation according to the PAC-QOL scale one year after surgery.

Materials and Methods: PAC-QOL scores were studied in 107 patients with chronic slow-transit constipation (main group) before and one year after total colectomy (57), subtotal colectomy (29) and colectomy with low rectal resection (21). 70 patients were included into the reference group. Open surgery was performed in 70 (65.4%) patients, while laparoscopic access - in 37 (34.6%) patients.

Results: Despite the long-term conservative treatment PAC-QOL scores for all subscales significantly exceeded the reference values in all patients before surgery (all $p<0,05$). After surgery PAC-QOL scores decreased to reference values and were statistically lower than preoperative values (all $p<0.01$): physical component - from 2.78±0.52 to 1.01±0.32; psychological component - from 1.90±0.48 to 0.83±0.41; warries and concerns - from 1.99±0.31 to 0.72±0.34; the satisfaction component - from 2.35±0.60 to 0.84±0.47; total PAC-QOL score - from 2.14±0.23 to 0.82±0.35.

Conclusions: Surgical treatment – total or subtotal colectomy in patients with chronic slow-transit constipation resistant to conservative treatment provides a significant reduction of all PAC-QOL scores to reference values and provides full social and functional adaptation.

KEY WORDS: chronic slow-transit constipation, PAC-QOL score, quality of life

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INTRODUCTION

Chronic constipation (CC) is a common condition characterized by the heterogeneity of etiological factors and pathophysiological mechanisms, which can occur across all age groups. Patients with CC typically have a long disease history, often starting in childhood, with a progressive course. Many patients had multiple courses of conservative therapy, the outcomes remain variable and unpredictable. According to studies, the prevalence of CC in general population ranges 3% - 27%. The overall prevalence of constipation was higher in women than in men, increased with age among men, and was inversely related to family income. Female gender, low socioeconomic status, stroke, nervous system disease, anal fissures, fistulae, hemorrhoids, previous anorectal surgery in anamnesis are significantly associated with constipation [1]. The average global prevalence is estimated to be approximately 16%, while among individuals aged 60 to 110 years, may reach 33.5%. With a global prevalence of 15%, chronic constipation is among the most common gastrointestinal conditions diagnosed in outpatient clinics and is a frequent reason for referrals to gastroenterologists and colorectal surgeons in the United States [2].

The annual use of laxatives results in significant financial costs up to millions of dollars. Prevalence rates of CC vary between countries. For instance, in Australia, the prevalence among adults, based on the Rome criteria, reached 24% [3], while in Brazil, previous studies showed a range from 14% to 26% [1, 4-11].

Chronic constipation significantly impacts patients' quality of life, often causing additional issues such as mental health disorders [11-14]. Despite the significance of the problem, contemporary literature lacks sufficient data on the impact of various treatment methods on the quality of life of patients with CC. While total or subtotal colectomy has been proven for slow-transit constipation (STC) treatment, its impact on defecation function and quality of life (QOL) remains insufficiently studied [14].

The SF-36 scale was commonly used to assess the quality of life in patients with CC, [13, 14], although

it is not disease-specific. Disease-specific PAC-QOL (Patient Assessment of Constipation Quality of Life Questionnaire) scale was increasingly advised by some authors [9], [11]. However, there are currently no studies investigating the impact of surgical treatment on the quality of life of patients with slow-transit chronic constipation (STCC) using this scale.

Standardized diagnostic approaches are necessary for effective treatment and evaluation of patients with CC. At present, information regarding the quality of life of such patients in Ukraine is limited. Comprehensive treatment should aim not only to alleviate individual symptoms but also to improve the overall quality of life.

AIM

The aim to assess the impact of surgical treatment on quality of life in patients with chronic slow transit constipation according to the PAC-QOL scale one year after surgery.

MATERIALS AND METHODS

107 patients with CSTC were studied and operated at the surgical department of the Saint Michael Clinical Hospital – clinical base of O. O. Bogomolets National Medical University in the period 2011 — 2023 (group O). 70 patients without CSTC were included into the reference group (group R). The Rome IV criteria were used to diagnose CSTC [15].

INCLUSION CRITERIA

- Age over 18 years.
- CSTC that does not respond or poorly respond to modern conservative treatment methods for at least 6 months.
- Low QoL.
- Consent for surgical treatment.
- Consent to complete a QoL questionnaire.

EXCLUSION CRITERIA

- Age under 18 years.
- Severe comorbidities.
- Patients with mental disorders.
- Pregnancy.
- Oncological diseases.
- Harmful habits.
- Refusal to complete the QoL questionnaire.
- Proctogenic constipation.
- Irritable bowel syndrome and/or constipation of secondary specific etiology (associated with an underlying condition).
- Drug-induced constipation.

The patients in the study groups did not differ in gender, average age, and body mass index. In both groups, women were predominant: 102 (95.3%) in group O and 65 (92.9%) in the group R, p=0.486. The average age was 43.1±13.6 years and 41.5, p=0.436; the body mass index was 22.9±4.5 kg/m^2 and 22.2±2.0 kg/m^2, p=0.227 respectively.

QUALITY OF LIFE ASSESSMENT

The quality of life was evaluated using the disease-specific PAC-QOL questionnaire, developed and validated by Marquis et al. [8] in 2005. The questionnaire includes 28 items grouped into 4 subscales:

- Worries and concerns (11 items),
- Physical discomfort (4 items),
- Psychosocial discomfort (8 items), and
- Satisfaction with treatment (5 items).

Each item is assessed using a 5-point Likert scale ranging from 0 (not at all/never) to 4 (very much/all the time) over the previous 2-week period. A higher score indicates a worse QoL due to constipation. Total PAC-QOL scores and subscale scores were calculated according to the original PAC-QOL documentation for every patient [8]. QoL was assessed before surgery and one year after the surgery.

CSTC was manifested at various ages, with an average onset in patients of 21.5±16.3 years (ranging from 1 year to 67 years) according to the anamnestic data. The duration of the disease before treatment in our clinic was on average 20.7±13.2 years, ranging 5 - 53 years. The delay in bowel movements before clinic consultation was on average 9.4±5.1 days (from 3 days to 30 days). The stool consistency according to the Bristol Stool Form Scale [16] was Type I in 67 (62.6%) patients, Type 2 in 28 (26.2%), Type 3 in 8 (7.5%), Type 4 in 3 (2.8%), and Type 5 in 1 (0.9%). More than a quarter of bowel movements required manual assistance in 60 (56.1%) patients. The sensation of incomplete evacuation was reported by 101 (94.4%) patients, and the sensation of a blockage in the rectum during more than a quarter of bowel movements was reported by 97 (90.7%). Abdominal bloating and pain were registered in 80 (74.8%) patients.

Before visiting the clinic, all patients were continuously undergoing courses of conservative therapy, which gradually became less effective over time. They used a high-fiber diet in 103 (96.3%) cases, pharmacological agents in 107 (100.0%), and cleansing enemas in 92 (85.9%).

In O group 29 (27.1%) patients underwent subtotal colectomy, 57 (53.3%) underwent total colectomy, and 21 (19.6%) underwent colectomy with low rectal resection. Open surgery was performed in 70 (65.4%) patients, while laparoscopic access - in 37 (34.6%) patients.

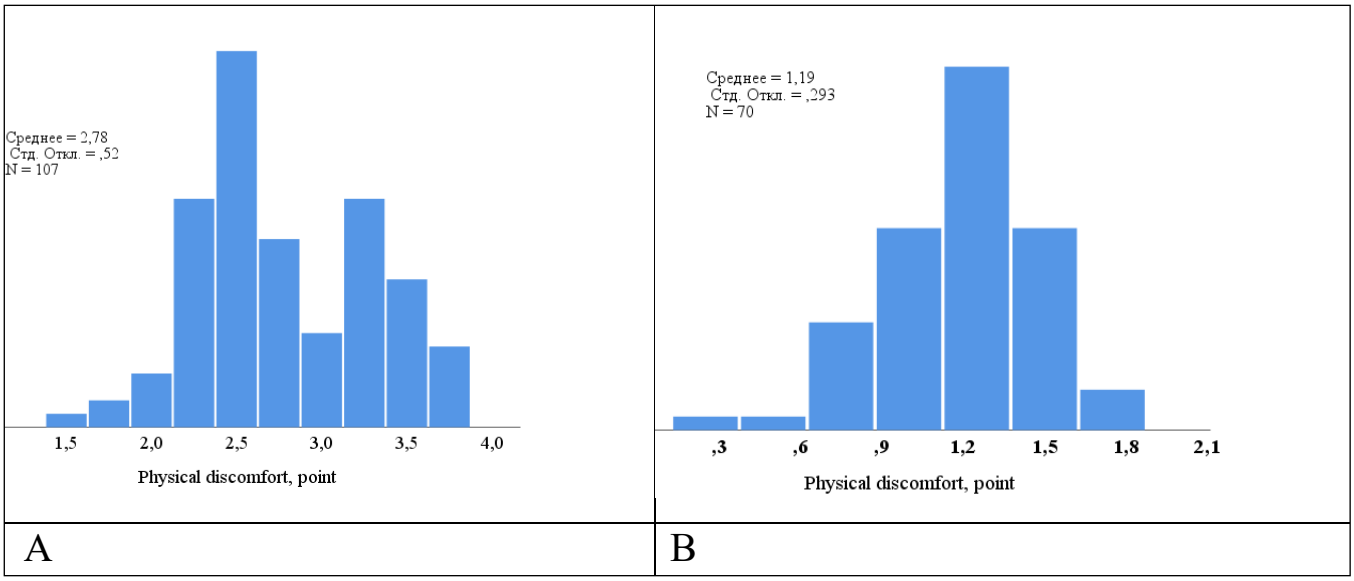

Fig. 1. The distribution of the average score for the Physical discomfort subscale in patients of group O (A) before surgery and in the respondents of group R (B).

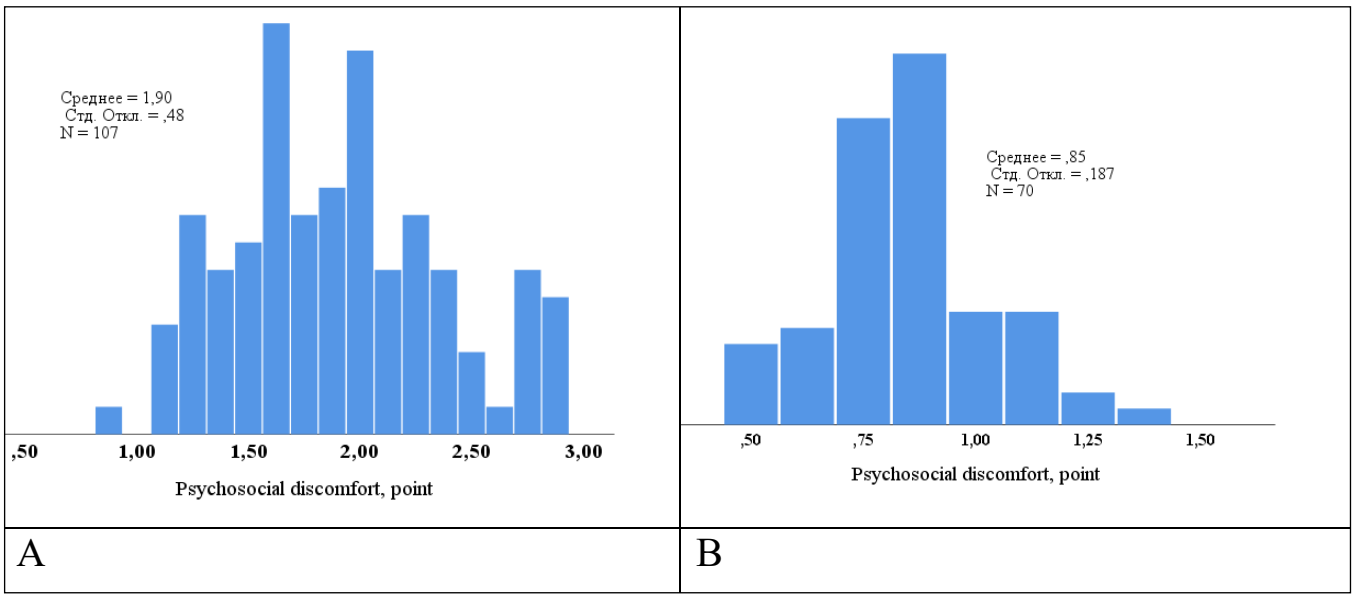

Fig. 2. The distribution of the average score for the Psychosocial discomfort subscale in patients of group O (A) before surgery and in respondents of group R (B).

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM SPSS Statistics, version 22. Descriptive statistics were calculated. Data normality was assessed using the Shapiro– Wilk test. Mean values were presented as M±SD. Categorical data were expressed as counts (%). The Student's t-test was used to compare variables between groups when the data distribution wasn't differ from normal; in other cases, the Wilcoxon-Mann-Whitney test was used. Comparisons of relative frequencies were performed using Pearson's chi-square test. The null hypothesis of equality of variables was rejected at $p<0.05$.

RESULTS

Despite the long history of conservative treatment, patients of group O had unsatisfactory quality of life scores in all PAC-QOL subscales, which significantly exceeded the reference values.

Thus, for the physical discomfort subscale, the average score in group O was 2.78±0.52, ranging 1.5 - 3.78 points (Fig. 1A), while in the reference group, it was 1.19±0.29, ranging from 0.25 to 1.75 points ($p<0.001$), (Fig. 1B).

The average score for the psychosocial discomfort subscale in group O was 1.90±0.52, ranging 0.88 - 2.88 (Fig. 2A), while in the R group, it was 0.85±0.18, ranging (0.5 - 1.38) ($p<0.001$), (Fig. 2B).

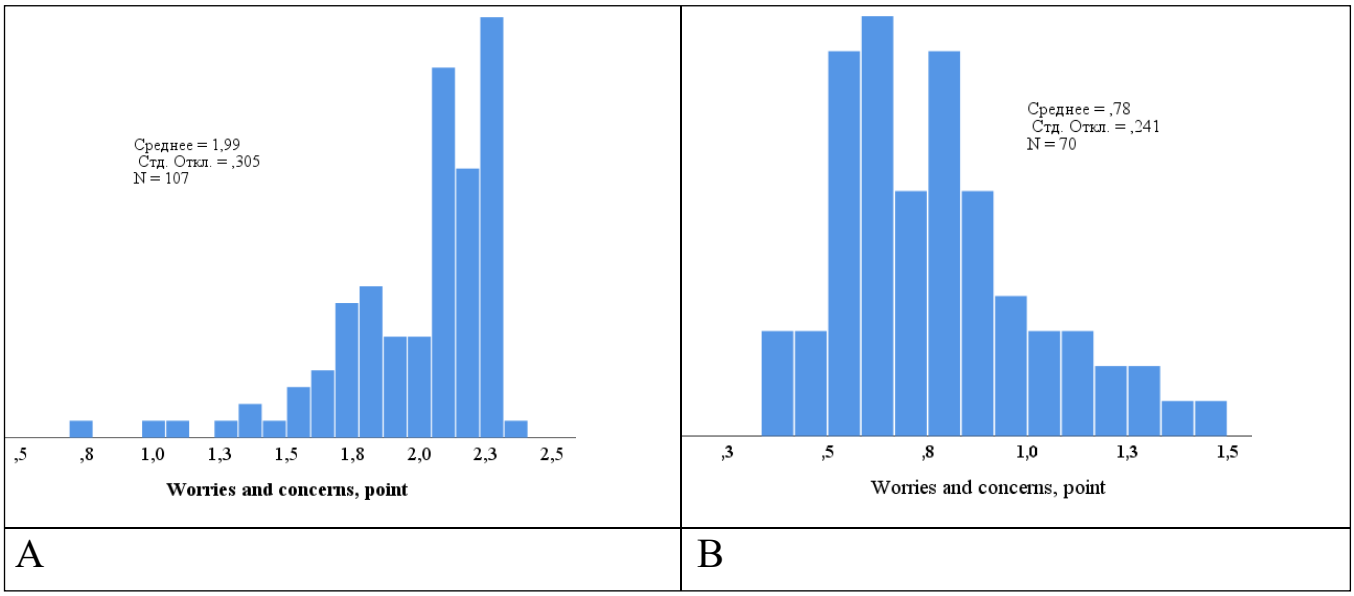

Fig. 3. The distribution of the average score for Worries and concerns subscale in patients of group O (A) before surgery and in group R (B).

Table 1. Postoperative reduction in PAC-QOL scale and subscale scores (in %) after surgical treatment

PAC-QOL scales	Mean	SD	Min	Max
Physical discomfort	63,1	11,2	30,0	81,8
Psychosocial discomfort	56,7	16,0	5,5	78,6
Worries and concerns	64,0	15,8	7,8	82,4
Satisfaction	81,2	7,9	33,3	88,2
PAC-QOL	61,5	14,9	6,2	77,2

According to the worries and concerns subscale, the average score in group O was 1.99±0.31, ranging from 0.73 to 2.36 points (Fig. 3A), while in the R group, it was 0.77±0.24, ranging from 0.36 to 1.45 points ($p<0.001$), (Fig. 3B).

For the satisfaction with treatment subscale, the average score in group O was 2.36±0.58, ranging 0.8 - 3.4 (Fig. 4A), while in the reference group - 0.86±0.28, ranging 0.20 - 1.60 ($p<0.001$), (Fig. 4B).

According to the total PAC-QOL score, the average value in group O was 2.13±0.23, ranged 1.57 - 2.71, fig. 5 A, while in group R it was 0.87±0.12, ranging 0.61 - 1.18 ($p<0.001$), fig. 5 B.

PAC-QOL scale scores were decreased by 56.7% to 81.2% in all patients after surgical treatment, Table 1.

The average scores in all PAC-QOL subscales were significantly lower compared to preoperative values one year after surgery. Specifically, the physical discomfort scores decreased from 2.78±0.52 to 1.01±0.32 ($p<0.01$); the psychological discomfort scores - from 1.90±0.48 to 0.83±0.41 ($p<0.01$); the warries and concerns scores - from 1.99±0.31 to 0.72±0.34 ($p<0.01$); the satisfaction with treatment score decreased from 2.35±0.60 to 0.84±0.47; and the total PAC-QOL score - from 2.14±0.23 to 0.82±0.35.

At the same time, the mean scores of all PAC-QOL subscales in postoperative patients did not statistically differ from those in the reference group (all $p>0.05$), (Fig.6).

DISCUSSION

Surgical treatment of chronic slow-transit constipation (CSTC) remains one of the most important aspects of gastroenterology, requiring a proffessional approach. Patient selection, the choice of optimal intervention methods, and the assessment of long-term outcomes are still subjects of active scientific discussions despite advancements in surgical techniques. The impact of surgery on patients' quality of life is one of the main aspects, it allows to evaluate treatment success by patients themselves.

The obtained results demonstrate a significant impact of CSTC on patients' quality of life, as evidenced by high scores across all subscales of the PAC-QOL scale compared to the reference group. High scores for

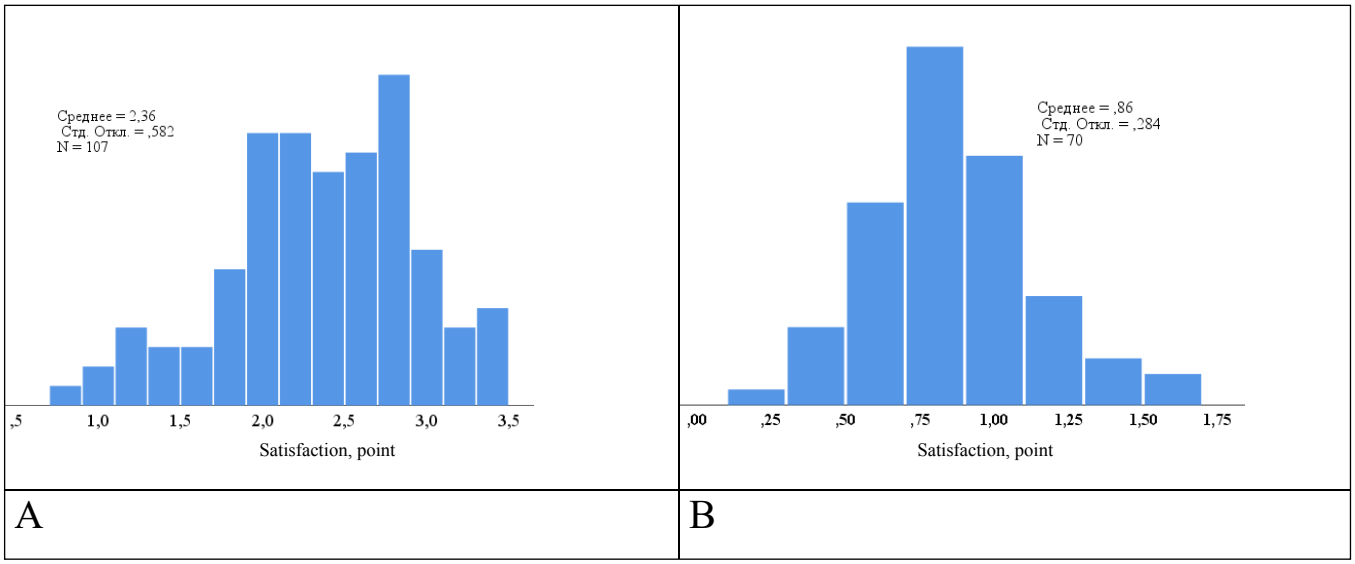

Fig. 4. Distribution of the average score for the Satisfaction subscale in patients of group O (A) before surgery and in the respondents of group R (B).

physical discomfort (2.78±0.52), psychosocial discomfort (1.90±0.52), warries and concerns (1.99±0.31), and satisfaction (2.36±0.58) indicate that CSTC significantly restricts both the physical functioning and social and emotional well-being in patients. Our results align with previous studies showing significantly worse quality-of-life indicators in patients with CSTC than the general population [5, 17-20].

Studies about SF-36 questionnaire reported a significant deterioration in the physical and mental components among patients with chronic constipation [20].

For instance, studies using the PAC-QOL and SF-36 scales showed similar trends in quality-of-life deterioration across various components. A 2015 study analyzed data using PAC-QOL and SF-36 questionnaires in patients with chronic functional constipation and irritable bowel syndrome with constipation (IBS-C) according to the Rome III criteria. The PAC-QOL survey included 43 patients (14% with IBS-C, 37% with functional constipation, and 49% with unclassified constipation), while the SF-36 survey included 93 patients (23% with IBS-C, 27% with functional constipation, and 51% with unclassified constipation).

The SF-36 questionnaire results indicated that patients with irritable bowel syndrome had a lower quality of life compared to those with functional and unclassified constipation. Statistically significant differences were observed between IBS-C patients and those with functional constipation in the fatigue/energy scale in favor of the latter (41.67 ± 3.386 vs. 55.20 ± 4.383, p = 0.0221), as well as in the pain scale between IBS-C patients and those with unclassified constipation (p = 0.0362, 49.64 ± 5.290 vs. 63.62 ± 3.673).

The PAC-QOL questionnaire also showed worse results in patients with IBS-C compared to those with functional constipation for physical component (p = 0.0029; 10.00 ± 1.125 vs. 4.938 ± 0.8086), psychological component (p = 0.0278; 14.33 ± 2.704 vs. 7.438 ± 1.469), warries score (p = 0.0379; 17.33 ± 4.410 vs. 9.375 ± 1.494), satisfaction (p = 0.0180; 16.50 ± 0.4282 vs. 10.31 ± 1.440), and overall PAC-QOL score (p = 0.0034; 2.077 ± 0.2704 vs. 1.146 ± 0.1391) [11].

In our study, significant improvements in quality of life were observed across all components using the disease-specific PAC-QOL scale in patients with CSTC. Unlike the findings described by M.C. Ruiz-López, other authors evaluated the quality of life in 30 patients with slow-transit constipation after colectomy using the Gastrointestinal Quality of Life Index (GIQLI) and SF-36. It was found that GIQLI scores statistically improved (P < 0.05) after surgery during the study period (3 months, 6 months, 1 year, 2 years) as follows: 77.8 ± 17.5 before surgery, 109.7 ± 21.2 in 3 months, 115.0 ± 20.7 in 6 months, 121.3 ± 20.3 in 1 year, and 123.6 ± 17.5 in 2 years. Additionally, SF-36 questionnaire results showed significant improvements in six health domains after colectomy in 3, 6, 12, and 24 months, respectively: Physical role (32.5 ± 46.0 vs. 66.7 ± 44.7, 78.3 ± 38.7, 74.4 ± 37.7, 76.7 ± 38.9). Emotional role (30.0 ± 46.6 vs. 67.8 ± 44.2, 87.8 ± 28.4, 87.8 ± 30.9, 81.6 ± 36.3). Physical pain (59.2 ± 31.8 vs. 72.2 ± 24.7, 77.4 ± 28.7, 83.9 ± 25.6, 88.9 ± 23.5). Vitality (49.0 ± 28.9 vs. 71.7 ± 27.4, 73.5 ± 25.6, 78.0 ± 26.1, 80.9 ± 23.0). Mental health (49.5 ± 26.5 vs. 74.0 ± 25.9, 76.3 ± 26.6, 83.7 ± 25.1, 86.2 ± 21.3). General health (29.4 ± 26.6 vs. 55.6 ± 27.5, 56.6 ± 28.3, 66.6 ± 33.0, 71.1 ± 30.0).

Thus, this study demonstrated that total or subtotal colectomy for slow-transit constipation is not only an effective method for eliminating constipation-associat-

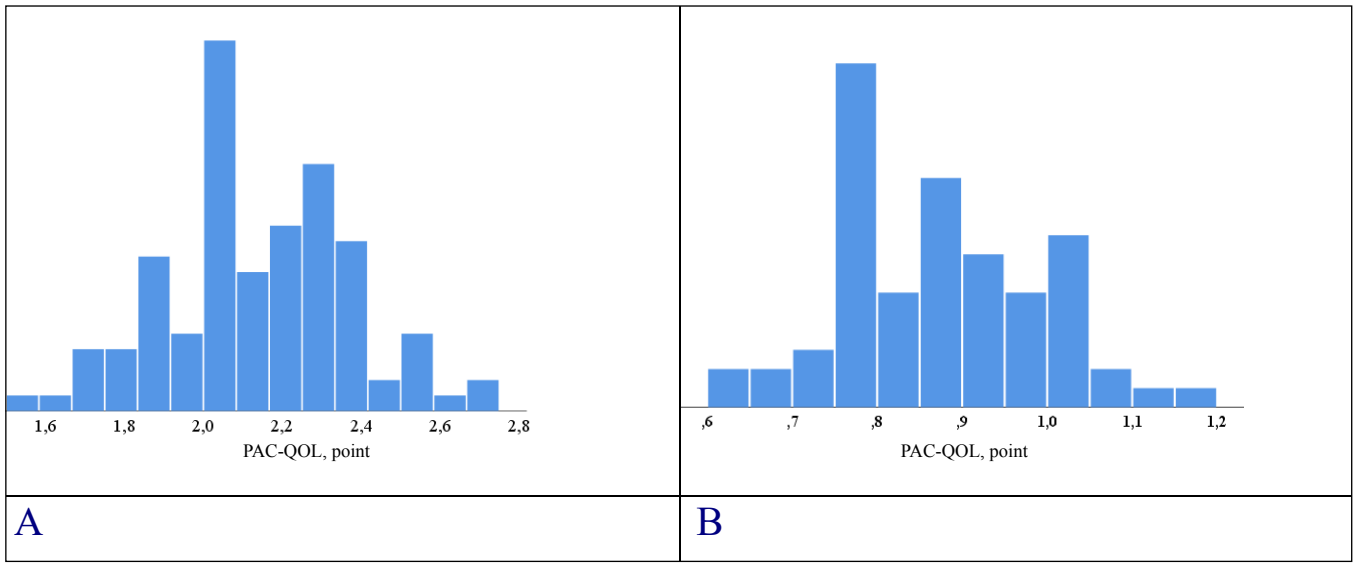

Fig. 5. Total PAC-QOL score distribution in patients of group O (A) before surgery and in the respondents of group R (B).

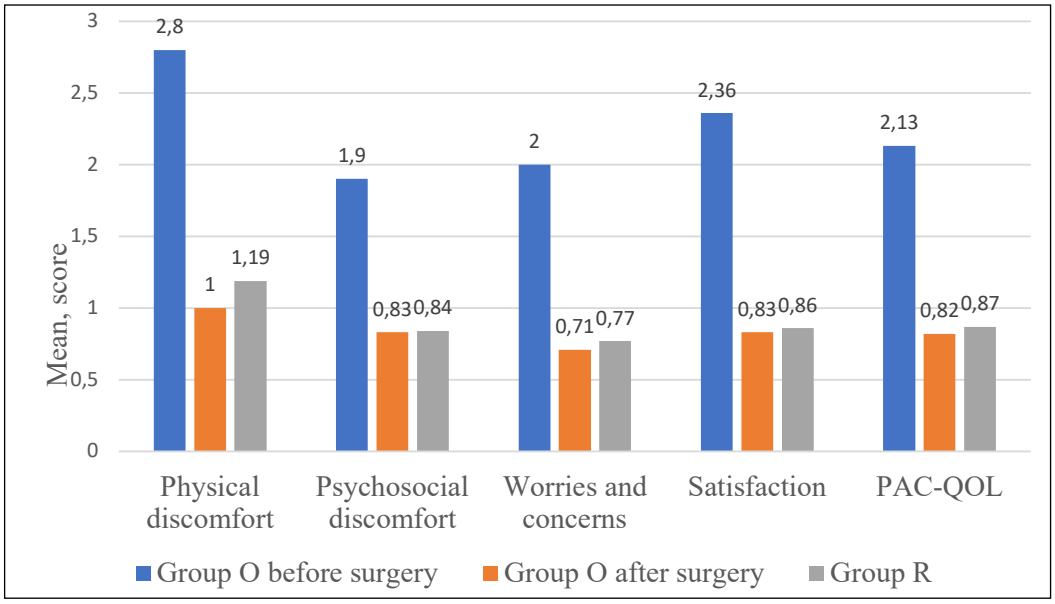

Fig. 6. Average quality of life scores in patients of group O before and after surgery, and in respondents of the reference group.

ed symptoms but also significantly improves patients' quality of life [14].

One of the most debated issues is the criteria for selecting patients for surgical intrusion. Surgery is typically considered as a last-resort treatment when conservative therapy has failed and the patient's quality of life has significantly deteriorated [28]. However, even under these conditions, it is not always clear whether surgery is the best decision for every individual patient. For instance, manometric studies and colon transit time assessments are valuable tools, but they do not guarantee an accurate prediction of surgical success [2, 21, 22]. The choice of surgical procedure is also complex. The most common surgery techniques are total colectomy with ileorectal anastomosis and subtotal colectomy [19]. Total colectomy is effective for most patients with severe CSTC but carries risks of complications such as chronic diarrhea or pelvic organ dysfunction [2, 24]. Subtotal colectomy with ileorectal anastomosis is considered the standard surgical procedure for CSTC. When strict patient selection criteria are applied, the overall success rate exceeds 80% [2, 25]. Although subtotal colectomy is less invasive, it can still be associated with complications. According to some authors, a high incidence of small bowel obstruction was observed, leading to impaired peristalsis in some cases. Eight patients required conversion to an ileostomy. In long-term follow-up, 15 patients experienced worsening fecal incontinence ($p < 0.01$), stool consistency became softer ($p < 0.01$), and

stool frequency decreased ($p < 0.01$) [26]. These factors highlight the necessity of an individualized approach when selecting a surgical method. For example, studies using the Wexner scale and GIQLI scores showed similar trends of poorer quality-of-life scores before treatment. The clinical effectiveness of laparoscopic total colectomy with ileorectal anastomosis and laparoscopic subtotal colectomy with an antiperistaltic cecorectal anastomosis in adults with slow-transit constipation has been demonstrated. These methods were found to significantly improve patients' quality of life in the long-term postoperative period [24].

The issue of long-term surgical effectiveness remains a topic of debate. While many studies show significant improvements in the physical and psychosocial components of quality of life following surgery, some patients develop new problems, such as fecal incontinence or social isolation due to side effects [27, 28]. These findings highlight the necessity for a comprehensive approach to evaluate treatment outcomes, considering both objective clinical indicators and the patient's subjective experience. Alternative methods, such as biofeedback or intestinal electrostimulation, are also gaining interest as potential ways to avoid radical surgery. However, current data on their effectiveness in patients with severe CSTC remain limited, necessitating further research to define their role in managing these patients [29].

The postoperative reduction in PAC-QOL scale scores by 56.7–81.2% confirms the high effectiveness of surgical treatment in improving the quality of life for patients with CSTC. The most significant changes were observed in the physical component (decreasing from 2.78±0.52 to 1.01±0.32; $p<0.01$) and the warries and concerns component (decreasing from 1.99±0.31 to 0.72±0.34; $p<0.01$). These findings align with those of other authors [14], where substantial improvements in physical and emotional parameters after total or subtotal colectomy were also registered.

Interestingly, one year after surgery, the average scores across all PAC-QOL subscales did not differ from those in the reference group ($p>0.05$). This suggests that surgery not only significantly reduces symptoms but also restores quality of life to a level comparable to healthy individuals. However, even when these improvements are achieved, some patients may remain dissatisfied due to minor but impactful postoperative issues, such as changes in bowel habits. Despite the clear benefits of surgical treatment, it is not without disadvantages. Some patients experience postoperative complications such as diarrhea, fecal incontinence, or electrolyte imbalances. Additionally, CSTC patients tend to have high levels of anxiety, which may affect their adaptation in postoperative period. Patient selection for surgical treatment remains debatable, particularly regarding the necessity to standardize approaches for determining the severity of CSTC and the optimal timing for intrusion. In our study, patients with a long CSTC history had significantly worse initial quality-of-life scores, which may have influenced treatment outcomes. Overall, surgical treatment of CSTC is an important tool for managing patients with refractory constipation, but it presents with significant challenges that require careful analysis. The impact of surgery on patients' quality of life should be a key factor in determining treatment strategies. Future research should focus on establishing optimal selection criteria, improving surgical techniques, and evaluating long-term outcomes.

CONCLUSIONS

Surgical treatment – total or subtotal colectomy in patients with chronic slow-transit constipation resistant to conservative treatment provides a significant reduction of all PAC-QOL scores to reference values and provides full social and functional adaptation.

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The work performed in accordance with the research work plan at the surgery chair with the emergency and vascular surgery course at the O.O. Bogomolets National Medical University.

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Beyond traditional lipid markers: why lipoprotein(a) screening matters

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ABSTRACT

Aim: To assess the correlation between lipoprotein(a) levels and traditional lipid profile markers in statin-naive men and women without established atherosclerotic cardiovascular disease.

Materials and Methods: Sixty-seven statin-naive adult patients without a prior history of established atherosclerotic cardiovascular disease were included in the study. Lipoprotein(a) levels were determined using nephelometry in all patients.

Results: According to the results of the correlation analysis, it was found that there is no statistically significant correlation between lipoprotein(a) level and traditional parametres of lipid profile in both groups ($p>0.05$). Reliable direct correlation of moderate strength was observed between lipoprotein(a) and age in the group A ($R=0.46$, $p=0.04$).

Conclusions: Elevated lipoprotein(a) levels, independent of other lipid profile parameters, can significantly contribute to cardiovascular risk, emphasizing the importance of routine lipoprotein(a) screening in clinical practice. It is particularly noteworthy that lipoprotein(a) concentrations tend to increase after menopause, potentially placing postmenopausal women at an elevated risk for cardiovascular events. Consequently, it is imperative to monitor lipoprotein(a) levels in females, especially during the peri-menopausal and postmenopausal stages, to more accurately assess and manage cardiovascular risk in this population.

KEY WORDS: Lipoprotein(a), atherosclerotic cardiovascular disease, lipid profile

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INTRODUCTION

Lipoprotein(a) (Lp(a)) has garnered increasing attention in the medical community due to its potential role as a significant biomarker and therapeutic target in cardiovascular and renal diseases. Recent studies have provided substantial evidence linking elevated Lp(a) levels with various health conditions, including calcific aortic valve stenosis (CAVS), chronic kidney disease (CKD), and atrial fibrillation (AF). The findings from several recent studies suggest that Lp(a) may be a modifiable risk factor in these diseases, opening new avenues for prevention and treatment strategies.

A recent systematic review and data analysis examined the relationship between elevated Lp(a) levels and the progression of CAVS. The study revealed a significant association between higher Lp(a) concentrations and accelerated CAVS progression, suggesting the potential for targeting Lp(a) as part of therapeutic strategies for managing this condition. As CAVS continues to rise in prevalence, understanding the underlying mechanisms may provide new insights into its treatment and management [1-3].

Lp(a) is a large macromolecular complex composed of an low-density lipoproteins (LDL) particle containing apolipoprotein B-100 (apoB-100) and a large, highly variable glycoprotein known as apolipoprotein(a) (apo(a)), which is produced by the liver. Apo(a) contains kringle domains, triple-loop structures, which play a crucial role in the particle's structure. A disulfide bond links one of the kringle domains in apo(a) to apoB-100, forming the Lp(a) complex. Lp(a)'s plasma concentration is highly variable, with significant differences between individuals, populations, and even ethnic groups. Lp(a) concentrations range from less than 0.1 mg/dl to over 200 mg/dl, with levels in individuals of African descent being 2–3 times higher than those in Asian and European populations [4,5]. Lipoprotein(a)'s concentration is largely genetically determined, and it

is believed to have atherogenic, proinflammatory, and prothrombotic properties [6].

In renal health research, several studies have explored the link between Lp(a) levels and kidney disease. A Mendelian randomization study investigated the causal relationship between elevated Lp(a) levels and CKD, utilizing genetic variants associated with Lp(a). Analysis of data from large population cohorts showed that higher genetically determined Lp(a) levels were linked to an increased risk of CKD, supporting the notion that Lp(a) may be a causal factor in kidney disease and highlighting its potential as a modifiable risk factor for CKD prevention and treatment.

Another study analyzed the relationship between Lp(a) levels, renal function indicators, and CKD risk in a large cohort of 329,415 participants. With a median follow-up of 12.5 years, it found that elevated Lp(a) levels were associated with a 32% increased risk of CKD, particularly in individuals with high-normal urine albumin-to-creatinine ratio (UACR). These findings underscore the importance of considering both Lp(a) and UACR when assessing CKD risk, offering valuable insights for early detection and prevention strategies [7, 8].

In the cardiovascular field, research explored the role of Lp(a) as a risk factor for cardiovascular events in both diabetic and non-diabetic populations. Analysis of clinical records indicated that elevated Lp(a) levels were independently linked to an increased risk of cardiovascular events in both groups, with a stronger association seen in individuals without diabetes. This highlights the importance of monitoring Lp(a) levels in non-diabetic individuals for early cardiovascular risk assessment and intervention [9].

The potential link between elevated Lp(a) levels and atrial fibrillation (AF) was also explored through a systematic review and meta-analysis of Mendelian randomization studies. The findings revealed a significant association between higher genetically determined Lp(a) concentrations and an increased risk of AF, suggesting a causal relationship. This emphasizes the need to consider Lp(a) in cardiovascular health, particularly in the prevention and management of arrhythmias like AF [10, 11].

Together, these studies contribute to a growing body of evidence supporting the role of Lp(a) as a crucial biomarker and potential therapeutic target in both cardiovascular and renal diseases. Elevated Lp(a) levels are associated with increased risks of CAVS, CKD, cardiovascular events, and AF, underscoring the importance of including Lp(a) in routine clinical assessments. Future research focused on the mechanisms behind these associations could lead to more effective prevention and treatment strategies, ultimately improving patient outcomes.

AIM

To assess the correlation between lipoprotein(a) levels and traditional lipid profile markers in statin-naive men and women without established atherosclerotic cardiovascular disease (ASCVD).

MATERIALS AND METHODS

Sixty-seven statin-naive adult patients without a prior established atherosclerotic cardiovascular disease were included in the study: group A – females (n=34), group B – males (n=33). The study groups did not differ statistically in age. Among the examined patients, 50.7% (34/67) were women, while 49.3% (33/67) were men. The average age of the patients of group A was 48.06±13.67 and the patients of group B – 42.12±6.25 years. Exclusion criteria were established atherosclerotic cardiovascular disease, organic heart pathology, arrhythmias, familial hypercholesterolemia and pregnancy. Peripheral blood was collected from each participant via venipuncture. Lipoprotein(a) levels were determined using nephelometry, a technique that measures the concentration of particles in a sample by detecting the scattering of light. In this method, a sample containing lipoprotein(a) is mixed with specific antibodies that bind to the lipoprotein particles. When light passes through the sample, the scattered light is detected by a photodetector. The intensity of the scattered light correlates with the concentration of lipoprotein(a) in the sample, allowing for quantitative measurement. This technique is highly sensitive and specific, providing accurate results for lipoprotein(a) determination [12].

The results were statistically analyzed using Office Excel 2010 and the Statsoft Statistica 12.0 software on a personal computer. A discrepancy was deemed significant if the probability value was 95% or greater ($p<0.05$). Variational statistics were employed to analyze the data, with average values and standard error (M±m) taken into account. The analysis of the relationship between two features in the presence of a normal distribution of data was carried out according to the data of the Pearson correlation coefficient (r), in the case of a distribution different from the normal - the nonparametric Spearman rank correlation coefficient (R) was calculated. The correlation coefficient was evaluated according to the criteria generally accepted in statistics: $r<0.3$ - weak connection; 0.3-0.49 - moderate; 0.5-0.69 - significant; 0.7-0.89 - strong; >0.9 is very strong, close to a functional relationship [13].

RESULTS

Despite the study group consisting of patients aged 25 to 72 years with no prior history of atherosclerotic cardiovascular disease, the average total cholesterol levels in groups

Table 1. Parameters of lipid profile and age of examined patients (M ± m)

Parameters	Group A (n=34)	Group B (n=33)	p
Age, years	48.06±13.67	42.12±6.25	p=0.06
Total cholesterol, mmol/l	6.74±1.70	6.20±1.53	p=0.28
HDL, mmol/l	1.69±0.31	1.34±0.77	p=0.06
LDL, mmol/l	4.23±1.44	3.85±1.35	p=0.34
VLDL, mmol/l	0.62±0.56	0.78±0.63	p=0.47
Triglycerides, mmol/l	1.22±0.61	1.90±1.56	p=0.39
Lipoprotein(a), mg/dl	46.85±47.20	29.78±42.99	p=0.04*

p – reliability of correlation; * - statistically reliable correlation.

Table 2. Correlation between lipoprotein(a), indicators of lipid profile and age of examined patients

Parameters	Group A (n=34)		Group B (n=33)	
	Spearman R	p	Spearman R	p
Age, years	0,46	0,04*	0,35	0,08
Total cholesterol, mmol/l	0,01	0,97	0,08	0,71
HDL, mmol/l	0,27	0,15	0,17	0,46
LDL, mmol/l	-0,11	0,64	0,29	0,19
VLDL, mmol/l	0,09	0,68	-0,12	0,61
Triglycerides, mmol/l	-0,01	0,96	0,04	0,86

p – reliability of correlation; R-correlation coefficient; * - statistically reliable correlation.

A and B were (6.74±1.70) mmol/l and (6.20±1.53) mmol/l, respectively, suggesting the presence of hyperlipidemia. Regarding HDL levels, the average value in females was (1.69±0.31) mmol/l, whereas in males it was lower at (1.34±0.77) mmol/l, though no statistically significant difference was observed. LDL levels were elevated in both groups, with average values of (4.23±1.44) mmol/l in group A and (3.85±1.35) mmol/l in group B. The triglyceride level was somewhat higher in men, averaging (1.90±1.56) mmol/l, compared to (1.22±0.61) mmol/l in women. No statistically significant differences were found in the traditional lipid profile parameters between groups A and B (p>0.05). This suggests that despite variations in lipid levels, the two groups had comparable lipid profiles overall. Regarding the average lipoprotein(a) levels, a statistically significant difference was observed between groups A and B. In females, the average lipoprotein(a) level was higher, reaching (46.85±47.20) mg/dl, while in males, it was lower, with an average of (29.78±42.99) mg/dl (Table 1). This difference suggests a potential gender-related variation in lipoprotein(a) concentrations, which could have implications for cardiovascular risk assessment and treatment strategies.

According to the results of the correlation analysis, it was found that there is no statistically significant correlation between lipoprotein(a) level and traditional parametres of lipid profile in both groups (p>0.05) (Table 2). Reliable direct correlation of moderate strength was observed between lipoprotein(a) and age in the group A (R=0.46, p=0.04).

The results of the correlation analysis revealed that there was no statistically significant correlation between lipoprotein(a) levels and traditional lipid profile parameters in both groups (p>0.05) (Table 2). However, Reliable direct correlation of moderate strength was observed between lipoprotein(a) and age in group A (R=0.46, p=0.04). This finding suggests that while lipoprotein(a) is generally considered genetically determined, it appears that in women, lipoprotein(a) levels may increase with age. This highlights the potential role of aging in influencing lipoprotein(a) concentrations, which could have significant implications for cardiovascular risk assessment, particularly in postmenopausal women, who may experience an increase cardiovascular risk due to hormonal changes.

DISCUSSION

Lipoprotein(a) is a genetically determined lipoprotein that has been identified as an independent risk factor for cardiovascular disease. Elevated levels of Lp(a) are closely associated with an increased risk of atherosclerotic cardiovascular diseases, including heart attacks and strokes, making Lp(a) a crucial biomarker for assessing cardiovascular risk. Genetic factors predominantly influence Lp(a) concentrations, with approximately 70% to ≥90% of interindividual variability attributed to genetic determinants. Notably, Lp(a) levels remain relatively constant throughout an individual's life and are not

significantly affected by lifestyle factors or conventional lipid-lowering therapies.

It is important to measure Lp(a) levels in individuals with a personal or family history of premature ASCVD. Lp(a) levels can be elevated independently of other lipid parameters, such as total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides. This characteristic makes Lp(a) a unique cardiovascular risk factor that can remain hidden unless specifically tested for, even when other lipid markers are within normal ranges. Recognizing elevated Lp(a) levels can aid in identifying individuals at increased risk for ASCVD, facilitating early interventions and personalized treatment strategies [14-16].

Anagnostis, P. et al. suggest in their study that menopause can influence Lp(a) concentrations in women, potentially contributing to their increased cardiovascular risk. They examined the impact of menopause on Lp(a) levels, finding that the transition to menopause is associated with an increased cardiovascular risk, primarily attributed to atherogenic dyslipidemia. However, the study did not establish a clear conclusion regarding the specific effect of menopause on Lp(a) levels, leaving this aspect of the relationship unclear [17].

In contrast, a study by Aljawini, N. et al. explored the relationship between age, menopause, and Lp(a) levels in Saudi women. The findings revealed that Lp(a) concentrations increased significantly after the age of 50, with postmenopausal women exhibiting markedly higher levels than their premenopausal counterparts. This suggests that menopause could be a contributing factor to the elevation of Lp(a) levels in this population, pointing to a potential link between hormonal changes and lipid metabolism during menopause [18].

Additionally, Simony, S. B. et al. examined sex differences in Lp(a) levels and their association with cardiovascular risk. The study found that plasma Lp(a) levels increased with age, with a notable rise around age 50 in women. Postmenopausal women exhibited Lp(a) levels that were 22% higher compared to premenopausal women, underscoring the significant increase in Lp(a) concentrations after menopause [19].

Taken together, these studies suggest that menopause may be associated with increased Lp(a) levels, contributing to the heightened cardiovascular risk observed in postmenopausal women. However, further research is needed to better understand the mechanisms underlying this association and its clinical implications for cardiovascular risk assessment and management in this population.

Understanding the impact of menopause on Lp(a) levels could ultimately guide more precise cardiovascular risk stratification and personalized interventions for postmenopausal women.

CONCLUSIONS

While traditional lipid profile parameters are valuable in assessing cardiovascular risk, they do not encompass the full spectrum of lipid-related risk factors. Elevated Lp(a) levels, independent of other lipid profile parameters, can significantly contribute to cardiovascular risk, emphasizing the importance of routine Lp(a) screening in clinical practice. It is particularly noteworthy that Lp(a) concentrations tend to increase after menopause, potentially placing postmenopausal women at an elevated risk for cardiovascular events. Consequently, it is imperative to monitor Lp(a) levels in females, especially during the peri-menopausal and postmenopausal stages, to more accurately assess and manage cardiovascular risk in this population.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Pathomorphological characteristics of the supravaginal part of the cervix depending on the echogenicity ratios of the cervix to the uterine body

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ABSTRACT

Aim: To analyze the morphological features of the supravaginal part of the cervix depending on the echogenicity ratios of the cervix to the body of the uterus.
Materials and Methods: In 87 reproductive-age patients (30–40 years) with uterine leiomyoma (>14 weeks gestation), morphological features of the supravaginal cervix were analyzed in 23 hysterectomy specimens based on echogenicity ratios: Group I (n = 10): Cervical echogenicity > uterine body. Group II (n = 8): Cervical echogenicity = uterine body. Group III (n = 5): Cervical echogenicity < uterine body.
Results: Histological analysis revealed that increased cervical echogenicity corresponded to a predominance of collagen fibers over smooth muscle bundles. Conversely, when cervical echogenicity was equal to or lower than the uterine body, smooth muscle bundles dominated. These specimens also exhibited destructive changes, connective tissue disorganization, and dystrophic alterations, which are pathognomonic signs of potential lower uterine segment failure during pregnancy.
Conclusions: 1. Comparative studies show that in cases of excess echogenicity of the cervix over the body of the uterus, pathomorphological changes in the supravaginal part of the cervix were not detected. 2. Equal or reduced cervical echogenicity was associated with connective tissue disorganization and dystrophic changes in smooth muscle, indicating structural inferiority. 3. A change in the ratio of echogenicity of the cervix to the body, which is closely related to the morphological structure of the isthmus of the uterus, can serve as one of the criteria for predicting the failure of the lower segment of the uterus in women.

KEY WORDS: Echogenicity, cervix, uterine body

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INTRODUCTION

The increasing frequency of surgical interventions in the lower segment requires improvement of criteria for assessing the morphofunctional state of this anatomical structure even before pregnancy [1-4].

However, only a few scientific works have been devoted to the study of the morphostructure of the lower uterine segment in comparison with the echogenicity of the cervix and uterine body [5].

At the same time, it is known that one of the main contributing factors to inferiority of the lower uterine segment is pathomorphological changes in the area of the lower edge of the uterine body and isthmus, especially after cesarean section [6, 7].

Some authors have established [8] that in conditions of hypo- or hypercollagenosis , the structure of connective tissue may change, becoming more or less elastic in its structure, which affects its elasticity and echo signal reflectivity during ultrasound scanning [9].

Therefore, there are many outstanding issues in early diagnosis of changes in the structural elements of the isthmus of the uterus, which does not allow to improve the prediction of lower uterine segment insufficiency and improve measures to prevent scar incompetence, especially after cesarean section.

AIM

To analyze the morphological features of the supravaginal part of the cervix depending on the echogenicity ratios of the cervix and the body of the uterus.

MATERIALS AND METHODS

Sonographic determination of echogenicity ratios of the tissues of the cervix and uterine body before surgery and pathomorphological examination of the removed uterus and its isthmus after hysterectomy in women of reproductive age (30-40 years) who were diagnosed with uterine leiomyoma > 14 weeks of gestation. According to the ratio of echogenicity of the cervix to the body, three representative study groups were identified in terms of age and uterine pathology:

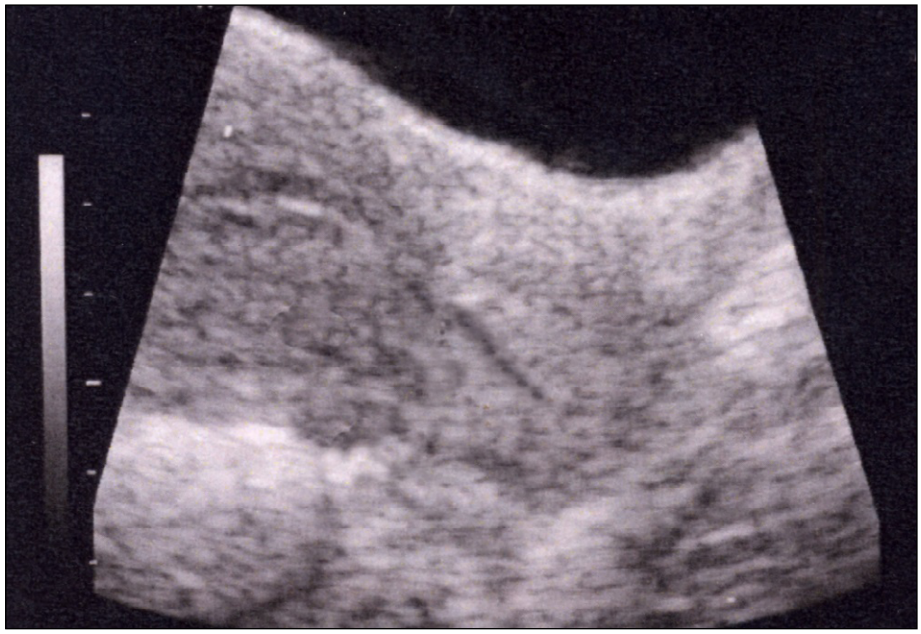

Fig. 1. Echogram of the uterus. The echogenicity of the cervix is higher than the echogenicity of the body of the uterus.

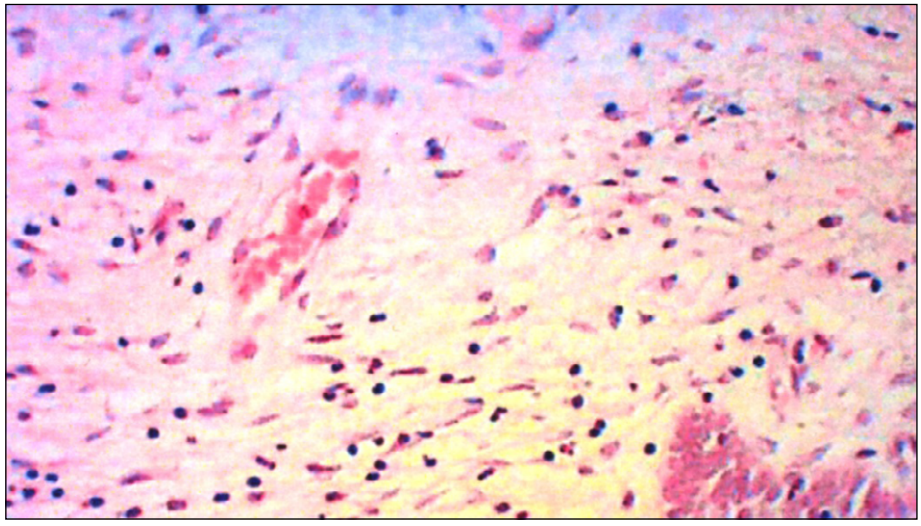

Fig. 2. Biopsy sample supravaginal part of the cervix (isthmus). *Hematoxylin and eosin staining. Predominance of connective tissue (collagen fibers) over smooth muscle bundles. Enlargment: oculus 10, lens 20.

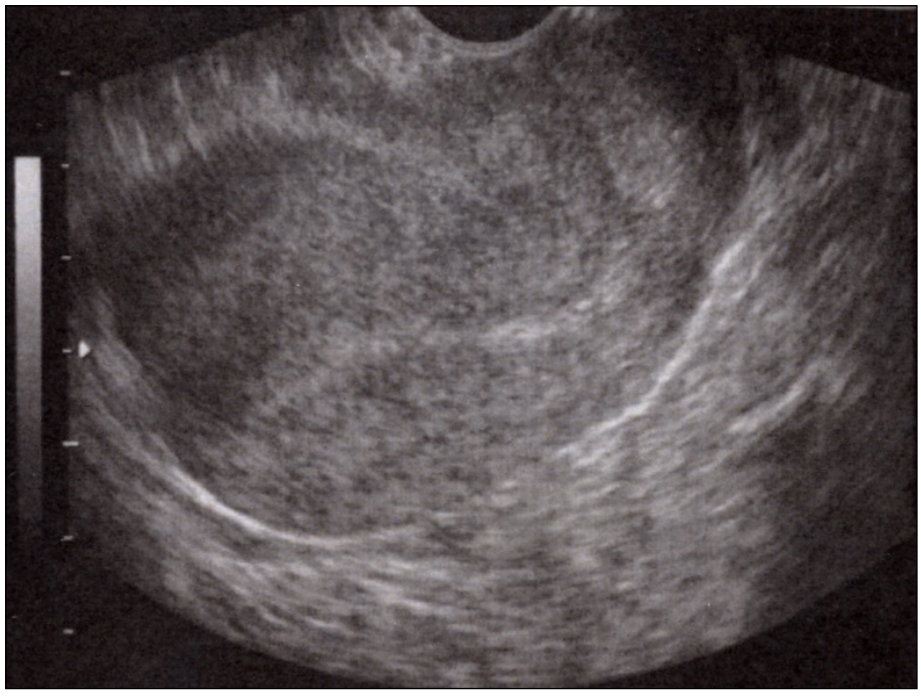

Fig. 3. Echogram of the uterus. The echogenicity of the cervix is the same as the echogenicity of the body of the uterus.

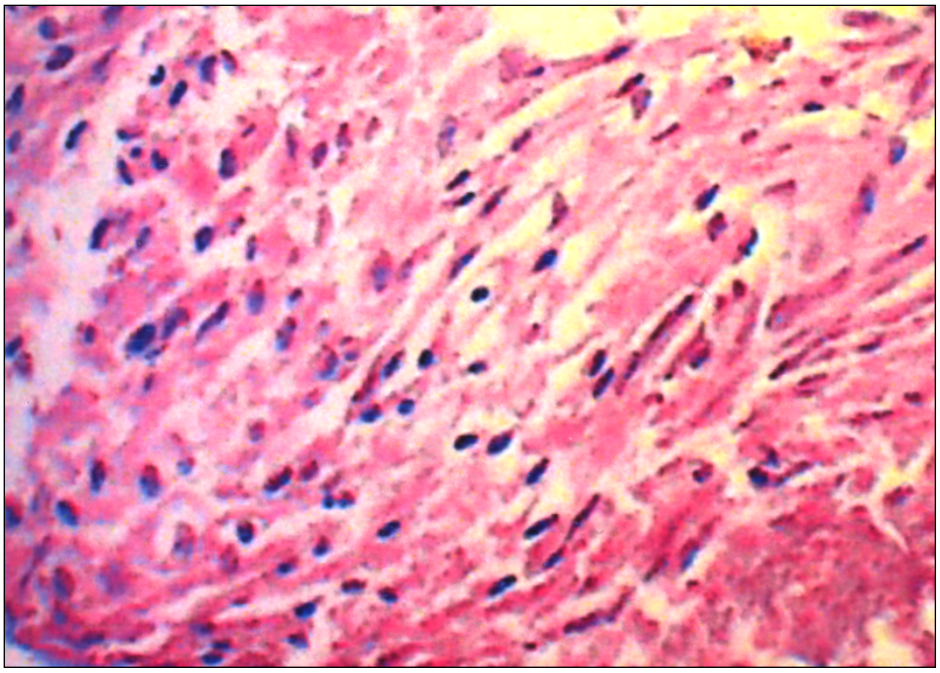

Fig. 4. Biopsy sample supravaginal part of the cervix (isthmus). Staining with hematoxylin and eosin. There is an increase in smooth muscle components over connective tissue components. Enlargment: oculus 15, lens 20.

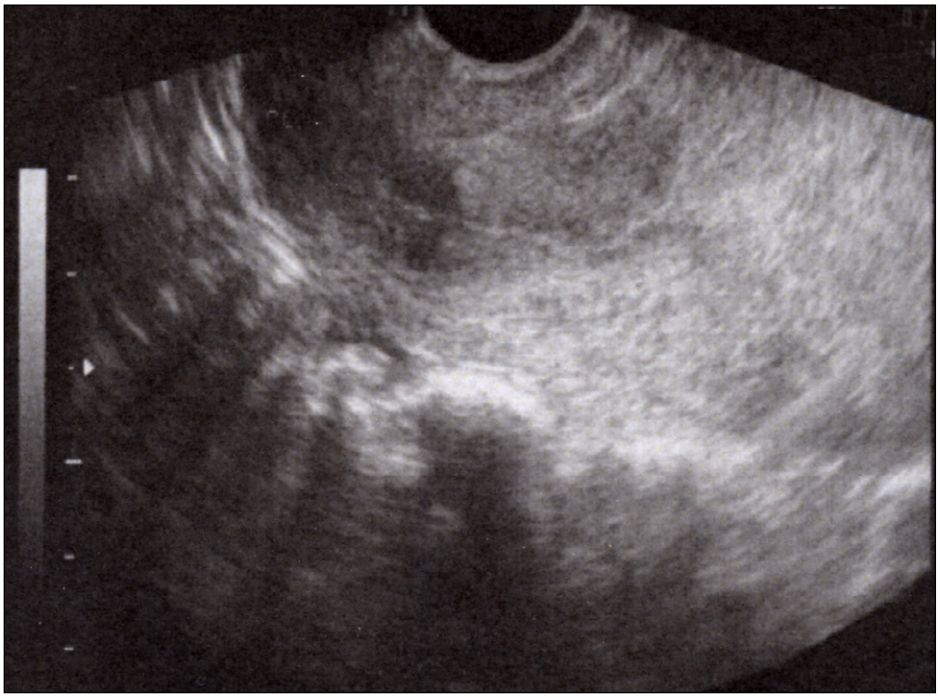

Fig. 5. Echograms of uterine leiomyoma . The echogenicity of the cervix is lower than the echogenicity of the uterine body.

Group I (n = 10) patients whose echogenicity of the cervix prevailed over the echogenicity of the uterine body;

Group II (n = 8), where the echogenicity of the cervix and uterine body coincided with each other;

Group III (n = 5), patients whose echogenicity of the cervix was lower than that of the body.

For the purpose of pathomorphological examination, a biopsy specimen was taken from the supravaginal part of the cervix from the removed uterus. The obtained material was fixed in a 10 percent solution of neutral buffered formalin (pH 7.9) for 24-36 hours, followed by embedding in paraffin blocks.

Features of the morphological structure of the uterine isthmus were studied in histological sections stained with hematoxylin and eosin depending on the echogenicity ratios of the cervix and uterine body in patients after hysterectomy. uterine leiomyomas . To assess the connective tissue component, sections were additionally stained according to Van Gieson and Masson (Trichrome Stain Kit).

RESULTS

In the first observation group with increased echogenicity of the cervix above the body of the uterus (Fig. 1), in hysterosalpingograms (Fig. 2) of the supravaginal part of the cervix, smooth muscle bundles were found in the form of a thin layer or individual cellular elements.

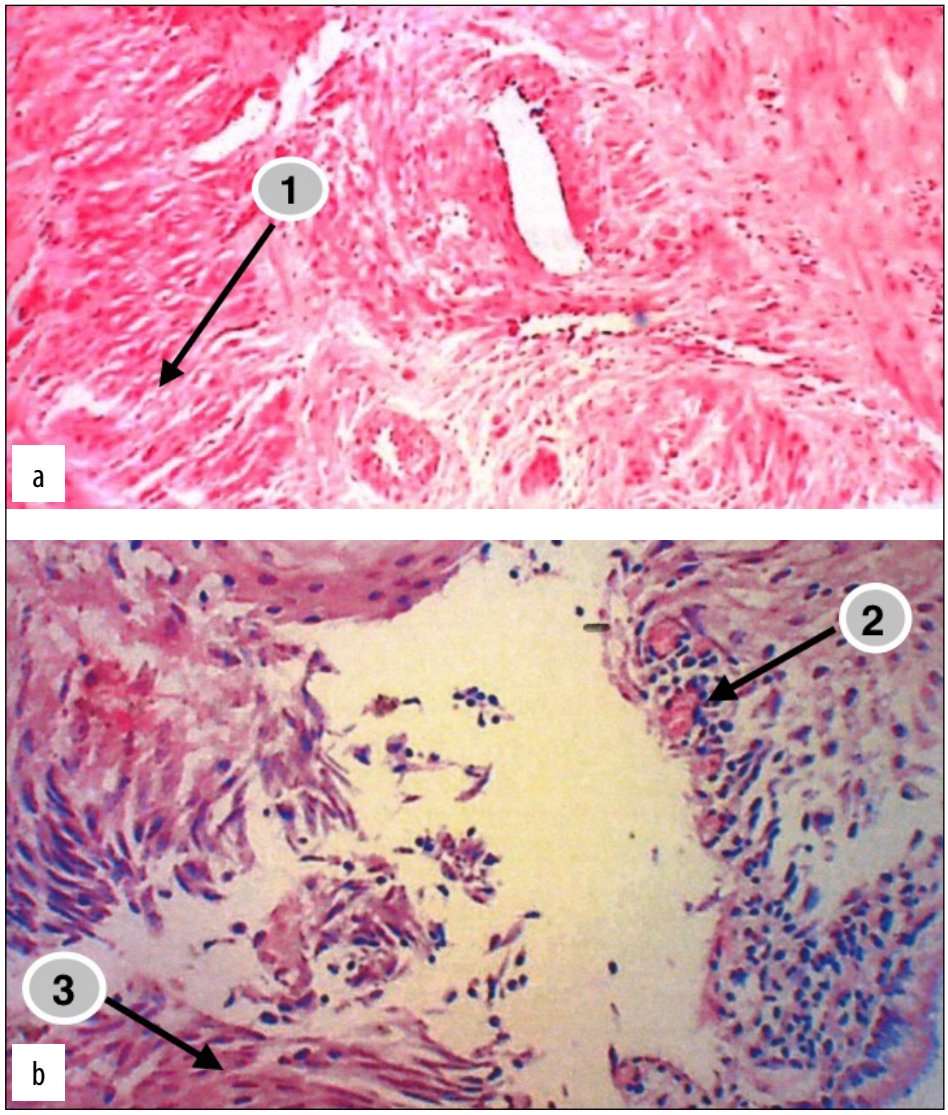

Fig. 6. a, b. Biopsy sample supravaginal part of the cervix with a violation of the morphological structure of the connective tissue in the form of an increase in the spaces between collagen fibers (1), hyperemia of the vessels of the connective tissue with the presence of foci of angiogenesis (2) and a significant number of smooth muscle fibers (3). Staining with hematoxylin and eosin. Enlargment: oculus 10, lens 20.

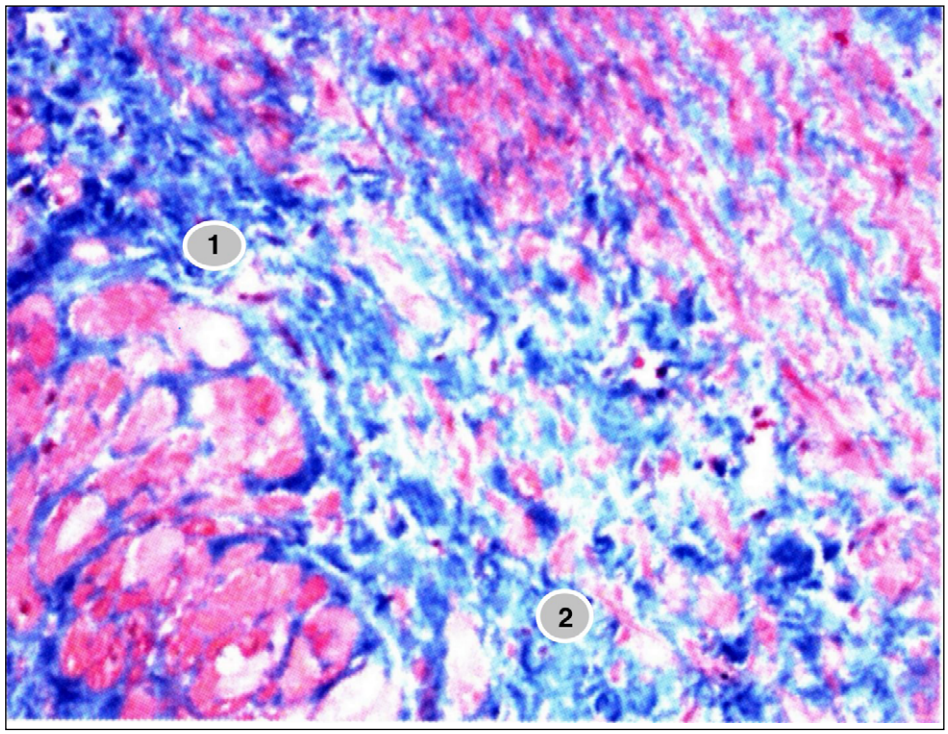

Fig. 7. Biopsy sample supravaginal part of the cervix from a multiparous patient . The histopathology shows a chaotic arrangement of connective tissue fibers with heterogeneous areas of hypochromia (1) and hyperchromia (2) and foci of degeneratively altered smooth muscle bundles. Staining according to Van Gieson and Masson. Enlargment: oculus 10, lens 20.

Smooth muscle bundles were separated by connective tissue fascia, where connective tissue elements predominated, creating a typical paravasal environment through which the functional activity of myocytes is ensured [10].

According to the results of the study, in the second group, both the cervix and the body of the uterus had the same echogenicity (Fig. 3).

Histomorphological data indicate a relative increase in the muscle component over the connective tissue component of collagen and elastic fibers, which predominated by 20-25% over smooth muscle bundles (Fig. 4).

In women of group III with echogenicity of the cervix less than the echogenicity of the uterine body (Fig. 5). on histological specimens. In the supravaginal part of the cervix, there is disorganization of the connective tissue with the presence of individual smooth muscle fibers, hyperemia of the connective tissue, and the presence of angiomatosis (Fig. 6).

In this case, the collagen tissue of the supravaginal part of the uterus, especially in multiparous patients, is stained heterogeneously with individual areas of hypo- and hyperchromia with the presence of foci of altered smooth muscle bundles (Fig. 7).

DISCUSSION

The results of our study indicate that in the supravaginal part of the cervix, when the echogenicity of the tissues of the cervix exceeds that of the body, the predominance of the muscular component over the connective tissue component is noted. In this case, the smooth muscle bundles are supported by connective tissue, which creates a typical paravasal environment between the muscle bundles, through which the functional activity of myocytes are ensured [10, 11].

In all women with the same echogenicity of the cervix relative to the body of the uterus or lower, disorganization of connective tissue was noted in histological preparations and dystrophic changes were observed in the cells of smooth muscle bundles, which changed the reflective activity of the echo signal by the structural elements of the cervix and body of the uterus and isthmus. The obtained data are consistent with the literature [11].

In some studies, there is evidence indicating that under conditions of chronic hypoxia, the processes of both collagenogenesis and lithogenesis are disrupted. Therefore, the disorganization of connective tissue and dystrophic changes in the cells of smooth muscle bundles that we have discovered, especially in multiparous patients, indicate the consequence of hypoxia in structural reorganization after traumatization of the lower uterine segment during childbirth.

In the literature there are some scientific works [11,12], which indicate that the intensity of collagen synthesis and the formation of collagen fibers occurs due to autoregulation of the processes of collagen synthesis and breakdown. This can be realized in two ways: pathological, when collagen fibers appear inside the cells in the cytoplasm of fibroblasts and myofibroblasts or by replacing damaged smooth muscle cells in bundles with connective tissue in case of impaired reparative processes [12], which is confirmed by histomorphological changes in histological specimens of multiparous women.

CONCLUSIONS

1. Comparative studies show that in cases of excess echogenicity of the cervix over the body of the uterus, pathomorphological changes in the supravaginal part of the cervix are not detected.
2. With the same or reduced echogenicity of the cervix compared to the body of the uterus, disorganization of connective tissue and dystrophic changes in the smooth muscle bundles of the supravaginal part of the cervix were observed in histological specimens.
3. A change in the ratio of echogenicity of the cervix to the body, which is closely related to the morphostructure of the isthmus of the uterus, can serve as one of the criteria for predicting the failure of the lower uterine segment in women.

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ORIGINAL ARTICLE

CONTENTS

Peculiarities of complex oral rehabilitation of young patients with juvenile idiopathic arthritis and malocclusion

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ABSTRACT

Aim: The aim of the study was to analyze the efficiency when planning complex oral rehabilitation, conducting proper preparation, occlusal analysis and adjustment during the stages of orthodontic treatment in young patients with Juvenile Idiopathic Arthritis.

Materials and Methods: 11 young patients with diagnosis of Juvenile Idiopathic Arthritis (according to ILAR criteria) aged from 18 to 34 years (mean age 22.64±4.82) who included 6 females and 5 males were examined and underwent orthodontic treatment at Dental Medical Center of Bogomolets National Medical University during 2021-2024 years. The orthodontic treatment was carried out after oral cavity sanation and elimination of temporomandibular disorders manifestations using occlusal splints and was combined with local medication therapy (mouth rinse using medicinal composition of highly dispersed silica gel and bacteria strains of Bacillus subtilis B-7812(AX20) and Bacillus licheniformis IMB B-7811(EA22)). Occlusal analysis and adjustments were carried out during all stages of rehabilitation.

Results: Provided orthodontic treatment in combination with application of bite blocks and direct dental hard tissues restorations allowed to expand the dental arches, align the midline and normalize the occlusal contacts of the teeth in static and dynamic occlusion in all patients. There were no signs of premature contacts, occlusal overload, deterioration of periodontal lesion or temporomandibular disorder manifestation recurrence.

Conclusions: Our study showed that the proposed complex rehabilitation of patients with JIA including proper occlusal analysis and adjustment and probiotics usage during orthodontic treatment within the framework of a personalized approach during their rehabilitation ensures minimization of risks and is the key to achieving optimal results.

KEY WORDS: juvenile idiopathic arthritis, dental occlusion, temporomandibular joint, orthodontics, periodontitis

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INTRODUCTION

Juvenile idiopathic arthritis (JIA) is a collective term that combines a number of systemic connective tissue diseases with a currently unclear etiology and a predominant localization of the inflammatory process in the musculoskeletal system, lasting at least 6 weeks and developing in children under 16 years of age. The prevalence of this disease in Ukraine is estimated at 0.37 cases per 1000 children under 17 years of age with a tendency to increase. In total, more than 3000 children with JRA have been registered in Ukraine. The disease has a progressive course, does not end with complete recovery and has a high degree of disability [1, 2]. Therefore, JIA is an important medical and social problem.

In patients with JIA, the clinical situation in most cases becomes comorbid. Therefore, comprehensive rehabilitation of such patients with the involvement of specialists of various profiles is of great importance. Unfortunately, many questions related to the treatment of patients with JIA still remain unanswered, including the features of complex oral rehabilitation of such patients.

The nature of the course of JIA depends not only on its clinical variant, disease activity and the degree of involvement of certain target organs in the pathological process, but also on the therapy performed, dose, its effectiveness and duration. It is known that most often side effects develop in patients due to prolonged use of glucocorticoids and against the background of the lack of an adequate response to basic disease-modifying synthetic or immunobiological drugs. The use of corticosteroids can increase the risk of root resorption, which may complicate orthodontic movement and the overall stability of teeth after treatment. Drugs like methotrexate can cause mucosal ulcers, dry mouth, or oral infections, which need to be managed during rehabilitation. Patients undergoing Disease-Modifying Anti-Rheumatic Drugs therapy may be more prone to infections or delayed wound healing after dental pro-

cedures. Immunobiological drugs and TNF inhibitors may alter the immune response and increase the risk of periodontal diseases or other oral infections, necessitating close monitoring and care during oral rehabilitation. JIA's activity can fluctuate, so oral rehabilitation plans should remain flexible, with frequent reassessment to adjust treatment plans as needed, based on the patient's rheumatologic status.

In patients with JIA all the joints can be affected, including the temporomandibular joint (TMJ). The incidence of TMJ involvement according to literature data ranges from 17% to 87%, depending on the studied population, JIA subtypes and diagnostic method [3]. In addition to direct inflammatory lesions, deformation of the TMJ and severe pain syndrome, JIA may have an indirect effect on the development of musculoskeletal dysfunctions and occlusal disorders. During the growth and formation of the maxillofacial system, even a minor inflammatory process in the synovial membrane leads to impaired development of the TMJ, which is the growth zone of the lower jaw, which contributes to delayed development of the jaw bones, the formation of incorrect occlusal relationships and as a result leads to further destruction of the articular process of the lower jaw.

During the initial stage of the disease, this manifests itself in the form of dysfunction, which, according to various authors, occurs in 50-94% of patients. During later stages of the disease, muscle weakness, decreased chewing efficiency and limited mobility in the TMJ are being noted [4]. With further damage to the TMJ, the movements of the lower jaw become limited and painful. The patient cannot open his mouth wide and eating becomes difficult. The impaired motor skills due to damage to the musculoskeletal system, pain symptoms, limited mouth opening, possible side effects of drug therapy, limited access to qualified dental care contribute to a decrease in the quality of oral hygiene and negatively affect the patient's oral health. With further progression, the patient cannot open his mouth wide, eating becomes difficult, and oral hygiene deteriorates, which can lead to the development and progression of periodontal tissue lesions and the formation of occlusal pathology [5]. At the same time, mandibular micrognathia – underdevelopment of the lower jaw develops, which leads to the development of malocclusion (in particular, II class by Angle, facial asymmetry), which, can lead to development of periodontal lesions and the formation of occlusal pathology. Semi-retention of the third permanent molars may be often observed in patients with JIA, which can be associated with violations of the mechanism of tooth eruption.

Damage to the temporomandibular joint as well as the presence of chronic inflammation, long-term use of glucocorticoids and cytostatic therapy in patients with JIA leads not only to orthodontic disorders, but also to a deterioration in the periodontal condition of the patients. Chronic inflammation, medications, and limited oral hygiene due to pain or jaw restrictions can make patients with JIA more susceptible to periodontal disease. Periodontitis is caused by a group of periodontal pathogenic microorganisms, and its continuation depends on the inflammatory and immune reactions of the body that can be induced by JIA. According to the literature, laboratory diagnostics of the levels of IL-1, IL-6 and IL-17, MMP-8 and tumor necrosis factor α (TNF-α) is an important characteristic of the course and an indicator of the effectiveness of treatment of systemic connective tissue diseases, in particular juvenile idiopathic arthritis, which is often a comorbid background for the development of periodontal diseases [6].

Elimination of occlusal disorders at a young age can eliminate or reduce the clinical manifestations of TMJ dysfunction as well as reduce the clinical manifestations of masticatory muscle parafunctions and normalize the muscle's activity. The combination of therapeutical, surgical, orthodontic and prosthetic methods allows to reach the best results of complex rehabilitation [7, 8].

That's why the patients with JIA require a personalized approach and special tactics for treatment of dental pathology as important part of complex rehabilitation. This is especially important for the treatment of young patients with malocclusion, since such patients, against the background of systemic damage to connective tissue and reduced bone mineralization during tooth movement, have an increased risk of developing complications from periodontal tissues, as well as accelerated bone resorption.

AIM

The aim of the study was to demonstrate the importance and analyze the efficiency of considering the general somatic status and bone tissue condition in young patients with Juvenile Idiopathic Arthritis when planning complex oral rehabilitation, conducting proper occlusal analysis and adjustment during at the stages of treatment to minimize possible risks and achieve optimal results.

MATERIALS AND METHODS

11 young patients with diagnosis of Juvenile Idiopathic Arthritis (according to International League of Associations for Rheumatology criteria) and malocclusion aged from 18 to 34 years (mean age 22.64±4.82) who included 6 females and 5 males were examined and

underwent orthodontic treatment at Dental Medical Center of Bogomolets National Medical University during 2021-2024 years. The patients with skeletal severe forms of dentofacial deformities, ankyloses and severe temporomandibular disorders were excluded from the study.

In order to plan the treatment and monitor its effectiveness in addition to the generally accepted clinical examination and orthopantomography, the results of dual-energy X-ray absorptiometry obtained using the Hologic Discovery DXA System, analysis of jaw bone density according to cone-beam computed tomography obtained using the MyRay Hyperion X9 PRO 3D/2D tomograph were analyzed. Digital occlusal analysis was performed with Medit Occlusion Analyzer v.1.02 software using intraoral scans of the jaws and bite records obtained with Medit i500 intraoral scanner.

All the patients during the pre-orthodontic stage of treatment were introduced to basic oral hygiene skills and underwent professional oral hygiene using an ultrasonic apparatus complex. Also all the patients underwent the sanation of oral cavity including caries treatment, replacement of inappropriate direct restorations, periodontal treatment and extraction of impacted third molars when it was necessary.

The orthodontic treatment was applied in case of absence or after elimination of temporomandibular disorders manifestations using mandibular miorelaxing and stabilizing occlusal splints. It included the expansion of the dental arches of the upper and lower jaws, normalization of the position of the teeth using low forces and fixed orthodontic appliances (a bracket system using a straight arch, starting from 12) with subsequent restoration of the abraded hard tissues of the teeth and final correction of the occlusal relationships based on the data of the occlusal analysis at the final stage of rehabilitation. In order to prevent bone resorption and complications from periodontal tissues it was recommended to use low forces and conduct control examinations and corrections at intervals of 2 weeks. After the expansion of the dental arches, in order to normalize the occlusal relationships in statics and dynamics, a repeated intraoral scan with occlusal analysis was performed and occlusal overlays first to the first premolars of the upper jaw, and then to the second molars, as well as direct restoration of the abraded tooth tissues was carried out. Occlusal analysis and adjustments were carried out every 4 weeks during active teeth movement stage and every 2 weeks at the final stage of rehabilitation.

Orthodontic treatment was combined with local medication therapy in the form of mouth rinse using proposed medicinal composition of highly dispersed silica gel and bacteria strains of Bacillus subtilis B-7812(AX20) and Bacillus licheniformis IMB B-7811(EA22) (1g of the mixture contains silica gel and 2,5 x 109 CFU of live microbial cells in equal parts). Patients diluted the mixture with boiled water in a ratio of 1 to 10 and rinsed oral cavity once a day during the entire treatment period. In order to analyze the efficiency of probiotics usage, periodontal and oral hygiene status was evaluated using Russell's Periodontal Index (PI) and Greene-Vermillion Simplified Oral Hygiene Index (OHI-S), which were calculated before and after orthodontic treatment.

RESULTS

The study revealed that the caries prevalence among examined patients was 100%, mean DMFT index was 8.75±4.94. Overall periodontitis prevalence was 100%, while only 4 (21%) had severe periodontitis. All patients shown certain signs of traumatic occlusion such as gingival recessions, abfractions or pathological mobility of teeth of I-II degree. 2 patients (18%) had I class bite, 6 patients (55%) - II class bite, 3 patients (27%) – III class bite. 9 patients (82%) had teeth crowding. 8 patients (73%) had signs of TMJ disorders such as clicking, decreased mouth open or pain.

The results of X-ray absorption densitometry indicated that 8 patients (73%) had osteoporosis and osteopenia. When analyzing cone-beam computed tomography, a significant decrease in the radiological density of the spongy substance of the alveolar processes of the upper (119.99±105.01 Hounsfield units) and lower (227.01±150.67 Hounsfield units) jaws was detected, while the density of the cortical bone on the upper (1550.55±117.33 Hounsfield units) and lower (1932.86±152.92 Hounsfield units) jaws was high.

Digital occlusal analysis revealed that 9 patients (82%) had premature contacts in maximal intercuspal position, 6 patients (55%) – during protrusion and 7 patients (64%) – during laterotrusion.

Positive changes in the position of the TMJ condyles, confirmed by CBCT, were observed in all patients after the use of occlusal splints. These changes served as an indicator for progressing to the next stage of dental treatment, including orthodontic correction. Occlusal equilibration and the normalization of occlusal contact balance were successfully achieved in all patients (Fig. 1).

Provided orthodontic treatment in combination with application of bite blocks and direct dental hard tissues restorations allowed to expand the dental arches, align the midline and normalize the occlusal contacts of the teeth in static and dynamic occlusion in all patients (Fig. 2).

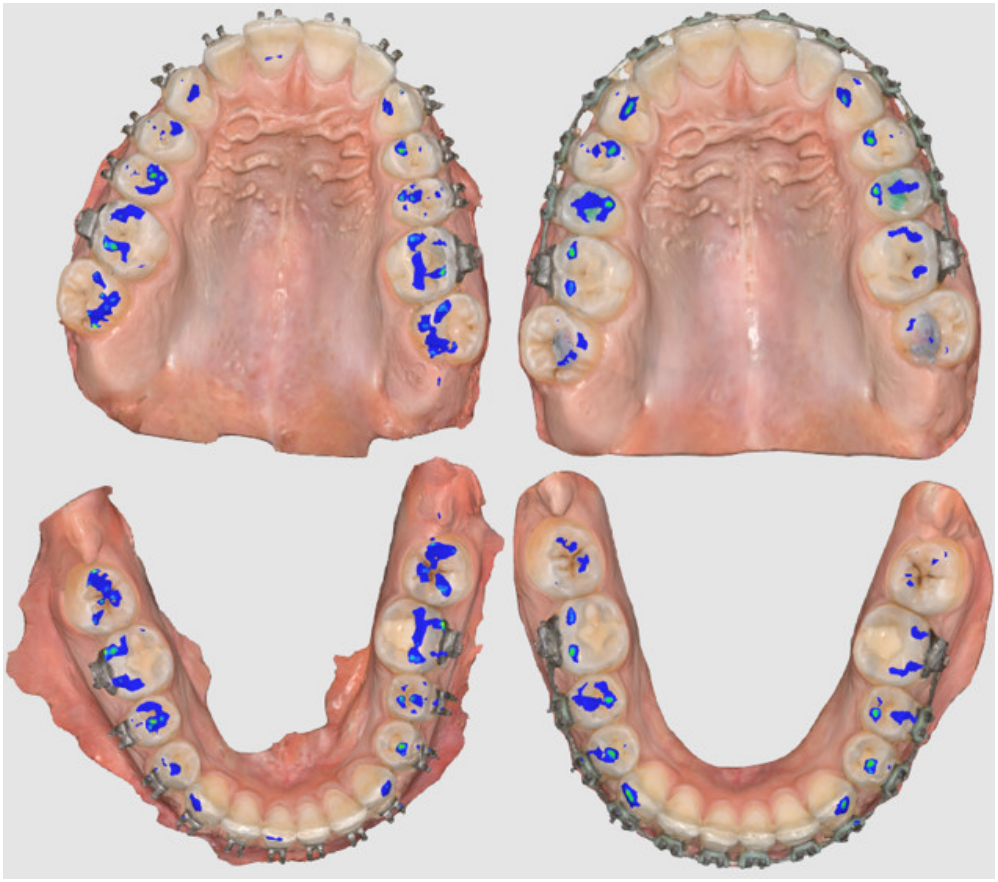

Fig. 1. Dynamics of occlusal contacts changes during the stages of complex oral rehabilitation of a patient with JIA and malocclusion.

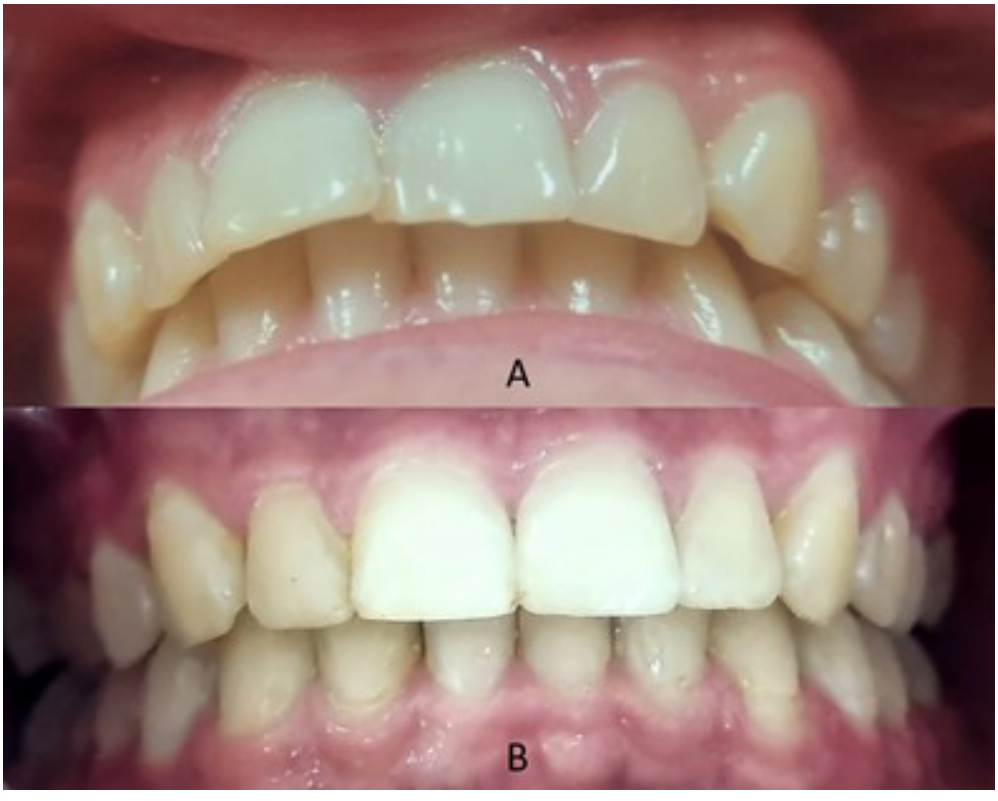

Fig. 2. Dental rows of a patient with JIA and malocclusion before (A) and after (B) complex oral rehabilitation.

There were no signs of premature contacts, occlusal overload, deterioration of periodontal lesion or temporomandibular disorder manifestation recurrence. A follow-up cone-beam computed tomography did not reveal any signs of osteoporosis progression, decreased bone density, or bone resorption following orthodontic treatment (Fig. 3).

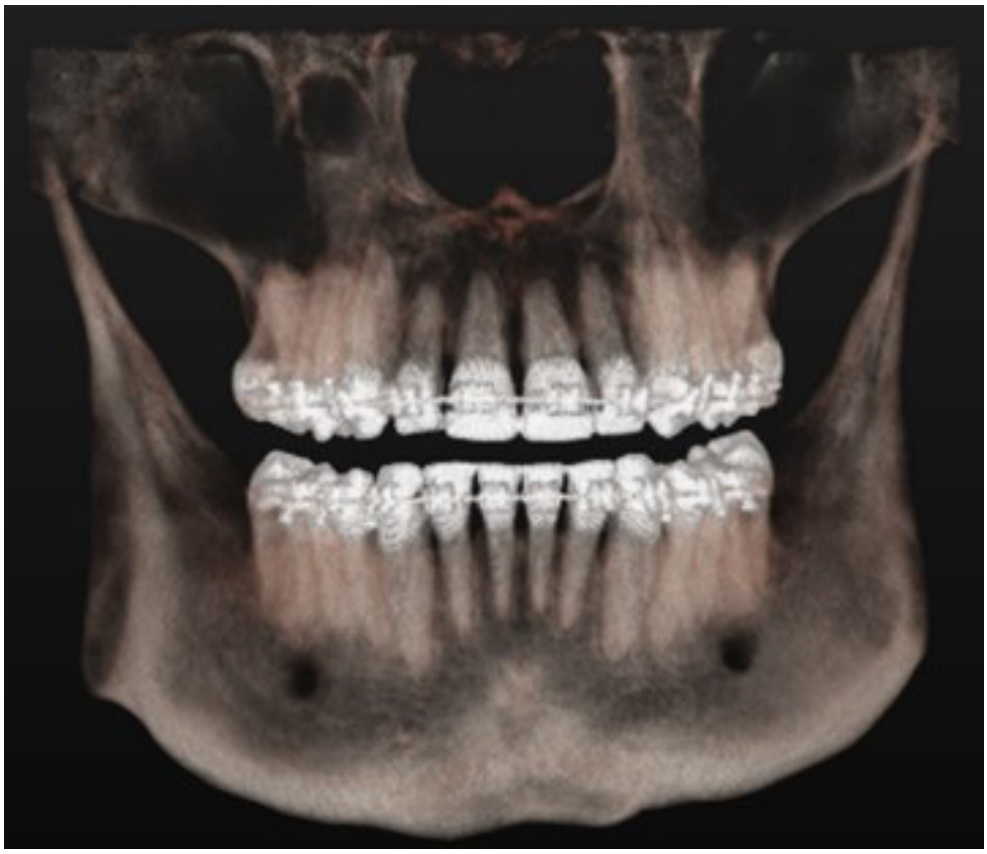

Fig. 3. CBCT of a patient with JIA and malocclusion.

Table 1. Periodontal and oral hygiene status of patients with JIA before and after orthodontic treatment

Indicators	M±m		p
	Before treatment (n=11)	After treatment (n=11)	
Russell's Periodontal Index (PI)	5,63±0,33	2.95±0.36	<0,05
Greene-Vermillion Simplified Oral Hygiene Index (OHI-S)	2.98±0,26	0.49±0.16	<0,05

The analysis of periodontal and oral hygiene status in patients after using a medicinal mixture of silica gel and bacteria B. subtilis B-7812(AX20) and B. licheniformis IMB B-7811(EA22) has showed a pronounced reduction in the manifestations of periodontal inflammation and an improvement in the hygienic condition of the oral cavity. The mean value of PI decreased significantly from 5.63±0.33 before the probiotic usage to 2.95±0.36 after and the mean value of OHI-S decreased from 2.98±0,26 to 0.49±0.16 after the treatment (Table 1).

DISCUSSION

The results of the study show that significant levels of caries, periodontal lesions were revealed in young patients with juvenile idiopathic arthritis. Almost all examined patients had certain signs of occlusal disorders and TMJ dysfunctions. That's why such patients may need a comprehensive complex of dental prophylaxis and rehabilitation measures in order to keep their oral health and prevent the development or progressing of occlusal and TMJ disorders. This emphasizes the importance of the development of interdisciplinary protocols for the management of patients with Juvenile Idiopathic Arthritis and malocclusions which can be complicated by inflammatory periodontal diseases. The rehabilitation of such patients should extend beyond orthodontic treatment and be founded on an interdisciplinary approach, involving pediatric rheumatologists, maxillofacial surgeons, orthodontists, radiologists, pediatric dentists, prosthodontists, physiotherapists, and orofacial pain specialists [7, 8].

The goals of TMJ arthritis management are timely diagnosis, reduction of TMJ inflammation, decreasing orofacial symptoms, optimization of orofacial function, normalization of dentofacial growth and correction of dentofacial deformities. Occlusal splints are commonly used to help support and balance both TMJs and to prevent further pain and discomfort to the TMJ complex during orthodontic treatment. Occlusal splints are safe,

low cost and allow the patient to have even contacts when the teeth occlude in all ranges of motion including biting and side to side jaw movements, which can result in decreased TMJ arthritis-related manifestations and improved mandibular function. Proposed mechanisms for these outcomes include the repositioning of the condylar head in the TMJ, reduction of excessive pressure on the joint surfaces, a temporary decrease in masticatory muscle activity, a reduction in bruxism, achieving balanced occlusion and the potential placebo effect [8-10].

Modern scientific studies show that there is evidence that periodontal disease and rheumatoid arthritis are linked by common immunoinflammatory reactions of imbalance in the pathogenetic basis of both diseases, have similarity of allelic genes and a common imbalance of the state of the cytokine network. There is a hypothesis according to which the mechanism of bone resorption in periodontitis also underlies the progression of joint lesion in patients with rheumatoid arthritis [11].

Our own observations convincingly indicate a significantly higher proportion of patients with generalized periodontitis and moderate destructive changes and inflammatory signs in the periodontium, which may indicate the onset of rapidly progressive periodontitis in young patients with JIA and serve as an additional motivating factor for the prevention and treatment of the primary disease.

Pronounced antimicrobial properties of probiotic strains make it possible to consider the possibility of using drugs based on them as an alternative to antibiotics, which is especially relevant in the era of the rapid spread of resistant forms of pathogenic microorganisms and the decrease in the effectiveness of a number of antimicrobial agents [12]. As a result of numerous preclinical and clinical studies of Bacillus subtilis and Bacillus licheniformis strains, good perspectives of these microorganism's usage for the treatment and prevention of periodontal tissue diseases were established [12]. Previously conducted microbiological studies have shown that the proposed mixture of silica gel and bacteria strains of B. subtilis and B. licheniformis has a pronounced antimicrobial activity both on the test strains of microorganisms and on the mixed microbial flora of the periodontal pockets of patients with generalized periodontitis [14]. Our study has also shown that probiotics usage has a pronounced therapeutic effect on periodontal tissues and significantly improves the local hygienic status of patients with chronic periodontitis who undergo orthodontic correction with fixed appliances, therefore it can be recommended for use in order to reduce the risks of developing inflammatory complications during the complex treatment of such patients.

By addressing the specific challenges caused by JIA such as TMJ dysfunction, bone health, medication effects, and psychosocial factors dentists can help manage the complexities of treatment while improving the functional and aesthetic outcomes for the patient. Orthodontic treatment in young patients with juvenile idiopathic arthritis requires careful planning, individualized strategies, and a multidisciplinary approach. Orthodontists should work closely with the patient's rheumatologist to ensure that JIA is under control during orthodontic treatment. Medication adjustments or changes in disease activity can impact the treatment plan, so close coordination is necessary. Regular monitoring, ongoing collaboration with other healthcare professionals, and a focus on both the patient's oral health and overall well-being are key to successful orthodontic management.

CONCLUSIONS

Our study showed that the proposed algorithm of complex rehabilitation of patients with Juvenile Idiopathic Arthritis including implementation proper occlusal analysis and adjustment and probiotics usage during orthodontic treatment of the patients with Juvenile Idiopathic Arthritis within the framework of a personalized approach during all the stages of their rehabilitation ensures minimization of risks and is the key to achieving optimal results.

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CONFLICT OF INTEREST

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ORIGINAL ARTICLE

CONTENTS

AI-driven rehabilitation: evaluation of ChatGPT-4o for generating personalized physical rehabilitation plans in comorbid patients

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ABSTRACT

Aim: To evaluate the performance of ChatGPT-4o in creating personalized physical rehabilitation plans for comorbid patients.

Materials and Methods: ChatGPT-4o was employed to generate physical rehabilitation plans for 50 clinical cases of comorbid patients. These plans were evaluated independently by two experts according to 6 criteria using a 5-point Likert scale. Experts also classified each plan regarding its suitability for use into 3 categories: "Completely unsuitable for use", "Suitable for use with corrections", "Completely suitable for use". Statistical analysis included the Mann–Whitney U test, intraclass correlation coefficient (ICC) and linear weighted Cohen's kappa (k_w). The statistical significance was set at $p<0.05$

Results: The overall mean score of ChatGPT-4o generated rehabilitation plans was 4.30±0.28 with the highest scores for respiratory and musculoskeletal pathology (4.37±0.36 and 4.33±0.24, respectively). Among the evaluation criteria, the highest indicators were observed for Clinical accuracy and Safety (4.59±0.59 and 4.41±0.71, respectively). 72.00% of the generated plans were classified as "Suitable for use with corrections". None of the plans were identified as "Completely unsuitable for use". The agreement percentage ranged from 84% to 90%, ICC values were 0.80-0.86, and overall suitability k_w was 0.77

Conclusions: LLM-generated rehabilitation plans show promise as supportive tools in clinical practice, but they are not yet at a stage where they can be implemented without expert review and modification. The high overall inter-rater reliability provides confidence in the evaluation process, while also highlighting areas for improvement in both the LLM's performance and the assessment methodology.

KEY WORDS: ChatGPT-4o, large language model, performance, physical rehabilitation

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INTRODUCTION

Over the past few years, the phrases "Artificial Intelligence" and "Large Language Models" have become increasingly common both in everyday conversation and in the pages of scientific journals. According to Cambridge dictionary, the large language model (LLM) is "a complex mathematical representation of language that is based on very large amounts of data and allows computers to produce language that seems similar to what a human might say" [1]. These models represent a significant advancement in artificial intelligence, particularly in the realm of natural language processing (NLP). They have been trained on extensive datasets using deep learning algorithms, particularly transformer architectures. LLMs are capable of performing various NLP tasks, including recognizing, generating, translating, and summarizing text. The training process involves learning statistical relationships from vast amounts of text data, allowing the model to understand context, syntax, and semantics inherent in human language. The architecture of LLMs typically includes multiple layers of neural networks that process input data in parallel, significantly enhancing their efficiency compared to earlier models like recurrent neural networks (RNNs).

Over the last 60 years LLMs made a great journey from the first pattern matching ELIZA to modern GPT-4 and LLaMA that are pushing the boundaries of AI and are capable of writing, conversing, summarizing, and translating with a sophistication that closely mirrors human intelligence. The number of released LLMs is constantly rising every year. Thus, in only 2023 more than 20 such models were introduced [2, 3].

LLMs are successfully used in various fields of activity from media and entertainment to financial management. The adoption of LLMs is projected to increase significantly across various industries from 2022 to 2026. While the technology sector leads with an expected adoption rate of 85% by 2026, other industries such as finance, retail, and manufacturing are also anticipated to see substantial growth in LLM integration [4].

Table 1. Evaluation criteria and 5-point likert scale for assessing ChatGPT-4o-generated rehabilitation plans

Criterion and it's description	5-point Likert scale
Individualization: The extent to which the plan was tailored to the specific needs and conditions of the patient.	1 - No personalization evident 2 - Minimal consideration of patient-specific factors 3 - Basic tailoring to patient needs 4 - Well-tailored plan with some unique considerations 5 - Highly personalized plan addressing all patient-specific factors
Clinical accuracy: The correctness of the interventions proposed, based on current clinical guidelines and best practices.	1 - Contains significant clinical errors 2 - Some clinical inaccuracies present 3 - Generally accurate but lacks depth 4 - Clinically sound with minor oversights 5 - Demonstrates high-level clinical knowledge and accuracy
Safety: Assessment of potential risks and the inclusion of appropriate safety precautions.	1 - Potentially harmful recommendations 2 - Inadequate safety considerations 3 - Basic safety measures included 4 - Comprehensive safety protocols with minor gaps 5 - Exceptional attention to safety, covering all potential risks
Progressive design: The logical progression of rehabilitation activities over the one-month period, ensuring a gradual increase in intensity and complexity.	1 - No progression in difficulty or intensity 2 - Minimal progression evident 3 - Basic progression structure present 4 - Well-structured progression with some refinement needed 5 - Optimal progression design tailored to patient capabilities
Feasibility and accessibility: The practicality of implementing the plan given the patient's likely resources, environment, and support system.	1 - Impractical or inaccessible for the patient 2 - Limited consideration of patient's circumstances 3 - Moderately feasible and accessible 4 - Highly feasible with minor accessibility concerns 5 - Perfectly aligned with patient's resources and circumstances
Focus on the result: The clarity and specificity of the expected outcomes, with a focus on measurable improvements in the patient's condition.	1 - No clear goals or outcome measures 2 - Vague or inappropriate goals 3 - Basic outcome-oriented approach 4 - Clear, relevant goals with some room for improvement 5 - Comprehensive, patient-centered goals with clear outcome measures

The use of this technology in healthcare is also promising. It was shown that LLMs can provide differential diagnoses and suggest potential treatments, improve the accuracy and speed of medical decision-making, transcribe and summarize patient interactions, answer patient queries, provide health advice, identify new drug candidates and predict their potential efficacy, create personalized treatment plans by analyzing individual patient data etc [5-9]. The evaluation of the LLMs application in health care is carried out by researchers from different positions. Thus, the most common task is assessing medical knowledge, while making diagnoses and educating patients are less common. More than 80% of studies focused on question answering, with fewer on summarization, conversational dialogue, and translation. Accuracy of LLMs responses is the primary focus in the majority of studies, while fairness, bias, toxicity, robustness, and deployment considerations are less frequently measured. Among all medical specialties LLMs application in internal medicine, surgery, and ophthalmology were the most studied areas [6]. Physical rehabilitation is one of the medical fields that is often overlooked by LLMs researchers.

Physical rehabilitation plays a vital role in the comprehensive treatment approach for patients across a wide spectrum of diseases and conditions. It serves as a critical component in restoring function, improving quality of life, and promoting overall well-being. Effective rehabilitation can significantly reduce healthcare costs by preventing complications, reducing hospital readmissions, and decreasing dependency on long-term care services.

Physical rehabilitation specialists encounter several challenges in their practice, which impact patient care and outcomes. This includes but is not limited to complexity and diversity of patient conditions, resource limitations, patient adherence to rehabilitation programs, telerehabilitation techniques, designing personalized rehabilitation plans [10, 11]. Involving the LLMs in routine practice of physical rehabilitation specialists may help to overcome these issues and significantly increase the efficacy of rehabilitation programs. Although the integration of such tools offers promising advantages, it is crucial to consider potential drawbacks and constraints associated with their use. Paramount among these considerations is

Table 2. ChatGPT-4o performance in generating rehabilitation plans across various pathology classes and evaluation criteria, M±SD

Pathology class	Individualization	Clinical accuracy	Safety	Progressive design	Feasibility and accessibility	Focus on Results	Overall Mean
Musculoskeletal	4.35±0.60	4.55±0.61	4.60±0.61	4.30±0.75	4.00±0.71	4.20±0.60	4.33±0.24
Nervous	4.35±0.81	4.70±0.47	4.60±0.68	4.00±0.79	3.95±0.69	4.00±0.73	4.27±0.23
Respiratory	4.40±0.75	4.75±0.44	4.55±0.60	4.10±0.72	3.85±0.75	4.55±0.69	4.37±0.36
Cardiovascular	4.50±0.51	4.45±0.60	4.50±0.69	4.30±0.57	4.05±0.83	3.95±0.89	4.29±0.26
Digestive	4.20±0.70	4.50±0.76	3.80±0.70	4.35±0.75	4.60±0.6	4.00±0.65	4.24±0.30
Overall Mean	4.36±0.67	4.59±0.59*	4.41±0.71#	4.21±0.71	4.09±0.75	4.14±0.74	4.30±0.28

Note. * - the difference is statistically significant compared to all other criteria ($p<0.05$),
- the difference is statistically significant compared to all criteria except Individualization ($p<0.05$).

the necessity to guarantee the accuracy, effectiveness, and clinical appropriateness of rehabilitation programs developed with LLM assistance.

AIM

The aim of this study was to evaluate the performance of ChatGPT-4o in creating personalized physical rehabilitation plans for comorbid patients.

MATERIALS AND METHODS

A total of 50 clinical cases were utilized in this study. Each case contained a comprehensive description of the patient's current condition, past medical history, primary diagnosis, and accompanying comorbidities. The cases were carefully selected to represent a diverse range of medical conditions, with an equal distribution across five major physiological systems: musculoskeletal system (10 cases), nervous system (10 cases), respiratory system (10 cases), cardiovascular system (10 cases), digestive system (10 cases). All patient data used in the clinical cases were anonymized to protect privacy.

LLM ChatGPT-4o (OpenAI) was employed to generate individualized physical rehabilitation plans for all 50 clinical cases. It was prompted to create plans that had to cover a one-month period and be tailored to each patient's specific condition and needs.

Two experts in physical rehabilitation independently evaluated the rehabilitation plans generated by the LLM on six key criteria: Individualization, Clinical accuracy, Safety, Progressive design, Feasibility and accessibility, and Focus on result. A 5-point Likert scale was employed for each criterion (Table 1).

To ensure consistency in scoring, the experts were provided with detailed rubrics describing the expectations for each score level across all criteria. They underwent a calibration session before the evaluation process to align their understanding of the scoring system.

In addition to the detailed evaluation, the experts provided a final conclusion regarding the overall suitability for use of each rehabilitation plan using the following scale: "Completely unsuitable for use", "Suitable for use with corrections", "Completely suitable for use". If the experts' conclusions differed, the worst one was taken into account when calculating the final frequency of the rehabilitation plan's overall suitability.

Statistical analysis was performed to assess the ChatGPT-4o performance. To analyze the effectiveness of the studied LLM in the generation of physical rehabilitation plans, mean scores were calculated. The mean scores presented as M±SD. The Mann–Whitney U test was used to compare the differences between the evaluation criteria. Additionally, the frequency of each overall suitability assessment was tabulated.

Inter-rater reliability was assessed using agreement percentage, intraclass correlation coefficient (ICC) with 95% confidence intervals (CI) and linear weighted Cohen's kappa (k_w) to ensure consistency between the two experts' evaluations. ICC values <0.5 were considered as poor reliability, 0.5-0.75 – moderate reliability, 0.75-0.9 – good reliability, and >0.90 – excellent reliability [12]. k_w 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 [13].

Mean (M) and standard deviations (SD) were calculated using statistical package Statistica 12 (TIBCO Software Inc., USA). k_w values were calculated using Microsoft Excel 2016 software (Microsoft Corporation, Redmond, WA, USA), while the ICCs with their 95% CIs were computed using the web-based tool StatsToDo. (https://www.statstodo.com/IntraclassCorrelation.php). The statistical significance of all tests was set at $p<0.05$.

RESULTS

The physical rehabilitation plans generated by ChatGPT-4o, based on the provided clinical case descriptions,

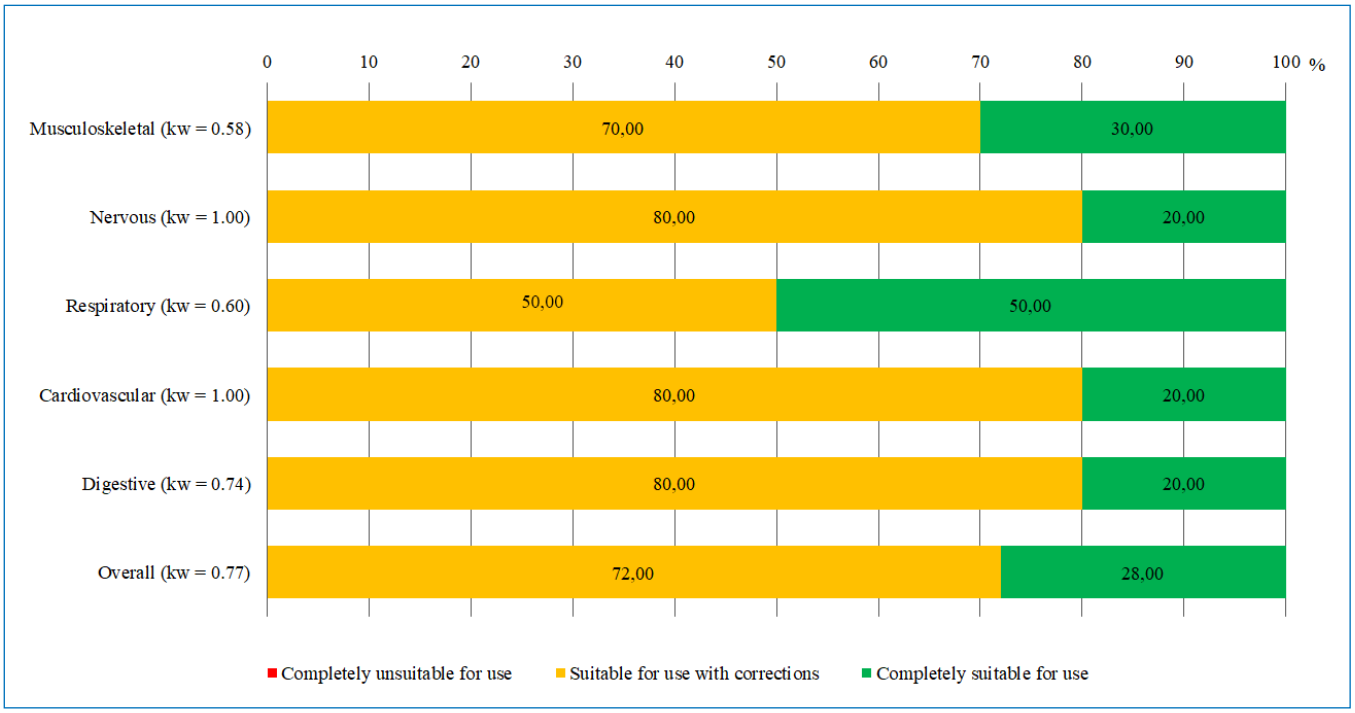

Fig. 1. Frequency distribution of rehabilitation plans' overall suitability assessments across the pathology groups (%) with inter-rater agreement (Cohen's weighted kappa, k_w).

were highly evaluated by the experts. The average ratings were consistently above 4.0 on the proposed 5-point Likert scale, both across the pathology classes and evaluation criteria with the overall mean score of 4.30±0.28 (Table 2).

In terms of pathology classes, LLM showed the highest efficiency in developing rehabilitation plans for patients with primary pathology of respiratory and musculoskeletal systems (4.37±0.36 and 4.33±0.24, respectively). The effectiveness in the development of rehabilitation plans for pathologies of other systems was slightly worse. However, there was no statistically significant difference between the obtained indicators ($p<0.05$).

Among evaluation criteria, the Clinical accuracy received the highest overall mean score (4.59±0.59, $p<0.05$) surpassing all other criteria. The difference was statistically significant. Clinical accuracy was also the highest for plans developed for the rehabilitation of patients with the respiratory and nervous systems pathology (4.75±0.44 and, 4.70±0.47 respectively).

The overall mean score of Safety criterion was somewhat lower (4.41±0.71). However, the average score obtained by this criterion was statistically significantly higher compared to the Progressive design, Feasibility and accessibility, and Focus on result criteria ($p<0.05$). In terms of pathology classes, the Safety criterion for plans for diseases of the musculoskeletal system was rated the highest, and the digestive system was the lowest (4.60±0.61 and 3.80±0.70, respectively).

The overall scores for the criteria Individualization, Progressive design, Feasibility and accessibility, and Focus on result were also quite high, but there was no statistically significant difference between them ($p>0.05$). It is worth noting that the Feasibility and accessibility criterion had the lowest overall mean score (4.09±0.75), but was rated the highest by experts regarding rehabilitation plans for digestive system pathology.

According to the study design the experts also were prompted to make a conclusion on the suitability of physical rehabilitation plans for use. In cases of disagreement between experts' opinions, the final distribution of frequencies took into account a more conservative conclusion (Fig. 1).

The general analysis of the conclusions obtained in this way showed that in most cases (72.00%) these plans were classified as "Suitable for use with corrections". 80.00% of the plans generated for cases of nervous, cardiovascular and digestive systems pathology received this rating. In only 14 (28.00%) cases, both experts independently identified the proposed rehabilitation plans as "Completely suitable for use". Most often (50.00% of cases) such a high rating occurred among rehabilitation plans for patients with respiratory system pathology. It is important to note that none of the plans was classified as "Completely unsuitable for use", indicating a baseline level of acceptability for all plans created by the studied LLM.

The level of agreement between the assessments provided by the experts was high across all evaluation

Table 3. Inter-Rater Reliability Metrics for Expert Evaluations

Evaluation Criteria	Agreement, %	ICC, 95% CI
Individualization	84.00	0.83, 0.71-0.90
Clinical accuracy	86.00	0.80, 0.65-0.89
Safety	86.00	0.86, 0.77-0.92
Progressive design	86.00	0.86, 0.77-0.92
Feasibility and accessibility	88.00	0.84, 0.74-0.91
Focus on Results	84.00	0.85, 0.76-0.91
Overall Suitability for use	90.00	0.78, 0.64-0.87

Note. ICC – intraclass correlation coefficient, CI – confidence interval

criteria. The agreement percentage ranged from 84.00% to 90.00% (Table 3).

In terms of evaluation criteria, the highest percentage of agreement was observed for the Feasibility and accessibility criterion (88.00%). ICC values ranged from 0.80 (CI: 0.65-0.89) to 0.86 (CI: 0.77-0.92). k_w values for the overall suitability of rehabilitation plans across pathology classes ranged from 0.58 to 1.00 with the overall k_w of 0.77 (Fig.1).

DISCUSSION

Upon its release in open access, ChatGPT has been the subject of numerous scientific studies aimed at exploring its performance in a wide variety of medical tasks [14, 15]. The findings obtained in our study highlight the potential of LLMs in creating effective rehabilitation plans even for patients with various comorbidities. The rehabilitation plans proposed by ChatGPT-4o for patients with various pathologies have been highly rated by experts across various evaluation criteria which in turn underscores the sufficient quality of these plans. The highest mean scores of Clinical accuracy and Safety criteria indicate that the model has been trained on a sufficient amount of modern medical data in this field and can effectively use it. On the other hand, it is able to anticipate and mitigate potential risks associated with rehabilitation activities. These findings are extremely important to ensure the quality of the proposed interventions. The high overall score obtained by ChatGPT-4o in this study suggests that it can be used effectively in clinical settings, especially in the case of limited human resources. The studied LLM showed equally high efficiency in the rehabilitation plans development for patients with pathologies of various systems. This suggests a certain level of versatility of its application and the possibility of use in different clinical scenarios. Such high performance, as found in our research, is supported by other studies with similar objectives but different designs and versions of this LLM [16-18].

Despite ChatGPT-4o has demonstrated a strong ability to generate individualized, accurate, and safe rehabilitation plans, there remains room for improvement in ensuring consistent progressive design and aligning rehabilitation plans with patient resources and environments.

There is no doubt that evaluating rehabilitation plans against pre-defined criteria is important and reflects a more formalized approach. However, it is equally important to take into account the expert's general impression of the analyzed rehabilitation plan about its suitability for use as criterion referenced assessment cannot fully reflect all the nuances. On the other hand, creating a generalized conclusion requires the expert not only to use certain knowledge, but also professional experience, which is extremely important. As noted earlier, a conservative approach was used in calculating the final frequency distribution of usability judgments in cases of disagreement between experts. Although this approach may slightly underestimate the final result, this decision was made deliberately, giving priority to patient safety and the quality of the plan. Under these circumstances, the significant number of plans categorized as "Suitable for use with corrections" suggests that although the LLM can create relevant and potentially beneficial rehabilitation plans, there remains a considerable necessity for professional evaluation and adjustment to ensure these plans meet the specific needs of individual patients. The higher rate of completely suitable plans for respiratory conditions (50%) could be due to the more standardized nature of pulmonary rehabilitation practices or a larger volume of used training data in this domain.

Inter-rater reliability indicators play an important role in the analysis of the results of studies with raters involvement. A high percentage of agreement between experts' assessments obtained in our research was revealed, both on separate criteria and on the indicator of the general suitability of rehabilitation plans for use. These results were confirmed by fairly high ICC values, indicating good reliability of the re-

sults. The relatively narrow 95% confidence intervals for these ICCs further confirm the robustness of the agreement. These results suggest that the peer review process was reliable, providing a solid basis for evaluating the quality of rehabilitation plans produced by ChatGPT-4o.

The observed variability in inter-rater agreement across pathology classes, as evidenced by the weighted Cohen's kappa values ranging from moderate (k_w = 0.58) to almost perfect agreement (k_w = 1.00), warrants careful interpretation. This heterogeneity in agreement levels may be attributed to several factors such as small sample size within each pathology class, complexity of pathology-specific rehabilitation, expertise bias or quality variability in LLM output and underscore the needs of further research in this field. Despite this, the overall k_w of 0.77 demonstrates substantial agreement and suggests that the experts generally concurred in their evaluations of the rehabilitation plans generated by ChatGPT-4o, despite the variability across different pathology classes. However, the discrepancies within specific classes highlight the necessity for continued refinement in both the LLMs' output and the evaluation criteria, particularly in addressing the nuances of complex cases.

CONCLUSIONS

This study provides valuable insights into the potential of ChatGPT-4o in generating physical rehabilitation plans across various pathology classes. The overall performance of LLM according to the evaluation criteria was 4.30±0.28 points out of 5.00. Rehabilitation plans generated for patients with respiratory system diseases received the highest rating (4.37±0.36). Clinical accuracy and Safety showed the best results among all criteria (4.59±0.59 and 4.41±0.71, respectively).

None of the created rehabilitation plans was rated by experts as "Completely unsuitable for use". At the same time, 72% were categorized as "Suitable for use with corrections" which underscores the continued necessity of expert oversight. Across different pathology classes, respiratory and musculoskeletal rehabilitation plans received the highest rate of overall suitability for use.

The inter-rater reliability analysis showed strong agreement between experts in their assessments, as confirmed by high values of agreement percentage, ICC and k_w.

The findings of our study demonstrate both the promise and the current limitations of LLMs usage in the field of physical rehabilitation and aligns with the current understanding that AI-generated medical content should not replace but rather augment clinical expertise.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Pathomorphological features of systemic manifestations of severe acute pancreatitis complicated by abdominal compartment syndrome

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ABSTRACT

Aim: To classify systemic pathomorphologic changes in severe acute pancreatitis (SAP) and to identify histologic features associated with abdominal compartment syndrome (ACS).

Materials and Methods: The retrospective cohort examination of 53 patients with SAP, who died due to progressive organ failure. Microscopic specimens of the heart, lungs, liver, kidneys and intestines have been reviewed, divided by the proposed four levels of pathological changes and comparable between the two groups, depending on the presence of ACS (25 and 28 patients).

Results: When comparing in two groups the levels of lesions of each studied organ, according to given levels of change, a statistically significant difference between groups in the level of kidney and intestine lesions ($P < 0.05$, Mann-Whitney U-Test) was obtained. The changes in moderate and severe levels were significantly dominating in the microscopic specimens of the kidneys and intestines of group A compared to group B ($p < 0.001$).

Conclusions: Histopathological changes in the examined organs of patients who died from severe acute pancreatitis complicated by abdominal compartment syndrome were characterized by more pronounced ischemic and inflammatory damage in the kidneys and intestines. The variability of extrapancreatic pathological changes in SAP patients depended on intra-abdominal pressure levels. Monitoring and timely correction of intra-abdominal hypertension aimed at preventing ACS in SAP patients may influence disease progression and treatment outcomes.

KEY WORDS: Severe acute pancreatitis, Abdominal Compartment-Syndrome, Pathology

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INTRODUCTION

Acute pancreatitis (AP) is one of the most common emergency diseases of the gastrointestinal tract [1-3]. Approximately 10-30% of patients develop severe acute pancreatitis (SAP) characterized by persistent organ failure and pancreatic necrosis [4-5]. The mortality rate in SAP reaches 50%, which is almost ten times higher than the mortality rate from other forms of AP [5].

Intra-abdominal hypertension (IAH) complicates the course of AP in 50-60% and in 15-30% progresses to abdominal compartment syndrome (ACS), which is characterized by an increase in intra-abdominal pressure (IAP) above 20 mm Hg and a decrease in abdominal perfusion pressure of less than 60 mm Hg [6,7]. The consequence of a critical increase in IAP is intra- and extra-abdominal organ perfusion disorders, which, in turn, lead to a new "wave" of organ failure (OF) and increase mortality by up to 83% [7]. Consequently, ACS is considered a marker of adverse treatment outcomes in patients with AP, which requires constant monitoring of IAP, timely recognition and correction of IAH [7-9].

Multiorgan failure inherent in SAP is characterized by clinical variability and pathomorphologic polymorphism, and in patients with SAP complicated by ACS may have characteristic manifestations.

AIM

To classify systemic pathomorphologic changes in severe acute pancreatitis and to identify histologic features associated with abdominal compartment syndrome.

MATERIALS AND METHODS

A retrospective single-center cohort study was conducted at the Bogomolets National Medical University

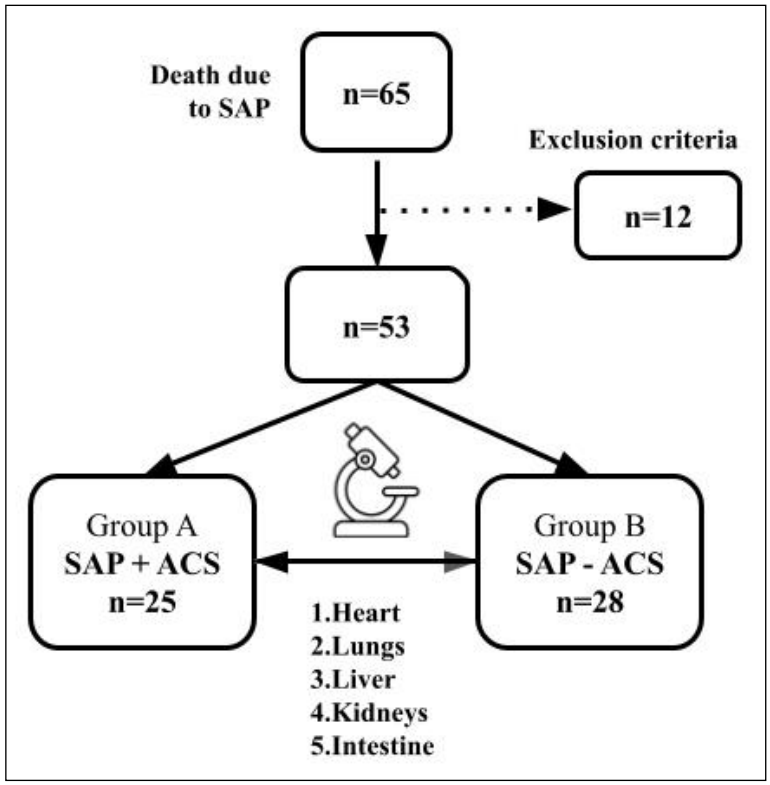

Fig. 1. Flowchart of the study design.

Department of General Surgery No. 1 in the Kyiv City Clinical Hospital No. 10. In the course of the given study 65 cases of patients with the main diagnosis of severe acute pancreatitis (K-85) were selected, who died in the early phase of the disease (up to 14 days from the onset of complaints) due to progressive multiorgan failure in the period 2017-2023. The diagnosis and severity of acute pancreatitis (AP) in patients were determined according to the criteria of the 2012 revised Atlanta classification [10].

All patients underwent autopsy in the pathology department, followed by pathologists' description of the microscopic changes in the selected sectional material. The obtained descriptions of the organs were stratified into four levels of pathological changes.

Out of 65 case histories, 12 patients were excluded, resulting in 53 patients included in the study groups. Criteria for exclusion from the study were the following:

- patients without an autopsy due to the recorded refusal of relatives/legal representatives to perform an autopsy (N=5);
- patients with significant chronic pathology that distorted the morphologic assessment (liver cirrhosis, chronic kidney disease, chronic obstructive pulmonary disease, significant cardiosclerosis, etc.) (N=7).

The study included 33 (62%) men and 20 (38%) women, with a mean age of 55 ± 14.5 years. The median (QI - QIII) Charlson comorbidity index was 3 (2-5) points. The median (QI - QIII) time from onset to hospitalization was 8 (7-12) hours. The median (QI - QIII) length of stay in hospital was 10 (8-12) bed days, i.e. the period from the moment of hospitalization to the moment of death. Of 53 patients included, 19 were diagnosed with AP of alcoholic origin (36%), 14 (26%) with biliary pancreatitis, 10 (19%) with hypertriglyceridemic pancreatitis, 3 (6%) with postoperative pancreatitis, and 7 (13%) with idiopathic acute pancreatitis.

The average BMI was 31.8±4.2 kg/m^2. Comorbidities were mainly represented by cardiovascular disease and chronic liver disease, whereas arterial hypertension was observed in 41 (77%), steatohepatosis in 39 patients (73.5%). Obesity was also common in the cohort - 34 (64.2%) cases. Diabetes mellitus was observed in 8 (15%) patients. The median (QI - QIII) length of hospital stay (duration of CAP from hospitalization to patient death) was 10 (8-12) days.

The selected patients included to the study were divided into two groups:

Group A: severe acute pancreatitis complicated by abdominal compartment syndrome (n = 25); Group B: severe acute pancreatitis not complicated by abdominal compartment syndrome (n = 28).

The study design, represented in the form of a flowchart, is shown in Fig. 1.

The level of IAP in patients was recorded in each group in the medical records every 8-12 hours of ob-

Table 1. Comparative characteristics of clinical and epidemiologic data of patients in group A (SAP+ACS) and group B (SAP - ACS)

Parameters		Group indicators		P
		ACS "+" (A)	ACS "-" (B)	
Age, years,X ± SD		53,7 ± 12,8	56,5 ±15,9	0,47[a]
Gender, n (%)	Men	18 (72)	15 (54)	0,25[b]
	Women	7 (28)	13 (46)	
BMI, kg/m2,X ± SD		32,2 ± 4,3	31,4 ± 4,1	0,51[a]
Comorbidity index, points, Me (Q_I - $Q_{(III)}$))		3 (3 - 5)	4 (3 - 5)	0,6[c]
Etiology, n (%)	Alcoholic	8	11	0,89[b]
	Biliary	8	6	
	GTG-associated	5	5	
	Postoperative	1	2	
	Idiopathic	3	4	
Time to hospitalization, hours, Me (Q_I - $Q_{(III)}$))		8 (7 - 12)	8 (6 - 12)	0,67[c]
Length of inpatient stay, bed days, Me (Q_I - $Q_{(III)}$))		9 (8 - 11)	11 (8,75 - 12.25)	0,015[c]

a - Student's t-test
b - Fisher's exact test
c - Mann-Whitney U test.

Table 2. Comparison of the levels of organ damage between groups 1 and 2 (Mann-Whitney U-test)

Body	Level of change	Group A, n (%)	Group B, n (%)	p-value
Heart	Absent	4 (16%)	6 (22%)	0,48
	Mild	16 (64%)	18 (64%)	
	Moderate	4 (16%)	4 (14%)	
	Expressed	1 (4%)	0	
Lungs	Absent	0	0	0.14
	Mild	10 (40%)	6 (22%)	
	Moderate	13 (52%)	18 (64%)	
	Expressed	2 (8%)	4 (14%)	
Liver	Absent	0	2 (7%)	0,09
	Mild	7 (28%)	12 (43%)	
	Moderate	17 (68%)	13 (46%)	
	Expressed	1 (4%)	1 (4%)	
Kidneys	Absent	0	1 (4%)	<0,001
	Mild	0	8 (28%)	
	Moderate	11 (44%)	18 (64%)	
	Expressed	14 (56%)	1 (4%)	
Intestines	Absent	0	9 (32%)	<0,001
	Mild	3 (12%)	17 (61%)	
	Moderate	16 (64%)	2 (7%)	
	Expressed	6 (24%)	0	

Note: All results were considered statistically significant at $p < 0.05$

servation. The classical indirect method was used to measure intra-abdominal pressure [11]. Group A (SAP, complicated by ACS) included patients with a recorded IAP level above 20 mmHg.

The study used archival data on the results of sectional examinations of the given patients, and the autopsy was performed at the Pathological and Anatomical Department of the Kyiv City Clinical Hospital No. 10. Sectional material was collected no later than 6 hours after the death of the patients. Pathological changes were assessed under a light microscope after hematoxylin-eosin staining.

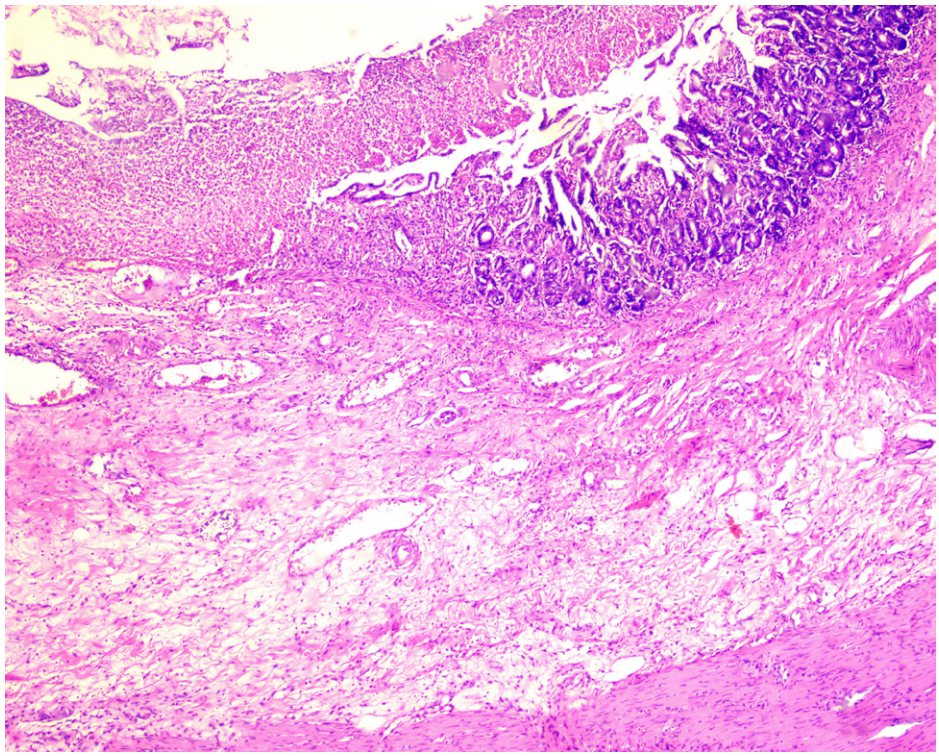

Fig. 2. Small intestine microscopic specimen with moderate changes. Small intestine wall with focal necrosis of the mucosa and leukocyte infiltration. Explicit submucosal edema with venous fullness. Magnification: 4x10, hematoxylin-eosin stain.

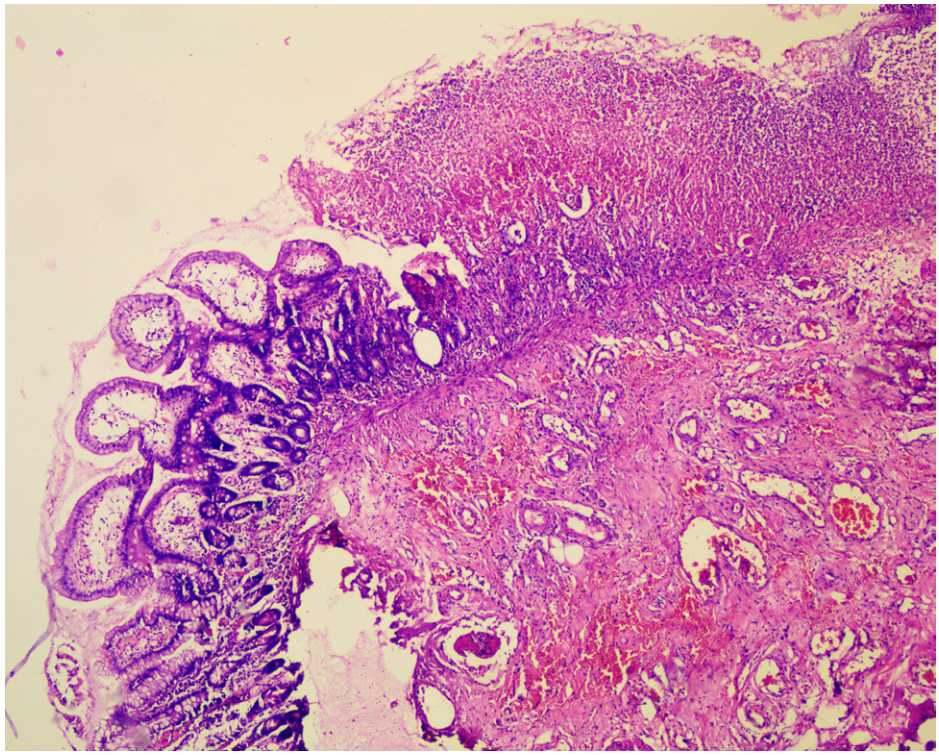

Fig. 3. Small intestine microscopic specimen with moderate changes. The wall of the small intestine with severe edema and mild leukocyte infiltration of the villi stroma, venous hemorrhage of the submucosal layer, and an acute ulcer with inflammatory infiltration of necrotic masses. Magnification: 10x10, hematoxylin-eosin stain.

In case with the morphological study, tissue pieces were fixed in a 10% solution of neutral formalin, subjected to standard paraffin wiring through alcohols of increasing concentration (70%, 80%, 96% (1), 96% (2) alcohol), xylene 1, xylene 2, "porridge" (xylene + paraffin 50/50) 1, "porridge" 2 (xylene + paraffin 50/50), paraffin 1, paraffin 2, and then embedded in paraffin. Serial sections of 4-5 mm thickness were made from the blocks prepared in that particular way.

The endpoints of the study were pathomorphologic microscopic changes in the heart, lungs, kidneys, liver, and intestines. Records of the examination of named organs were selected for the study from the documented autopsy data and microscopic evaluation, where the severity of edema, inflammation, ischemia, and necrosis was assessed. To assess objectively and compare morphologic changes in organs in patients of the two

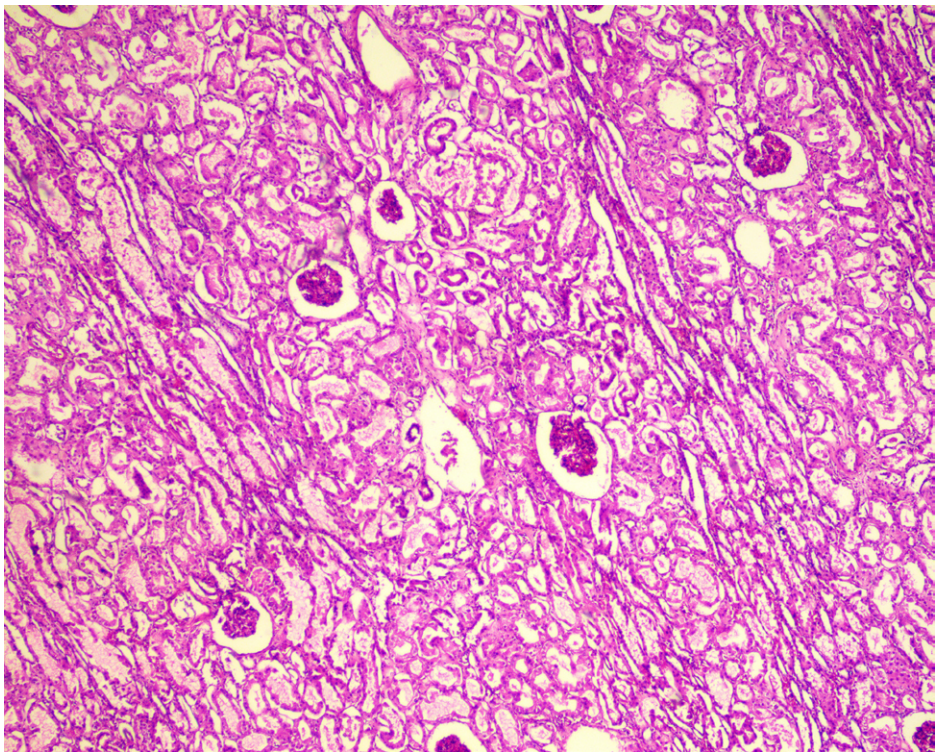

Fig. 4. Microsection of the kidney with a mild level of changes. Interstitial edema of the kidney with granular dystrophy of the convoluted tubule epithelium and glomerular hemorrhage: 4x10, hematoxylin-eosin stain.

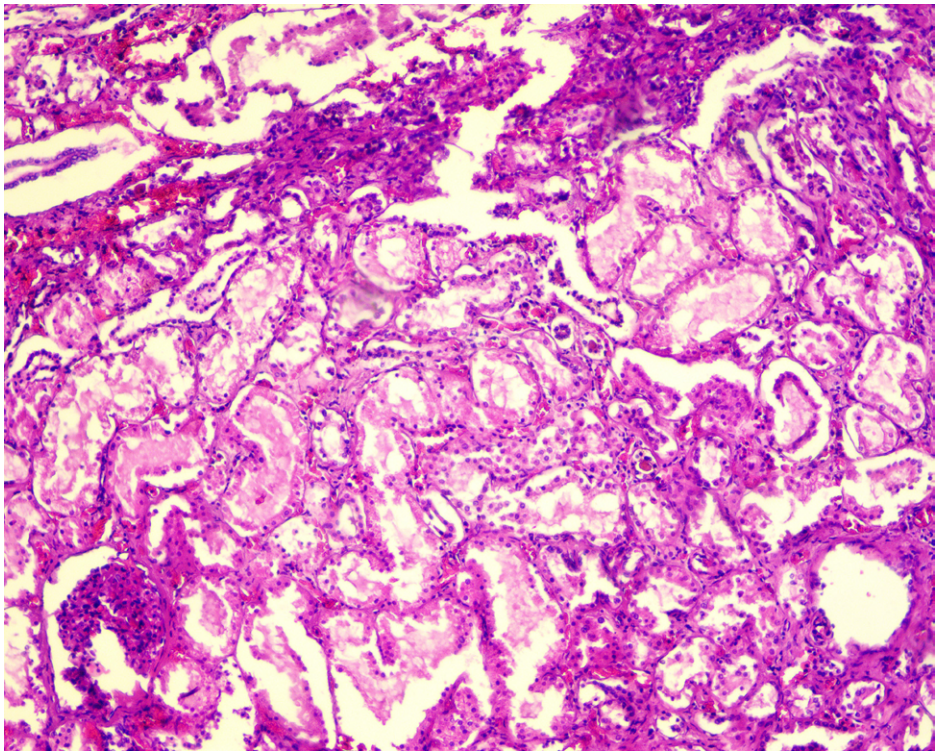

Fig. 5. Microscopic specimen of a kidney with moderate changes. Granular dystrophy of convoluted tubules and foci of vacuolar dystrophy. Magnification 10x10, hematoxylin-eosin stain.

groups, changes in organs were reviewed according to the proposed levels of damage.

1. Heart: assessment of cardiomyocyte necrosis, inflammatory infiltration and fibrosis, depending on the severity of the changes, in accordance with the four levels (0 - no changes, 1 - mild changes, 2 - moderate changes, 3 - severe changes).
2. Lungs: assessment of the severity of interstitial edema, alveolar collapse, necrosis and inflammatory infiltration, depending on the severity of the changes, in accordance with the four levels.
3. Liver: assessment of venous congestion and edema, necrosis in different zones of acinus, depending on the severity of the changes, in accordance with the four levels.
4. Kidneys: assessment of venous stasis, dystrophy, tubular necrosis in accordance with the four levels.

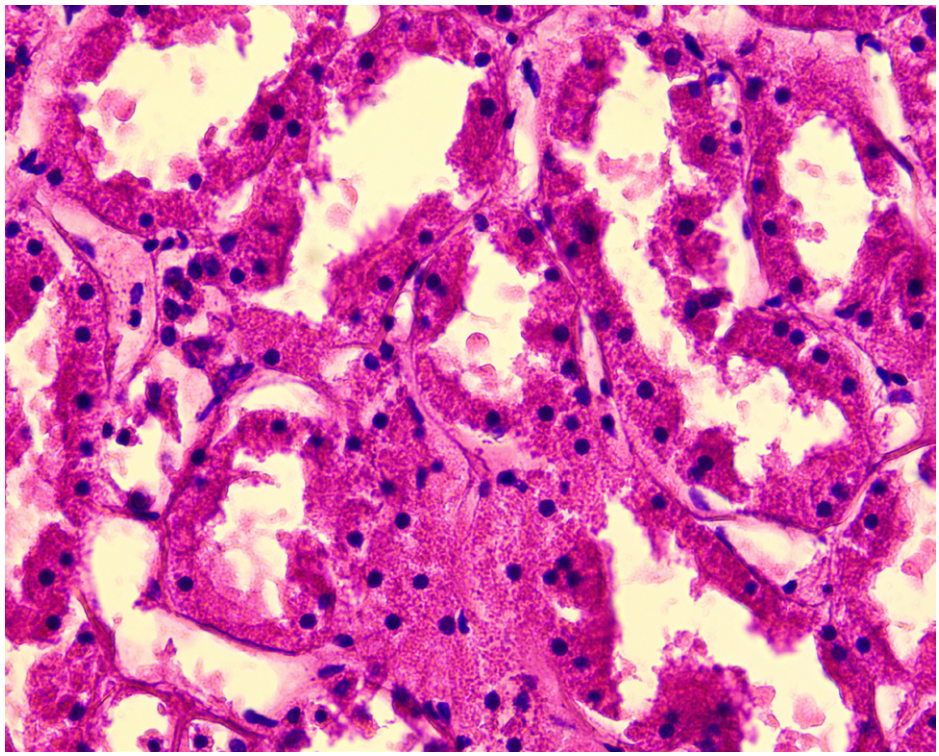

Fig. 6. Microscopic specimen of the kidney with a severe level of changes. Irreversible processes in the form of hyaline droplet dystrophy. Magnification 20x10, color: hematoxylin-eosin.

5. Intestines: assessment of the severity of ischemic changes in the intestinal mucosa, venous congestion and inflammation, in accordance with the four levels.

STATISTICAL PROCESSING

The mean value ± standard deviation was used to present quantitative data in a normal distribution. The median and interquartile range (IQR) were given in a distribution other than normal. As for qualitative data, they were absolute numbers and percentage. Given the type and distribution of data and the limited number of observations, nonparametric methods of analysis were chosen to compare pathomorphologic changes in the study between the two groups of patients. The Mann-Whitney U-test was used to test the hypothesis on the differences between the two independent groups in the level of damage between the selected organs (heart, lungs, liver, kidneys, intestines). All results were considered statistically significant at a p value < 0.05. The standard Microsoft Excel 365 package was used to record the data from the medical records of the selected patients. The EZR (R-statistics) package was used to calculate and analyze the data [12].

RESULTS

When comparing the clinical and epidemiological data shown in Table 1, we found no statistically significant difference between patients in the two groups, except for the period from hospitalization to death: on average, patients in group A - acute severe pancreatitis complicated by abdominal compartment syndrome lived less ($p<0.05$).

The microscopic evaluation of the organs examined in the two groups of patients revealed different patterns described by pathologists according to the proposed scale. Table 2 shows the comparative characteristics of the degree of pathomorphologic changes in the organs for the two groups: those who died of SAP with ACS (group A) and those who died of SAP with no ACS (group B).

In the assessment of cardiac injury, mild changes were observed in group A in 64% of patients, and similarly in 64% of patients in group B. In the assessment of lung damage, moderate changes were the most common in both groups (52% and 64%, respectively). When assessing microscopic changes in the liver, moderate changes also prevailed in the groups (68% and 46%, respectively). The microscopic description of kidney damage in group A showed 56% of severe changes and 44% of moderate changes, with no patients without any changes, while in group B, the kidneys were moderately affected in 64% of patients. When describing changes in the intestinal wall, moderate changes (64%) prevailed in group A and mild changes (61%) in group B.

A statistically significant difference between the groups in the level of damage to the kidneys and intestines was obtained ($p<0.05$, Mann-Whitney U-test) when comparing the levels of damage to each organ in the two groups, according to the specified levels of change.

Thus, in the microscopic specimens of the kidneys and intestines of group A, compared to the changes in group B, significantly moderate and severe changes prevailed (Table 2). Below are microphotographs of the examined microscopic speciments of the intestine (Fig. 2, Fig. 3) and kidneys (Fig. 4, Fig. 5) of patients of both groups with moderate and severe changes.

DISCUSSION

The patterns of significant damage to selective organ systems in SAP complicated by ACS revealed in our study are consistent with previously published data, including modeling of prolonged significant IAH in animals. This underscores the importance of preventing the progression of MOF by rapidly correcting elevated IAP.

The kidneys are considered a target organ in case of critical increase in IAP due to early clinical manifestations of morphological changes [13]. Abdominal compartment syndrome and SAP are etiologic factors of pre-renal acute kidney injury (AKI) resistant to fluid resuscitation, and prolonged IAH with hypoperfusion leads to damage to the tubular epithelial cells, which leads to the adhesion of internal (structural) AKI [14]. The mortality rate from AKI in SAP has decreased by about three times as of 2018 [15]. AKI in SAP occurs as a result of a decrease in the volume of circulating fluid from the vascular space caused by fluid extravasation, accompanied by a complex interaction of inflammatory, vascular and humoral factors [16].

The intestine is extremely sensitive to ischemia caused by increased IAP due to its anatomical and physiological features: a decrease in mesenteric blood flow is caused by arterial vascular compression and venous stasis. Intra-abdominal hypertension significantly reduces microcirculatory blood flow in the intestinal mucosa, increases intestinal permeability, leads to endotoxemia and irreversible mitochondrial damage and necrosis of the intestinal mucosa. [17] Severe abdominal microcirculatory disturbances may remain masked during successful stabilization of systemic blood pressure and cardiac output. [18] Artificially induced intra-abdominal hypertension in pigs with a decrease in abdominal perfusion pressure by 12-18 mmHg, reduced intestinal mucosal blood flow by 45-63% and diuresis by 50-80%. [19] In the experiment on female rats, induced intra-abdominal hypertension significantly worsened the histological picture of the colon mucosa, mucosal permeability and the balance of pro- and antioxidants, and an increase in IAP even below threshold values also demonstrated negative effects [20].

CONCLUSIONS

1. Pathologic changes in the studied organs of the group of patients, who died due to acute severe pancreatitis complicated by abdominal compartment syndrome, were characterized by more pronounced ischemic and inflammatory damage in the kidneys and intestines ($p<0.001$).
2. Pathologic changes in the heart, lungs and liver of patients, who died of acute severe pancreatitis, did not differ significantly, depending on the presence of abdominal compartment syndrome. ($p > 0.05$)
3. According to the results of the study, the variability of extra-pancreatic pathomorphologic changes in patients with severe acute pancreatitis depended on the level of intra-abdominal pressure. Complicated by abdominal compartment syndrome, severe acute pancreatitis led to the most pronounced irreversible changes in organs that are extremely sensitive to intra-abdominal pressure. Monitoring and timely correction of intra-abdominal hypertension aimed at preventing abdominal compartment syndrome in patients with acute severe pancreatitis may affect the course of the disease and the outcome of treatment.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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

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ORIGINAL ARTICLE

CONTENTS

Assessment of olfactory and gustatory dysfunction in post-COVID dentists by a modified CCCRC

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ABSTRACT

Aim: This study aimed to evaluate the prevalence of sense loss and their deterioration within and post infection with COVID-19.

Materials and Methods: A systematic informative questionnaire was filled out for each participant. Chemosensitive symptoms were recorded by using n-butyl alcohol and environmentally realistic odorants and calculated in a modified way for the detection of olfactory function. The gustatory test scoring was done using the four standard primary tastes.

Results: Out of 133 participants, dentists were more commonly females (81, 61%) with the majority below 35 years of age (97, 72%). Half of the participants experienced smell and/or taste loss during the COVID-19 pandemic; whereas almost all reported having had olfactory dysfunction (132, 99.2%) and (105, 78.2%) gustatory dysfunction. Males reported a higher significant rate of taste loss (P=0.009) and females showed a higher recovery rate within the first two weeks after the onset than males. The Pfizer vaccine showed a significantly more frequent smell loss than other vaccinations (P=0.038).

Conclusions: Gender variation was noticed with taste and smell loss. Females recovered faster from dysfunction. Pfizer fully vaccinated participants were more prone to lose smell compared to others.

KEY WORDS: healed dentists, smell, and taste dysfunction, CCCRC

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ABBREVIATIONS

CCCRC: Connecticut Chemosensory Clinical Research Center
TLR: Toll-Like Receptors

INTRODUCTION

Hyposmia, anosmia, and hypogeusia, ageusia were some of the symptoms of COVID-19 infection [1]. The sudden onset of these symptoms was recognized by the European Centre for Disease Prevention and Control and WHO [2]. In Iraq, the first case of COVID-19 appeared in February 2020 [3] with a careless recording of these olfactory/gustatory symptoms. COVID-19 infected cells through the angiotensin-converting enzyme-2 protein (ACE-2) receptor, along with (Transmembrane protease, serine 2) TMPRSS2 [4]. Olfactory sensory neurons and mitral cells in the olfactory bulbs do not express ACE-2, but sustentacular and microvillar cells in the olfactory mucosa do. ACE-2 receptors are expressed in the basal region of filiform papillae on the tongue [5]. COVID-19 was caused by either conductive and/or sensorineural disorders. Obstruction anosmia occurs in 95% of patients and recovers within one month; while neuroinvasive anosmia related to coronavirus causes direct infection, injury, and death of neuronal cells [6]. In COVID-19, sustentacular cells are infected, leading to immune cell infiltration and desquamation of the olfactory epithelium, and cilia loss causing anosmia symptoms. Similarly, activation of toll-like receptors (TLR) and interferon (IFN) receptors in taste buds by inflammatory cytokines may limit taste cell regeneration, resulting in ageusia. However, positive ACE2 signals in inflammatory cells are expressed in less than 20% of cases [7]. Chemical senses during COVID-19 can be assessed subjectively using questionnaires or objectively using standardized tests like the Connecticut Chemosensory Clinical Research Center (CCCRC) through quantitative threshold determination and semiquantitative odor identification [8]. Olfactory and taste disorders in COVID-19 appear to differ from other post-viral olfactory disorders, with symptoms starting suddenly [9]. Dental and oral health

professionals are at greater risk for several occupational hazards and harmful agents, biological agents living in patients' saliva and blood, contaminated instruments, and bioaerosols in their working environment [10]. This study's hypothesis was to detect the deterioration of both senses after the pandemic and to assess the prevalence of this dysfunction among dentists even if this loss of sense had not occurred during early symptoms of infection using a modified CCCRC test.

AIM

This study aimed to evaluate the prevalence of sense loss and their deterioration within and post infection with COVID-19.

MATERIALS AND METHODS

SAMPLE COLLECTION

According to STROBE guidelines for cross-sectional studies [11], this study was conducted on Iraqi volunteer dentists after a thorough explanation of the study aims and procedures from Baghdad City working in health centers and clinics, from 9 March-21 September 2022 after receiving approval from the Ethics Committee for Research from the College of Dentistry, University of Baghdad, Baghdad, under protocol number (460722). A consent form was signed. Dentists with previous surgery, radiotherapy, pre-existing alterations of smell and taste, ahead trauma, allergic rhinitis, chronic rhinosinusitis, and psychiatric disorders were excluded by one examiner, a questionnaire (was validated by experts and included demographic information, medical history, COVID-19 infection status, onset, duration, chemo-sensitive symptoms, and vaccination date and type) was filled.

OLFACTORY FUNCTION ASSESSMENT

The CCCRC was used to assess the olfactory threshold using n-butyl alcohol (l-butanol) as the odorant [12], the average of both nostril scores was done. All scores 7 and higher were scored as 7 for each test and expressed as a composite threshold (0-50). Identification: environmentally realistic odorants are particularly suitable and have three necessary ingredients: odorants or trigeminal stimuli and distractors e.g., garlic [13]. The second correct answer canceled a previous error [14]. An average of both nostrils was recorded (0-7) and expressed as composite identification (0-50) [15]. When the average threshold or identification resulted in a number not recorded by (Cain, 1988) Ex. (average threshold=4.5; and identification score=3.5) in which composite score was put? For this reason, a modification in the threshold, identification, and sum scores was done in this study, to prevent the loss of accurate results for both nostrils reading and an accurate final score.

GUSTATORY FUNCTION

Four primary tastes: sweet, salty, sour, and bitter were used [16], scoring from 0-4; 0-ageusia and 4-normal [17].

STATISTICAL ANALYSIS

Data was expressed using mean/standard deviation and frequency/percent according to the type of the variable. Chi-square test and Fisher's exact test were used alternatively to assess the relationship between categorical variables. Correlation tests were used to assess the strength and direction of relationship between the studied variables. A confidence level of 95% with P-value equal or less than 0.05 was considered significant, by using SPSS version 26.

RESULTS

DEMOGRAPHY AND CLINICAL FINDINGS

Out of 133 Iraqi dentists who were included in this study, sex, age mean and groups, and habits like smoking were all recorded in Table 1, show the relation between Age and sex with social findings beside the descriptive data.

Information regarding infection status, frequency, duration from the last infection, loss of smell and taste as early signs and symptoms, and the recovery time were recorded from the questionnaire, Table 2.

Loss of smell and taste sensation as early symptoms of infection were found in 32% out of 67 participants with a history of loss, Table 3. A significant relation and positive correlation varied from weak to relatively strong were detected between variables, in Table 3.

CONNECTICUT CHEMOSENSORY CLINICAL RESEARCH CENTER TEST (CCCRC)

For all participants, an objective clinical assessment was done; the majority showed the score (2-3.9) of the olfactory threshold, and the score (1-2.9) of olfactory identification. The sum composite scores of both threshold and identification resulted in a score (>10-40). This was represented clinically as severe hyposomnia, Table 3.

Table 1. Demography and social findings

variable		Frequency	percent	p-value	p-value	p-value
Gender	Male	52	39%	Chi Seq. between gender systemic dis. & age groups	Chi Seq. between gender smoking & age groups	Chi Seq. between gender smoking status & age groups
	Female	81	61%			
Age	Mean	31years				
	SD	±9.09				
Age groups	<=35years	97	73%	0.59	0.33	0.29
	>35 years	36	27%	0.4	0.3	0.3
Systemic diseases	Yes	24	18%			
	No	109	82%			
Smoking	Yes	26	20%			
	No	107	80%			
smoking status	Cigarette <1 pack	11	8%			
	Cigarette ≥1 pack	10	11%			

Table 2. The infection status, loss of sense, and recovery time

Infection status	Total sample	Frequency of infection			Duration from the last infection				Loss of sense			Recovery time		
History of infection	N= 133	Once	Twice	< twice	≤6 months	>6 months-1 year	>1-2 years	>2 years	Smell	Taste	Both	1-15 days	>15-30 days	>30 days
Positive PCR	80 60%	45 34%	23 17%	12 9%	20 15%	30 23%	25 19%	5 4%	11 8%	8, 6%	48 36%	36 27%	15 11%	16 12%
Negative PCR	44 33%	53 (40%)			53 (39%)				66 (50%)			66 (50%)		
No. PCR	9 7%													
r & p-value		r=-0.73 P=0.000			r=-0.44 P=0.000				r=0.2 p=0.014			r=0.4 p=0.000		
			p=0.000 (Fisher Exact)			p=0.000 (Fisher Exact)								
		Frequency loss p=0.000 (Fisher Exact) Frequency Recovery P=0.000 (Fisher Exact) r=0.4 p=0.000												
		Duration loss p=0.000 (Chi-square), r=0.33 p=0.000 Duration Recovery p=0.000 (Chi-square), r=0.44, p=0.000												
Sex Female 61%	p-value								p=0.013 (Fisher Exact) r=0.19 p=0.028			p=0.038 (Fisher exact) r=0.22 p=0.010		
Sex Male 39%														

SMELL THRESHOLD AND IDENTIFICATION ACCORDING TO AGE AND SEX

Regarding the sex a weak positive correlation was determined between composite threshold and identification with sex (Pearson correlation=0.18, p=0.049), Table 3. The mean of both threshold and Identification of the female was higher than that of males, making the female more affected by olfactory dysfunctions than the male, Fig. 1.

Table 3. The modified CCCRC test scores

CCCRC composite threshold		CCRC composite identification		Composite score threshold+ identification		Clinically	
	N=133		N=133		N=133		
0-1.9	26 (19%)	0-0.9	6 (6%)	≤10	14 (10%)	Anosmia	
2-3.9	27 (20%)	1-2.9	57 (43%)	>10-40	57 (43%)	Severe	
4-4.9	26 (20%)	3-3.9	38 (29%)	>40-60	43 (32%)	Moderate	hyposomnia
5-5.9	18 (18%)	4-4.9	18 (13%)	>60-80	18 (14%)	Mild	
6-6.9	12 (9%)	5-5.9	10 (7%)	>80-100	1 (1%)	Normo-sonmia	
≥7	24 (18%)	6-7.	4 (3%)				
Sex	Female 61%			Pearson r=0.18 p=0.049		Pearson r=0.17 p=0.041	
Sex	Male 39%						

Table 4. Gustatory dysfunction according to age and sex

Variables Severe		Hypogeusia				p-Value
		Moderate	Mild	Normal		
Age	≤ 35	11 (69%)	25 (66%)	40 (82%)	20 (69%)	0.436[1]
	>35	5 (31%)	13 (34%)	9 (18%)	9 (31%)	
Sex	Male	10 (63%)	20 (53%)	17 (35%)	5 (17%)	0.009[1]
	Female	6 (37%)	18 (47%)	32 (65%)	24 (83%)	
Infection	pos. PCR	13 (81%)	18 (47%)	35 (71%)	14 (48%)	0.012[1]
	neg. PCR	3 (19%)	14 (37%)	14 (29%)	12 (41%)	
	no PCR		6 (16%)		3 (10%)	
Frequency of infection	once	5 (31%)	11 (29%)	19 (39%)	10 (35%)	0.03[1]
	twice	3 (19%)	3 (8%)	13(27%)	4 (14%)	
	>twice	5 (31%)	4 (10%)	3 (6%)		
	none	3 (19%)	20 (53%)	14 (29%)	15 (52%)	
Duration from last infection	≤ 6 month	4 (25%)	7 (18%)	9 (18%)	5 (17%)	0.863
	6mon-1 year	6 (38%)	6(16%)	14 (29%)	8 (28%)	
	>1-2 years	3 (19%)	10 (26%)	11 (22%)	5 (17%)	
	>2 years	1 (6%)	1 (3%)	2 (4%)	1 (3%)_	
	none	2 (13%)	14 (37%)	13 (27%)	10 (35%)	
Recovery time	1-15 days	6(38%)	8 (21%)	16 (33%)	6 (21%)	0.566
	>15-30days	1 (6%)	2 (5%)	9 (18%)	3 (10%)	
	>30days	2 (13%)	5 (13%)	4 (8%)	5 (17%)	
	none	7 939%)	23 (61%)	20 (41%)	15 (52%)	

[1] Significant relation between infection, frequency of infection, and gustatory dysfunction (p=0.012, 0.03), respectively.

Regarding age, no significant correlation was detected between both age groups in this study and both threshold and identification. The mean of both threshold and identification of the age group >35 years were higher than those of the age group ≤ 35; Fig.2. The threshold and identification tests had a validity between (0.51-0.82) which was within an acceptable range of validity (0.3-0.7) and high reliability as (Alpha Cronbach's=0.879). The clinical expression of olfactory function: is shown in, Table 3. The mean of olfactory dysfunction within this study was (41.43), 95% CI= (37.92-44.94). The prevalence of olfactory dysfunction was 99.2%. A weak significant correlation was detected between olfactory dysfunction and sex, Table 4. Concerning infection status, loss of sense, and recovery no significant relation was detected with olfactory dysfunction.

TASTE SCORING

Regarding gustatory dysfunction, Table 4.

The mean taste score was (2.66), 95% confidence interval (2.50-2.84). The prevalence of the taste disorder

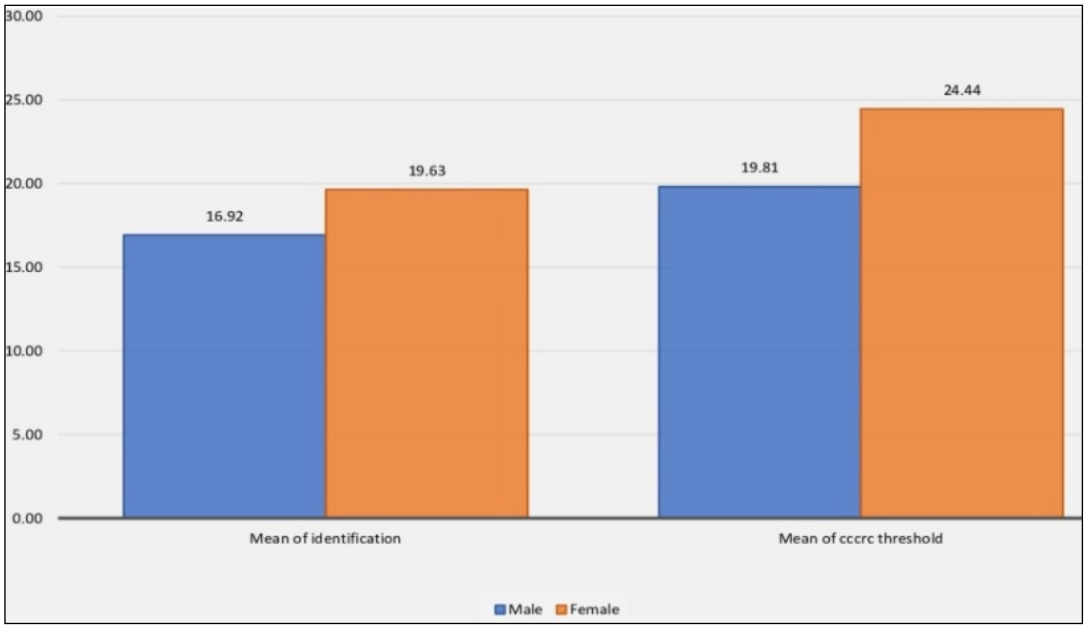

Fig. 1. Threshold and identification composite mean according to sex.

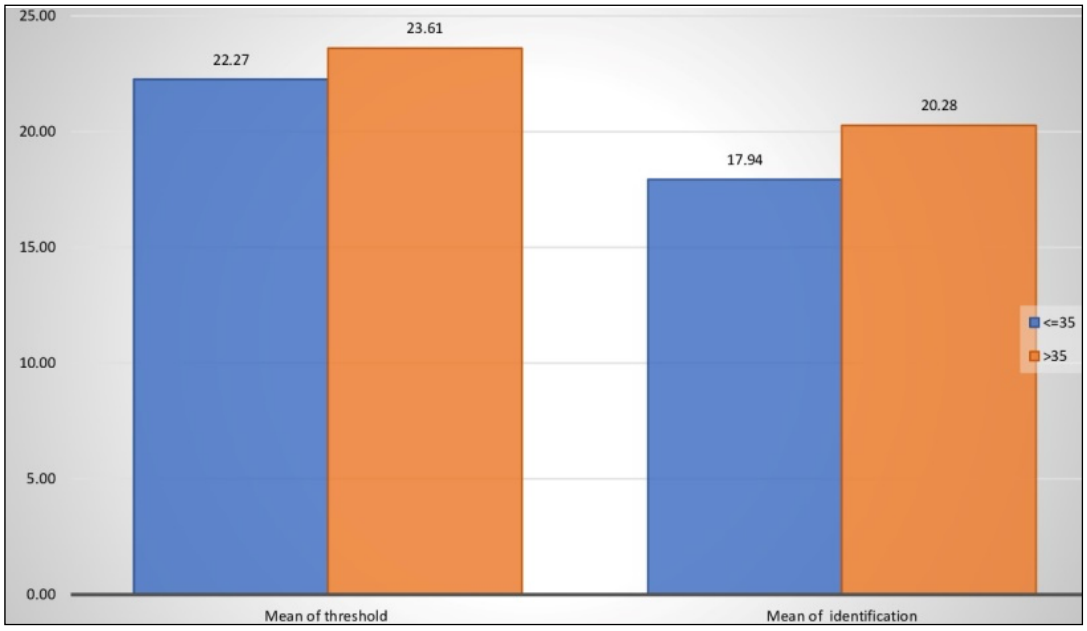

Fig. 2. Threshold and identification mean according to age.

in this study was 78.2%. There was a significant relation between taste disorder and gender (p =0.009); the male gender experienced moderate and severe hypogeusia more than the females. Age is not significantly related to taste disorder although; all types of hypogeusia in the first age group were more. There was no significant correlation between smell and taste dysfunction.

RECOVERY PERIOD WITH GUSTATORY AND OLFACTORY DYSFUNCTION:

There was a significant relation between time to recovery of both olfactory and gustatory disorder and sex (p=0.034) high percentage of the female gender recovering within the first 2weeks; Fig 3.

TYPES OF VACCINES

Medical staff received Pfizer type rather than other types of vaccines, a significant relation between Pfizer type and olfactory dysfunction (p=0.038) while no such relation with gustatory dysfunction was shown as in Table 5.

DISCUSSION

Dentists are in a high-risk group for being infected, once infected [18]. In contrast to a systematic review involving over 4000 participants from 40+ countries, our study included 133 participants from a single region. Both studies independently indicated a reduced sense of smell associated with the onset of infection

Table 5. Compare between olfactory and gustatory in relation to vaccine type

Dysfunction			Type of vaccine		
			Pfizer (83)	AstraZeneca (23)	P--Value
Olfactory	Hypo-somnia	Ansonia	6	2	0.038(Chi-Seq.)
		Sever	37	5	
		Moderate	29	10	
		Mild	10	6	
		Normal	1	0	
Gustatory	Hypoge-usia	Agusia	0	0	0.88(Chi-Seq.)
		Sever	9	3	
		Moderate	25	7	
		Mild	30	9	
		Normal	19	4	

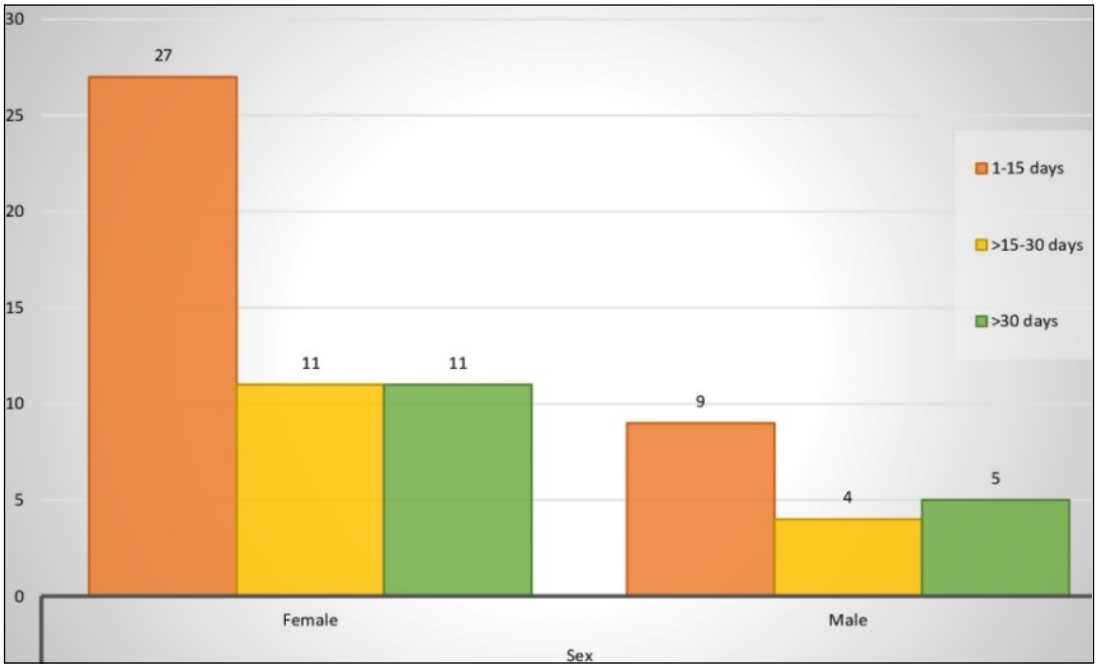

Fig. 3. Recovery time according to sex.

signs and symptoms [8]. In a post-infected study, 102 participants with demographics similar to this study (mean age: 39.1± 9.09 years, 60.9% women) were included [19]. Variation may result from differences in ethnicity, sample size, and categories like "children or adults, hospitalized, non-hospitalized, with nasal symptoms, subjective, objective assessment, and disease severity" [20]. The prevalence of olfactory and gustatory dysfunctions in this study was similar to (83%; 89%) [21], (85.6%; and 88%) [22], respectively; but was higher than that of the multi-centric case (41% and 61.2%) [21]. Objective methods 77% show higher prevalence than subjective (44%), suggesting subjective measures may miss critical COVID-19 symptoms, resulting in lower reported prevalence [1]. Geographical differences may affect symptom prevalence [23]. Higher ≤ 35 prevalence, more dysfunction in younger, non-hospitalized; younger age predisposes symptoms' appearance [24]. Milder infections in high immunity; symptoms more common in milder patients [23]; Older patients report lower CCCRC, taste scores, influenced by age-related chemoperception reductions [2].This study revealed females preferred smell and taste disturbance, with males having lower threshold anosmia. More females experienced mild dysfunction, while severe COVID-19 conditions were linked to males, possibly influenced by social factors [26]. This study identified a significant relationship between sex and gustatory dysfunction, potentially attributed to the predominantly female study population. Hyposmia significantly relates to reduced taste scores [27]. Viral invasion impairs taste buds retrogradely from an infected nerve. Anosmia results from direct virus impact on the olfactory epithelium and ACE2, expressed outside taste papillae [7]. The high prevalence of olfactory compared to gustatory is

due to the higher renewal and faster turnover rate of taste buds than olfactory neuron receptors [28]. Using odorless and colorless agents for 'Sweet', 'Sour', 'Salty', and 'Bitter' indicates viral invasion to the gustatory system, avoiding retro-nasal aroma taste due to taste bud dysfunction [27]. Smoking hurts and severe the clinical outcome, lower study smokers agrees with the study [21]. No significant association was found regarding olfactory dysfunction. Olfactory dysfunction was more common in positive COVID-19 infections, coinciding with Kurdistan Iraqi study [29]. No significant link between dysfunction and COVID-19's three waves (Jan. 2021-2022) [30]. In contrast, a significant relation was found between gustatory dysfunction and COVID-19 infection frequency, coinciding with milder forms that do not require hospitalization [31]. COVID-19 may cause dysgeusia, like ACE2 inhibitors, and cause additional damage to the olfactory epithelium [32] initiated by cytokine and antiviral storms, causing apoptosis of olfactory receptor neurons and loss of cilia and sense dysfunction [33]. Both sense's dysfunction prevalence was higher than [34] and lower than [19]. Mild COVID-19 is linked to a significant relation between this dysfunction and infection or its frequency [31]. Smell and taste disorders seem to be an early symptom, in agreement with Lauer and colleagues [18] becoming warning signs even in oligosymptomatic individuals. This aligns with Heidari et al but at a higher percentage [35], suggesting the olfactory epithelium's role as the first line of defense against viruses. In milder cases, sense defects prevent viral spread into the lower respiratory system [34]. Most participants recovered within two weeks, aligning with studies reporting a rapid symptom recovery within the same timeframe [31]. However, it is yet to occur in others [1]. In Europe and the USA, olfactory dysfunctions persisted in half of the patient's even 8 weeks post-symptom onset [20]. Virus near CNS leads to potential long-term neurological effects. Younger ages recover faster; over 40, prolonged effects, influenced by increased transmembrane serine protease 2 with age [36]. Females recover from sense loss 2:1 faster than males within two weeks. Other studies suggest longer recovery for females. Anosmia and ageusia in males usually resolve within 7 days [37]. Controversial results arise from diverse assessment methods for smell and taste dysfunction, potential sex-related inflammatory differences, ethnic variations, faster recovery in Asia, and genetic influences [38]. ACE-2 invasion causes mild temporary anosmia, while NRP1-mediated damage causes more persistent loss. Viral binding with oral mucosal cells induces inflammation, abnormal turnover, and reduced taste bud sensitivity, resulting in sensorineural dysgeusia after COVID-19 [39]. Pfizer vaccine recipients showed a significant association with olfactory dysfunction, similar to the initial finding with influenza vaccine in 0.19% of patients [40]. Post-vaccine inflammation may cause transient smell disorder. Virus presence in neuroepithelium or olfactory bulbs could lead to antibody-related inflammation, causing temporary anosmia. Serum antibodies are higher in severe COVID-19, while nasal antibodies are higher in milder cases. Investigation in vaccinated individuals is essential [41]. The study's strength is in the precise, unbiased assessment with the objective CCCRC method, ensuring accuracy through blind testing, however, the pandemic complicated finding unaffected control participants. Further studies are needed to explore sense dysfunction recovery time relating to COVID-19.

CONCLUSIONS

1. High olfactory dysfunction prevalence was found in this study in comparison to other previous studies
2. Olfactory dysfunction prevalence was higher than the gustatory one within the same sample.
3. Although there were no significant changes regarding age groups and olfactory threshold and identification, the mean of both were higher in age group >35 years old.
4. Sex disparity was detected as more severe scores of olfactory dysfunctions shown in males but higher proportion and milder form of this dysfunction in females.
5. Fast recovery from the dysfunction occurred in females.
6. High significant correlation was detected between hypogeusia and female sex, beside the positive PCR tested participants with one time of infection showed a significant relation with hypogeusia.
7. Pfizer may be more related to olfactory dysfunction as one of its complications.

LIMITATION AND STRENGTH

Study strength was that use of the objective modified CCCRC assessment for this dysfunction is more accurate and with decreased bias results due to the blind testing of the participants and precise scoring values.

Study was limited by the pandemic making it very difficult to find normal, non-infected control participants for comparison purposes.

Follow-up the sense dysfunction recovery time related to COVID-19 by using repeatable CCCRC test could be a very valuable step.

Dentists as a sample not selected in the previous studies to compare these study findings with.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Experience in clinical application of various protocols in bone insertion channel preparation for dental implants

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ABSTRACT

Aim: To analyze and compare the results of clinical usage of two protocols of bone drilling during the surgical stage of dental implantation.

Materials and Methods: The clinical study group was formed from 30 patients. All selected patients underwent dental implantation in the lateral segments of the jaws using a delayed two-stage technique, using the standard drilling protocol (1000 rpm) and slow (50 rpm). The study included measuring implants' torque during the installation, bone density, and marginal bone resorption after 1,5 years.

Results: The slow bone tissue preparation protocol showed the higher torque level during the installation, and did not cause significant changes in the structure of the bone tissue and level of sauserisation compared to the standard protocol for preparation.

Conclusions: The usage of two different protocols showed a significant difference at the moment of installation. However, it did not reveal any statistically significant differences between the two sgroups of patients during the long-term follow-up.

KEY WORDS: dentistry, implantation, bone, osseodensification, osseointegration

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INTRODUCTION

Restoration of dentition in patients with partial and complete edentulousness using dental implant-supported structures is now recognized as an effective and predictable treatment method, a serious alternative to partial and complete removable prosthetics. The basic designs of dental implants and materials for their manufacture were invented from the 60s to the 90s of the XX century and have undergone mostly minor modifications to date. But since the 90s of XX century, clinical implantation protocols have developed significantly. Knowledge has been significantly enriched regarding the process of osseointegration, long-term use of dental implants, problems of repair of orthopedic structures and "rescue-rehabilitation" of osseointegrated structures in various fractures, protocols and approaches to bone bed preparation, etc. [1-4]. In the trend of the listed research areas, the osseodensification protocol proposed by S. Huwais also looks original and promising in clinical application. The use of the osseodensification protocol showed promising results in terms of improving osseointegration, increasing bone density in the preparation area, reducing the risk of Schneiderian membrane injury and increasing the transverse size of the alveolar crest, reducing the level of bone resorption during sauserisation. A standard high-speed protocol for implant bed preparation (osteotomy, with the use of aggressive cutting surgical burs at high speeds clockwise with constant irrigation of the surgical field) leads to bone loss. The developed osseodensification protocol involved using a unique non-aggressive design of burs that prepared counterclockwise bone tissue [5-7].

The main ideas of the "adepts" of osseodensification were that such a phenomenon provides a denser and closer contact of the bone and the implant surface, which increases the torque during the insertion of the implant itself and reduces the time to achieve osseointegration of the dental implant. The osseodensification protocol increases the primary stabilization of the implant and allows for the installation of larger diameter implants compared to the standard protocol since the process of bone compaction increases the volume

of the alveolar crest. The osseodensification protocol proposed by S. Huwais was performed with modified surgical burs with the function of bone densification (reduced number of cutting edges and their design), and such burs could be used at a speed of 800-1200 rpm counterclockwise - mode without osteotomy, and clockwise – cutting mode [5,8,9].

In modern conditions, the trend towards the use of osseodensification techniques has affected a significant number of dental implantation systems, which has manifested itself in the improvement of the design of milling cutters, which are becoming more universal – at high speeds, they operate in osteotomy mode, and at low speeds – 50-70 rpm – they perform osseodensification. These modifications began to spread from 2018-2019 and, accordingly, long-term results of the use of such new surgical dental implantation protocols have not yet been accumulated. Accordingly, this area of dental implantology requires further research at various levels – from experimental to clinical [10,11].

AIM

The objectives of study were to analyze and compare the results of clinical usage of two protocols of bone drilling during the surgical stage of dental implantation – standard and slow protocols.

MATERIALS AND METHODS

The clinical study group included 30 patients (15 men and 15 women) from a private dental healthcare institution. Two clinical groups were randomly selected alternately, using the standard speed of bone tissue preparation and the slow speed. The average age of the patients was 36.10±5.68 years (M=36.50). All patients underwent intraosseous dental implantation in the lateral segments of the jaws using a delayed two-stage technique with "DENTIUM" "SuperLine NEW" system (Korea). The following bone tissue preparation protocols were used: standard high-speed - 1000 rpm at a torque of 35 N×cm with water cooling; slow – formation of the primary hole with guide mills at a speed of 1000 rpm at a torque of 35 N×cm with water cooling and subsequent use of final mills at a speed of 50 rpm at a torque of 35 N×cm without water cooling. After installation, the torque during fixation was measured using a torque wrench (three times, the average value was entered into the intermediate tables). All patients were prescribed a prophylactic course of amoxicillin/clavulanate, for the prevention of pain syndrome – ibuprofen. 6–7 months after the surgical stage, the dental implant was opened to install a gingival former. The strength of the product's fixation in the bone tissue was measured using an XNTW torque wrench – the maximum torque of the product was 70 N×cm. After a year and a half, the patients were called to the clinic, the oral cavity was examined, and the clinical status of the tissues around the structure fixed on the implant was determined. The patients were referred for a repeated CBCT (or panoramic study) of the jaws. Using archival data, the relative radiographic density of the bone tissue around the installed dental implant (at 12 arbitrary positions on the image) was compared in a computer program with the indicators of the same segment before dental implantation. The depth of bone resorption around the implant neck was measured, considering that all products were installed at the level of the alveolar bone edge. The analysis of the obtained data was carried out using Microsoft Excel 2016 and the software package "BioStat LE" (version 7.6.5), where descriptive statistics and comparative statistics methods were applied with the calculation of ANOVA and Student's criteria.

RESULTS

The analysis of the results of measuring the torque of dental implants achieved during installation showed that its level differed between groups (Table 1). Overall, the torque level during dental implant placement was 38.90±7.92 N×cm (M=39.50), with a minimum value of 29.00 N×cm and a maximum of 50.00 N×cm. In the first group of patients (where the standard high-speed implant bed preparation protocol was used), the average implant torque during placement was 31.33±1.95 N×cm (M=31.00), with a minimum value of 29.00 N×cm and a maximum value of 35.00 N×cm. In the second group, the average implant torque during placement was 46.47±1.89 N×cm (M=46.00), with a minimum value of 44.00 N×cm and a maximum value of 50.00 N×cm. Using comparative statistics methods showed a statistically significant difference between the torque values in the two groups. The value of the ANOVA criterion was 5.42×10^{-19} and the Student t-test was 3.97×10^{-11}.

At the time of opening the implants, the third of the installed products was partially covered by an overgrown cortical plate of newly formed bone tissue, which had to be carefully removed mechanically with a ball-shaped surgical bur with water cooling at minimum speed. After removing the plug screw, the internal canal of the implant was washed with a 0.05% chlorhexidine bigluconate solution, after which a manual implant driver was inserted into the implant canal, to which the torque wrench ring was fixed. The torque of the installed implant was recorded by attempting to turn the "leg" of the torque wrench - up to a maximum of 50.00 N×cm.

Table 1. Implants' torque level at moment of insertion, N×cm

Index	Group 1	Group 2	General
Torque	31.33±1.95 (M=31.00)	46.47±1,89 (M=46.00)	38.90±7,92 (M=39.50)
Min.	29.00	44.00	29.00
Max.	35.00	50.00	50.00

Note: $p<0.05$

Table 2. The level of marginal bone resorption around the implants' cervices after the one year of prosthetic stage, mm

	Group 1	Group 2	General
M±m (Median)	0.46±0.10 (M=0.45)	0.47±0.09 (M=0.45)	0.47±0.10 (M=0.45)
Min.	0.35	0.35	0.35
Max.	0.60	0.60	0.60

Note: $p=0.35$

Table 3. The average x-ray jaw bone density among patients, dHu

	Group 1		Group 2	
	Before implantation	1.5 years after implantation	Before implantation	1.5 years after implantation
M±m (Median)	719.93±43.10 (M=720.00)	722.53±27.49 (M=721.00)	710.00±28.52 (M=704.00)	713.80±20.46 (713.00)
Min.	645.00	690.00	678.00	692.00
Max.	789.00	780.00	765.00	754.00

It was determined that all the implants installed withstood such a load without rotation, regardless of the clinical group of the study. No great effort was made to avoid damaging the thread of the implant's internal canal. One year after the fixation of the orthopedic structure, the patients were examined in the clinic. In the entire study group, the state of the implant-osseous relationship around the installed orthopedic structures was satisfactory, regardless of the level of tissue keratization. An analysis of the radiographs was performed to determine the bone resorption level near the implants' neck (Table 2).

In general, in the study group, the level of bone resorption was 0.47±0.10 mm (M=0.45), the minimum value was 0.35 mm, and the maximum – 0.60 mm. In the first group, the depth of resorption was 0.46±0.10 mm (M=0.45), the minimum and maximum values were 0.35 and 0.60 mm, respectively. In the second group, the average level of sauserisation was 0.47±0.09 mm (M=0.45), the minimum and maximum values were also 0.35 and 0.60 mm, respectively. Applying comparative statistics methods did not reveal a significant difference between the two groups in the above indicator (p=0.93).

The analysis of the relative radiographic density of the jaw bone tissue in the areas of dental implantation before and one and a half years after the installation of dental implants did not reveal a significant difference in this indicator between the two groups of the study. Thus, in the first group of patients at the time of installation of dental implants, the average conditional radiographic density of bone tissue was 719.93±43.10 dHu (M=720.00), the minimum value was 645.00 dHu, and the maximum – 789.00 dHu (Table 3). After one and a half years, when approximately a year had passed since the beginning of the functioning of the dental implant as a support for the orthopedic structure, the average value of the indicator was 722.53±27.49 dHu (M=721.00), the minimum density was 690.00, and the maximum – 780.00 dHu. The comparative statistical tests did not reveal significant differences in bone density before implantation and one and a half years after the operatio. The comparative statistical tests also did not reveal any significant differences in the values before implantation and one and a half years after implantation. In group 2, the relative radiographic bone density level before implantation was 710.00±28.52 dHu (M=704.00), the minimum value was 678.00 dHu, and the maximum – 765.00 dHu. One and a half years after dental implantation, the values changed uncritically. The average density was 713.80±20.46 dHu (M=713.00), the minimum value was 692.00 dHu, and the maximum – 754 dHu.

DISCUSSION

Similarly, the same studies were performed on the conditional radiographic bone density values between two groups of patients - before the operation and one and a half years after it. The results also did not reveal statisti-

cally significant differences between the groups. Such data suggest that using both protocols for preparing the implant bed in the alveolar bone caused the same reactions from the bone tissue. The results largely coincide with the known works performed in many other countries. In the experimental work of J. Calvo-Guirado (2015) it was also shown that new hybrid protocols for preparing the implant bed (including slow, without water irrigation) in the alveolar bone tissue do not lead to significant changes in the primary stabilization of dental implants [10]. Experimental studies of bone regeneration in animals after the installation of screw-shaped intraosseous dental implants using different rapid preparation protocols by A. Sarendranath (2015) did not show significant differences in the quality of bone healing at the microscopic level [11]. Studies by H. Pellicer-Chover (2017) showed that in the clinic one year after the installation of dental implants using the rapid and "slow" protocols, there was no difference in marginal bone resorption, and the radiological structure of the surrounding bone tissue did not differ [12]. Comparative studies conducted by D. Simmons (2017) using a "soft" and standard implant bed preparation protocol showed that the implant survival rate for 1 year was 93.3% and did not depend on the preparation protocol, the level of radiographic bone resorption around the implant neck was approximately 0.5 mm and also did not differ in patients treated with different speed protocols [13]. Experimental studies by L. Witek (2019) showed that the osseodensification protocol enhances both primary and secondary stabilization (osseointegration) of tantalum intraosseous implants. Studies by A. Sultana (2020) in clinical conditions (6 months of patient follow-up) showed that osseodensification protocols have a generally favorable effect on dental implant outcomes [14]. Data from E. Pérez-Pevida (2020) on the results of using different preparation protocols for dental ceramic implants showed that with slow preparation, the levels of primary stabilization of the products are significantly higher [15]. Studies of the regenerative properties of bone tissue cells (A. Tabassum, 2020), which were collected during the preparation of the implant canal according to different protocols, showed that with a slow preparation speed, osteoblast-like cells had a higher potential for proliferation and differentiation than with a standard protocol [16]. The study by J. Bernabeu-Mira (2021) showed that the use of "slow" bone tissue preparation protocols, including osseodensification techniques, do not lead to a significant difference in the rate and intensity of wear of surgical burs for bone preparation, marginal bone resorption (sauserisation), the level of implantation success and the histomorphological structure of bone tissue after the onset of osseointegration. A clinical randomized trial of different speed protocols for alveolar bone preparation did not reveal a significant difference in the rates of marginal bone resorption 3 months after placement [7]. There were also no differences in the level of implantation success (A. Tabassum, 2021) [17]. A systematic review by X. Yu (2022) shows that three optional techniques for implant bed preparation (osseodensification, piezotomy, and the use of osteotomes) lead to a higher level of primary stabilization of dental implants [18]. A systematic review by C. Sigilião Celles (2023) indicated that no statistically significant difference was observed in the mechanical fixation of dental implants in the alveolar bone when using different high-speed implant bed preparation protocols [19]. S. Soler-Alcaraz (2023) conducted fractal measurements of bone regeneration after the use of two different speed protocols for implant bed preparation showed that bone tissue regenerated without significant differences, and the different speed protocols used did not affect the quality of implant osseointegration [20].

CONCLUSIONS

Using a slow protocol for implant channel preparation in clinical settings revealed that this approach, in addition to reducing bone tissue loss due to bone beam microabfractions and attrition, causes certain densification of bone tissue due to its condensation. The statistical calculations (comparative statistical tests) indicated the presence of a statistically significant difference between the torque values in the two groups. The slow bone tissue preparation protocol used in the clinic in the long term, one year after loading the implant with a superstructure, did not cause significant changes in the structure of the bone tissue compared to the standard protocol for preparation of the implant bed (as evidenced by the analysis of the relative radiographic density of the jaw bone tissue in the area of surgical intervention before and one and a half years after the installation of dental implants. Observation of the bone tissue resorption level in the implant's cervical area one year after the installation of the orthopedic structure (sauserisation) did not reveal statistically significant differences between the two groups of patients. Clinically, the bone tissue around the installed orthopedic structures was satisfactory in the entire study group, regardless of the level of tissue keratization. Such data may indicate that using a slow bone tissue preparation protocol without water cooling at the stage of implant bed formation allows for achieving clinical and radiographic treatment results that are identical to standard high-speed protocol with water cooling.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Clinical manifestation, laboratory and instrumental characteristics of infants born to mothers with a complicated anamnesis

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ABSTRACT

Aim: To improve early diagnosis by analyzing the pathological pattern of «mother-newborn» in newborns.

Materials and Methods: The study group included newborns with a diagnosis of "Infection specific to the perinatal period, unspecified" (P39,9, n=64), born to mothers (age 31,31 ± 2,08 years) with a complicated diagnosis and a control group (n=31) of infants.

Results: Clinical manifestations in newborns mainly included involvement of the central nervous system (57,8%), cardiovascular system (12,0%), congenital heart defects (2,8%), jaundice (11,0%), hepatosplenomegaly (5,2%), exanthema (9,0%), hypothermia (70,6%). Markers of inflammatory response confirmed an increase in the level of IL-1, a significant increase in IL-6 levels, the level of IL-8 in the studied contingent also significantly differs from the data of the control group, the level γ-IFN also exceeded the reference values by 2,4 times. Hypoxic-ischemic encephalopathy was detected in 63 infants (57,8%) and intracranial hemorrhage was diagnosed in 25 (22,9%) infants.

Conclusions: The values of cytokine profile parameters (IL-1, IL-6, IL-8, IL-10) on the first day of life varied within the reference values, but with significant differences from the values of the control group, which were 9,24; 20; 11 times, respectively. The levels of inflammatory mediators (γ-IFN, procalcitonin, neopterin, TNF-α, Pg E2) significantly differed from the data of the control group and exceeded the upper limit of reference values by 2,4; 40; 8,9; 25; 3,5 times, respectively.

KEY WORDS: newborns, infection specific to the perinatal period, early diagnosis

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INTRODUCTION

Given that the fetus develops in complex conditions of interaction with the mother's organism, if the mother has an infection, this is a risk factor for the development of severe pathological conditions of the fetus and newborn as well [1]. According to modern theories, infection of the fetus in the first trimester of pregnancy leads to the development of microcephaly, hydrocephalus, myocardial dysfunction, heart defects, defects of the gastrointestinal tract (GI), genitourinary system, skeleton, cataracts, deafness. Infection in the second and third trimesters causes hepatosplenomegaly, anemia, jaundice, hypotrophy, pneumonia, meningoencephalitis, sepsis in the fetus [2-4]. It has been established that the term of infection, the type of pathogen and the methods of its transmission can significantly affect the occurrence of clinical manifestations of intrauterine infections [5-7].

AIM

To improve early diagnosis by analyzing the pathological pattern of «mother-newborn» in newborns.

MATERIALS AND METHODS

The study group included infants with a diagnosis of "Infection specific to the perinatal period, unspecified" (P39,9, n=64), born to mothers (age 31,31 ± 2,08 years) with a complicated diagnosis and a control group (n=31) of infants. The average weight of preterm infants was 1477,69 ± 981,78 g (min – 600 g; max – 2450 g. Observation and treatment of newborns took place for 7 days (stay in the neonatology department and neonatal intensive care unit of the KNP "Uzhgorod City Maternity Hospital" of the Uzhgorod City Council).

RESULTS

Clinical manifestations in newborns mainly included involvement of the central nervous system (57,8%), cardiovascular system (12,0%), congenital heart defects (2,8%), jaundice (11,0%), hepatosplenomegaly (5,2%), exanthema (9,0%), hypothermia (70,6%) [8, 9, 10]. The most frequent components of the diagnosis were hypoxic-ischemic central nervous system damage and hypoxic-hemorrhagic central nervous sytem damage in the form of hypoxic-ischemic encephalopathy of the newborn (P91,6) in 57,8%, respiratory distress syndrome, severe respiratory distress, respiratory distress syndrome of the newborn (P22.0) – in 75,7%, intraventricular hemorrhage of various degree (P52) – 22,9%. Isolated cases of congenital pneumonia (P23) were detected less frequently – in 7,3% of newborns, neonatal meconium aspiration (P24.0) – in 2,8%. On the first day of life, jaundice (10%), gray skin color (80%), severe muscle hypotension (100%), hypothermia (95,6%), respiratory distress syndrome (75,7%), apnea (78,0%), hepatomegaly at birth (5.2%), CNS depression (47,8%), feeding problems (60%), inflammatory changes in the hemogram (100%), hypocalcemia (20,2%) and hypoglycemia (62%) were detected. Infants are often born with the background of intrauterine infection [4].

Hemogram data of the studied groups are considered in the following table (table 1).

According to the results of the hemogram, at the initial stage of the study, there are differences between the study group and the control group, except for insignificant differences between the levels of platelets (p2=0,19) and eosinophils (p2=0,34). An important point of the scientific study is the assessment of biochemical blood test parameters in infants with intrauterine infections (Table 2).

The study groups presented non-significant differences. Compared with the control group of infants, high levels of significance were observed for all studied parameters (p2<0.001). In particular, this is confirmed by a significant increase in the values of AST, ALT, Alkaline Phosphatase, Urea and Creatine Phosphokinase by 2-3 times compared with the data of the control group (p2<0,001), but within the reference values.

Indicators of electrolyte metabolism in children are considered in the following table (Table 3).

According to Table 4, no significant intergroup differences were observed compared to the control group. Also, insignificant differences were detected as for the parameter of Sodium level. Significant increases in potassium values were identified compared to the control group 5,50 ± 0,48 mmol/l, p2=0,0001). A significant decrease in Calcium levels was also found in infants compared to the control group (2,08 ± 0.20 versus 2,29 ± 0.21 mmol/l, p2<0.001).

Table IV presents the immunogram indicators of neonates.

IgM is a marker of the immune system, which provides the primary immune response. It does not pass through the placental barrier due to its large molecular structure and confirms the infection of the newborn, which is verified by our studies and presented by its increase in the group (3,68 ± 2,65 g/l versus the control group data of 0,71 ± 0,28 g/l, p = 0,96; p1 < 0,001; p2 < 0,001). On the contrary, IgG, by its structure, has the ability to overcome the placental barrier, that is, the newborn also receives maternal IgG. According to our data, there is a significant increase in the level of IgG by 2 times compared to the control group data (p2 = 0,0004).

The inflammatory response of the child's organism is a protective process that includes a multicomponent composition and diverse actions of components such as cytokines, chemokines, and other indicators of metabolic adaptation (Table 5).

According to Table 5, there is a significant increase in the level of IL-1 by 11 times (5,07 ± 1,44 pg/ml and compared to the data of the control group 0,63 ± 0,08 pg/ml, p2=<0,001), but within the reference values.

A significant increase in IL-6 levels by 24 times (22,31±14,43 pg/ml and compared with the control group of infants (0,78 ± 0,06 pg/ml, p<0,001) and by 2,2 times exceed the upper limit of reference values. The level of IL-8 in the studied contingent (8,97 ± 5,55 pg/ml) also significantly differs from the data of the control group (0,47 ± 0,09 pg/ml, p<0,001), almost 20 times, but the variation occurs within the reference range. There is also a significant difference in the values of IL-10 (17,58 ± 12,42 pg/ml from the values of the control group -1,42 ± 0,19 pg/ml, p<0,001), the ratio of which is about 11 times. The level γ-IFN (39,58 ± 39,04 pg/ml) also significantly differed (4 times) from the data of the control group (5,71 ± 0,23 pg/ml, p<0,001) and exceeded the reference values by 2,4 times. The value of procalcitonin (20,55 ± 18,51 ng/ml) significantly differed from the data of the control group (5,77 ± 0,49 ng/ml, p<0,001) and exceeded the upper reference limit by 40 times. The level of neopterin (85,71 ± 56,63 nmol/l) significantly differed (47 times) from the data of the control group (1,9 ± 0,04 nmol/l, p<0,001) and exceeded the upper reference limit by 8,9 times. The values of TNF-α levels (156,67 ± 20,45 pg/ml) significantly differed from the data of the control group (5,71 ± 0,13 pg/ml, p<0,001) and exceeded the upper reference limit by 25 times. The study of Pg E2, as one of the mediators of the inflammatory response in premature infants, also presented a significant difference in levels (1415,53 ± 172,2 pg/ml according to the groups and compared

Table 1. Hemogram results before the start of therapy (day 1)

Indicators	Studied group (n=64)	Control group (n=31)	Reliability of differences
Red blood cells, g/l	4,89 ± 0,77	5,58 ± 0,35	P<0,001
Hemoglobin, g/l	181,78 ± 33,15	196,89 ± 12,12	p=0,02
Platelets, g/l	207,56 ± 53,85	194,63 ± 20,04	p=0,19
White blood cells, g/l	20,06 ± 9,76	12,14 ± 1,15	p<0,001
Rod-nuclear neutrophils, %	7,64 ± 8,94	2,91 ± 0,93	p=0,004
Segmented neutrofils, %	55,56 ± 12,35	26,47 ± 4,41	p<0,001
Monocytes, %	12,31 ± 6,09	5,72 ± 1,69	p<0,001
Lymphocytes, %	25,88 ± 10,29	34,84 ± 3,75	p<0,001
Eosinophiles, %	3,11 ± 1,58	3,41 ± 1,15	p=0,34

Notes: p – probability of the difference between the parameters of the study and control groups.

Table 2. Assessment of biochemical parameters of the blood on the first day of life

Indicators	Studied group (n=64)	Control group (n=31)	Reliability of differences
Total protein, g/l	47,67 ± 8,44	60,99 ± 5,19	**p<0,001**
Urea, mmol/l	7,84 ± 3,44	3,59 ± 0,59	**p<0,001**
Glucose, mmol/l	4,19 ± 1,51	4,10 ± 1,02	**p=0,76**
AST, μmol/l	45,36 ± 18,75	10,22 ± 3,17	**p<0,001**
ALT, μmol/l	34,67 ± 29,17	10,49 ± 3,63	**p<0,001**
Alkaline phosphatase U/l	159,84 ± 55,74	88,99 ± 20,95	p<0,001
Creatine phosphokinase, U/l	151,74 ± 65,62	108,95 ± 9,51	**p=0,0005**
CRP, мг/л	10,40 ± 8,75	2,70 ± 1,19	**p<0,001**

Notes: p – probability of the difference between the parameters of the study and control groups.

Table 3. Parameters of electrolyte homeostasis in children (day 1)

Indicators	Studies group (n=64)	Control group (n=31)	Reliability of differences
K, mmol/l 3,5-5,5	6,53 ± 1,31	5,50 ± 0,48	p=0,0001
Na, mmol/l 135-145	139,35 ± 13,11	142,75 ± 6,8	p=0,18
Ca, mmol/l 2,2-2,6	2,08 ± 0,20	2,29 ± 0,21	p<0,001
Cl, mmol/l 101-111	106,47 ± 11,17	102,31 ± 4,20	p=0,05
Fe, mmol/l 17,9- 21,5	18,16 ± 2,70	21,36 ± 3,51	p<0,001

Notes: p – probability of difference between the parameters of the study and control groups.

to the data of the control group 379,79 ± 15,75 pg/ml, p<0,001), which was a significant difference in the data and exceeded the upper reference limit by 3,5 times.

Neurosonographic examination of brain structures was performed in all infants. Hypoxic-ischemic encephalopathy was detected in 63 infants (57,8%) and intracranial hemorrhage was diagnosed in 25 (22,9%) infants. We present the distribution of degrees of intraventricular hemorrhages in the brain in newborns according to the results of neurosonography (Fig. 1).

Intraventricular hemorrhages (IVH) are predictors of complications in prematurely born children, who, according to our data, constitute 22,9% of the studied group of infants. The number of cases of IVH of the II degree was the highest percentage: in the structure of the distribution I degree constituted 28%, II degree – 46%.

Let us consider the data of EchoCS and neurosonography in infants. This diagnostic study is performed in all infants. We present the most indicative figures of the study (Fig. 2, Fig.3, Fig.4)

Table 4. Immunogram results before treatment (day 1)

Indicators	Studied group (n=64)	Control group (n=31)	Reliability of differences
IgG, g/l 2,32-14,1	20,57 ± 16,55	9,58 ± 1,88	p=0,0004
IgM, g/l 0,03-1,45	3,68 ± 2,65	0,71 ± 0,28	p<0,001
IgE, IU/ml 0-87	1,79 ± 1,61	2,29 ± 1,66	p=0,16

Notes: p – probability of the difference between the parameters of the study and control groups.

Table 5. Markers of inflammatory response and metabolic adaptation of the child's organism on the first day of life

Indicators	Studied group (n=64)	Control group (n=31)	Reliability of differences
IL-1, pg/ml	5,07 ± 1,44	0,63 ± 0,08	p=<0,001
IL-6, pg/ml	22,31 ±14,43	0,78 ± 0,06	p<0,001
IL-8, pg/ml	8,97 ±5,55	0,47 ± 0,09	p<0,001
IL-10, pg/ml	17,58 ± 12,42	1,42 ± 0,19	p<0,001
γ-IFN, pg/ml	39,58 ± 39,04	5,71 ± 0,23	p<0,001
Procalcitonin, ng/ml	20,55 ± 18,51	5,77 ± 0,49	p_2<0,001
Neopterin, nmol/l	85,71 ± 56,63	1,9 ± 0,04	p<0,001
TNF-α, pg/ml	156,67 ± 20,45	5,71 ± 0,13	p<0,001
Pg E_2, pg/ml	1415,53 ± 172,2	379,79 ± 15,75	p<0,001

Notes: p – probability of the difference between the parameters of the study and control groups.

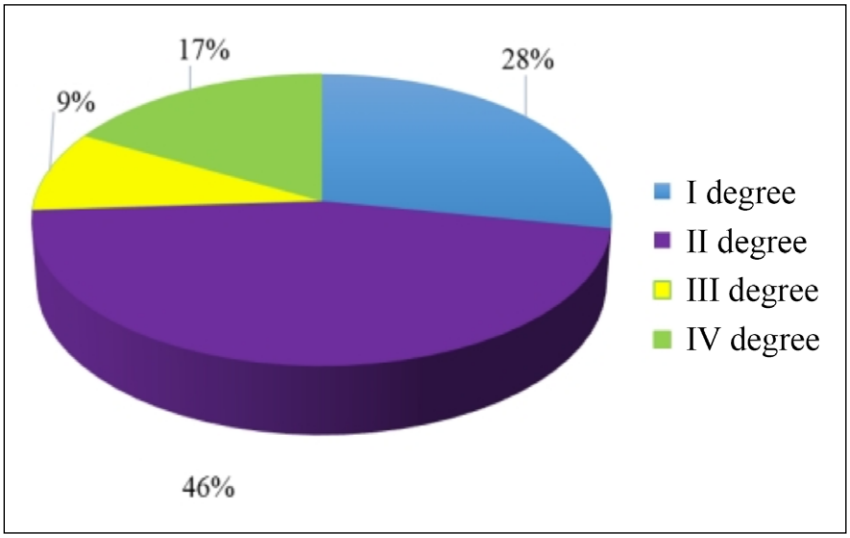

Fig. 1. Distribution of degrees of intraventricular hemorrhages in the brain in newborns, according to the results of neurosonography

It is also necessary to pay attention to the high level of hypoxic-ischemic encephalopathy in newborns (P91.6) diagnosed in our studied contingent (57,85%). Another statement, consistent with the conclusions of scientists, about the risks of mothers having a burdened history for infants [4,8].

DISCUSSION

It is known that the maternal immune system undergoes functional adaptation during pregnancy, which is considered physiological immunosuppression. This adaptation is crucial for creating a balance between the maternal and fetal immune systems, which is necessary for maintaining pregnancy itself and fetal development. When exposed to a viral infection, the balance is disrupted, the infection can spread and lead to negative consequences [9,10]. The reaction of the newborn to the influence of an infectious agent is determined by the physiological immaturity of all components that provide both nonspecific protection of the organism and its specific reactivity.

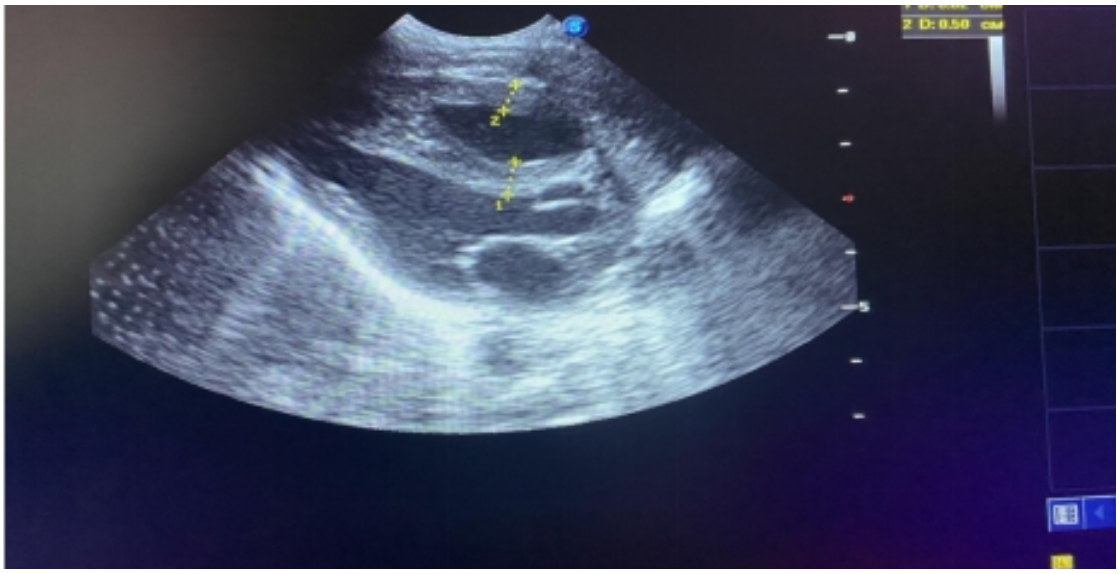

Fig. 2. ECHO of newborn M, 3 days old

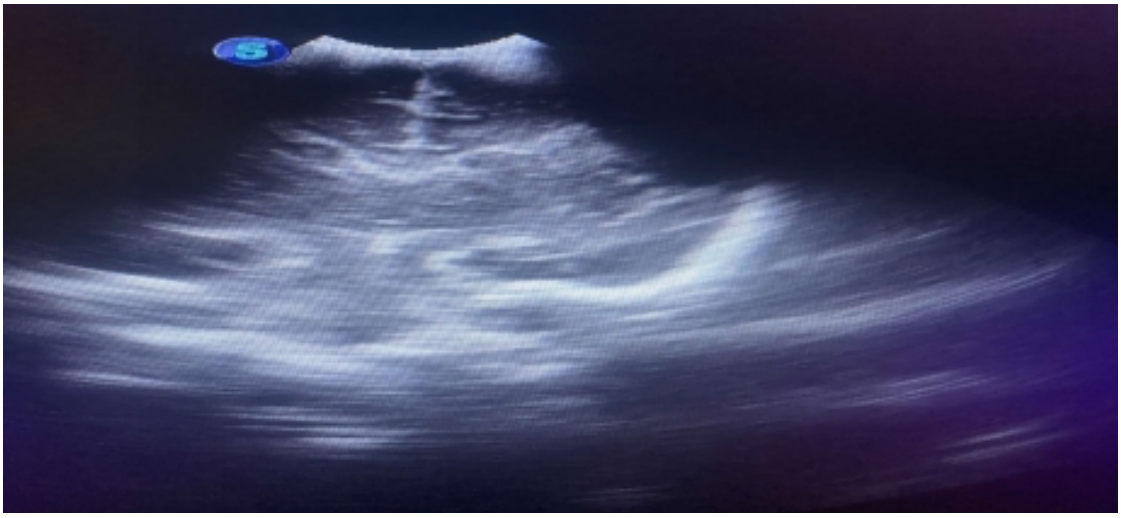

Fig. 3. Neurosonogram of newborn M, 3 days old

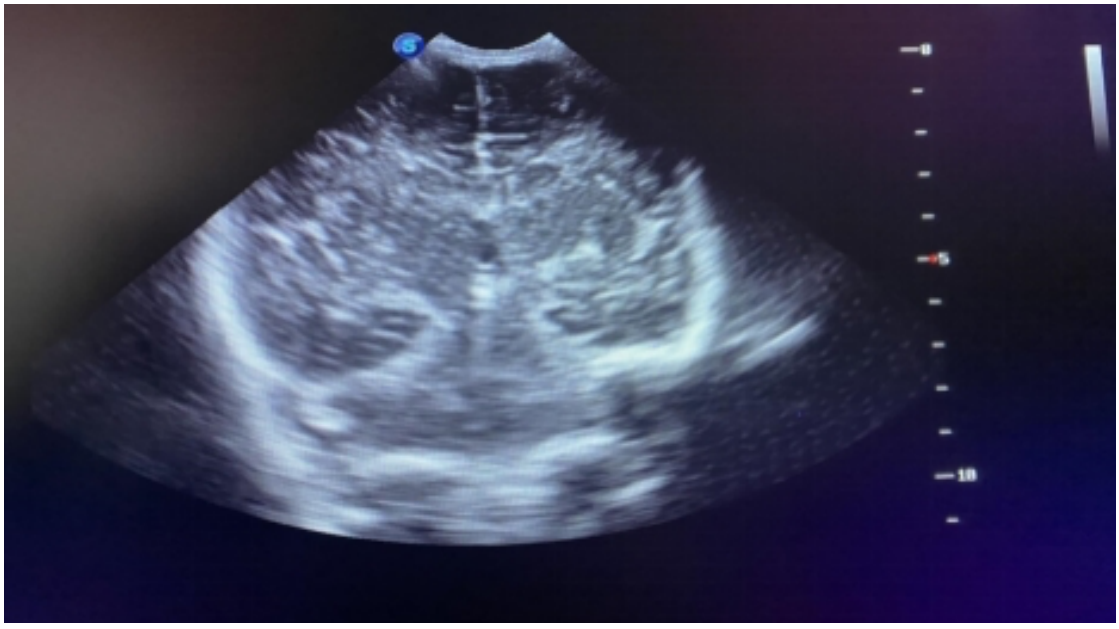

Fig. 4. Neurosonogram of newborn M, 3 days old

According to anatomical and physiological features, in infants, in particular, anatomical features of brain tissue, immaturity of autoregulatory mechanisms cause a high risk of developing central nervous system disorders in this population [11, 12].

It should be noted that ICH are not detected in all infants. Infectious and other inflammatory processes in the mother before childbirth or in the child after birth play a decisive influence on the development of the pathology. However, not all hemorrhages are associated with tissue trauma[13].

Cesarean section is one of the most common surgical measure in different countries. A WHO study showed that with a baseline cesarean section rate below 10%, maternal and neonatal mortality rates decrease if the frequency of this surgical intervention increases. If the cesarean section rate is 10–15%, then its further increase does not demonstrate a decrease in perinatal morbidity and mortality rates for infants born by cesarean section compared with infants born vaginally. Current data do not allow us to assess the association between maternal and neonatal mortality and cesarean section rates above 30%. Countries with a higher use of this intervention currently have higher neonatal morbidity and mortality rates [14]. Concerns have been raised about associations between cesarean section and a number of negative health outcomes for children [15,16].

Published studies have claimed that justified preterm delivery reduces the level of perinatal injuries. However, it should be noted that the improvement in neonatal outcomes occurs not only due to an increase in the frequency of cesarean sections, but also due to the use of modern technologies for managing the early neonatal period. [14,17].

CONCLUSIONS

1. The values of cytokine profile parameters (IL-1, IL-6, IL-8, IL-10) on the first day of life varied within the reference values, but with significant differences from the values of the control group, which were 9,24; 20; 11 times, respectively. A significant increase in IL-6 levels in both groups by 24 times (22,23 ± 14,79 and 22.31 ± 14.43 pg/ml) compared to the control group of infants (0.78 ± 0.06 pg/ml, p=0,98; p1<0,001; p2<0,001), and also by 2,2 times exceed the upper limit of reference values on the first day of life.
2. The levels of inflammatory mediators (γ-IFN, procalcitonin, neopterin, TNF-α, Pg E2) significantly differed from the data of the control group of infants and exceeded the upper limit of reference values by 2,4; 40; 8,9; 25; 3,5 times, respectively.
3. The presence of intrauterine infection of the mother and comorbid somatic pathology of an inflammatory nature cause functional changes in the cardiovascular system against the background of increased markers of inflammation in infants.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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

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ORIGINAL ARTICLE

CONTENTS

Prevention of oral diseases in the practice of primary care teams

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ABSTRACT

Aim: To analyze the use of oral disease prevention technologies by primary care physicians and nurses

Materials and Methods: A cross-sectional medical and sociological study was conducted in primary health care facilities in Ivano-Frankivsk region (western Ukraine) based on the original author's program. The study included 153 randomly selected physicians and 113 nurses who agreed to participate in the study by signing informed consent. The survey included questions about the use of evidence-based recommendations for the prevention of oral diseases by primary care providers in their medical practice and personal life.

Results: Primary care teams do not properly conduct screenings on oral cancer (40.8-41.2% of physicians and 61.1-64.6% of nurses), as well as do not advise patients on the frequency and necessity of dentist check-ups for children and adults (34. 0-36.6% and 38.1-42.5%, respectively). They do not consult patients on teeth (50.3-53.1% and 51.3-60.2%) and gums (52.3% and 54.0%) hygiene care and most of them do not have information about these issues in their healthcare facilities or on their social media pages. Half of the physicians and more than half of the nurses themselves either do not visit dentists for checkups at all (9.2% and 11.5%, respectively) or do so irregularly (39.2% and 44.2%). Every fourth doctor (26.8%) and nurse (25.7%) brush their teeth less than twice a day, most do not use flossers (65.4% of doctors and 79.6% of nurses) and interdental brushes (91.5% and 95.6%).

Conclusions: Primary care providers do not pay sufficient attention to oral diseases prevention among patients and themselves. Given the severity of the burden of oral diseases, a model for integrating basic dental prevention measures into primary health care should be developed and implemented at the state and regional levels.

KEY WORDS: oral health, primary health care, public health, prevention, oral hygiene, oral cancer screening

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INTRODUCTION

The most prevalent non-communicable diseases in the world are oral diseases, which embedded in overall health and contributes to physical, social and mental wellbeing [1-3].

The most prevalent and consequential oral diseases globally are dental caries (tooth decay), periodontal disease, tooth loss, and cancers of the lips and oral cavity [4]. Most of them are preventable, and yet, oral diseases, remain a substantial population health challenge, pose a significant public health problem and an economic burden globally [5], especially among the most vulnerable in society [6-8] in low-income and middle-income countries [4, 9]. However, dentistry has so far been unable to tackle this problem alone [3, 9].

Given the limited public funds, the most effective measures in this direction are: transformation of oral health systems away from a disease-based curative model to disease prevention [7, 10] and implementation of public health approach to the prevention and control of oral diseases at the population and individual levels [10, 11]. Equally important are measures to facilitate barriers to accessing dental care and maintaining good oral health [12], in particular, by implementing dentistry in the integrated healthcare system [9].

Given the importance of the problem of oral diseases and following a wide public consultation [2], WHO approves Global Strategy and Action Plan on Oral Health 2023-2030 [3]. Underlying the global oral health agenda are six guiding principles: a public health approach to oral health; integration of oral health into primary health care; innovative workforce models to respond to population needs for oral health; people-centred oral health care; tailored oral health interventions across the life course, and optimizing digital technologies for oral health [3].

In this document, the focus on the integration of dentistry with primary care services is extremely important, especially in countries and among people with limited access to dental care [9]. In the opinion

Table 1. Using of preventive dental technologies in the practice of primary care providers

Answer	Physicians, n=153				Nurses, n=113				p
	n	%	95% CI		n	%	95% CI		
			LL	UL			LL	UL	
Examination of the oral mucosa									
at every patient visit	58	37.9	30.1	45.7	61	54.0	44.7	63.3	
if there are risk factors	32	20.9	14.4	27.4	15	13.3	7.0	19.6	0.01461
if there are complaints	73	47.7	39.7	55.7	39	34.5	25.7	43.4	
never	5	3.3	0.4	6.1	8	7.1	2.3	11.9	
Palpation of head and neck lymph nodes									
at every patient visit	54	35.3	27.6	42.9	60	53.1	43.8	62.4	
if there are risk factors	37	24.2	17.3	31.0	19	16.8	9.8	23.8	0.00104
if there are complaints	72	47.1	39.1	55.0	31	27.4	19.1	35.7	
never	8	5.2	1.7	8.8	13	11.5	5.6	17.4	
Consultation on									
dental hygiene devices									
never	21	13.7	8.2	19.2	27	23.9	16.0	31.8	
very rarely	56	36.6	28.9	44.3	41	36.3	27.3	45.2	0.07456
sometimes	65	42.5	34.6	50.4	34	30.1	21.5	38.6	
often	11	7.2	3.1	11.3	11	9.7	4.2	15.3	
rules for tooth brushing									
never	30	19.6	13.3	26.0	21	18.6	11.3	25.8	
very rarely	52	34.0	26.4	41.6	37	32.7	24.0	41.5	0.82438
sometimes	54	35.3	27.6	42.9	38	33.6	24.8	42.4	
often	17	11.1	6.1	16.1	17	15.0	8.4	21.7	
rules for gum care									
never	34	22.2	15.6	28.9	11	44.0	24.3	63.7	
very rarely	46	30.1	22.7	37.4	9	36.0	17.0	55.0	0.86967
sometimes	64	41.8	33.9	49.7	2	8.0	0.0	18.7	
often	9	5.9	2.1	9.6	3	12.0	0.0	24.9	
frequency of dental check-ups									
never	13	8.5	4.0	13.0	12	48.0	28.2	67.8	
very rarely	43	28.1	20.9	35.3	6	24.0	7.1	40.9	0.11516
sometimes	75	49.0	41.0	57.0	2	8.0	0.0	18.7	
often	22	14.4	8.8	20.0	5	20.0	4.2	35.8	
dental prevention for children									
never	24	15.7	9.9	21.5	11	44.0	24.3	63.7	
very rarely	28	18.3	12.1	24.5	7	28.0	10.2	45.8	0.21076
sometimes	74	48.4	40.4	56.4	2	8.0	0.0	18.7	
often	27	17.6	11.5	23.7	5	20.0	4.2	35.8	

NB: 95%CI – 95% confidential interval; LL – lower limit; UL – upper limit.

of experts, primary care doctors and nurses can be involved in the prevention of dental diseases by disseminating knowledge about oral hygiene, diet and lifestyle changes among patients, providing screenings aimed at early detection of dental diseases, including cancer and precancerous diseases, referrals for dental care, etc. [7, 13-17]. At the same time, despite the considerable interest in this issue, there is still a lack of research on

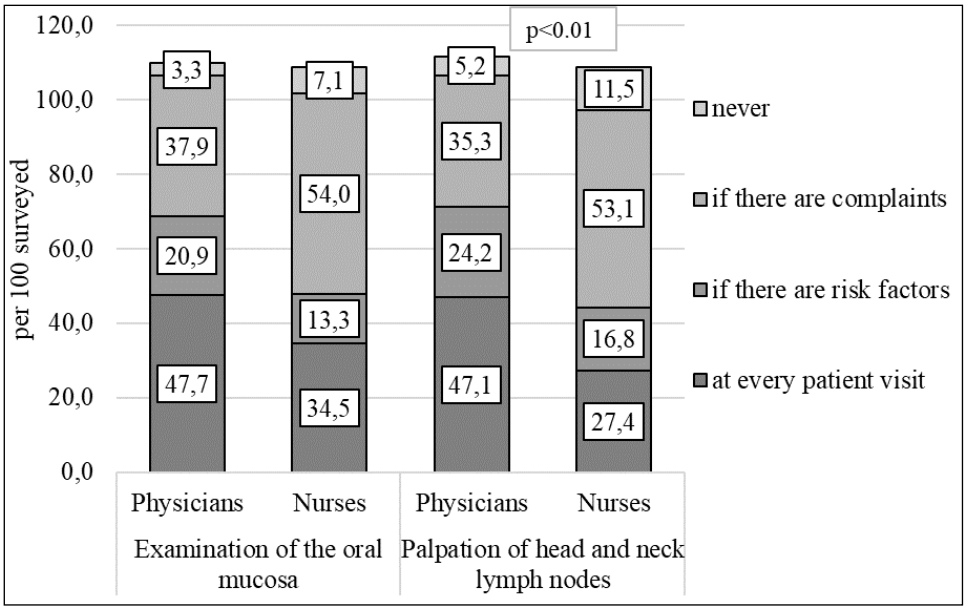

Fig. 1. Frequency and conditions of performing oral examinations and palpation of head and neck lymph nodes by primary health care providers.

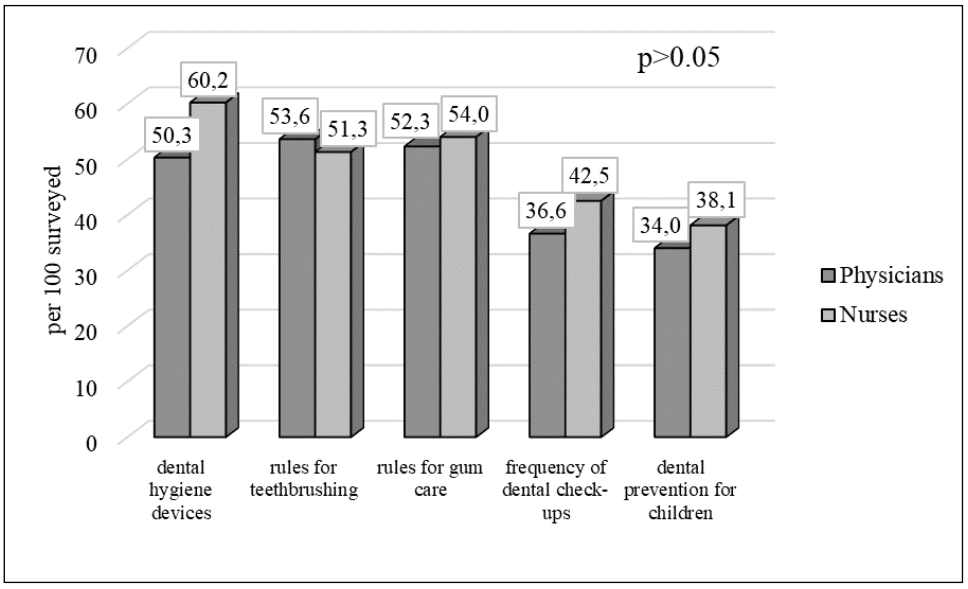

Fig. 2. Prevalence rate of negative answers of respondents regarding their consulting on different aspects of dental prevention.

models of involvement of primary care teams in the prevention of oral diseases.

AIM

The purpose of this study was to analyze the use of oral disease prevention technologies by primary care physicians and nurses.

MATERIALS AND METHODS

In 2024, a cross-sectional medical and sociological study was conducted in primary health care facilities in Ivano-Frankivsk region (western Ukraine) based on the original author's program. The study included 153 randomly selected physicians and 113 nurses who agreed to participate in the study by signing informed consent.

The survey included questions about the use of evidence-based recommendations for the prevention of oral diseases by primary care providers in their medical practice and personal life. To validate the questionnaire, it was first tested and adjusted on 15 volunteers to determine the time required to answer and the clarity of the answers.

The surveyed primary care physicians and nurses did not differ in age: the average age of physicians was 44.6±1.05 years, and of nurses – 44.8±0.99 years 44.8±0.99 years ($p>0.05$). The majority of respondents were female, and while there were only three male nurses (2.7%), there were a few more physicians – 17 (11.1%, $p<0.001$).

The design and program of the research were reviewed and approved by the Ethics Committee of Ivano-Frankivsk National Medical University (Protocol No. 129/22 of 20.09.2022).

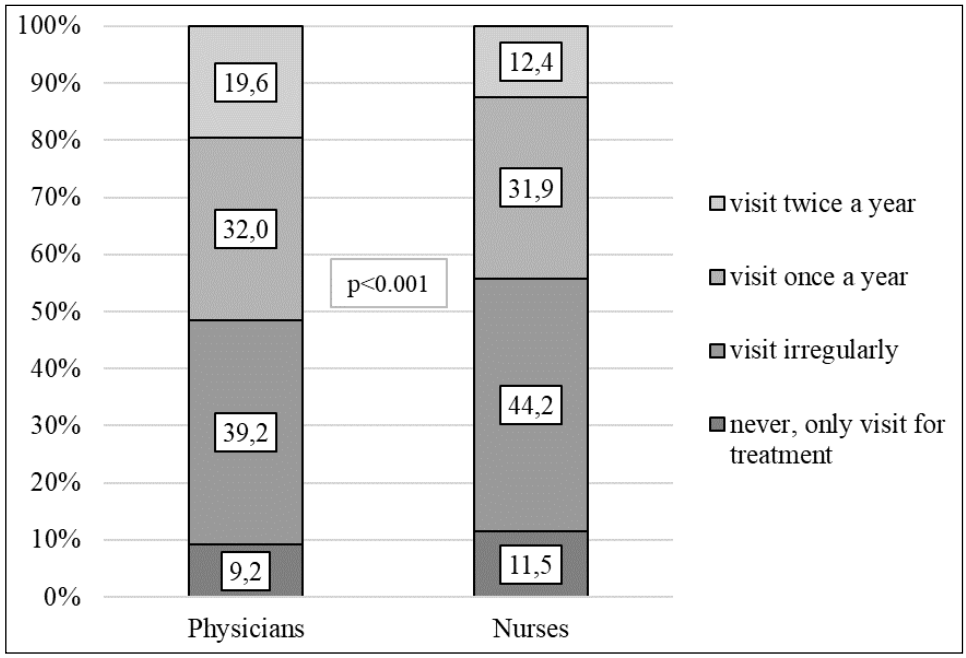

Fig. 3. Sources of information for patients about dental prevention in primary health care facilities.

Table 2. Using of preventive dental technologies by the primary care providers themselves

Answer	Physicians, n=153				Nurses, n=113				p
	n	%	95% CI		n	%	95% CI		
			LL	UL			LL	UL	
Frequency of preventive visits to dentists									
never. only visit for treatment	14	9.2	4.5	13.8	13	11.5	5.6	17.4	0.42336
visit irregularly	60	39.2	31.4	47.0	50	44.2	35.0	53.5	
visit once a year	49	32.0	24.6	39.5	36	31.9	23.2	40.5	
visit twice a year	30	19.6	13.3	26.0	14	12.4	6.3	18.5	
Frequency of tooth brushing									
a few times a week	1	0.7	0.0	1.9	2	1.8	0.0	4.2	0.66552
once a day	40	26.1	19.1	33.2	27	23.9	16.0	31.8	
twice a day	105	68.6	61.2	76.1	81	71.7	63.3	80.1	
after every meal	7	4.6	1.2	7.9	3	2.7	0.0	5.6	

NB: 95%CI – 95% confidential interval; LL – lower limit; UL – upper limit.

Statistical analysis of the received categorical data was carried out on the database created using Microsoft Excel by calculating the rate of characteristics per 100 respondents with 95% confidential interval. The reliability of the differences in the rates in different research groups (physicians, nurses) was evaluated by chi-square test (χ^2).

RESULTS

It was found that the less than half of primary care physicians perform oral examination (47.7 responses per 100 respondents) and palpation of head and neck lymph nodes (47.1%) at each patient visit, regardless of the reason for seeking medical care (Table 1, Fig. 1). In another 20.9% and 24.2% of cases, respectively, the surveyed physicians indicated that they performed these procedures when patients had cancer risk factors.

Primary care nurses chose the same answers much less than physicians. Thus, only about a third of them performed oral cavity examination (34.5 per 100 respondents, $p<0.05$) and palpation of lymph nodes (27.4%, $p<0.01$) at each patient visit. The presence of risk factors was the basis for these manipulations by the surveyed nurses in 13.3% and 16.8% of cases, respectively.

Among physicians, only a few respondents admitted that they never examine patients' oral cavities (3.3%)

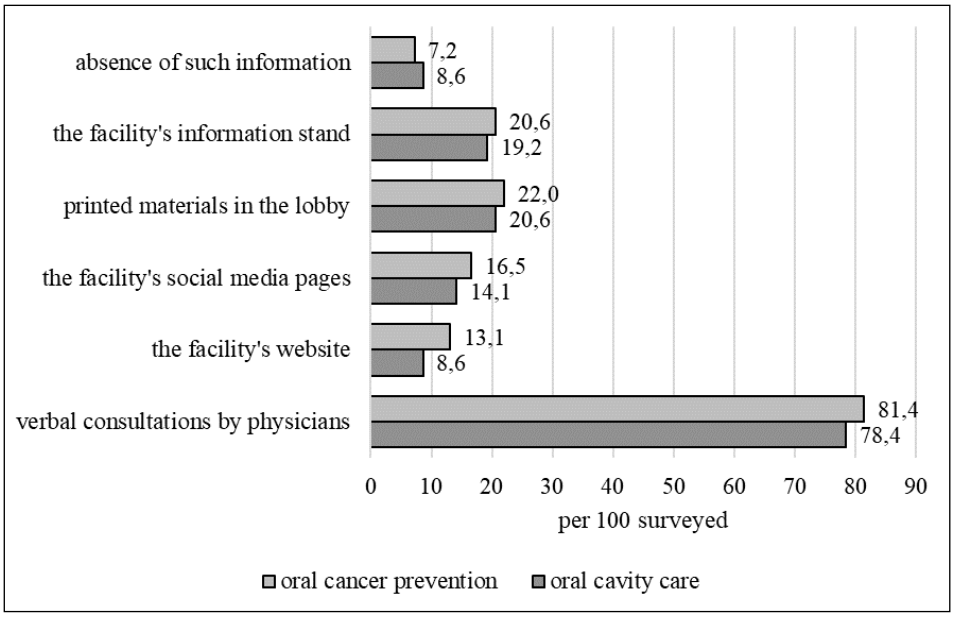

Fig. 4. Frequency of preventive visits to dentists by surveyed primary care providers.

and never palpate lymph nodes of the head and neck (5.2%). The number of such respondents among nurses was twice as high – 7.1% and 11.5%, respectively.

At the same time, about 35-40% of physicians (37.9% and 35.3%) and more than half of nurses (54.0% and 53.1%) reported they perform these procedures only if patients have complaints.

It was found (Fig. 2) that more than half of both surveyed physicians and nurses ($p>0.05$) do not or very rarely advise patients on the choice of oral hygiene devices (50.3% of physicians and 60.2% of nurses), rules for brushing teeth (53.1% and 51.3%, respectively), and gum care (52.3% and 54.0%). The rate of respondents advised their patients on the frequency of preventive visits to dentists or give advice on preventing dental diseases in children was somewhat higher. However, the share of those who do not do this was still quite significant: 36.6% and 34.0% among doctors and 42.5% and 38.1% among nurses.

The study of the possibility for patients to receive information about oral hygiene and prevention of oral cancer in the medical facilities where the respondents work showed (Fig. 3) that the majority of such information is obtained through verbal consultation of healthcare providers during patient visits (78.4-81.4%).

Only about 20% of primary care facilities place such data on information stands (19.2-20.6%) or place relevant printed materials in the lobby (20.6-22.0%). Even smaller share of healthcare facilities has such information on their social media pages (14.1-16.5%) or websites (8.6-13.1%). About 10% of facilities (8.6-7.2%) do not offer such information at all.

It was revealed that only 19.2% of primary care physicians and 12.4% of nurses visit dentists for check-ups twice a year, and another 32.0% and 31.9%, respectively, visit dentists once a year (Table 2, Fig. 4). In contrast, half of physicians and more than half of nurses ($p<0.001$) either make preventive visits to the dentist irregularly (39.2% and 44.2%, respectively) or do not make them at all and visit him/her only for treatment (9.2% and 11.5%).

Brushing teeth twice a day or more often was reported by 73.2% of primary care physicians and 74.3% of nurses ($p>0.05$), 61.4% and 51.3% of them ($p>0.05$) listen to dentists' advice when choosing toothpaste, and 36.6% and 32.7%, respectively ($p>0.05$), when choosing a toothbrush. Dental floss was used only by 34.6% of physicians and 20.4% of nurses ($p<0.01$), interdental brushes – 8.5% and 4.4%, respectively, and irrigators – 2.0% and 0.9%.

DISCUSSION

Despite the evidence-based effectiveness of oral cancer screening at the primary care level [16-17], our study found that 35-40% of doctors perform oral cavity examination and lymph node palpation only when patients complain, and a few 3-5% do not do so at all. At the same time the potential of primary health care nurses to conduct such screenings is poorly used as the same rates among them were 53-54% and 7-11%, respectively. Given that only emergency dental care for adults and dental care for children is covered by the National Health Coverage Program in Ukraine, this makes this type of care unavailable to the majority of the population, including such vulnerable groups as rural and elderly people [18]. This can result in a high proportion of oral cancer new cases being detected at

III-IV stage – 63% (2022), and share of patients lived less than one year since diagnosis at previous year – 34%, in the country [19]. Obviously, the solution to the problem could be wider involvement of primary care teams in screening for oral cancer and precancerous lesions.

The study also found that primary care teams do not sufficiently advise their patients on oral hygiene (50-52% of physicians and 52-60% of nurses) and on the need and frequency of preventive visits to dentists for adults and children (34-37% of physicians and 38-43% of nurses). While the scientific literature provides convincing evidence of the effectiveness of such information and education interventions [3, 7, 9, 15], in particular, by community nurses [13, 14]. In addition, it was found that in primary care facilities, information for patients on oral hygiene and cancer prevention is not sufficiently presented on information stands, in printed leaflets, and in social networks.

The study found that primary health care providers themselves do not sufficiently adhere to preventive recommendations for screenings and oral hygiene. Although there are ongoing discussions among scientists about the frequency of regular visits to dentists for check-ups, given the priority of the national health services towards prevention, the National Institute for Health and Care Excellence (NICE) in the UK insists in its current clinical guidelines on six-monthly dental intervals between oral health reviews [20]. Instead, there was found that only 19.2% of physicians and 12.4% of nurses comply with this. More often, but still insufficiently, respondents follow evidence-based recommendations for toothbrushing twice a day [3, 21] – 73.2% of physicians and 74.3% of nurses, as well as for choosing toothpaste (61.4% and 51.3%, respectively) and toothbrush (36.6% and 32.7%). The use of such evidence-based oral hygiene devices [22] as dental floss is also insufficient among primary care providers – a third of doctors (34.6%) and only a fifth of nurses (20.4%), and quite sporadically – interdental brushes and irrigators.

CONCLUSIONS

It was found that primary care teams do not pay enough attention to prevention of oral diseases among the population: do not properly conduct screenings on oral cancer (40.8-41.2% of physicians and 61.1-64.6% of nurses) and do not advise patients on the frequency and necessity of dentist check-ups for children and adults (34. 0-36.6% and 38.1-42.5%, respectively). They do not consult patients on teeth (50.3-53.1% and 51.3-60.2%) and gums (52.3% and 54.0%) hygiene care and most of them do not have information about these issues in their healthcare facilities or on their social media pages.

It has been shown that primary care physicians and nurses themselves do not sufficiently adhere to existing evidence-based recommendations for preventive visits to dentists and oral hygiene. Half of the physicians and more than half of the nurses in total either do not visit dentists for checkups at all (9.2% and 11.5%, respectively) or do so irregularly (39.2% and 44.2%). Every fourth doctor (26.8%) and nurse (25.7%) brush their teeth less than twice a day, most do not use flossers (65.4% of doctors and 79.6% of nurses) and interdental brushes (91.5% and 95.6%).

Given the severity of the burden of oral diseases, a model for integrating basic dental prevention measures into primary health care should be developed and implemented at the state and regional levels.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Tirzepatide therapy counters inflammatory and apoptotic responses induced by high-fat diet in rat liver

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ABSTRACT

Aim: To examine potential protective effect of Tirzepatide against obesity-induced metabolic dysfunction and hepatic inflammatory and apoptotic responses.
Materials and Methods: A total of 28 adult male Sprague-Dawley rats were employed and divided into four groups, normal control group involved seven rats fed a regular diet, while other rats received a high fat diet. Obese rats were separated into three groups after eight weeks of high fat diet: obesity, Tirzepatide (10 nmol/kg) s.c and vehicle groups, and treated for four weeks. Data regarding body weight, blood glucose, serum insulin, liver enzymes, and TNF-α, IL-1β and caspase-3 levels in the liver tissue were obtained.
Results: results revealed that Tirzepatide-treated obese rats exhibited significantly reduced body weight, blood glucose, serum insulin ALT, triglyceride, VLDL levels. Additionally, liver specimens from Tirzepatide group demonstrated lower levels of TNF-α, IL-1β and caspase-3 compared to obese untreated rats.
Conclusions: It concluded that Tirzepatide treatment mitigates the metabolic dysregulations induced by High Fat Diet, additionally; it ameliorates the inflammatory and apoptotic responses in hepatic tissue triggered by High Fat Diet.

KEY WORDS: Tirzepatide, obesity, inflammation, apoptosis, liver

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ABBREVIATIONS

HFD: High Fat Diet
T2DM: Type 2 Diabetes
GLP-1: Glucagon-Like Peptide-1

INTRODUCTION

Obesity is a widespread public health problem that affects people of all age groups [1]. The phenomenon has significant societal and economic influences, directly impacting individuals' well-being and overall standard of living. Accumulation of fat in the subcutaneous and visceral tissues results in obesity, this increment in body weight can negatively impact people health [2]. In addition to adipose tissue, the liver is also a common place for buildup of lipids and ectopic fat [3]. Avoiding and managing obesity involves controlling body weight and adiposity by conserving a negative energy balance, with diet and physical activities playing vital roles, however, as people's lifestyles have changed, with reduced physical activity and changes in eating habits, the investigation of alternate approaches to treating obesity, such as functional foods and bioactive substances, has become increasingly important [1]. The prevalence of obesity is steadily rising, suggesting that the existing treatments employed to manage this condition are inadequate and that further preclinical investigations are required. To examine the progression of obesity and its associated risk factors, scientists employ animal models of diet-induced obesity. Scientists prefer these models over genetic models because they replicate human obesity more accurately. Furthermore, controlled environments facilitate the comprehension of the findings in experiments conducted on animal models [4]. Obesity significantly increases the likelihood of developing many non-communicable diseases, such as sleep apnea, osteoarthritis, gout, dyslipidemia, gallbladder disease, T2DM, coronary heart disease, hypertension, and stroke, which primarily affect the lungs, joints, metabolism, and cardiovascular system [5]. Excessive energy consumption generates visceral fat in non-fat tissues and the enlargement and multiplication of fat cells, leading to liver and cardiovascular diseases. Also, adipokines and inflammatory cytokines made by adipose tissue may influence the environment, causing high blood sugar and insulin resistance and starting up signaling pathways related to inflammation. This increases the likelihood of developing and

exacerbating obesity-related diseases [6]. The human body accumulates excess energy in its adipose tissues which commonly occurs when the number of calories consumed exceeds the energy expended. Obese people have a high abundance of adipocytes ad excess energy intake can lead to continuous proliferation of adipocytes ultimately leading to the onset of obesity [7]. The correlation between obesity and inflammatory disorders can be attributed to several processes; for instance, adipose tissue in overweight individuals makes more pro-inflammatory adipocytokines, which create reactive oxygen species (ROS). Moreover, the elevated level of oxidative stress changes adipose tissue in important ways that cause a systemic, low-grade inflammatory response affecting the whole body [8], while a range of stressors can upset homeostasis and trigger inflammation as a natural physiological response to restore it, excessive or persistently high levels of inflammation can be detrimental to health. It is thought that overeating is the initial signal of inflammation and that the pathway originates in tissues involved in metabolism, such as adipose tissue, liver, and muscle, which triggers the inflammatory [9-10]. Previous reports have linked hepatic steatosis, insulin resistance, and the recruitment of macrophages into adipose tissue to the increase in adipose tissue associated with obesity. Adipocyte apoptosis is common in obese individuals and animals where activation of certain apoptosis mechanisms was seen in the adipose tissue of dietary models of obesity. Blocking these processes prevented hepatic steatosis, insulin resistance, and macrophage infiltration of adipose tissue, as well as adipocyte apoptosis [11]. Tirzepatide is a glucose-dependent insulinotropic polypeptide (GIP) receptor and GLP-1 receptor agonist that is recommended to help persons with T2DM manage their blood sugar levels in addition to diet and exercise [12]. It activates GLP-1 receptors to stimulate glucose-dependent insulin secretion, suppress glucagon release, and slowdown gastric emptying. As a result, it produces hypoglycemic effects that are comparable to those of selective GLP-1 agonists. It also suppresses food consumption and the desire to eat simultaneously [13]. Conversely, it increases the responsiveness of islet β cells to GIP, facilitating GIP function in stimulating initial insulin release and enhancing insulin sensitivity, resulting in a more efficient and consistent reduction in blood sugar levels [14]. Recent studies have demonstrated that the concurrent administration of GLP-1 and GIP can provide mutually enhancing effects, leading to improved regulation of blood sugar levels and a greater reduction in body weight. This can significantly protect against cardiovascular and cerebrovascular ailments [15]. In addition to their effects on diabetes mellitus and reduction of body, GLP-1 act to decrease oxidative stress where GLP-1 receptor agonists have anti-inflammatory and anti-apoptosis properties [16-17].

AIM

This study aims to examine the potential protective effect of Tirzepatide against obesity-induced metabolic dysfunction and hepatic inflammatory and apoptotic signals.

MATERIALS AND METHODS

Tirzepatide (CAS no.:2023788-19-2) from Hangzhou Go Top Peptide Biotech Co., Ltd., China. The glucometer and test strip from on call plus, USA. Chemical analyzer type Cobas from Roche, Germany. Tumor necrosis factor-α (TNF-α) assay kit, interlukin-1β (IL-1β) assay kit, caspase-3 assay kit and insulin assay kit from SUNLONG BIOTECH CO. LTD, China.

STUDY DESIGN

Animals were housed in the animal facilities, Faculty of Pharmacy, University of Kufa in a temperature-controlled environment at 24±2°C with 12-hour light and dark cycles. The investigation lasted for 12 weeks. Twenty-eight mature male Sprague-Dawley rats weighing 250±5 grams were employed. Rats were randomly divided into four groups, each consisting of 7 rats: control, obesity, vehicle, and Tirzepatide groups. For 12 weeks, the rats in the control group received a regular pellet whereas the other groups were fed HFD (30% fat). In the last four weeks, high-fat fed animals were treated with Tirzepatide at a daily dose of 10 nmol/kg s.c or its vehicle D.W or left untreated as an obesity control group.

COLLECTION OF BLOOD AND TISSUE SAMPLES

Ketamine and xylazine 75 mg/kg were used to euthanize the animals, and then they were sacrificed after collecting the blood samples. A midline incision was performed to access the liver then the tissue was kept at -80°C. On the analysis day, the liver was homogenized in a fresh PBS and ELISA approach was performed.

MEASUREMENT OF BODY WEIGHT

Throughout the 12-week investigation, animal weights were measured and recorded once weekly using an animal balance.

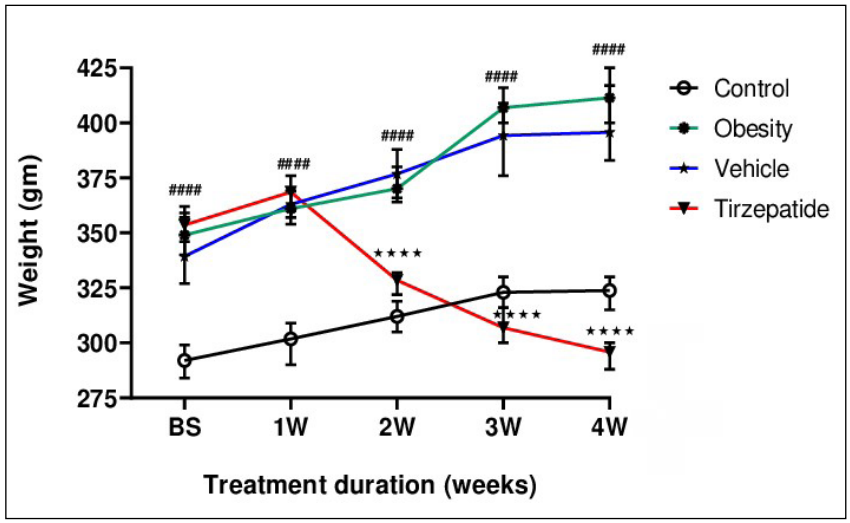

Fig. 1. Effect of tirzepatide on body weight in HFD-fed rats. ****p <0.0001. ####p <0.0001.

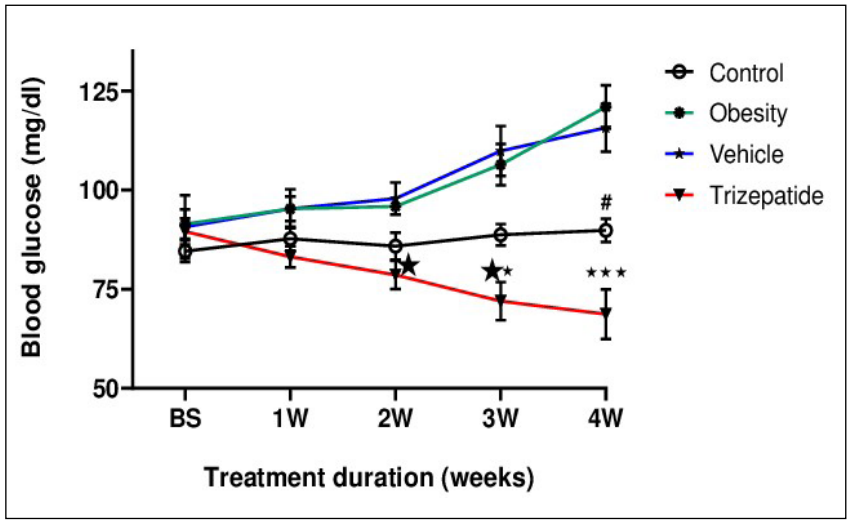

Fig. 2. Effect of tirzepatide on fasting blood glucose in HFD-fed rats. ***p<0.001. #p<0.05.

MEASUREMENT OF FASTING BLOOD GLUCOSE (FBG)

After the completion of eight weeks of HFD, fasting blood glucose was estimated once weekly using a glucometer once weekly throughout the 4-week treatment period.

MEASUREMENT OF INSULIN, LIPID PROFILE AND LIVER FUNCTION TESTS

Blood samples were drawn from rats under anaesthesia via heart puncture using a 5 cm syringe using a gel tube. Blood samples were centrifuged at 3000 rpm for 15 minutes. Chemical analyzer was employed to obtain the lipid profile parameters and liver function tests. Insulin concentration was measured by ELISA sandwich technique.

MEASUREMENT OF INFLAMMATORY AND APOPTOSIS BIOMARKERS

Liver tissue samples were homogenized on ice and then used to measure TNF-α, IL-1β and caspase-3 by the ELISA sandwich technique using commercially available kits.

STATISTICAL ANALYSIS

GraphPad Prism (version 9.0.0) was used for data analysis and presentation. Data were showed as mean plus or minus the standard error of the mean (SEM). Depending on data, either one-way or two-way Analysis of Variance (ANOVA) was used followed by Tukey's multiple comparison tests. Statistical significance was set at $P<0.05$.

ETHICAL APPROVAL

The study received ethical approval from Kufa University, central ethics committee (under no. 6684) on 10 March 2024.

RESULTS

EFFECT OF TIRZEPATIDE ON BODY WEIGHT

After eight weeks of HFD and before commencing the treatment (baseline), there was no significant difference between the obesity, vehicle and Tirzepatide groups, whereas the normal control group differed significantly

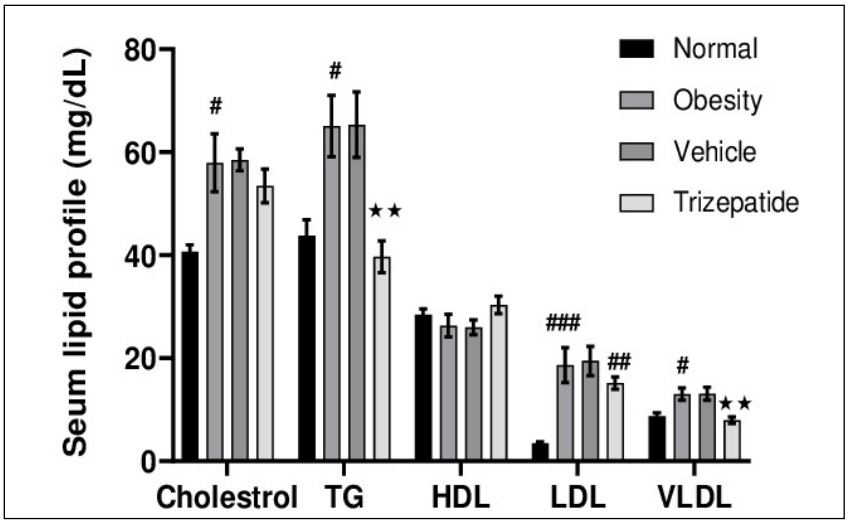

Fig. 3. Tirzepatide improves lipid profile parameters in HFD-fed rats. **p<0.01. ###p<0.001.

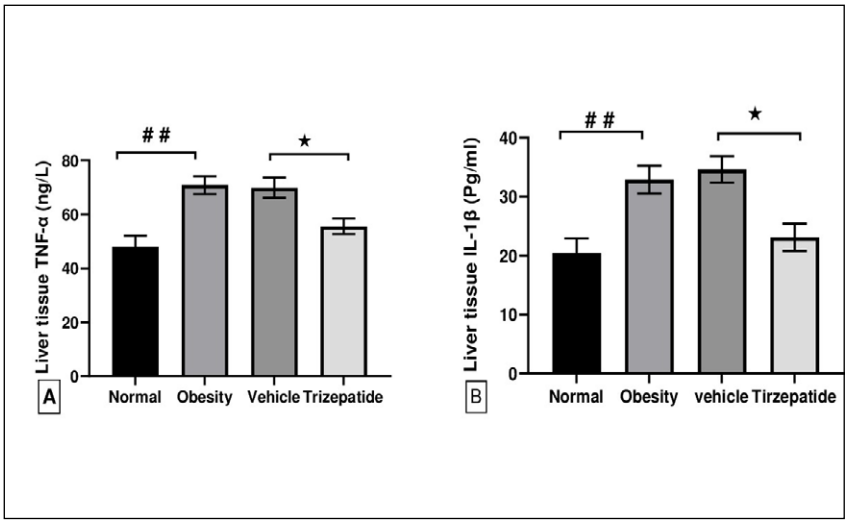

Fig. 4. A)Effect of tirzepatide on hepatic TNF-α, B) IL-1β levels in HFD-fed rats. *p<0.05. ##p<0.01.

from all the other groups p<0.0001 as shown in (Fig. 1). At the end of treatment period, the mean body weight for tirzepatide treated group decreased significantly compared to the vehicle group p<0.0001 as shown in (Fig. 1).

Rats fed HFD were treated with tirzepatide 10 nmol/kg daily for four weeks. In vehicle group, obese rats were administered tirzepatide vehicle. BS: baseline. Data are presented as mean ± SEM of seven rats in each group, p <0.0001 compared with vehicle group, p< 0.0001 versus normal group.

EFFECT OF TIRZEPATIDE ON FASTING BLOOD GLUCOSE IN HFD-FED RATS

After eight weeks of HFD, there was an insignificant difference in FBG between all the treatment groups, however, after twelve weeks of HFD, a significant increment (p<0.05) in FBS occurred between rats on regular diet and untreated rats fed HFD as shown in (Fig. 2). In contrast, the average FBG in the tirzepatide group decreased considerably compared to the vehicle group (p<0.001) (Fig. 2).

Rats fed HFD were treated with tirzepatide 10 nmol/kg daily for four weeks. In vehicle group, obese rats were administered tirzepatide vehicle. Data are presented as mean ± SEM of seven rats in each group, p<0.001 compared with vehicle group, p<0.05 versus normal group.

IMPACT OF TIRZEPATIDE ON SERUM INSULIN AND LIVER FUNCTION IN HFD-FED RATS

The level of serum insulin in the obesity group increased significantly compared to the normal control p<0.05, however, tirzepatide treatment reduced serum insulin level in obese animals to an insignificant level compared to normal rats as shown in (Table 1). Regarding liver functions, data showed that ALT and AST enzymes in the obesity group increased significantly compared to the normal group p<0.05, whereas obese animals treated with tirzepatide demonstrated lower level of ALT compared to vehicle group (p<0.05) as shown in (Table 1).

Table 1. Effect of tirzepatide on insulin level and liver functions in HFD-fed rats

Biochemical parameter	Groups			
	Normal	Obesity	Obesity+vehicle	Obesity+Tirzepatide
Serum insulin	0.869±0.163	1.413±0.092#	1.446±0.117	0.996±0.041*
ALT	30.57±6.963	52.01±4.819#	54.39±3.296	35.24±4.492*
AST	111.1±10.44	203.3±21.69#	212.8±26.17	161.2±18.94

Data are presented as mean ± SEM of seven rats in each group, *p<0.05 compared with vehicle group, #p<0.05 versus normal group.

Fig. 5. Effect of tirzepatide on hepatic caspase-3 level in HFD-fed rats. *p<0.05. ##p<0.05.

TIRZEPATIDE IMPROVES LIPID PROFILE PARAMETERS IN HFD-FED RATS

Feeding animals' high-fat diet resulted in significantly higher levels of lipid parameters: total cholesterol p<0.05, LDL p<0.001, triglyceride p<0.05 and VLDL p<0.05 compared to rats fed regular diet as shown in (Fig. 3). On the other hand, tirzepatide therapy lowered the TG and VLDL to an insignificant level compared to normal rats as shown in Fig. 3. LDL level remained significantly higher than normal p<0.01 even after tirzepatide administration, whereas no significant difference was observed in serum HDL measurement between all the studied groups throughout the experiment.

Rats fed HFD were treated with tirzepatide 10 nmol/kg daily for four weeks. In vehicle group, obese rats were administered tirzepatide vehicle. TG: triglyceride. Data are presented as mean ± SEM of seven rats in each group, p<0.01 compared with vehicle group, #p<0.05, p<0.001 versus Normal control.

TIRZEPATIDE ATTENUATED THE HEPATIC INFLAMMATORY RESPONSE IN HFD-FED RATS

In obese untreated animals, TNF-α and IL-1β levels were increased significantly compared to the normal group p<0.01, while after treatment with tirzepatide, the hepatic TNF-α and IL-1β content decreased significantly p<0.05 compared to vehicle-treated group as shown in (Fig. 4: A-B).

Rats fed HFD were treated with tirzepatide 10 nmole/kg daily for four weeks. In vehicle group, obese rats were administered tirzepatide vehicle. Data are presented as mean ± SEM of seven rats in each group, p<0.05 compared with vehicle group, p<0.01 versus normal group.

TIRZEPATIDE ADMINISTRATION COMBATED APOPTOSIS IN HEPATIC TISSUE OF FED HFD RATS

As illustrated in (Fig. 5), high fat intake caused a marked increment in the hepatic apoptosis level compared to normal rats. Caspase-3 estimation in liver homogenate revealed a significant elevation in obesity and vehicle-treated groups compared to normal control p<0.05. In contrast, administration of tirzepatide significantly decreased hepatic caspase-3 level p<0.05 compared to vehicle treated rats.

Rats fed HFD were treated with tirzepatide 10 nmol/kg daily for four weeks. In vehicle group, obese rats were administered tirzepatide vehicle. Data are presented as mean ± SEM of seven rats in each group, p<0.05 compared with vehicle group, p<0.05 versus normal group.

DISCUSSION

Obesity is prevalent in both affluent and emerging countries, impacting the health of around 500 million

people globally [18]. Various behavioral, environmental, and socioeconomic factors contribute to the accumulation of fat in adipose tissue, which also influences the prevalence of obesity. GLP-1 agonists have demonstrated multiple beneficial effects in obesity; therefore, our study investigated the impact of tirzepatide on obesity-induced metabolic dysregulation and hepatic inflammatory and apoptotic status. Obesity was induced in animals by feeding rats a high-fat diet. Rats are valuable animal models in this field of research due to their extensive usage in studies on obesity to understand the underlying mechanisms, genetic factors, and potential therapies for obesity [19]. High-fat diet can lead to obesity as a result of multiple factors, including heightened calorie consumption, hormonal disparities, genetic predisposition, and environmental impacts [20]. The association between high blood glucose and obesity is due to decreased muscle glucose absorption and increased glucose synthesis in the liver by gluconeogenesis and glycogenolysis. A condition that causes insulin resistance, elevated blood sugar, weakened anabolic and anti-inflammatory capabilities, muscular protein loss, increased susceptibility to infections, and a worsened inflammatory response [21]. Previous studies have shown that when high-fat diet is present insulin becomes less efficient [22]. Insulin resistance is a defining feature of both obesity and the metabolic syndrome [23-24]. AST and ALT are two important markers of hepatocellular injury, specifically related to liver function. High fat diet induces hepatic insulin resistance, leading to increased oxidative stress and lipid peroxidation. In animal model of nonalcoholic fatty liver disease, feeding animals' high fat diet resulted in significantly higher levels of liver enzymes, such as AST and ALT [25]. The elevated TNF-α and IL-1β content in rat liver might be attributed to high fat intake. Consuming a diet high in lipids is linked to elevated levels of leptin in the bloodstream. Adipocytes, which are primarily responsible for producing leptin, also create other mediators, particularly inflammatory ones like TNF-α and IL-1β [26]. Leptin additionally affects the immune system by promoting the generation and movement of white blood cells in the bone marrow. Additionally, it enhances the synthesis of pro-inflammatory cytokines and promotes the attachment and engulfment of macrophages, while also stimulating the growth of T lymphocytes. Recent research has indicated that obesity leads to a reduction in blood flow to adipose tissue, resulting in a condition called hypoxia. This lack of oxygen triggers an inflammatory response [27]. Adipose tissue functions as an endocrine organ, releasing chemicals such as TNF-α. These factors can disrupt food consumption and the body's nutrient balance. Obesity upsets the balance by causing insulin resistance and increases the proinflammatory factors, such as TNF-α. Insulin primarily decreases lipolysis in adipose tissue. This entails the hydrolysis of triglycerides into glycerol and free fatty acids to produce energy. This process reduces the concentration of fatty acids in the blood and promotes the production of fatty acids and triacylglycerols in the body's tissues. It also enhances the absorption of triglycerides from the bloodstream into adipose tissue, leading to an increase in fat storage in fat cells when there is an excess of fat in the body, and it triggers inflammatory processes [28]. HFD stimulates the NLRP3 inflammasome via the AMPK-autophagy-ROS signaling pathway, while ceramides, which are metabolites of fatty acids, can activate NLRP3-Caspase-1 and release IL-1β in macrophages [29-30]. Additionally, it is plausible that other lipid constituents in HFD can trigger the activation of inflammasomes and the generation of IL-1 [31]. In a dietary model of obesity, adipose tissue triggers two main pathways for apoptosis, the extrinsic one which is regulated by death receptors on the cell surface, and the intrinsic one which is activated via mitochondrial pathway. Stopping these pathways stops adipocytes from dying and protects against macrophages getting into adipose tissue, liver steatosis, and insulin resistance [32-33]. Tirzepatide is a novel hypoglycemic medication that functions as a dual antagonist of the GIP-1 and GIP receptors. Tirzepatide therapy has shown significant weight reduction in overweight and obese individuals [34]. The GLP-1/GIP dual receptor agonists demonstrated superior weight loss compared to GLP-1RA alone; however, prolonged use of GIPRA does not lead to a reduction in body weight [35]. Tirzepatide administration leads to appetite suppression and increased energy expenditure, increasing concentrations of GIP. The combined actions of GIP and GLP-1 receptors may occur at the central nervous system level. When GLP-1 and GIP were given together to people with anorexia nervosa, their POMC genes were turned up. This led to a decrease in appetite and food consumption [36-37]. Tirzepatide activates GLP-1 receptors, which stimulate insulin secretion in response to glucose, suppresses glucagon release, and reduces the speed at which the stomach empties. Consequently, it generates hypoglycemic effects that are similar to those of selective GLP-1 agonists. On the other hand, it improves the ability of islet β cells to respond to GIP, enabling GIP to contribute to the stimulation of early insulin release and the improvement of insulin sensitivity. This results in a more effective and uniform decrease in blood glucose levels [38-39]. Both in vivo and in vitro investigations have demonstrated that GLP-1 in pancreatic beta cells

promotes the production of insulin. GLP-1 not only increases insulin production but also lowers glucose levels by slowing down stomach emptying, improving the body's response to insulin, and reducing glucagon release. These actions can lead to a decrease in the generation of glucose by the liver [40]. Therefore, Tirzepatide treatment has shown significant improvements in biomarkers related to β-cell activity, insulin sensitivity, glycemic control, and body weight reduction. The benefits of simultaneous GLP-1R agonism in lowering glucose may quickly restore GIP sensitivity, making the benefits of lowering glucose even better. Tirzepatide doses resulted in a notable decrease in biomarkers linked to non-alcoholic steatohepatitis (NASH), a condition stemming from obesity. Multiple investigations evaluated the effects of tirzepatide on AST and ALT enzyme activity, as well as adiponectin levels. Tirzepatide therapy significantly decreased ALT levels. GLP-1 agonists have strong inhibitory effects on indicators of oxidative stress after delivery, whereas treatment with GLP-1 agonists markedly increased the antioxidative indicators. Oxidative stress triggers degenerative processes, such as inflammatory and apoptotic signals. Previous evidence has shown that GLP-1 counteracts the increment in apoptotic cells that occurs due to chronic hyperglycemia and inhibits caspase-3 activity in pancreatic β-cells. The administration of GLP-1 considerably reduced the release of TNF-α and IL-1β decreased the number of macrophages infiltrating the body and the number of inflammatory cytokines that macrophages secrete, such as TNF-β and IL-1β.

CONCLUSIONS

The findings of this study suggest that consumption of high-fat diet causes metabolic dysregulations manifested by higher body weight, blood glucose, insulin level and worsened serum lipids. However, tirzepatide administration mitigates these metabolic dysfunctions. Moreover, treating obese animals with tirzepatide ameliorates the inflammatory and apoptotic responses in hepatic tissue triggered by HFD.

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CONFLICT OF INTEREST
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ORIGINAL ARTICLE

CONTENTS

The role of lipid metabolism disorders and dysbiotic changes in metabolically dysfunction-associated steatotic liver disease combined with acne vulgaris

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ABSTRACT

Aim: To determine the features of hepatic lipid metabolism disorders and intestinal dysbiotic changes in patients with acne vulgaris and metabolic dysfunction-associated steatotic liver disease (MASLD)

Materials and Methods: The study included 109 patients: 59 with a combination of acne vulgaris and MASLD and 50 with acne only.

Results: It was found that patients with acne and MASLD are more likely to be overweight and have more pronounced changes in hepatic lipid metabolism. Also, patients with combined pathology showed a decrease in the number of the colonic normoflora , as well as an increase in the number of hemolytic forms of E. coli, Enterobacter, Citrobacter, Staphylococcus, Klebsiella, Clostridium, Proteus, Candida spp.

Conclusions: Our study highlighted that lipid profile determination and anthropometric examination are important factors in the diagnosis of patients with common acne and MASLD. Our data on changes in the composition of the colonic microflora in patients with comorbidities confirmed the existence of a relationship between the presence of intestinal dysbacteriosis and impaired skin homeostasis.

KEY WORDS: acne vulgaris; hepatic lipid metabolism disorders; intestinal dysbacteriosis; metabolic dysfunction-associated steatotic liver disease; microbiota; obesity

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INTRODUCTION

Metabolic dysfunction-associated steatotic liver disease (MASLD), previously termed non-alcoholic fatty liver disease is now the most common cause of chronic liver disease in the Western world with a prevalence ranging from 5 to 33%[1]. This disease is characterized by the accumulation of fat in the liver. The histological changes are identical to alcoholic liver disease, but patients do not drink alcohol or drink it minimally [2]. Epidemiological studies [3] support a trend toward an increased incidence of MASLD in patients with insulin resistance (IR), which occurs in the context of obesity, type 2 diabetes mellitus (DM), and the presence of metabolic syndrome (MS). Some studies have shown that MASLD precedes the development of MS [4]. The literature has shown that MS is associated with inflammation, as well as increased levels of pro-inflammatory cytokines such as IL-6, TNF-α, and C-reactive protein[5,7]. The chronic inflammatory process of acne vulgaris is known to be associated with the development of MS [7,8]. In addition, other skin diseases with a similar pathogenesis as acne, such as psoriasis, rosacea and hidradenitis suppurativa, already have a well-established association with MS [7,8].

According to the literature, the pathogenesis of acne is explained by four key factors: excess sebum production, hyperproliferation of the bacteria *Cutibacterium acnes* (C. acnes, formerly called *Propionibacterium acnes*), hyperkeratinization of the sebaceous follicle, and inflammatory mechanisms [9]. Excess sebum production is mediated by several hormones, such as androgens, insulin, and insulin-like growth factor 1 (IGF-1) [10]. Moreover, there is increasing information about the relationship between acne and insulin resistance [11,12]. Interestingly, MASLD is found in patients with insulin resistance without obesity and without diabetes, which means that when developing in individuals with normal weight, it can be a predictor of early metabolic disorders and diseases [13,14].

The diseases mentioned above share common pathogenetic factors in their development and progression, therefore, they can combine and potentiate the development of each other.

AIM

Our study aimed to determine the features of hepatic lipid metabolism disorders and intestinal dysbiotic changes in patients with acne vulgaris and MASLD.

MATERIALS AND METHODS

Patients with acne vulgaris and MASLD were enrolled in the study. It was conducted from January 2019 to September 2024 at the clinical base of the Department of Propedeutics of Internal Diseases of the State Educational Institution "Uzhhorod National University".

The inclusion criteria were:

- age of at least 18 years;
- a diagnosis of mild or moderate acne vulgaris (2-3 points on the IGA scale) with the presence of symptoms for more than 6 months;
- a diagnosis of MASLD with or without liver fibrosis.

Exclusion criteria included: pregnancy; current use of any acne medications; use of oral or topical antibiotics or retinoids within last 2 months; alcoholic, viral (hepatitis B, C, D viruses), autoimmune liver fibrosis; Wilson-Konovalov's disease; hemochromatosis; chronic inflammatory bowel diseases (Crohn's disease, nonspecific ulcerative colitis); lactose intolerance; celiac disease; intestinal surgeries (including appendectomy with a duration of up to 6 months); colon cancer; doligosigma; colon diverticulosis; positive test for toxins A and B of Clostridium difficile bacteria in feces; type 1 diabetes mellitus; type 2 diabetes mellitus (decompensation stage); pulmonary tuberculosis (active form); psychiatric diseases; pregnancy and lactation; systemic autoimmune diseases; HIV; oncology.

STUDY DESIGN

109 participants were enrolled in the study with informed consent. 34 men (31.2 %), with an average age of 30.8±6.5 years and 75 women (68.8 %), with an average age - of 29.9±6.7 years.

Patients were divided into two groups:

- group 1 (n= 59) — patients with mild/moderate acne vulgaris and MASLD;
- group 2 (n= 50) — patients with mild/moderate acne vulgaris.

All participants were scheduled for the following examinations:

- assessment of the severity of acne using the Investigator's Global Assessment (IGA) scale;
- standardized photo-fixation of the face in three projections;
- blood lipidogram (plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), very low-density lipoproteins (VLDL), triglyceride (TG), atherogenic index) and insulin-like growth factor-1 (IGF-1) ;
- the liver function tests — alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), serum bilirubin;
- assessment on scales for the degree of liver fibrosis — NFS (NAFLD fibrosis score), FIB-4 (Fibrosis-4 index), Fibrotest, FibroIndex, Forns, APRI (AST to platelet ratio index);
- liver transient elastography;
- microbiological examination of feces;
- abdominal and pelvic ultrasonography.

The severity of acne was assessed by certified dermatologists who had previous experience with the scales that were used in the study. Diagnosis of MASLD and assessment of fibrosis severity was performed by a certified gastroenterologist according to scales and sonography in adherence to EASL–EASD–EASO clinical recommendations. Whole venous blood for lipid

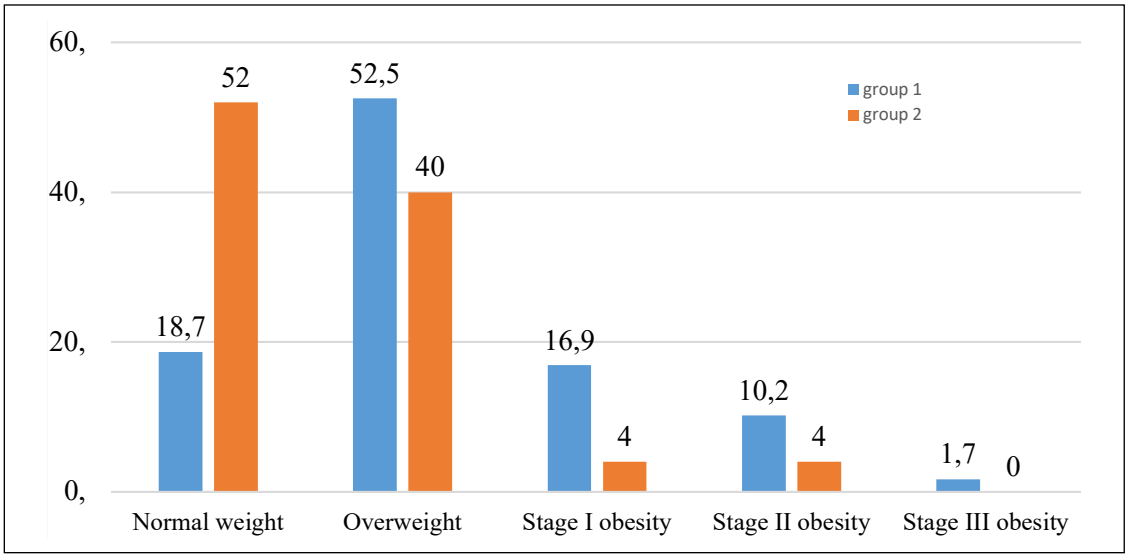

Fig. 1. Changes in BMI in patients with acne vulgaris and MASLD.

Table 1. Changes of indicators of quantitative and qualitative composition of colon microbiota

Indicator	Group 1 (n=59)	Group 2 (n=50)
***Bifidobacterium*: Control group 100.0 % (8.65±0.26)**		
frequency, %	67.8 %**	80.0 %*+
lg CFU/gr	5.88±0.09**	7.76±0.10*++
***Lactobacillus*: Control group 100.0 % (6.78±0.22)**		
frequency, %	66.8 %**	85.0 %*+
lg CFU/gr	5.01±0.10**	6.06±0.11*+
***E.coli* (with normal enzymatic properties): Control group 93.3 % (7.87±0.15)**		
frequency, %	71.2 %*	80.0 %*+
lg CFU/gr	5.28±0.07**	6.87±0.07*++
***E.coli* (haemolytic form): Control group 3.3 % (1.12±0.07)**		
frequency, %	20.3 %**	10.0 %*+
lg CFU/gr	4.77±0.15***	2.51±0.23**++
***Enterococcus*: Control group 90.0 % (7.56±0.11)**		
frequency, %	44.1 %***	70.0 %**++
lg CFU/gr	6.25±0.16*	7.08±0.08*+
***Enterobacter*: Control group 23.3 % (1.15±0.12)**		
frequency, %	42.4 %*	34.0 %*+
lg CFU/gr	4.66±0.09***	1.98±0.06**++
***Citrobacter*: Control group 26.7 % (1.47±0.09)**		
frequency, %	52.5 %**	30.0 %++
lg CFU/gr	3.56±0.12**	2.98±0.09*+
***Staphylococcus*: Control group 26.7 % (3.48±0.22)**		
frequency, %	64.4 %**	34.0 %++
lg CFU/gr	5.54±0.16**	4.12±0.06*++
***Klebsiella*: Control group 16.7 % (1.29±0.09)**		
frequency, %	45.8 %**	20.0 %+++
lg CFU/gr	3.29±0.18**	2.38±0.10**++
***Clostridium*: Control group 13.3 % (4.22±0.18)**		
frequency, %	32.2 %**	18.0 %++
lg CFU/gr	5.80±0.15**	4.98±0.09*+
***Proteus*: Control group 6.7 % (0.55±0.09)**		
frequency, %	28.8 %**	12.0 %*+
lg CFU/gr	2.50±0.08***	1.56±0.10**+
***Candida*: Control group 3.3 % (2.89±0.20)**		
frequency, %	15.3 %*	8.0 %
lg CFU/gr	4.98±0.09**	3.77±0.11*+

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

profile determination was collected using appropriate vacutainers and the analysis was performed by the certified laboratory no later than one day after blood collection on Dimension EXL200. 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 inoculated 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 (10^{-1}-10^{-9}). Changes in the quantitative and qualitative composition of the colon

Table 2. Severity of colon dysbiosis

The degree of CD	group 1 (n=59)	group 2 (n=50)
I degree of CD	20.3 %	64.0 %+
II degree of CD	64.4 %+	32.0 %
III degree of CD	15.3 %	4.0 %

Note: the difference between the indicators in patients by groups 1 and 2 is significant: + - p<0.01.

Table 3. The indicators of liver function in blood serum

Indicator	Group 1 (n=59)	Group 2 (n=50)
ALT, U/l	93.41±0.44**	32.14±0.32**
AST, U/l	76.14±0.56***	27.36±0.44***
TB, mmol/l	34.12±0.11*	28.29±0.27*
GGT, U/l	82.03±0.48**	30.67±0.38**
ALP, mmol/l	134.15±0.32**	124.16±0.42*

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

Table 4. The indicators of blood lipid metabolism

Indicator	Group 1 (n=59)	Group 2 (n=50)
TC, mmol/l	3.56±0.08**++	2.09±0.07*
TG, mmol/l	7.84±0.09**++	5.98±0.08*
LDL, mmol/l	3.80±0.10**+	2.23±0.07*
VLDL, mmol/l	1.98±0.07**++	0.97±0.11*
HDL, mmol/l	0.99±0.07**+	1.44±0.09*

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

microflora were determined using the classification of intestinal dysbiosis by Kuvaeva-Ladodo (1991).

The analysis and processing of the results was carried out using the computer program Statistics for Windows v.10.0 (StatSoft Inc, USA) using parametric and nonparametric methods of evaluating the results.

RESULTS

In both groups, there were more women than men but the difference between them was not statistically significant (P=0.5). In Group 1 39 (66,1%) were female and 20 (33.8%) were male, Group 2 were 36 (68.9%) and 14 (31.1%), respectively. The mean ages of patients were 29.7±6.3 and 29.5±5.1, respectively, and there was no statistically significant difference (P=0.61). The age of participants ranged from 18 to 45 years.

BODY MASS INDEX

An anthropometric measurement found an increase in BMI in the vast majority of patients with acne vulgaris and MASLD (Fig. 1). Normal body weight was more often observed in patients from Group II (52% of patients). According to the results obtained, it was found that patients with acne vulgaris and MASLD are more likely to be overweight — obesity of all stages prevailed in Group 1.

CHANGES IN COLON MICROBIOTA

Changes in the quantitative and qualitative composition of the colonic microbiota were diagnosed during microbiological examination of feces. The study revealed a decrease in the number of normoflora (*Bifidobacterium and Lactobacterium, Escherichia coli* with normal enzymatic activity), as well as an increase in the number of hemolytic forms of *E. coli, Enterobacter, Citrobacter, Staphylococcus, Klebsiella, Clostridium, Proteus. and Candida spp* in patients from Group 1.

The changes in the quantitative and qualitative composition of the colon microbiota are presented in Table 1.

THE DEGREE OF COLON DYSBIOSIS

Our study showed that the majority of patients in Group 1 had an II degree of colon dysbacteriosis (CD), while the majority of patients in Group 2 had an I degree of

CD. The degree of colon dysbacteriosis in both groups is presented in Table 2.

THE LIVER FUNCTION TESTS

The results of biochemical blood tests indicate impaired liver function in patients with combined pathology (Group 1). An increase in cholestatic syndrome indicators (levels of ALP, GGT, TB) was observed in Group 1. Interestingly, in Group 2, an increase in serum bilirubin levels was detected while normal levels of AST and ALT (Table 3).

BLOOD LIPIDOGRAM

According to the results of the study, dyslipidemia was detected in both groups. However, these changes were more pronounced in patients with combined disease (Group 1). Table 4 shows the blood lipid metabolism indicators in patients of both study groups.

DISCUSSION

The immunomodulatory potential of the gut microbiota and its influence on distant organs has been increasingly investigated in recent years. Of particular interest are the gut-brain [16], gut-lung [17], gut-liver [18] and gut-skin axes [19,20]. Gut microbes can influence systemic inflammation, oxidative stress, glycemic control, and tissue lipid content [15] Epidemiological studies have assessed the distribution of the gut microbiota between healthy individuals and patients with MASLD. Some studies have observed a decrease in bacterial α- or β-diversity [21-23] microbial taxa, a meta-analysis of 54 studies (8894 participants) found a depletion of anti-inflammatory microbes (i.e. Ruminococcaceae and Coprococcus) and an enrichment of pro-inflammatory microbes (i.e. Fusobacterium and Escherichia) in patients with MASLD [14, 15]. Acne vulgaris has been postulated to have a gastrointestinal mechanism; however, little is known about gut microbiota dysfunction in this condition [16]. The results of our study also confirmed the information about changes in the quantitative and qualitative composition of microbiota in patients with combined pathology of acne vulgaris and MASLD. Statistically reliable data were obtained on a decrease in the number of normoflora (Bifidobacteria and Lactobacteria, Escherichia coli with normal enzymatic activity), as well as an increase in the number of hemolytic forms of E. coli, Enterobacter, Citrobacter, Staphylococcus, Klebsiella, Clostridium, Proteus and Candida spp.

According to the literature, it is known that liver dysfunction plays a significant role in the pathophysiology of chronic inflammatory skin disease. [24,25] Scientists actively discuss the relationship between MASLD and chronic inflammatory dermatological diseases. [24] One of the important indicators of MASLD is the progression of dyslipidemia. This disease leads to the development of the "lipid quartet" — a variant of highly atherogenic dyslipidemia with high titers of triglycerides, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and low high-density lipoproteins (HDL) and high Plasma concentration of particles dangerous for the endothelium-intermediate density lipoproteins, the molecular weight of which is between VLDL and LDL [26, 27].

Dyslipidemia was observed in both study groups, which confirms the need for assessing serum lipid levels during acne treatments. According to our results, patients with combined pathology (acne and MASLD) have a worse serum lipid profile compared to patients who have only acne vulgaris.

According to clinical guidelines, the presence, duration and severity of obesity are associated with an increased risk of disease progression in MASLD [28]. Almost all patients are found to be obese (BMI>30 kg/m2) or overweight, which correlates with the degree of hepatic steatosis [29]. According to the literature, acne vulgaris is also known to be significantly associated with changes in lipid profile and body mass index (BMI) [30].

The results of our study also confirmed the relationship between increased BMI and impaired hepatic lipid metabolism. Anthropometric measurements revealed obesity in the vast majority of patients with combined pathology of acne vulgaris and MASLD. This suggests the need for additional examinations in patients with overweight and acne.

The main limitations of the research were the small sample size and cross-sectional study design. Future prospective multi-center studies with larger sample sizes are necessary to validate the results of our study.

CONCLUSIONS

Our study highlighted that altered serum lipid levels are common in patients with acne vulgaris and MASLD, indicating that lipid profile testing is an important factor in the diagnosis of these pathologies. As well as the importance of anthropometric examination, since most patients in the combined pathology group had an increased BMI. Noteworthy, our data obtained on changes in the quantitative and qualitative composition of the colonic microflora in patients with acne vulgaris and MASLD confirmed the relationship between the presence of intestinal dysbacteriosis and impaired skin homeostasis.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Surgical prevention of venous thromboembolic complications of thrombosis in the basin of the small saphenous vein

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ABSTRACT

Aim: To evaluate the results of surgical prophylaxis of venous thromboembolic complications in superficial vein thrombosis in the basin of the small saphenous vein.

Materials and Methods: The study evaluated the results of treatment of 134 patients with superficial vein thrombosis in the basin of the small saphenous vein, who were divided into two groups: Group I - 86 (64.2%) patients who were operated on for thrombosis in the basin of the small saphenous vein and Group II - 48 (35.8%) patients who received only conservative treatment.

Results: In the postoperative period, no recurrence of superficial or deep vein thrombosis and pulmonary embolism was detected in patients of group I within 12 months. The regression of symptoms of chronic venous insufficiency was noted in 66.7% of patients, and no patient was diagnosed with a decompensated form of chronic venous insufficiency. Over the course of the year, 12.2% of patients in group II showed an increase in the manifestations of chronic venous insufficiency. Recurrence of superficial or deep vein thrombosis in group II was detected in 14.6% and 4.9% of patients, respectively.

Conclusions: Surgical treatment of superficial vein thrombosis in the basin of the small saphenous vein prevented pulmonary embolism and recurrence of venous thrombosis in all patients, and in 66.7% of patients contributed to the regression of decompensated forms of chronic venous insufficiency. Recurrence of superficial and deep vein thrombosis during conservative treatment was observed in 14.6% and 4.9% of cases, respectively, which led to pulmonary embolism in 4.9% of patients.

KEY WORDS: superficial vein thrombosis, saphenopopliteal junction, surgical intervention, conservative treatment, pulmonary embolism

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INTRODUCTION

The treatment of superficial vein thrombosis as a complication of varicose veins is an ongoing debate among the surgical community. There is a widespread belief in the benign clinical course of superficial vein thrombosis, which requires only symptomatic treatment [1].

The German registry of superficial venous thrombosis shows that conservative treatment remains the only treatment, but there is no standardized regimen and duration of anticoagulant therapy [2]. Patients are prescribed heparin, oral anticoagulants, or no anticoagulants at all [2]. At the same time, the authors note that despite the high percentage of anticoagulant use, there is a significant risk of venous thromboembolic complications and recurrent thrombosis within three months [2], which reaches up to 10.2% of cases [3].

Bauersachs R. et al. (2021), based on a prospective study (INSIGHTS-SVT) involving 1150 patients with acute isolated superficial vein thrombosis, reported that despite different anticoagulant therapy, the incidence of pulmonary embolism and its recurrence was 1% and 5%, respectively [4].

The authors' studies differ in the frequency of venous thromboembolic complications in superficial vein thrombosis. In 2016, the authors, based on a meta-analysis of 21 studies including 4358 patients, found deep vein thrombosis in 18.1-18.2% of patients with superficial vein thrombosis [5]. A similar meta-analysis of 11 studies (2484 patients) found pulmonary embolism in 6.9-8.2% of patients with superficial vein thrombosis [5].

A retrospective cohort study conducted by the National Health Insurance Service of Taiwan, which included two huge groups of patients: the main group - 212,984 patients with varicose veins and the control group, which included a similar number of people without varicose veins, revealed a significantly higher risk of deep vein thrombosis and pulmonary embolism in the main group: 6.55 vs. 1.23 cases and 0.48 vs. 0.28 cases per 1000 person-years, respectively [6]. based on data that revealed a significant incidence of deep vein thrombosis in the setting of varicose veins [6].

The incidence of venous thromboembolic complications in superficial vein thrombosis remains high, which refutes the statement about the benign course of the disease [5-8]. Thus, 25% of patients with superficial vein thrombosis have asymptomatic pulmonary embolism [2]. This is confirmed by the common risk factors for deep vein thrombosis and pulmonary embolism, which are also characteristic of superficial vein thrombosis [2, 5, 8, 9]. At the same time, the annual cost of treating such a «benign» disease as superficial vein thrombosis in the United States alone is $3 billion annually [10].

Thus, the issue of treatment of deep vein thrombosis remains controversial. Even with anticoagulation therapy, the incidence of venous thromboembolic complications in superficial vein thrombosis remains high.

AIM

To evaluate the results of surgical prophylaxis of venous thromboembolic complications in superficial vein thrombosis in the basin of the small saphenous vein.

MATERIALS AND METHODS

The study evaluated the results of surgical and conservative treatment of 134 patients with superficial vein thrombosis in the basin of the small saphenous vein. Depending on the method of treatment, patients were divided into two groups:

- Group I (main) - 86 (64.2%) patients who were operated on for thrombosis in the basin of the small saphenous vein;
- Group II (control) - 48 (35.8%) patients who received only conservative treatment.

The diagnostic algorithm for the examination of patients included a general blood and urine test, glycemic testing, biochemical blood tests, coagulation test, and detection of hepatitis markers. If necessary, additional laboratory tests were prescribed. Particular importance was attached to ultrasound examination of patients. An electrocardiogram, consultation with a cardiologist, and ultrasound examination of the veins of both lower extremities and pelvis were considered mandatory. The latter was performed in all patients without exception at their initial visit for medical care, which was repeated in patients of group I immediately before surgery, and subsequently in patients of both groups before discharge from the hospital. In addition, in 3 (4.6%) patients of group I and 5 (22.7%) patients of group II , ultrasound examination was performed more often during inpatient treatment, as indicated.

The statistical processing of the study results was performed using Microsoft Excel 2010 computer programs with the Statistica 5.0 for Windows application package. The mean values and relative indicators were compared using Mann-Whitney criteria and Pearson's parametric correlation analysis.

The study was conducted in accordance with the provisions of the Declaration of Helsinki of the World Medical Association «Ethical Principles for Medical Research Involving Human Subjects» (revision 2008) and approved by the Bioethics Committee of the School of Medicine of the Uzhhorod National University. All patients signed an informed consent to participate in the research work.

RESULTS

All patients underwent ultrasound examination of the veins of both lower extremities during the initial visit for medical care to detect venous thrombosis of the contralateral lower extremity. The superficial, deep and communicating veins of the lower extremity were examined sequentially, including ultrasound examination of the iliac veins and inferior vena cava. The most common cause of superficial vein thrombosis in the basin of the small saphenous vein was varicose veins in 95 (70.9%) patients, post-thrombotic changes in the small saphenous vein or the absence of any pathological changes in the venous wall were detected in 22 (16.4%) and 17 (12.7%) patients, respectively. Pathologic changes in the venous system of the contralateral lower extremity were detected in 45 (33.6%) patients, in particular:

- varicose veins - in 27 (60%);
- post-thrombotic changes in superficial veins - in 5 (11.1%)
- superficial vein thrombosis - in 4 (8.9%)
- deep vein thrombosis - in 8 (17.8%)
- simultaneous thrombosis of superficial and deep veins - in 1 (2.2%) case.

Significant attention was paid to the detection of the sapheno-popliteal junction or its absence during ultrasound examination of the affected limb, in particular, the following anatomical variants of the inflow of the small saphenous vein were found:

- in the popliteal vein (sapheno-popliteal cofemoral) - in 78 (58.2%);
- extension of the small popliteal vein into the vein of Giacomini with a branch to the popliteal vein - in 33 (24.6%);
- extension of the small popliteal vein into the vein of Giacomini without communication with the popliteal vein - in 12 (9.0%);

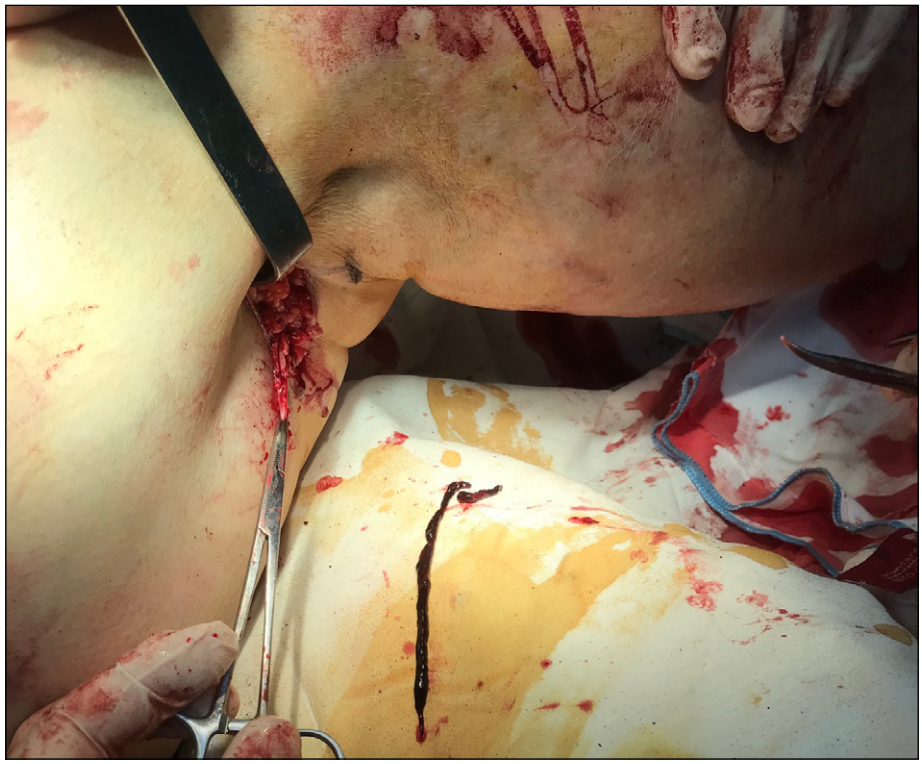

Fig. 1. Treatment of the sapheno-popliteal junction, thrombectomy from the vein of Giacomini.

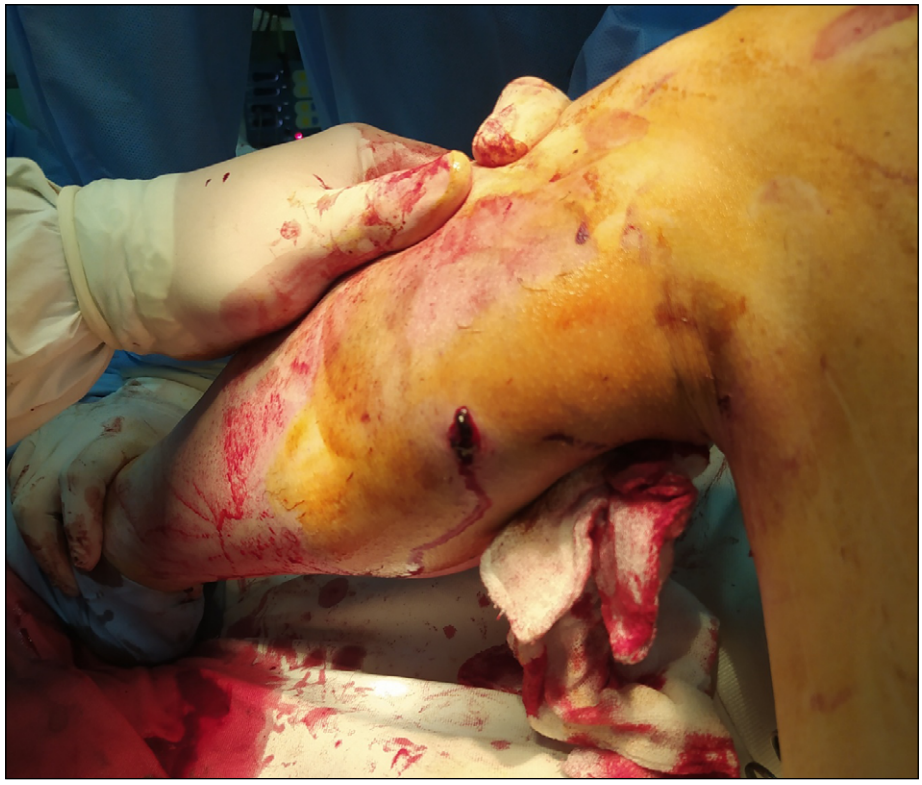

Fig. 2. Removal of thrombosed tributaries of the small saphenous vein.

- merger of the small saphenous vein with the medial bilateral venous sinus and a single trunk flowing into the popliteal vein - in 3 (2.2%);
- extension of the small saphenous vein into the veins of the muscles of the posterior thigh - in 7 (5.2%);
- inflow of the small saphenous vein into the great saphenous vein - in 1 (0.8%) observation.

Thrombosis of the trunk of the small saphenous vein was detected in 129 (96.3%) of 134 patients, with the apex of thrombotic masses in 82 (63.6%) patients localized in the upper third of the lower leg, and in 18 (14.0%) in the popliteal fossa, which posed a significant threat of venous thromboembolic complications.

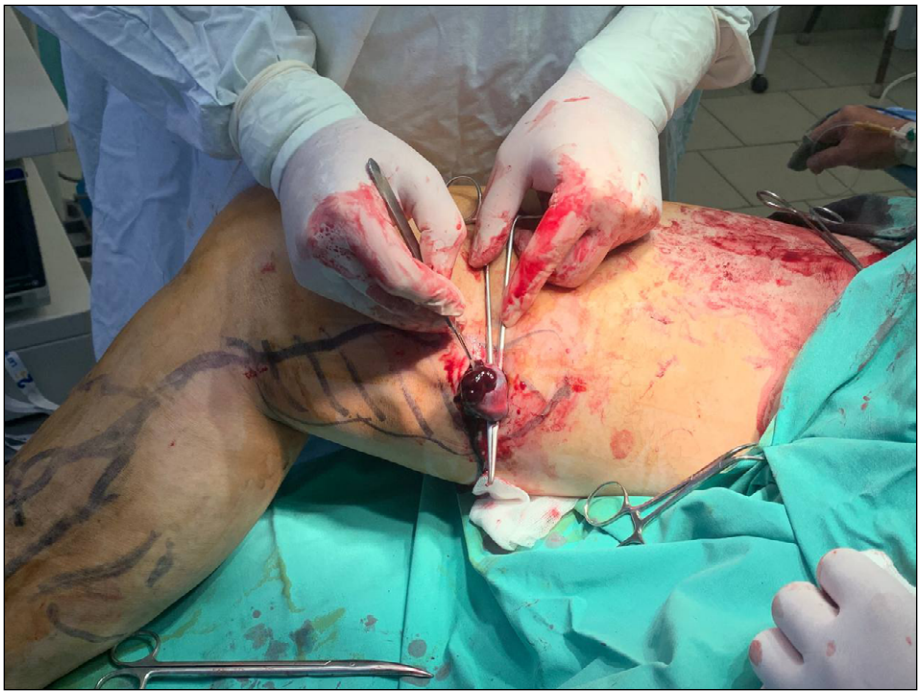

Fig. 3. Removal of the thrombosed trunk of the great saphenous vein.

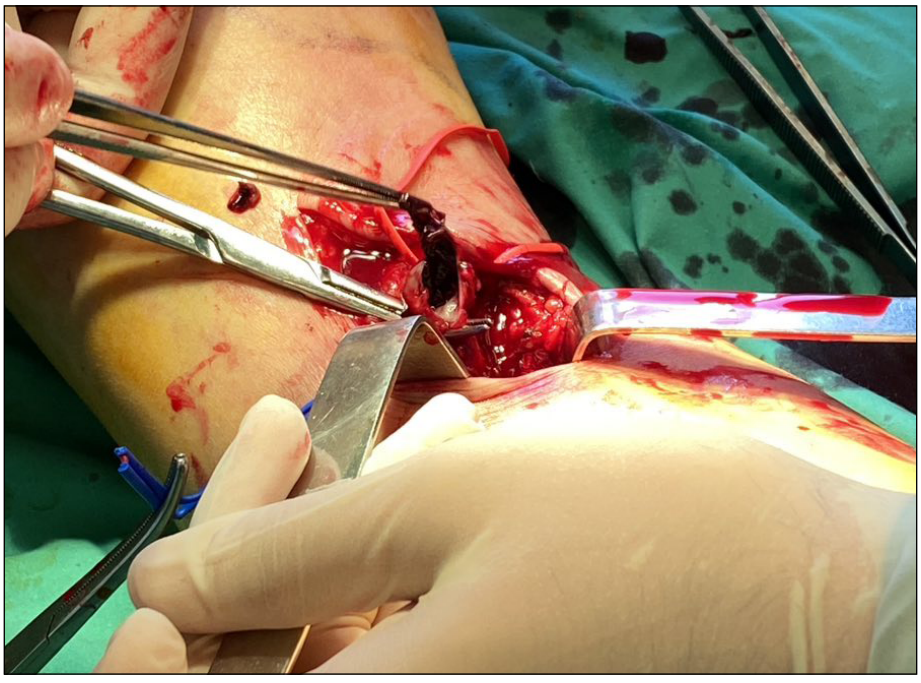

Fig. 4. Open thrombectomy from the popliteal vein.

In 5 (3.7%) of 134 patients, thrombotic lesions of the tributaries of the small saphenous vein were detected without the transition of thrombotic masses to the trunk of the superficial highway. Simultaneous lesions of the trunk of the small saphenous vein and its tributaries were detected in 53 (39.6%), including 10 (18.9%) with thrombotic lesions of the intersaphenous veins connecting the small and large saphenous veins. A combination of thrombotic lesions of the basin of the small and large saphenous veins was detected in 12 (9.0%) of 134 patients, with 92 (68.7%) patients with thrombosis of the small saphenous vein having varicose ectasia in the basin of the large saphenous vein

A patient with thrombosis of the superficial veins of the basin of the small saphenous vein was potentially considered as a candidate for urgent surgical intervention for the effective prevention of venous thromboembolic complications. However, the criteria for exclusion of the patient from the main group were as follows:

- the presence of thrombosis or post-thrombotic changes in the deep veins of the affected limb;

Table 1. Surgical interventions in patients of group I

No.	Surgical intervention	Quantity
1.	Ligation of SPC + phlebectomy of SSV	3
2.	Ligation of the SPC + phlebectomy of the SSV and its tributaries	5
3.	Phlebectomy of SSV and LSV + CE	24
3.	Ligation of SPC + phlebectomy of SSV and LSV + CE	46
4.	TE from the mouth of the SSV + ligation of the SPC + phlebectomy of the SSV and its tributaries	4
5.	TE from the mouth of the SSV + ligation of the SPC+ phlebectomy of the SSV and LSV + CE	1
6.	TE with SV + SPC ligation + phlebectomy of SSV and LSV + CE	2
7.	TE and ligation of the medial bilateral sinus + phlebectomy of the SSV and its tributaries	1
Total:		86

Notes: SPC - sapheno-popliteal connection
SSV - small saphenous vein
CE - crossectomy
LSV - large saphenous vein

- thrombotic lesions of the superficial and/or deep veins of the contralateral limb;
- severe comorbidities and/or contraindications to anesthesia;
- categorical refusal of the patient from the proposed surgery.

In patients of the main group, the surgical intervention involved the removal of the thrombotically affected trunk and tributaries of the small saphenous vein, but in the presence of varicose veins in the basin of the great saphenous vein, the volume of the operation was increased to eliminate valve insufficiency and venous reflux in both superficial highways of the affected limb. Immediately before the surgery, a control ultrasound examination was performed, which included marking the following important anatomical landmarks:

- the place where the small saphenous vein enters the popliteal vein, the medial bilateral venous sinus or the great saphenous vein, extending into the vein of Giacomini or the thigh muscles;
- the course of the trunk of the small saphenous vein;
- localization of failed piercing veins;
- localization of thrombosed and/or varicose tributaries of the small saphenous vein;
- localization of thrombosed and/or varicose intersaphenous veins.

Similarly, thrombosed and/or varicose veins of the great saphenous vein basin were labeled.

The main stages of the surgical intervention were as follows:

- treatment of the sapheno-popliteal junction - in 48 (55.8%) (Fig. 1);
- removal of thrombosed or varicose vein trunk of the small saphenous vein - in 86 (100%);
- removal of thrombosed or varicose tributaries of the small saphenous vein - in 47 (54.7%) (Fig. 2);
- removal of thrombosed or varicose intersaphenous veins - in 7 (8.1%);
- removal of thrombosed or varicose veins and the trunk of the great saphenous vein - in 73 (84.9%) (Fig. 3);
- thrombectomy from the mouth of the small subcutaneous vein, popliteal vein (Fig. 4) or medial bilateral venous sinus - in 5 (5.8%), 2 (2.3%) and 1 (1.2%) cases, respectively.

The main task of surgical treatment in group I was to eliminate the thrombotic process and prevent venous thromboembolic complications. The operation began with ligation of the sapheno-popliteal junction or venous connection of the small saphenous vein with the popliteal vein in its extension into the vein of Giacomini, the next step was the removal of the trunk of the small saphenous vein and, if necessary, its tributaries. In case of spreading thrombotic lesions or varicose changes in the basin of the IVC, crossectomy and phlebectomy of the great saphenous vein and its tributaries were performed.

Patients with the presence of the apex of thrombotic masses at the mouth of the small saphenous vein (n=5) and popliteal vein (n=2) required special attention. Surgical intervention began with open thrombectomy from the mouth of the small saphenous vein or popliteal vein. In both cases, thrombectomy from the popliteal vein was performed without venotomy through the mouth of the small saphenous vein.

In the presence of an open trophic ulcer - in 11 (12.8%) patients of group I, the operation was completed by surgical treatment of the trophic ulcer. Discharge from the trophic ulcer was sent for bacterial culture and antibiotic susceptibility testing. Antibiotic therapy was prescribed for 7 days.

Surgical interventions in patients of group I are presented in Table 1.

In group I, patients in the preoperative period

were prescribed injections of low-molecular-weight heparins in a therapeutic dose to prevent thromboembolic complications, which were continued in the postoperative period until discharge from the hospital with subsequent transfer to oral anticoagulants in a prophylactic dose for 1 month. Along with anticoagulants, complex phlebotropic agents (diosmin 400 mg/day + hesperidin 600 mg/day) and elastic knitwear of compression class II-III were prescribed, depending on the degree of chronic venous insufficiency.

In the immediate postoperative period before discharge from the hospital and at 1 month of follow-up, the following postoperative complications were observed (n=6) serous discharge from the postoperative wound - in 5 (5.8%) patients and marginal necrosis of the postoperative wound - in 1 (1.2%) patient. The average length of hospital stay was 4.8±1.6 days ($p \leq 0.05$). Within 1 month after surgery, trophic ulcers healed in all 11 operated patients. Not a single case of venous thromboembolic complication was noted in patients of group I within 1 month after surgery

Within 12 months after surgery, 78 (90.7%) of 86 patients were followed up. No recurrence of superficial or deep vein thrombosis or pulmonary embolism was noted in any patient during the follow-up year. The regression of symptoms of chronic venous insufficiency was noted in 52 (66.7%) of 78 patients, and no patient was diagnosed with a decompensated form of chronic venous insufficiency.

Conservative treatment in patients of group II consisted of the administration of low-molecular-weight heparins at a therapeutic dose for 10 to 14 days (mean 12.3±1.9 days ($p \leq 0.005$)), followed by transfer to oral anticoagulants at a therapeutic dose for 2 to 5 months (mean 3.2±0.7 months ($p \leq 0.05$)); in addition, complex phlebotropic agents (diosmin 400 mg/day + hesperidin 600 mg/day), non-steroidal anti-inflammatory drugs (paracetamol 500 mg) and elastic knitwear of compression class II - III, depending on the degree of chronic venous insufficiency, were prescribed.

In the presence of a trophic ulcer, compression dressings with antiseptics were prescribed in 5 (10.4%) patients of group II. Discharge from the trophic ulcer was sent for bacterial culture and antibiotic susceptibility testing. Antibiotic therapy was prescribed for 7 days. On average, the period of trophic ulcer healing was 28.4±5.6 days ($p \leq 0.05$).

Conservative treatment in patients of group II was mainly performed on an outpatient basis - 39 (81.3%) of 48 patients. Inpatient treatment for 5-7 days was required in patients (n=9) with severe comorbidities.

We managed to follow up 41 (85.4%) of 48 patients for 12 months. No patient showed regression of chronic venous insufficiency symptoms. In 5 (12.2%) patients, there was an increase in the manifestations of chronic venous insufficiency, with 4 patients developing trophic ulcers during the year. Recurrence of superficial or deep vein thrombosis was detected in 6 (14.6%) and 2 (4.9%) patients, respectively. In 1 (2.4%) patient, simultaneous thrombosis of superficial and deep veins of the affected limb was observed. Pulmonary embolism was detected in 2 (4.9%) patients, including 1 patient who died.

DISCUSSION

The high incidence of venous thromboembolic complications in superficial vein thrombosis is explained by the transition of thrombotic masses to the deep venous system through the saphenofemoral or sapheno-popliteal junction [5, 11], so the question of treatment of superficial vein thrombosis localized in the area of the saphenofemoral or sapheno-popliteal junction remains open [12]. Casian D. et al. (2022) conducted a prospective study comparing the results of conservative and surgical treatment of superficial vein thrombosis [13]. The authors treated 190 patients and concluded that surgical methods are not inferior to anticoagulant therapy [13]. Opponents of surgical treatment of superficial vein thrombosis argue that a high risk of pulmonary embolism is associated with the occurrence of deep vein thrombosis after surgical treatment of varicose veins [14]. Therefore, retrospective studies that do not take into account the results of varicose veins treatment can be misleading because they do not take into account the actual number of venous thromboembolic complications [15, 16]. At the same time, European guidelines state that the incidence of venous thromboembolic complications in patients almost does not depend on the presence of varicose veins [17, 18].

At the same time, other authors claim a decrease in the incidence of venous thromboembolic complications in patients with varicose veins after its surgical treatment [19], according to some reports, the risk of postoperative deep vein thrombosis is below 1%, although the literature data vary considerably [20, 21]. In addition, the absence of systematic ultrasound examination of patients in the postoperative period after open or endovenous interventions does not allow us to estimate the actual incidence of venous thromboembolic events [22].

Popovych Y.M. and co-authors (2023) state that the introduction of surgical treatment of superficial vein thrombosis effectively prevented the recurrence of the

thrombotic process in the superficial and deep veins of the lower extremity, pulmonary embolism, while in isolated conservative treatment their frequency was 5.1%, 3.4% and 3.4%, respectively [11]. In addition, active surgical tactics in patients with superficial vein thrombosis reduced the incidence of decompensated chronic venous insufficiency from 27.1 to 7.0%, and the manifestations of postthrombotic syndrome in the deep veins of the lower extremity from 100 to 3.7% [11]

Ultrasound monitoring of patients treated with anticoagulant therapy for superficial vein thrombosis during the first week after diagnosis revealed a tendency for the thrombotic process to spread proximally and distally in 23.1% and 9.6% of cases, respectively [23]. The ultrasound proximal and distal boundaries of the thrombotic process exceeded the clinical ones by an average of 15.26±1.21 cm and 7.94±1.32 cm, respectively [23]. In all patients with progression of the thrombotic process, the thrombotic masses were disorganized, and when they spread to the superficial venous highway, an unfixed apex of the thrombotic masses was detected in 85.7% of cases [23].

Thus, the issue of surgical prevention of venous thromboembolic complications in superficial vein thrombosis remains unexplored.

CONCLUSIONS

1. Surgical treatment of superficial vein thrombosis in the basin of the small saphenous vein can effectively prevent pulmonary embolism and recurrence of venous thrombosis in all patients, and in 66.7% of patients it promotes regression and prevents the development of decompensated forms of chronic venous insufficiency.
2. Recurrence of superficial and deep vein thrombosis during conservative treatment was observed in 14.6% and 4.9% of cases, respectively, and a simultaneous combination of superficial and deep vein thrombosis was detected in 2.4% of patients, which led to pulmonary embolism in 4.9% of patients.
3. Conservative treatment in no case led to a regression of chronic venous insufficiency, and in 12.2% of the observations, decompensated forms of chronic venous insufficiency were observed.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Histological manifestations in the structures of the anterior abdominal wall after implantation of the acellular dermal matrix using the sublay method

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ABSTRACT

Aim: The objective of this study was to assess the level of vascularization, integration of the acellular dermal matrix, as well as the development of granulation tissue and collagen fibers following implantation on the anterior abdominal wall ("sub lay") in pigs over the course of 7, 14, and 21 days.

Materials and Methods: The experiment was conducted on six pigs of the Ukrainian White steppe. Under general anesthesia, an acellular perforated dermal matrix from pigs, produced by the «Institute of Biomedical Technologies», was implanted into the anterior abdominal wall, between the aponeurosis and muscles. The quality of engraftment, degree of angiogenesis, condition of granulation tissue, and graft structure were assessed. The postoperative period was uncomplicated, with each pig receiving one intramuscular dose of ceftriaxone (1.0). Material was collected from two pigs on the 7th day, two more on the 14th day, and the final two on the 21st day after implantation. Pathomorphological analysis was carried out in the laboratory of I. Horbachevsky Ternopil National Medical University. Sections of the implanted material, stained with hematoxylin-eosin, were evaluated for local inflammation reactivity, vascularization levels, and granulation tissue replacement.

Results: The research findings revealed that, after 7 days of implantation, the acellular skin matrix "sub lay" led to infiltration caused by acute inflammation, accompanied by the formation of granulation tissue, numerous microcirculatory vessels, and collagen fibers. By day 14, the signs of inflammation had reduced, and there was an increase in fibroblasts and blood vessels. On day 21, the implantation process intensified, marked by a rise in collagen fibers and vascularization, along with a decrease in macrophages and lymphocytes. No signs of infection were observed.

Conclusions: Acellular dermal matrix after «sub lay» implantation causes acute inflammation with slow vascularization in the early period with a moderate increase up to 14 and 21 days. The implant acts as a base to support the migration of natural cells and subsequent replacement by granulation tissue, as a result of strengthening this area. No signs of infection were detected, which gives hope for the use of implants in previously infected environments after complications due to the implantation of meshes made of artificial materials before.

KEY WORDS: Acellular dermal matrix, pigs, "sub lay" implantation, microscopy

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INTRODUCTION

Surgical reconstruction of the abdominal wall is one of the most common needs in hernia surgery due to both tissue deficiency and high suture line tension, which leads to postoperative hernias [1]. Incisional hernias are a common complication of laparotomy incisions and a major healthcare burden. The management of incisional hernias are challenging and complex. This activity reviews preoperative optimization, techniques for repair, mesh use, and complications of incisional hernia repair and highlights some strategies to reduce the rate of incisional hernia.

Treatment strategy for hernia involves surgical repair or conservative treatment. The decision to choose between the two options depends on a few factors like symptoms, the size of a hernia, complications and patients' preference. Open, laparoscopic technique has been commonly used to repair incisional hernias. Mesh provides the strength for the repair and scaffold for the healing tissue. Meshes are characterized as permanent vs. absorbable, and synthetic vs. biologic.

The use of polypropylene mesh for the surgical treatment of hernia defects of the abdominal wall helps to reduce recurrence [2, 3]. However, this is accompanied by several complications, such as increased postoperative pain, abdominal adhesions, and areas of fibrosis, and an increased risk of infection due to prosthesis placement [1, 4].

Since the beginning of the last century, the use of prostheses has become a constant in the surgery of abdominal wall hernias especially inguinal hernias [4]. The study and search for alternative materials, such as

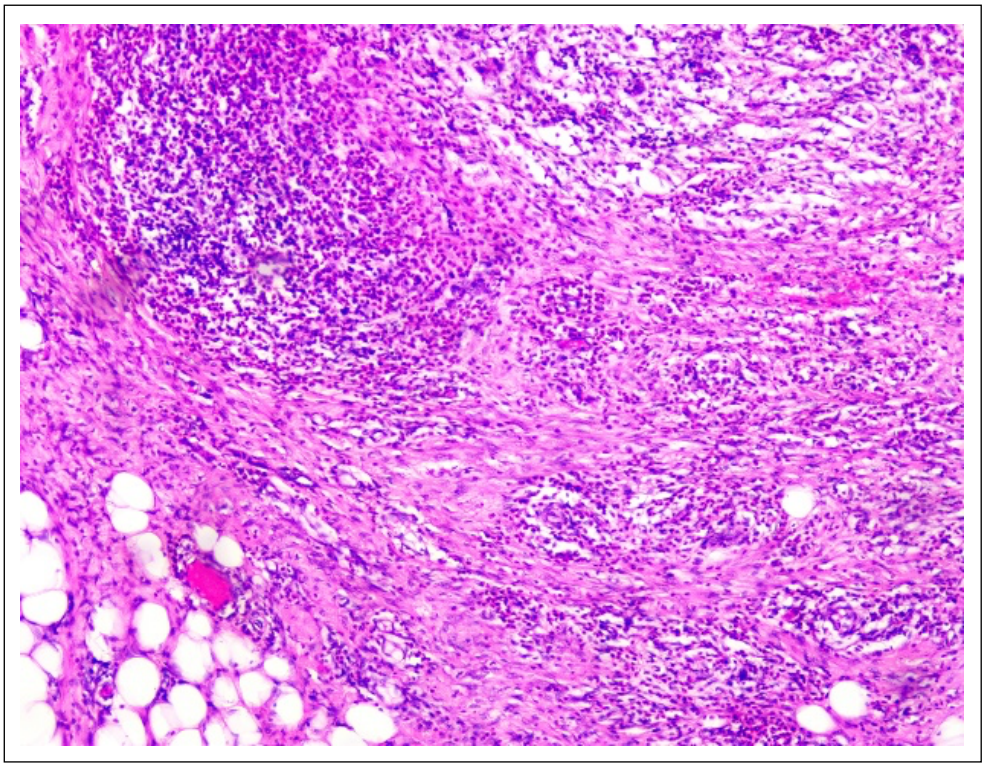

Fig. 1. The area of aponeurosis treated with a porcine skin xenograft using the sublay method after 7 days of experiment. Inflammatory infiltration of granulation tissue with the formation of leukocyte infiltration foci. Hematoxylin and eosin, x 100.

bovine pericardium, dermal matrix from pig skin, human skin, have proven to be one of the options for hernia repair and treatment, leading to better results[5-7].

Complete evaluation of incisional hernias includes confirming the diagnosis, sizing the defect, identifying the herniated content, and assessing the abdominal cavity to plan the surgical treatment in complex hernias. CT scan imaging is useful for obtaining these details [8, 9].

However, there are insufficient data on the reaction of local tissues to the implant, the level of vascularization, integration and the formation of collagen fibers after implantation at different levels over some time [10, 11].

AIM

The objective of this study was to assess the level of vascularization, integration of the acellular dermal matrix, as well as the development of granulation tissue and collagen fibers following implantation on the anterior abdominal wall ("sub lay") in pigs over the course of 7, 14, and 21 days.

MATERIALS AND METHODS

The experiment was performed on 6 pigs of the Ukrainian White steppe. Under general anesthesia, acellular perforated dermal matrix of a pig produced by "Institute of Biomedical Technologies" (Chief Director Prof. Bigunyak V.V.) was implanted on the anterior abdominal wall in the space between the aponeurosis and muscles ("sub lay") The quality of engraftment, degree of angiogenesis, condition of granulation tissue and graft structure were estimated.

The postoperative period was uncomplicated, every pig received 1 dose of ceftriaxone 1.0 intramuscularly. From 2 pigs the material was collected on the 7th day, another 2 on the 14th day, and in the last 2 on the 21st day after implantation.

Pathomorphological research were done in the laboratory of I. Horbachevsky Ternopil National Medical University. Sections of the implanted material were stained with hematoxylin-eosin were evaluated concerning the reactivity of local inflammation, the level of vascularization, and replacement by granulation tissue.

RESULTS

HISTOLOGICAL CHANGES OBSERVED IN THE STRUCTURES OF THE ANTERIOR ABDOMINAL WALL FOLLOWING ACELLULAR DERMAL MATRIX IMPLANTATION USING THE «SUBLAY» TECHNIQUE OVER VARIOUS TIME PERIODS (7, 14, 21 DAYS)

The implantation of the acellular dermal matrix (lyophilized pig skin) was performed in the anterior abdominal wall, specifically in the space between the aponeurosis and muscles (sublay technique).

Seven days after the implantation, well-formed granulation tissue was noted, accompanied by intense inflammatory infiltration and focal areas of leukocyte infiltration (Fig. 1). A significant presence of microcir-

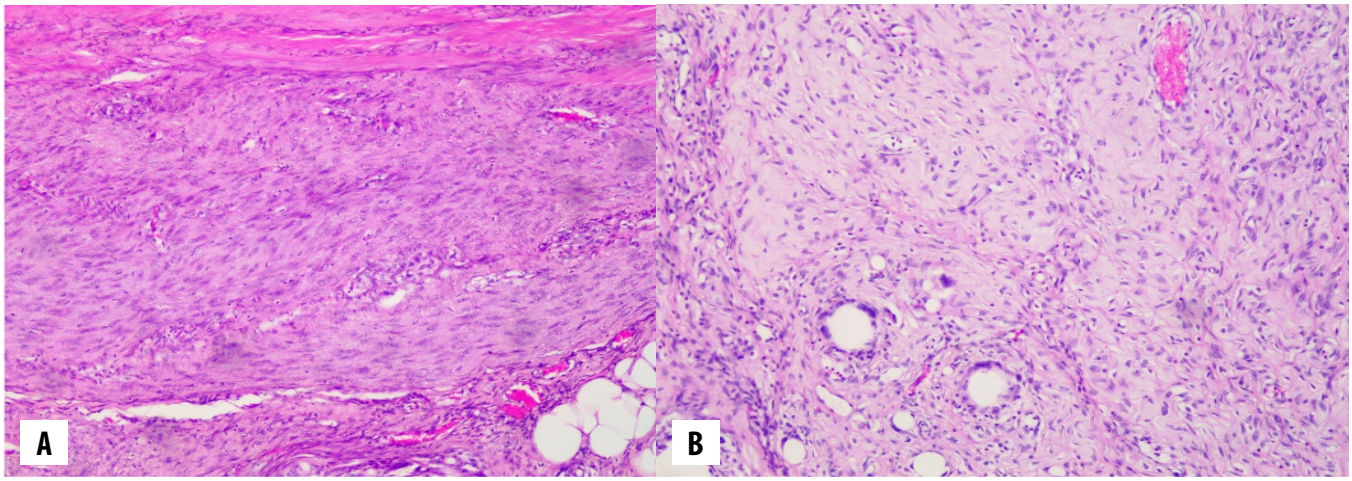

Fig. 2. The area of aponeurosis treated with a porcine skin xenograft using the sublay method after 14 days of experiment. Granulation tissue with mild inflammatory infiltration and an increased number of fibroblasts (A), the granulation tissue is formed with well-defined blood vessels (B).

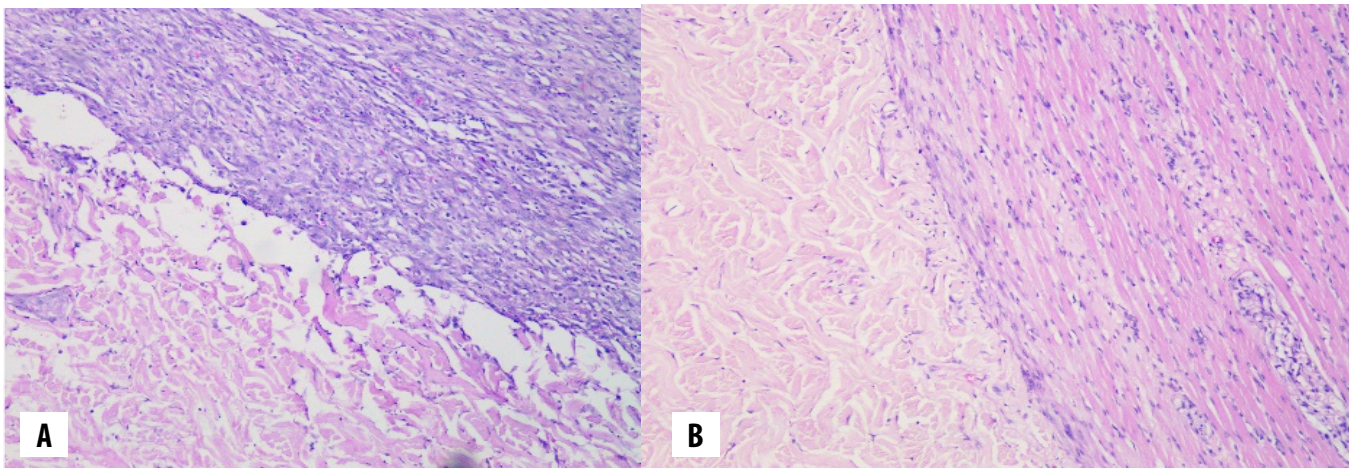

Fig. 3. The area of aponeurosis with the use of porcine skin xenograft according to the method (sublay) after 21 days of the experiment. Fibrinoid edema of collagen fibers with foci of necrosis (A), Collagen fibers are moderately organized. Moderate inflammatory infiltration of the stroma (B). Hematoxylin and eosin, x 100.

culatory vessels and collagen fibers was observed. The inflammatory response was particularly pronounced, with some areas showing necrosis at the graft contact points. The number of macrophages and fibroblasts remained low, while lymphocytic activity was moderate.

There was minimal inflammatory infiltration with clusters of polymorphonuclear leukocytes in the detected adipose tissue.

The graft`s structure was partially altered in the regions of contact with the tissue, as indicated by the presence of mucoid edema in the fibers. In other areas, the structure consists of well-preserved fibers arranged in an orderly manner, with minimal focal lymphocyte infiltration.

Histologic examination of the tissue 14 days after xenograft implantation revealed the formation of granulation tissue, including numerous microcirculatory vessels and a moderate amount of collagen fibers. The signs of inflammatory infiltration diminished, with lymphocytes and histiocytes being the predominant cells, while the number of the leucocytes significantly decreased (Fig. 2). Blood filling in the vessels was uneven, with some lumens still dilated, but no perivascular edema was noted. The overall density of microcirculatory vessels was was considerable.

Perivascular edema and moderate lymphohistiocytic infiltration, including occasional macrophages, were observed in the areas where the graft made contact. The inflammatory infiltration extended slightly into the surrounding tissue. Within the graft structure, individual capillaries surrounded by lymphocytes and histiocytes were visible.

Histologic examination of the aponeurosis area 21 days after acellular dermal matrix implantation showed a moderate reduction in inflammatory infiltration within the intervention zone. The granulation area had significantly redused, primarily due to decreasing inflammatory infiltration, perivascular edema and cap-

illary blood filling. The area of collagen fibers slightly increased, with the fibers arranged in a more orderly fashion (Fig. 3).

Macrophages, lymphocytes, histiocytes, and fibroblasts were observed among the fibers, with a reduction in the number of fibroblasts. Within the graft structure, the density of microcirculatory vessels sprouting from the granulation tissue moderately increased. In some areas, fibrinoid edema and focal necrosis of the collagen stroma were evident, while in other areas, focal inflammatory infiltration along the collagen stroma was visible. So, the implant acts as a base to support the migration of connective tissue cells and subsequent replacement by granulation tissue, as a result of strengthening this area.

DISSCUSION

Critical review of the literature revealed a large variety in mesh models; many different models, animal species, meshes, and parameters were assessed in the last decade leading to studies that were difficult to compare among each other. Identical models including all parameters were not found to be implemented by different centers, in other words all centers apparently use their own specific models. Due to the growing variety in existing and new concepts of meshes, preclinical animal research is necessary to assess biocompatibility and effectiveness of new meshes before implementing them in clinical practice [12-14]. Furthermore, many of the important mesh characteristics are derived from and can only be properly researched using animal models [15, 16]. However, for experimental research to have proper impact, research published by different research groups needs to be comparable and reproducible [17, 18].

In laparoscopic incisional hernia repair, direct contact between the prosthesis and abdominal viscera is inevitable and may lead to adhesions [19-21]. Despite the large variety of mesh prosthesis, little is known about their in vivo behavior. Biological meshes are considered to have many advantages, but due to their price they're rarely used. A rat model was used to assess biological and conventional synthetic meshes on their in vivo characteristics. Based on incorporation, adhesion surface, adhesion strength, mesh shrinkage, and the histologic parameters scaffold degradation, cellular infiltration, neovascularization, and extracellular matrix deposition, Strattice™ performed best in this experiment in a physiologic, non-contaminated rat model with intraperitoneal mesh placement.

Biological grafts, introduced as alternatives to synthetic commercial meshes for hernia repair, offer the potential for fewer post-operative complications compared to synthetic options [22-24]. These grafts are made through tissue decellularization and can be derived from human (allogenic) [25, 26] or animal (xenogenic) sources, including dermis from both species [27-29], porcine small intestinal submucosa [30-32], and bovine pericardium [33-35], or other organs. Decellularization methods – such as physical, chemical, and enzymatic processes – help prevent infections and foreign body reactions. Some commercially available biological tissue grafts for abdominal wall hernia repair include Permacol™, Surgisis®, SurgiMend™, XenMatrix™, FlexHD™, Veritas®, AlloMax™, Periguard®, and Alloderm®.

There are several basic methods for placing implants in reconstructive surgery of abdominal wall defects: Onlay (on the aponeurosis), Sublay (under the aponeurosis), PPT (preperitoneally), and several modifications of these methods (e.g., Inlay). All methods are highly effective and are commonly used in the practical work of surgeons. Each method has its own advantages and disadvantages [3, 15].

A significant number of complications are caused by the type of material used for the prosthesis [13]. The main aim of our research is to investigate the integration and engraftment of biological implants, specifically the acellular dermal matrix of pigs, when implanted at different levels of the abdominal wall. Here we showed the pathomorphological results of the implantation sublay of porcine dermal matrix.

Extracellular collagen matrices in biological grafts promote quick tissue ingrowth and neovascularization, encouraging strong, durable integration with the patient's own tissues. As the grafts integrate, they degrade gradually and remodel into newly regenerated tissue that supports the abdominal cavity. A study by Ghetti et al. assessed the post-operative morphological changes in hernia patients one year after receiving human decellularized matrices. Their findings revealed regenerative cellular recruitment, neovascularization, reduced inflammation, and a well-organized collagen matrix at the implant site [36]. In another study, Hoganson and colleagues developed an acellular porcine dermis matrix that successfully preserved ECM components such as proteins, glycosaminoglycans, and cytokines (e.g., VEGF, TGF-β), all of which aid in graft integration and wound healing [37]. Co-cultured fibroblasts showed typical spindle-like shapes and strong viability after one and two weeks. The presence of bioactive components and appropriate mechanical strength suggests that decellularized dermis materials have significant clinical potential. Additionally, biological meshes have been found to resist infection when implanted, with no need for removal even if infection occurs [37, 38]. For

this reason, biological meshes are particularly beneficial in addressing body trunk defects and complex cases where infection risks are high.

Faster vascularization and cell migration, with subsequent formation of granulation tissue, were observed after the Sublay method of surgery compared to the Onlay method. Moderate inflammation and the absence of infection following the Sublay technique are significant advantages for promoting high-quality healing.

Therefore, studies on the integration of biological implants after their implantation at different levels of the abdominal wall are promising for future research [38, 39].

CONCLUSIONS

Acellular dermal matrix after «sub lay» implantation causes acute inflammation with slow vascularization in the early period with a moderate increase up to 14 and 21 days. The implant acts as a base to support the migration of cells and subsequent replacement by granulation tissue, as a result of strengthening this area. No signs of infection were detected, which gives hope for the use of implants in previously infected environments after complications due to the implantation of meshes made of artificial materials before.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Correlation between MRNA IL1B and type, duration of infertility in women with endometriosis on the stage preparing to assisted reproductive technologies using probiotics

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ABSTRACT

Aim: To investigate the correlations between the level of IL1β mRNA gene expression and the type and duration of infertility and to study the levels of IL1β mRNA gene expression in women with endometriosis associated with infertility.

Materials and Methods: For mRNA gene expression analysis IL1β and determination of relative normalized mRNA expression IL1β used the real-time reverse transcription polymerase chain reaction method (RT-PCR).. Examined group consists of 30 infertile women undergoing assisted reproductive technologies. The main group consisted of 20 women diagnosed with endometriosis undergoing assisted reproductive technologies. The control group consisted of 10 healthy women.

Results: In the main group, the level of IL1β mRNA gene expression before preparing was 26.7877±0.01, which was significantly higher than the level after preparing (0.1610±0.01*).

Analized results, it's found out that Mean level of mRNA IL1β in women with endometriosis associated infertility 1-st degree is 8.53 c.u., at the same time Mean level of mRNA IL1β in women with endometriosis associated infertility 2-nd degree is 1.0 c.u.

Conclusions: The inclusion of probiotics in a comprehensive regimen of preparation for assisted reproductive technologies leads to a noticeable improvement in the patient's well-being.

KEY WORDS: endometriosis, probiotics, assisted reproductive technologies, infertility, IL1β

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INTRODUCTION

Endometriosis is a chronic inflammatory condition characterized by the growth of tissue similar to the lining of the uterus outside the uterus, usually in areas such as the peritoneum, ovaries, and cervix. Clinical symptoms often include progressive dysmenorrhea, chronic pelvic pain, profound dyspareunia, and infertility, which significantly affect the patient's quality of life [1]. It is estimated that approximately 10% of women of reproductive age suffer from endometriosis [2].

Although the exact cause and development of endometriosis remain unclear, the theory of retrograde menstruation proposed by Sampson in 1921 is widely accepted. Other hypotheses, such as coelomic metaplasia and vascular/lymphatic metastasis [3], have also been proposed, but cannot fully explain all forms of the condition. In addition, factors such as the immune system, hormones, genetics, and the environment are believed to play an important role in the pathogenesis of endometriosis [4].

Given the involvement of natural killer (NK) cells in endometriosis due to reduced toxicity, one potential treatment approach is to activate these cells. In animal models, intraperitoneal injections of Lactobacillus gasserii OLL2809, a probiotic that stimulates IL-12 production, resulted in NK cell activation and reduction of ectopic endometrioid lesions. A randomized, double-blind, placebo-controlled trial also showed that this probiotic can alleviate pain associated with endometriosis [5]. Adhesion of endometrial fragments to other tissues is considered to initiate a local inflammatory response that over time develops into chronic inflammation. The inflammatory response in the pelvic cavity largely involves the activation of macrophages, which produce a number of growth-regulating substances. There is evidence that some of the inflammatory factors also stimulate the growth of ectopic endometrial cells in the early stages of endometriosis [6]. These compounds can also affect fertility, as well as nociceptors, thus causing

infertility and pain. Cytokines are regulatory peptides or glycoproteins that can be produced by virtually every type of nucleated cell in the body and have pleiotropic regulatory effects on many cell types. Unlike hormones, cytokines usually act as paracrine and/or autocrine signals, only occasionally entering the circulation, where they can act as endocrine mediators [7]. Macrophages are among the major producers of cytokines, especially interleukins-1 and 6 (IL-1, IL-6) and tumor necrosis factor-α (TNFα); this is probably not the case under normal conditions, but after stimulation by various substances [8]. Interleukins are considered modulators of cell proliferation and as inducers of other cytokines, as a cascade in acute inflammation [9].

Cytokines produced in the uterine environment are involved in the regulation of endometrial growth through steroid-cell and cell-cell interactions [10]. Cytokines may also contribute to the pathophysiology of endometriosis in at least two ways, namely by enhancing the establishment and proliferation of ectopic endometrial implants and by influencing cytokine secretion by macrophages, which can lead to adverse changes. The cytokines IL-1β, IL-6 and TNFα are of great interest because they are partly hormonally regulated and play important roles as mediators of inflammation. IL-1 is involved in the regulation of the immune response and inflammation. There are two different forms of IL-1, α and β, with similar biological activities [7]. IL-1α is present in the endometrium, in both epithelial and stromal cells, at least in the late secretory phase. IL-1β has a similar distribution, usually appearing in lower amounts. IL-1β mRNA is expressed in the endometrium in the late secretory phase and corresponds to serum IL-1β levels, which vary throughout the cycle with maximum values during the secretory phase [11].

AIM

To study mRNA gene expression level IL1β and estimated correlation between IL1 mRNA β and type, duration of infertility in women with endometriosis on the stage of preparing for assisted reproductive technologies using probiotics

MATERIALS AND METHODS

For gene expression analysis IL1β mRNA and determination of relative normalized mRNA expression IL1β used the real-time reverse transcription polymerase chain reaction method(RT-PCR).The object for molecular genetic studies using RT-PCR was a fraction of mononuclear cells isolated from whole blood of patients with endometriosis. In this study, we conducted a retrospective analysis of case histories of 30 infertile women undergoing assisted reproductive technologies. The main group consisted of 20 women diagnosed with endometriosis who underwent assisted reproductive technologies. In addition to standard preparation for assisted reproductive technologies, women in the main group received a probiotic containing Lactobacillus 10^{10} manufactured by Unic Biotech Ltd, India. They took one tablet twice a day for one month as part of the general treatment before undergoing assisted reproductive technologies. We determined the level of IL1β expression before and after this stage of preparation. The control group consisted of 10 women who had tubal infertility due to a previous inflammatory disease, but according to the results of a comprehensive clinical and laboratory examination they were equated to healthy women. These women aged 21 to 42 years with a mean age of 29.75 years did not undergo our proposed preparation for ART with the inclusion of a probiotic. This study was conducted at the Bukovinian State Medical University and the "Medical Center of Infertility Treatment" clinic.

RESULTS

The average age of women in the control group (who did not take the probiotic – 28.78±5.09 years) and the main group (who took the probiotic) 29.54±2.04 ($p > 0.05$). Women in the main and control groups were examined and expression levels were determined IL1β mRNA genes. Expression level IL1β mRNA genes in whole blood in women before preparation for assisted reproductive technologies are given in Table 1

Examining the data presented in Table 1, we can distinguish two clear subgroups: the main group, consisting of women with endometriosis who received our proposed training for assisted reproductive technologies, including probiotics, before and after training, respectively. In the main group, the level of IL1β mRNA gene expression before training was 26.7877±0.01, which was significantly higher than the level after training (0.1610±0.01).

We performed analysis of level expression mRNA IL1β before treatment conditioning on group.

According to the presented table 2, when comparing of IL1β before treatment, statistically significant differences were revealed depending on group ($p < 0.001$) (applied method: Mann-Whitney U-test).

Analized results according Fig.1, Mean level of mRNA IL1β in women with endometriosis is 10.35 c.u., at the same time Mean level of mRNA IL1β in women control group is 1.0 c.u.

Table 1. Expression level IL1β mRNA genes in whole blood in women before preparation for assisted reproductive technologies (M±m)

Group	Expression level IL1β mRNA genes in whole blood		P
	Before preparing (treatment)	After preparing (treatment)	
Main	26,7877±0,01	0,1610±0,01	<0,001

Table 2. Analysis of level expression mRNA IL1β before treatment conditioning on group

Level expression mRNA IL1β before treatment					
Variable	Categories	Me	$Q_1 - Q_3$	n	p
Group	Control	1.00	1.00 – 1.00	10	< 0.001*
	Endometriosis	10.35	2.70 – 21.62	20	

* – differences are statistically significant (p < 0.05).

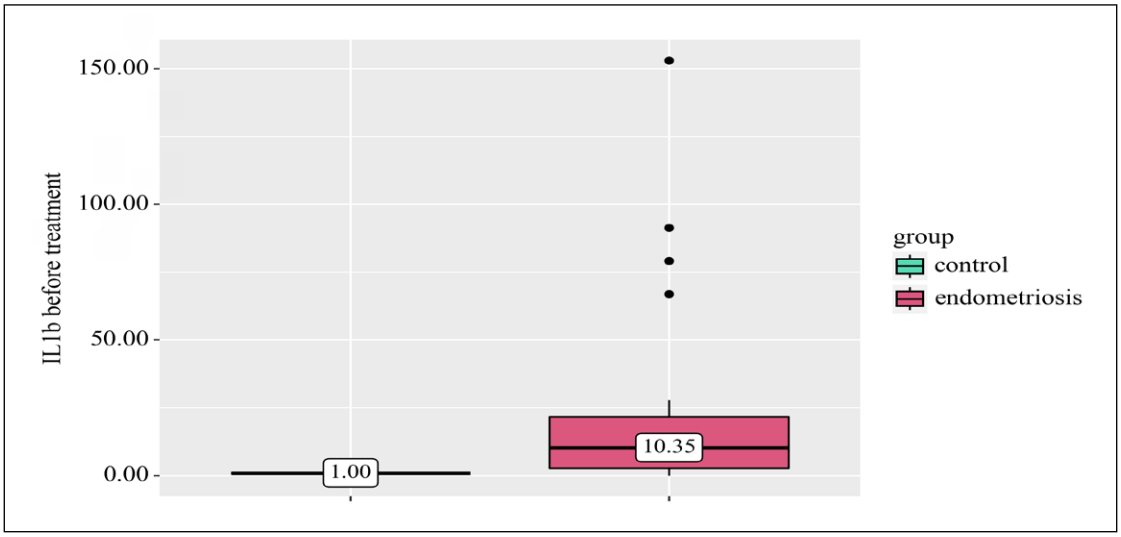

Fig. 1. Analysis of level expression mRNA IL1β before treatment conditioning on group.

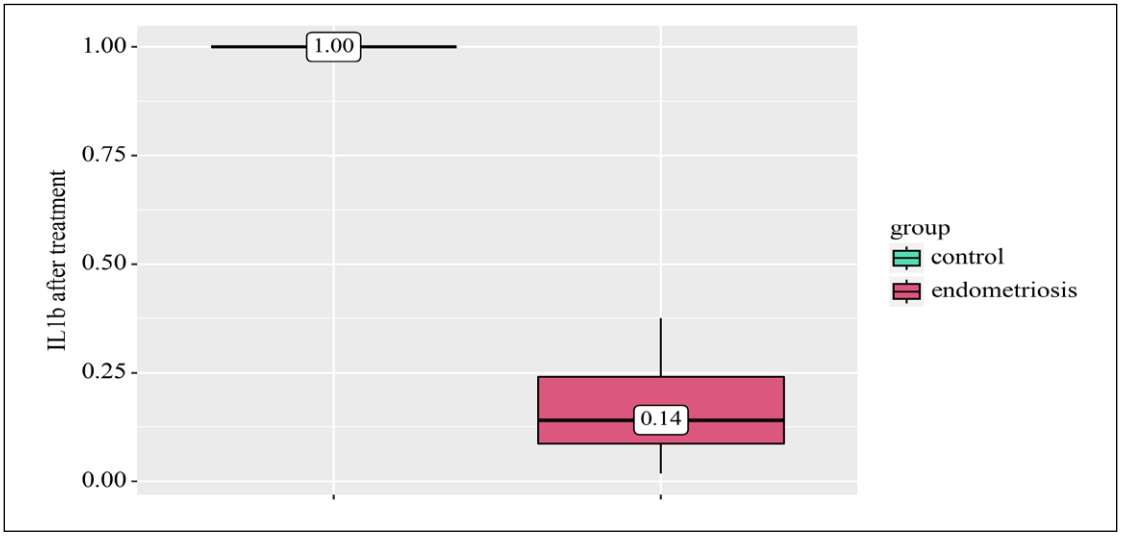

Fig. 2. Analysis of level expression mRNA IL1β after treatment conditioning on group.

We also performed analysis of IL1β after treatment conditioning on group.

According to the presented table 3, when comparing of level expression mRNA IL1β after treatment, statistically significant differences were revealed depending on group (p < 0.001) (applied method: Mann-Whitney U-test).

Analized results according Fig.2., Mean level of mRNA IL1β in women with endometriosis is 0.14 c.u., at the same time Mean level of mRNA IL1β in women control group is 1.0 c.u.

We also performed analysis of level expression mRNA IL1β before treatment conditioning on group.

In accordance with the presented table 4, when comparing of IL1β level before treatment, statistically significant differences were revealed depending on group (p < 0.001) (applied method: Pearson's chi-square test).

Analized results according Fig.3., normal level of mRNA IL1β in women with endometriosis is in 25% patients, high level is observed in 70% patients , low level is in only 5% patients., at the same time Mean level of mRNA IL1β in women control group is normal in 100 % patients.

Analysis of level expression mRNA IL1β level after treatment was performed conditioning on group.

In accordance with the presented table 5, when comparing of level expression mRNA IL1β after treatment, statistically significant differences were revealed depending on group ($p < 0.001$) (applied method: Fisher's exact test).

Odds of low were 59.182 times greater in women with endometriosis comparing with control group, the relative difference in odds was statistically significant (95% CI: 2.949 – 1187.719).

Analysis of level expression mRNA IL1β before treatment was performed conditioning on infertility degree (Fig. 4).

According to the data obtained when comparing of level expression mRNA IL1β before treatment statistically significant differences were revealed depending on infertility degree ($p = 0.050$) (applied method: Mann-Whitney U-test) (Table 6).

Analized results according Fig.5., Mean level of mRNA IL1β in women with endometriosis associated infertility 1-st degree is 8.53 c.u., at the same time Mean level of mRNA IL1β in women with endometriosis associated infertility 2-nd degree is 1.0 c.u.

Analysis of level expression mRNA IL1β after treatment was performed conditioning on infertility degree.

According to the presented table 7, when comparing of level expression mRNA IL1β after treatment, statistically significant differences were revealed depending on infertility degree ($p = 0.003$) (applied method: Mann-Whitney U-test).

Analized results according Fig.6., Mean level of mRNA IL1β after treatment in women with endometriosis associated infertility 1-st degree is 0.14c.u., at the same time Mean level of mRNA IL1β in women with endometriosis associated infertility 2-nd degree is 1.0 c.u.

Analysis of level expression mRNA IL1β before treatment was performed conditioning on infertility degree.

When comparing of level expression mRNA IL1β before treatment depending on infertility degree no statistically significant differences were revealed ($p = 0.075$) (applied method: Pearson's chi-square test) (Table 8).

Analized results according Fig.7., normal level of mRNA IL1β before treatment in women with endometriosis associated infertility 1-st degree is in 33,3%, high level – in 61,1%, low level in 5,6%, at the same time normal level of mRNA IL1β in women with endometriosis associated infertility 2-nd degree is in 75% and high level is in 25%.

We performed analysis of level expression IL1β level after treatment conditioning on infertility degree.

According to the presented table 9, when comparing of level expression IL1β after treatment, statistically significant differences were revealed depending on infertility degree ($p = 0.008$) (applied method: Fisher's exact test).

Odds of low were 13.000 times less in 2nd degree group than in 1st degree group, the relative difference in odds was statistically significant (OR = 0.077; 95% CI: 0.012 – 0.482).

Table 3. Analysis of level expression mRNA IL1β after treatment conditioning on group

Level expression mRNA IL1β after treatment					
Variable	Categories	Me	$Q_1 – Q_3$	n	p
Group	Control	1.00	1.00 – 1.00	10	< 0.001*
	Endometriosis	0.14	0.09 – 0.24	20	

* – differences are statistically significant ($p < 0.05$).

Table 4. Analysis of level expression mRNA IL1β before treatment conditioning on group

Variable	Categories	Group		p
		Control	endometriosis	
IL1β level before treatment	Normal	10 (100.0)	5 (25.0)	< 0.001*
	High	0 (0.0)	14 (70.0)	
	Low	0 (0.0)	1 (5.0)	

* – differences are statistically significant ($p < 0.05$).

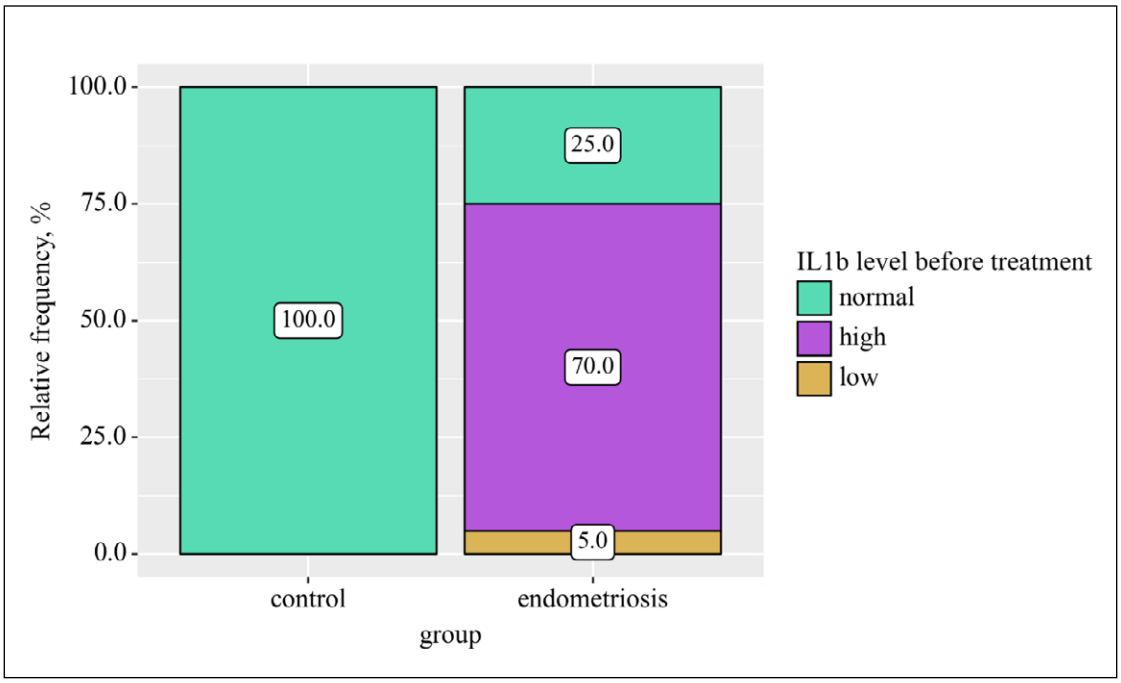

Fig. 3. Analysis of level expression mRNA IL1β level before treatment conditioning on group.

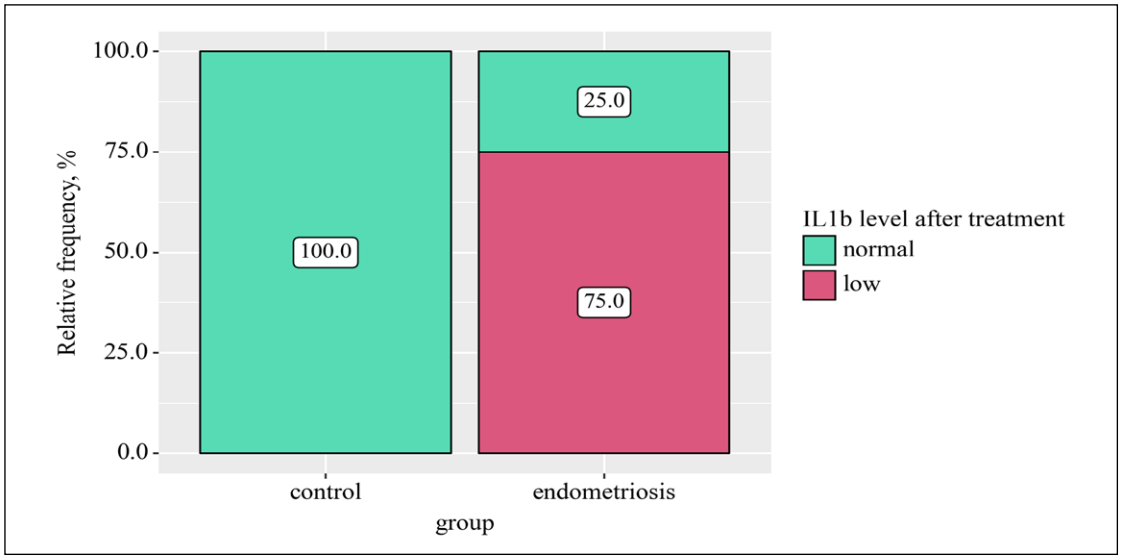

Fig. 4. Analysis of level expression mRNA IL1β after treatment conditioning on group.

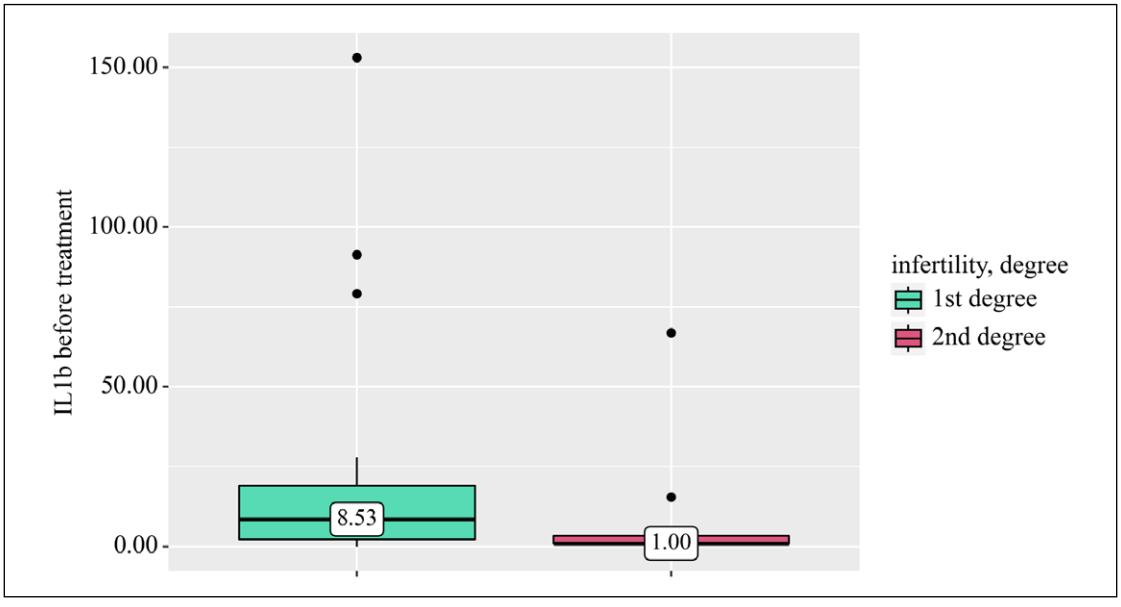

Fig. 5. Analysis of level expression mRNA IL1β before treatment conditioning on infertility degree.

Table 5. Analysis of level expression mRNA IL1β after treatment conditioning on group

Variable	Categories	Group		p
		Control	Endometriosis	
IL1β level expression mRNA after treatment	Normal	10 (100.0)	5 (25.0)	< 0.001*
	Low	0 (0.0)	15 (75.0)	

* – differences are statistically significant ($p < 0.05$).

Table 6. Analysis of level expression mRNA IL1β before treatment conditioning on infertility degree

Variable	Categories	Level expression mRNA IL1β before treatment		n	p
		Me	$Q_1 – Q_3$		
Infertility degree	1st degree	8.53	2.16 – 18.98	18	0.050*
	2nd degree	1.00	1.00 – 3.36	12	

* – differences are statistically significant ($p < 0.05$).

Table 7. Analysis of level expression mRNA IL1β after treatment conditioning on infertility degree

Variable	Categories	Level expression mRNA IL1β after treatment		n	p
		Me	$Q_1 – Q_3$	18	
Infertility degree	1st degree	0.14	0.10 – 0.24		0.003*
	2nd degree	1.00	0.30 – 1.00	12	

* – differences are statistically significant ($p < 0.05$).

Table 8. Analysis of level expression mRNA IL1β before treatment conditioning on infertility degree

Variable	Categories	Infertility degree		p
		1st degree	2nd degree	
Level expression mRNA IL1β before treatment	Normal	6 (33.3)	9 (75.0)	0.075
	High	11 (61.1)	3 (25.0)	
	Low	1 (5.6)	0 (0.0)	

Table 9. Analysis of level expressionIL1β level after treatment conditioning on infertility degree

Variable	Categories	Infertility degree		P
Level expression mRNA IL1β level after treatment	Normal	1st degree	2nd degree	0.008*
		5 (27.8)	10 (83.3)	
	Low	13 (72.2)	2 (16.7)	

* – differences are statistically significant ($p < 0.05$).

During our research we found out that there was no association between level expression mRNA IL1β before treatment and infertility duration.

Observed dependence of level expression mRNA IL1β before treatment from infertility, duration is described by a linear regression equation:

$$Y_{\text{IL1b before treatment}} = 4.283 \times X_{\text{infertility, duration}} - 4.223$$

With an 1 increase of infertility, duration 4.283 change of IL1β before treatment should be expected. According to the coefficient of determination R^2 of the resulting model, 5.9% of the observed variance of IL1β before treatment were explained.

We performed a correlation analysis of the association between infertility, duration and IL1β after treatment. A weak correlation positive association between level expression mRNA IL1β after treatment and infertility duration was estimated (Fig. 8).

Observed dependence of level expression mRNA IL1β after treatment from infertility duration is described by a linear regression equation:

$$Y_{\text{IL1b after treatment}} = 0.053 \times X_{\text{infertility, duration}} + 0.164$$

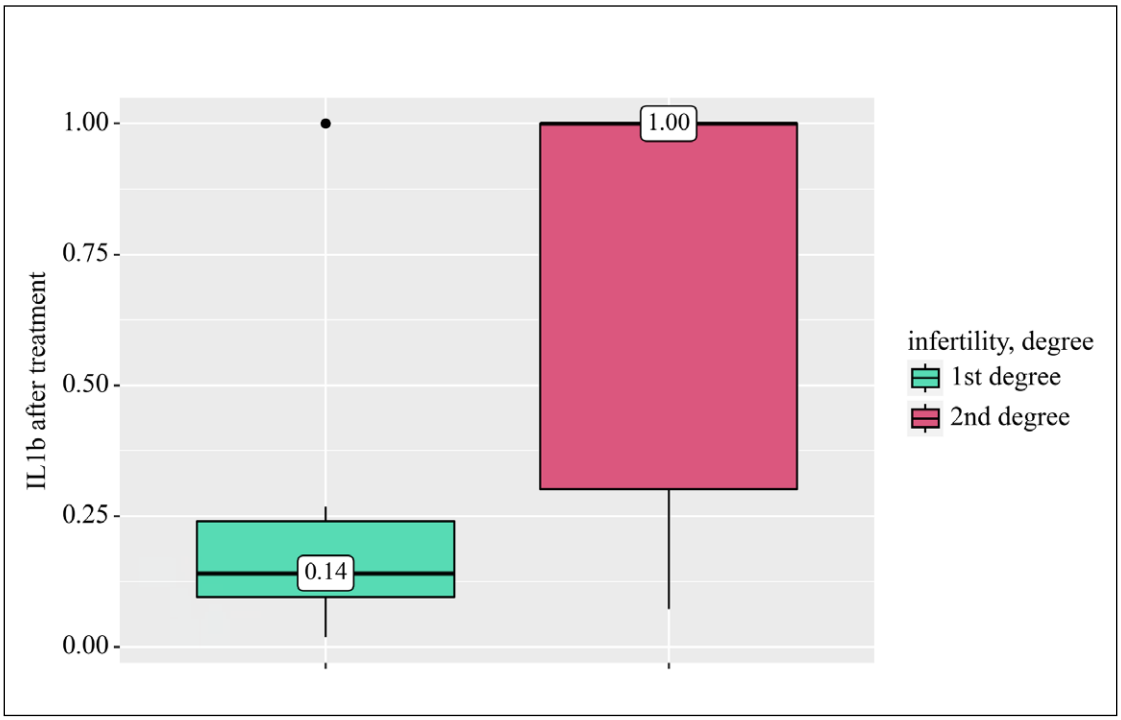

Fig. 6. Analysis of level expression mRNA IL1β after treatment conditioning on infertility degree.

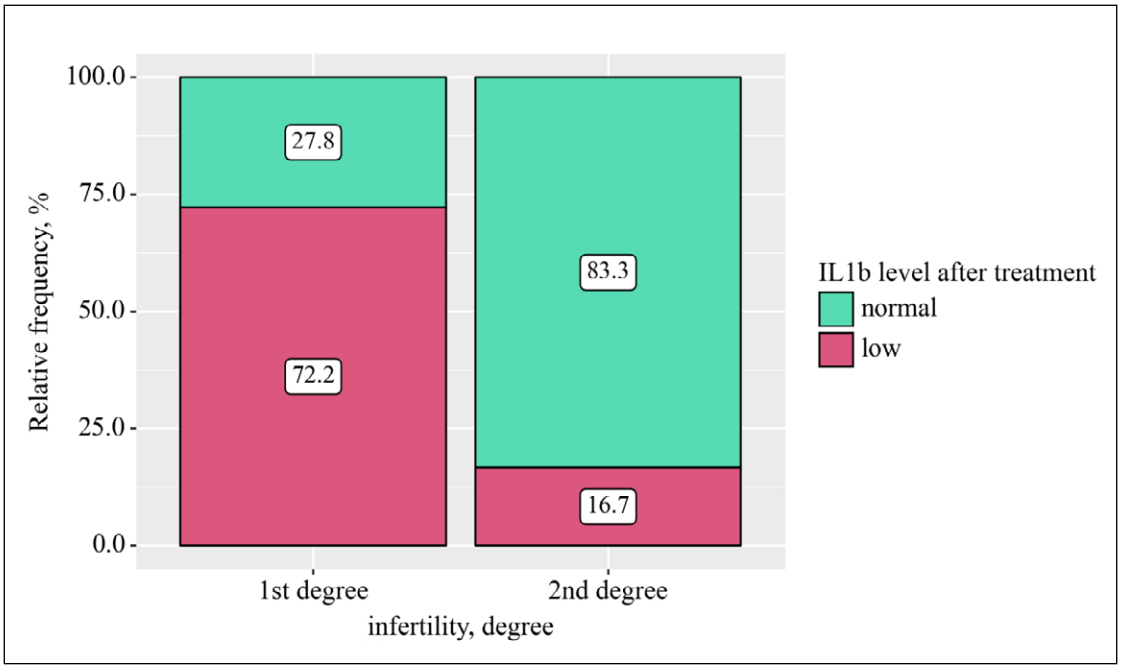

Fig. 7. Analysis of level expression mRNA IL1β before treatment conditioning on infertility degree.

With an 1 increase of infertility, duration 0.053 change of level expression IL1β after treatment should be expected. According to the coefficient of determination R^2 of the resulting model, 6.3% of the observed variance of level expression mRNA IL1β after treatment were explained(Fig. 9).

Therefore, our proposed preparation for assisted reproductive technologies with the inclusion of a probiotic is quite effective, as the levels of IL1β mRNA gene expression decreased sharply (Fig. 10).

DISCUSSION

In this study, our main aim was to examine gene expression levelsIL1β mRNAin whole blood in patients with endometriosis associated with infertility and to establish correlations between the type of infertility, its duration at the stage of preparation for assisted reproductive technologies, using a probiotic.

Endometriosis is a common benign gynecological disease characterized by the presence of ectopic endometrium, which causes dysmenorrhea, chronic

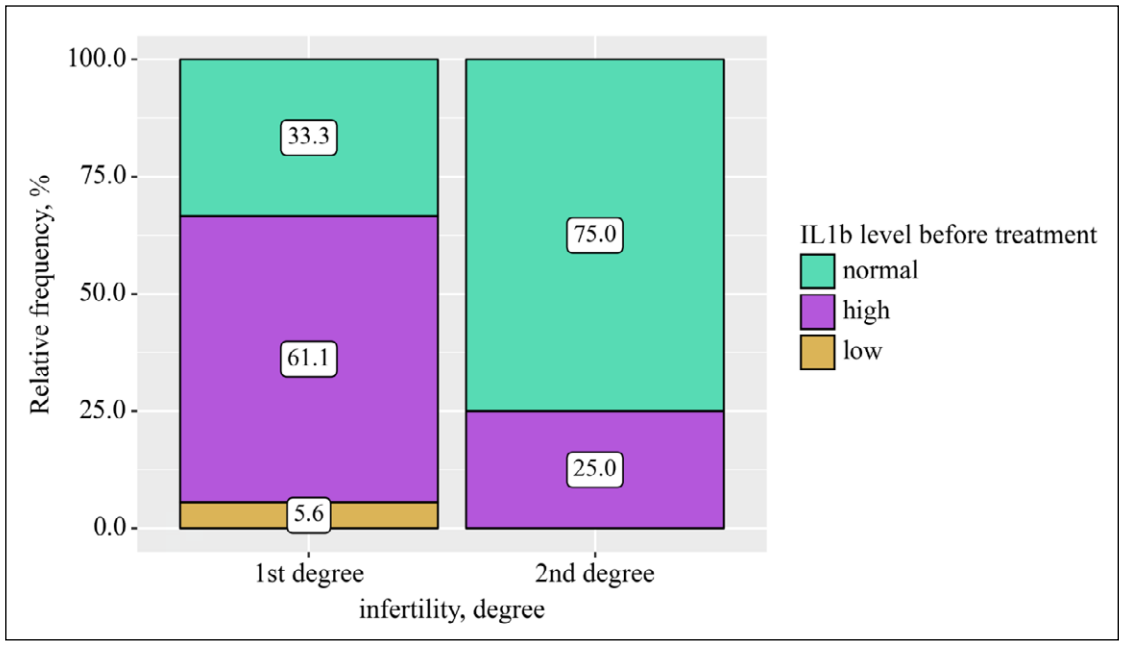

Fig. 8. Analysis of level expression mRNA IL1β after treatment conditioning on infertility degree.

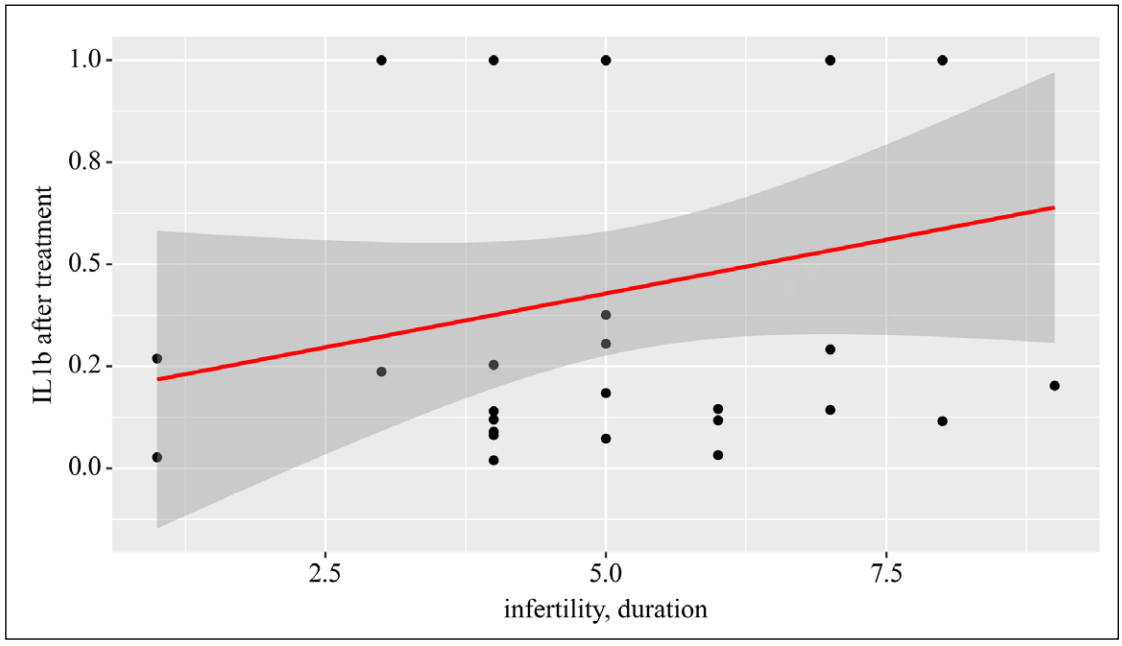

Fig. 9. Regression line characterizing the dependence of level expression mRNA IL1β before treatment from infertility duration.

pelvic pain, and infertility, and is associated with inflammation and immune disorders, as well as changes in ovarian steroid hormone production. The growth and maintenance of the endometrium and endometrioid tissue is regulated by several cytokines and growth factors, such as interleukin (IL) 6, 8, tumor necrosis factor (TNF)α, and vascular endothelial growth factor (VEGF). Retrograde menstruation into the abdominal cavity through the fallopian tubes plays an important role in the pathogenesis of endometriosis. Menstrual fluid is composed of blood cells, endometrial tissue, and waste products that are sources of endometrial cells. However, the profile of bioactive molecules in menstrual blood is unclear [8].

Therefore, our study aimed to investigate this relationship and its potential implications for clinicopathological features. Our results show that IL-1β mRNA gene expression levels have a negative correlation with the duration of infertility, significantly increased IL-1β mRNA gene expression levels in women with primary infertility than with secondary infertility, and may serve as non-invasive markers in women with endometriosis associated with infertility [9].

CONCLUSIONS

The extremely increased expression of IL1β mRNA genes indicates a close relationship between the

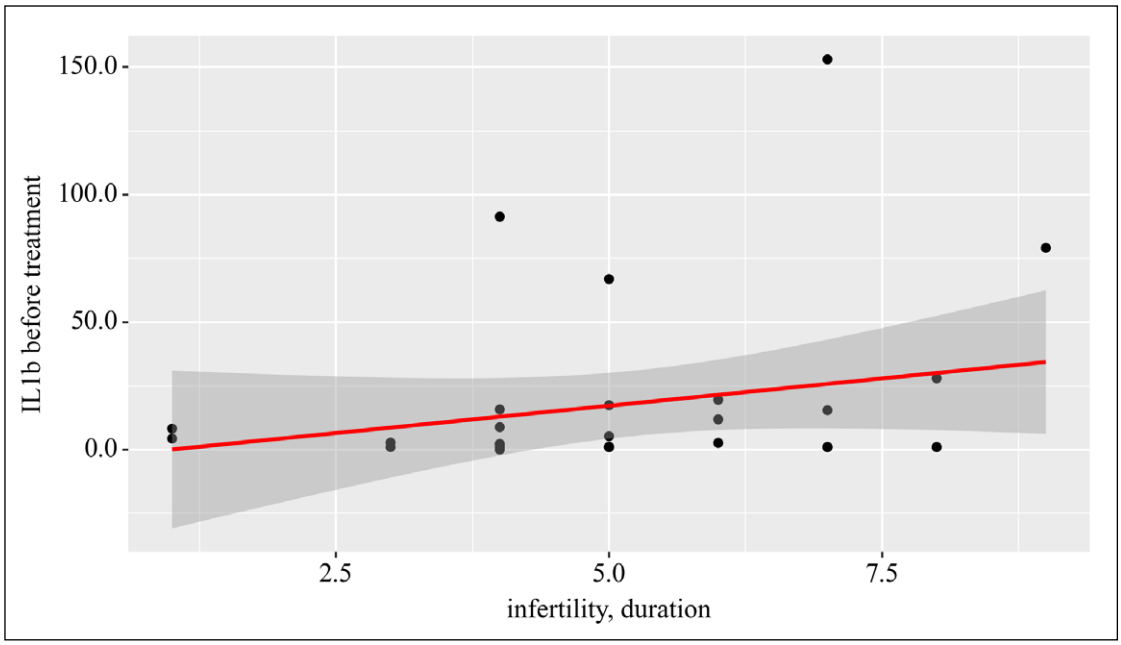

Fig. 10. Regression line characterizing the dependence of level expression mRNA IL1β after treatment from infertility duration.

pathogenesis of endometriosis and inflammation. The inclusion of probiotics in a comprehensive regimen of preparation for assisted reproductive technologies leads to a noticeable improvement in the patient's well-being and a significant decrease in IL1β mRNA gene expression. IL-1β mRNA gene expression levels have a negative correlation with the duration of infertility, significantly increased IL-1β mRNA gene expression levels in women with primary infertility than with secondary infertility .Therefore, we recommend the proposed preparation for assisted reproductive technologies with the inclusion of a probiotic.

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

CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Comprehensive approach to comparative characteristics of clinical and laboratory parameters of the study in children - in 6 months after Covid-19 treatment and 6 months after Covid-19 vaccination

Olesya M. Horlenko, Khrystyna A. Hechko, Lyubomyra B. Prylypko, Mykhaylo M. Hechko, Fedir V. Horlenko, Bohdan M. Galay
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ABSTRACT

Aim: To study and analyze data on clinical and laboratory parameters of investigated children in 6 months after treatment and 6 months after Covid-19 vaccination.
Materials and Methods: A prospective clinical and laboratory examination of a children group (group 1) with identified Covid-19 (n=68) was carried out in comparison with a group (n=31) of Covid-19 vaccinated children (group 2) after 6 months of observation.
Results: The data of Anti-SARS-CoV-2-S1-RBD IgG, non-significant intergroup differences in levels were found (344.71 ± 87.62 versus 315.67 ± 74.91 BAU/ml, p=0.11). Significant differences were found in the children group who was 6 months after Covid-19 treatment and the control group, as well as in the group before and 6 months after Covid-19 vaccination, in the following parameters: IL-2 (p6<0.01; p7<0.01, with a prevalence of 6 and 4 times, respectively); IL-4 (p6<0.01; p7<0.01with a prevalence of 6 and 4.6 times, respectively), IL-10 (p6<0.01; p7<0.01 with a prevalence of 6 and 4.8 times, respectively), Procalcitonin (p6<0.01; p7<0.01, 18 times and 1.4 times respectively).
Conclusions: In our study, according to the data of multivariate linear regression analysis, the level of Anti-SARS-CoV-2-S1-RBD IgG is the dependent variable, and D-dimer, Na level, Total Cholesterol, LF, IL-6, IgG are the influencing factors which determine the COVID-19 anti-infective protection.

KEY WORDS: Covid-19, vaccination, clinical laboratory investigation, children

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INTRODUCTION

COVID-19 vaccination programs have included children much later than adults due to the lack of early data on safety, efficacy, and the relatively mild disease profile in the pediatric population. The children's immune systems are functionally distinct from those of other age groups and the development of vaccines specifically for the pediatric population has largely been limited to titration of primarily vaccines doses developed for adults [1]. However, age-based de-escalation is now being used in the development of new vaccines: once safety and efficacy have been established in the adult population, younger cohorts are enrolled and studied [2].

Several COVID-19 vaccines have been approved for use in children and/or adolescents, including Comirnaty (Pfizer), Spikevax (Moderna), and CoronaVac (Sinovac Biotech). The Pfizer-BioNTech COVID-19 vaccine (BNT162b2) is the only vaccine approved by the Food and Drug Administration (FDA) for children ages 12–17 in the United States. Additionally, in June 2022, the FDA granted emergency use authorization for BNT162b2 and the Moderna vaccine for individuals ages 6 months to 5 years. Although the ingredients in these pediatric vaccines are the same as the adult formulations, the dosage has been adjusted based on age. Although the vaccines work somewhat differently, they all prime a person's immune system to prevent SARS-CoV-2 infection, if infected, to prevent severe curse [3,4].

AIM

To study and analyze data on clinical and laboratory parameters in children over a 6-month period: in those who have recovered from Covid-19 and those vaccinated against Covid-19, with subsequent prediction of anti-infective protection.

MATERIALS AND METHODS

A prospective clinical and laboratory examination of a children group (group 1) with identified Covid-19

Table 1. Anti-SARS-CoV-2-S1-RBD IgG levels in the children studied

Parameters	1 group (n = 68) M ± m	2 group (n = 31) M ± m	p
	6 months after treatment	6 months after vaccination	
Anti-SARS-CoV-2-S1-RBD IgG (BAU/ml)	344,71 ± 87,62	315,67 ± 74,91	0,11

Table 2. Metabolic pool indicators in dynamics

Parameters	Control group(n = 28) M ± m	1 group (n = 68) M ± m	2 group (n = 31) M ± m
		6 months after treatment	6 months after vaccination
Vitamn D3 (30-70, ng/ml)	35,27 ± 4,28	33,63 ± 5,17	34,68 ± 2,91 (p_1=0,54; p_2=0,29)
Zn (12-25, mkmol/l)	16,28 ± 3,05	14,75 ± 4,27	16,47 ± 4,35 (p_1=0,85; p_2=0,07)

Notes: p_1 - significance of differences between the values of indicators 6 months after vaccination and the control group; p_2 - significance of differences between the values of indicators 6 months after vaccination and indicators in patients with COVID 6 months after treatment.

(n=68) who were in the outpatient department of the city multidisciplinary clinical hospital in Uzhhorod was carried out in comparison with a group(n=31) of Covid-19 vaccinated children (group 2) after 6 months of observation. The control group included healthy children (n=28), identical in age and anthropometric parameters. Clinical and laboratory studies were conducted, which included biochemical, immunological examination, markers of inflammatory and endocrine regulation.

RESULTS

The SARS-CoV-2 mRNA vaccines (BNT162b2) and (mRNA-1273) have been approved by the European Medicines Agency and the US Food and Drug Administration (FDA) for use in children aged 6 months and older. These vaccines offer excellent protection against severe course diseases in children [5].

According to the Ministry of Health of Ukraine, by order No. 1477 dated 17.07.2021, the recommendations of the National Technical Group of Experts on Immunoprophylaxis had approved the possibility of children aged 12 and older vaccination with the Comirnaty, Pfizer-BioNTech vaccines, based on available scientific studies and WHO recommendations with appropriate changes to the instructions for the Comirnaty/Pfizer-BioNTech vaccine. Recommendations for the children from 12 years of age were administered 2 doses of the Comirnaty/Pfizer-BioNTech vaccine, 0.3 ml, with an interval of 21-28 days, with the necessary observance of a 14-day interval between the administration of the COVID-19 vaccine and other diseases, according to the developed [6].

Our study examined the indicators of the child's organism in response to vaccination against SARS-CoV-2 and in comparison with the transferred Covid-19 infection, after 6 months, by determining markers of inflammation, immune response, thyroid status and adipose tissue hormones. Vaccination was carried out as part of the primary vaccination series (i.e., the first two doses administered according to the recommended schedule) and administered before the registered infection. We compared the systemic levels of antibodies (Anti-SARS-CoV-2-S1-RBD IgG) in a subgroup of infected and vaccinated children (Table 1).

When comparing the antibodies data to the spike protein, insignificant differences between the levels were found (344.71 ± 87.62 versus 315.67 ± 74.91 BAU/ml, p=0.11), which indicates the effectiveness of the vaccination in children. Our data are consonant with the many scientists data [7].

Dynamic levels of metabolic pool indicators were investigated (Table 2).

According to Table 2, no significant differences were observed between the levels of vitamin D3 (33.63 ± 5.17 versus 34.68 ± 2.91 and compared with the data of the control group 35.27 ± 4.28 ng/ml, at p1=0.54; p2=0.29) and Zn values (14.75 ± 4.27 versus 16.47 ± 4.35 and compared with the data of the control group 16.28 ± 3.05 μmol/l, at p1=0.85; p2=0.07).

Table 3. Comparative dynamic characteristics of inflammation indicators in the groups of post-COVID and vaccinated children

Parametrs	Control group (n = 28) M ± m	1 group (n = 68) M ± m	2 group (n = 31) M ± m	
		6 months after treatment	Before vaccination	6 months after vaccination
IL-1 (0-11, ng/ml)	0,65 ± 0,06	0,66 ± 0,62	0,68 ± 0,07 (p_5=0,08)	0,67 ± 0,12 (p_6=0,69; p_7=0,43; p_8=0,93)
IL-2 (0-10, pg/ml)	0,34 ± 0,15	1,88 ± 0,89	0,40 ± 0,09 (p_5=0,07)	1,57 ± 0,65 (p_6<0,01; p_7<0,01; p_8=0,09)
IL-4 (up to 0,5, ng/ml)	0,17 ± 0,04	0,99 ± 0,61	0,20 ± 0,08 (p_5=0,08)	0,91 ± 0,42 (p_6<0,01; p_7<0,01; p_8=0,51)
IL-6 (0-10, ng/ml)	0,77 ± 0,04	2,53 ± 1,07	0,69 ± 0,32 (p_5=0,20)	2,18 ± 1,03 (p_6<0,01; p_7<0,01; p_8=0,13)
IL-10 (0-20, pg/ml)	0,48 ± 0,06	2,87 ± 1,92	0,51 ± 0,12 (p_5=0,24)	2,43 ± 1,51 (p_6<0,01; p_7<0,01; p_8=0,26)
γ-IFN (up to 15,0, pg/ml)	8,01 ± 0,32	6,23 ± 4,82	7,48 ± 1,71 (p_5=0,11)	6,73 ± 5,02 (p_6=0,43; p_7=0,18; p_8=0,64)
TNF-α (up to 6, pg/ml)	3,62 ± 0,31	3,17 ± 1,30	3,31 ± 0,86 (p_5=0,08)	3,09 ± 1,66 (p_6=0,52; p_7=0,10; p_8=0,79)
Neopteryn (up to 10, nmol/l)	7,61 ± 1,50	8,68 ± 6,39	6,97 ± 1,41 (p_5=0,10)	8,25 ± 5,74 (p_6=0,23; p_7=0,57; p_8=0,75)
CRP(<3, mg/l)	1,91 ± 0,53	1,64 ± 0,73	2,01 ± 0,39 (p_5=0,41)	1,58 ± 0,82 (p_6=0,01; p_7=0,08; p_8=0,72)
Procalcitonin (0-11, pg/ml)	1,61 ± 0,23	0,09 ± 0,01	1,49 ± 0,35 (p_5=0,13)	1,02 ± 0,02 (p_6<0,01; p_7<0,01; p_8<0,01)
Fibrinigen (2-4, g/l)	2,77 ± 0,33	2,97 ± 0,57	2,84 ± 0,29 (p_5=0,39)	3,02 ± 0,65 (p_6=0,16; p_7=0,07; p_8=0,70)
D-dimer (up to 0,5, mkg/ml)	0,33 ± 0,05	0,28 ± 0,14	0,35 ± 0,03 (p_5=0,07)	0,30 ± 0,09 (p_6=0,01; p_7=0,13; p_8=0,47)

Notes: p5 - significance of differences between the values of indicators before vaccination and the control group; p6 - significance of differences between the values before vaccination and after vaccination; p7 - significance of differences between the values of indicators 6 months after vaccination and the control group; p8 - significance of differences between the values of indicators 6 months after vaccination and the indicators in patients who had COVID, 6 months after treatment.

We will also consider a number of cytokines and other markers of the inflammatory reaction of the child'sorganism and their comparative dynamic characteristics in the studied groups (Table 3).

According to table 3, significant differences were found in the control and 6 months after treatment groups and in the group before vaccination and 6 months after in the following parameters: IL-2 ((p6<0.01; p7<0.01, with a prevalence of 6 and 4 times, respectively); IL-4 (p6<0.01; p7<0.01 with a prevalence of 6 and 4.6 times, respectively), IL-6 (p6<0.01; p7<0.01 with a prevalence of 3.3 and 3.2 times, respectively), IL-10 (p6<0.01; p7<0.01 with a prevalence of 6 and 4.8 times, respectively). The level of Procalcitonin presented significant differences in both groups (p6<0.01; p7<0.01;), in post-COVID children the level decreased in 18 times, of the vaccinated group in 1.4 times, which was a significant difference between the parameters, p8<0.01, but all values were corresponded to the reference values. A significant decrease in the C-reactive protein indicators (p6=0.01) and D-dimer (p6=0.01) were observed in 6 months after vaccination children, but, again, within the reference values. Let's consider the immunogram in children of the studied groups (Table 4)

According to Table 4, no significant differences between the immunogram indicators were found, which indicates intergroup identity or closeness of the immunogram values in children both after Covid-19 treatment and 6 months after Covid-19 vaccination. Many articles have been published on the topic of metabolic adaptation during COVID-19, so it is justified to study the level of adipose tissue hormones in the studied contingent (Table 5).

According to table 5, there are significant increases in Adiponectin levels (p6<0.01;p7<0.01;p8<0.01) in both groups and between them, with variation within the reference range. A significant increase in Ferritin levels (p7=0.002;p8=0.05) was noted in both groups and a significant decrease in Leptin values (p7=0.01; p8<0.01) For optimal interpretation and understanding of the obtained results of the study of children data in Covid-19 diagnosing multivariate linear regression analysis was performed. Regression analysis is a section of mathematical statistics devoted to methods of analyzing the dependence of one value to another. Unlike correlation analysis, regression analysis not only indicates the existence of a relationship between an independent variable and one or more dependent variables, but also allows us to determine this relationship quantitatively. Independent variables are called regressors or predictors, and dependent variables are called criteria. The terminology of dependent and independent variables reflects only the mathematical relationship between variables, and not the cause-and-effect relationships. Classical linear regression analysis is based on a system of provisions on the properties of the regression model, the implementation of which guarantees obtaining optimal estimates of the parameters and the regression function. Using the binary logistic regression method, it is possible to study the dependence of dichotomous (binary, i.e. those that have only 2 categorical values) variables on independent variables, the data can have any type of scale. Therefore, the relationships established during regression analysis can sometimes be mistakenly interpreted as cause-and-effect [8]. We present a developed mathematical model based on the statistically significant data we obtained and the ability to predict the dynamics of the dependent variable, in particular, the levels of ΔAnti-SARS-CoV-2-S1-RBD IgG, in accordance with the change in the studied influencing factors.

In our study, the Anti-SARS-CoV-2-S1-RBD test is the dependent variable, and D-Dimer, Na level, Total Cholesterol, APh, IL-6, IgG are the influencing factors. Formule:

ΔAnti-SARS-CoV-2-S1-RBD IgG = 28.08 + 6.93*D-Dimer – 0.17*Na + 2.09*Total Cholesterol – 0.05*APh + 0.76*IL-6 – 0.42*IgG.

An increase in the D-Dimer level by 1 μg/ml initiates an increase in the anti-SARS-CoV-2-S1-RBD IgG level by 6.93 BAU/ml; with an increase in the Na concentration by 1 mmol/l, the anti-SARS-CoV-2-S1-RBD IgG level will decrease by 0.17 BAU/ml; with an increase in the concentration of total cholesterol by 1 mmol/l, the level of anti-SARS-CoV-2-S1-RBD will decrease by 2.09 BAU/ml; an increase in the concentration of alkaline phosphatase by 1 U/l will be accompanied by a decrease in the level of anti-SARS-CoV-2-S1-RBD by 0.05 BAU/ml; with an increase in the concentration of IL-6 by 1 pg/ml, the level of anti-SARS-CoV-2-S1-RBD IgG will increase by 0.76 BAU/ml; an increase in the level of IgG by 1 g/l will contribute to a decrease in the level of anti-SARS-CoV-2-S1-RBD IgG by 0.42 BAU/ml.

DISCUSSION

Several previous studies of vaccine efficacy against long-term outcomes of COVID-19 have mostly demonstrated protective effects with a wide range of effect estimates, but some have not demonstrated an overall protective effect. [9,10] The methodology and data included in the previous studies were heterogeneous and had limitations. The study populations were rarely based on well-defined populations and often included small numbers of participants.[11,12] Analysis of the differential effects for different numbers of doses of the

Table 4. Dynamic indicators of the immunogram in the studied children

Parametrs	Control group (n = 28) M ± m	1 group (n = 68) M ± m	2 group (n = 31) M ± m	
		6 months after treatment	Before vaccination	6 months after vaccination
Ig M (0,31-1,79, g/l)	1,37 ± 0,06	1,53 ± 0,44	1,39 ± 0,24 (p_5=0,67)	1,41 ± 0,34 (p_6=0,79; p_7=0,54; p_8=0,18)
Ig G (6,98-15,49, g/l)	11,02 ± 0,07	10,21 ± 2,29	10,95 ± 0,46 (p_5=0,43)	10,08 ± 2,63 (p_6=0,08; p_7=0,06; p_8=0,80)
Ig E (Up to 120 IU/ml)	15,38 ± 5,07	13,61 ± 4,24	14,42 ± 6,01 (p_5=0,51)	14,63 ± 5,31 (p_6=0,89; p_7=0,58; p_8=0,31)
Ig A (0,61-3,48, g/l)	1,69 ± 0,43	1,75 ± 0,44	1,73 ± 0,52 (p_5=0,75)	1,77 ± 0,63 (p_6=0,79; p_7=0,58; p_8=0,86)

Notes: p5 - significance of differences between the values of indicators before vaccination and the control group; p6 - significance of differences between the values before vaccination and after vaccination; p7 - significance of differences between the values of indicators 6 months after vaccination and the control group; p8 - significance of differences between the values of indicators 6 months after vaccination and the indicators in patients who had COVID, 6 months after treatment.

Table 5. Analysis of metabolic homeostasis indicators in the studied groups of children

Parametrs	Control group (n = 28) M ± m	1 group (n = 68) M ± m	2 group (n = 31) M ± m	
		6 months after treatment	Before vaccination	6 months after vaccination
Feritin (7-140, ng/ml)	77,07 ± 10,40	96,81 ± 20,67	82,37 ± 12,11 (p_5=0,08)	88,41 ± 15,35 (p_6=0,09; p_7=0,002; p_8=0,05)
Adiponektin (5-18,6 mkg/ml)	7,73 ± 0,86	8,35 ± 8,62	8,95 ± 3,24 (p_5=0,06)	12,14 ± 2,61 (p_6<0,01; p_7<0,01; p_8<0,01)
Leptin (2,05-11,09, ng/ml)	6,97 ± 0,32	4,08 ± 0,61	6,53 ± 1,71 (p_5=0,19)	6,19 ± 1,17 (p_6=0,37; p_7=0,01; p_8<0,01)
C-peptide (0,81-3,85, ng/ml)	1,43 ± 0,08	1,56 ± 0,43	1,61 ± 0,62 (p_5=0,13)	1,58 ± 0,71 (p_6=0,86; p_7=0,27; p_8=0,86)

Notes: p5 - significance of differences between the values of indicators before vaccination and the control group; p6 - significance of differences between the values before vaccination and after vaccination; p7 - significance of differences between the values of indicators 6 months after vaccination and the control group; p8 - significance of differences between the values of indicators 6 months after vaccination and the indicators in patients who had COVID, 6 months after treatment.

COVID-19 vaccine has not always been performed.[13] A sound and informed understanding of the immune response nuances of to both, infection and vaccination, is essential for scientific research, which can be used to improve or develop new vaccines that are better able to control and prevent the spread of COVID-19 infection.[14].

The pediatric trial of the AstraZeneca COVID-19 vaccine was stopped after the detection of thrombosis with thrombocytopenia syndrome, a rare but serious adverse event

that affects mainly young people (3.4 per 100,000 people) [15]. Along with the deployment of vaccination programs for adolescents and children, vaccination of adults working in kindergartens, schools and health care facilities should be encouraged to provide indirect protection to children, minimizing transmission of SARS-CoV-2 [15].

It was noted that routine vaccination of healthy and non-contact children should not be stopped. On the contrary, it is necessary to continue primary vaccination of infants and young children according to routine programs to prevent the threat of outbreaks and epidemics, such as measles, polio, tetanus, diphtheria, etc. [16].

As described above, age-dependent molecular differences in the immune response to COVID-19 likely contribute to a milder course of infection in children. It is clear that adaptive immune responses change significantly with age. Studies should determine whether COVID-19 vaccination in children of different ages elicits different immune responses compared to adult patients to further optimize the efficacy of vaccination [16].

CONCLUSIONS

1. When comparing the data of antibodies to the Spike Protein, non-significant intergroup differences in levels were found (344.71 ± 87.62 versus 315.67 ± 74.91 BAU/ml, p=0.11), which indicates the effectiveness of the vaccination in children.
2. No significant differences were observed between the levels of Vitamin D3 (33.63 ± 5.17 versus 34.68 ± 2.91 and compared with the data of the control group 35.27 ± 4.28 ng/ml, at p1=0.54; p2=0.29) and Zn values (14.75 ± 4.27 versus 16.47 ± 4.35 and compared with the data of the control group 16.28 ± 3.05 µmol/l, at p1=0.85; p2=0.07).
3. Significant differences were found in the children group who was 6 months after Covid-19 treatment and the control group, as well as in the group before and 6 months after Covid-19 vaccination, in the following parameters: IL-2 (p6<0.01; p7<0.01, with a prevalence of 6 and 4 times, respectively); IL-4 (p6<0.01; p7<0.01with a prevalence of 6 and 4.6 times, respectively), IL-6 (p6<0.01; p7<0.01 with a prevalence of 3.3 and 3.2 times, respectively), IL-10 (p6<0.01; p7<0.01 with a prevalence of 6 and 4.8 times, respectively). The level of Procalcitonin presented significant differences in both groups (p6<0.01; p7<0.01;), in post-covid children the level decreased by 18 times, in the vaccinated group by 1.4 times, which was a significant difference between the indicators (p8<0.01), but all values corresponded to the reference values. A significant decrease in the indicators of CRP(p6=0.01) and D-Dimer (p6=0.01) was observed in post-vaccinated children after 6 months, but, again, within the reference values.
4. Significant increases in Adiponectin levels were found (p6<0.01; p7<0.01; p8<0.01) in both groups and between them, with variation within the reference range. A significant increase in Ferritin levels was noted (p7=0.002; p8=0.05) in both groups and a significant decrease in Leptin values (p7=0.01; p8<0.01) .
5. In our study, according to the data of multivariate linear regression analysis, the level of Anti-SARS-CoV-2-S1-RBD IgG is the dependent variable, and D-dimer, Na level, Total Cholesterol, LF, IL-6, IgG are the influencing factors that determine the value of the increase or decrease in the test of COVID-19 anti-infective protection.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

The significant impact of T-cell immunoglobulin and mucin domain 3(Tim-3) gene polymorphism on HCV infection and viral load

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ABSTRACT

Aim: To evaluate the role of -574 locus gene polymorphism in promotor region of TIM-3 gene in HCV infection and viral load.

Materials and Methods: The current study executed on 100 subjects (50 patients with HCV and 50 obviously healthy objects as control). Blood sample compiled from all participants. All samples underwent to diagnosis for HCV confirm by viral load measurement by real time-polymerase chain reaction (RT-PCR). Extraction the Genomic DNA performed from blood. The gene fragment corresponding the-574 locus (rs10515746) in TIM-3 gene was amplified and genotyping by allele specific - polymerase chain reaction (AS-PCR).

Results: In recessive model, in patients the frequency of GT-TT genotype was significantly higher than controls (86% vs. 62%) with a highly important variation (OR=3.76, 95% CI=1.41-10.05, p=0.008 at allelic level, T allele was more continual in patients than controls (60% contra 42%) with a considerable variation (OR=2.07, 95%CI=1.18-3.64, p= 0.015). About 42% of patients carrying TT genotype had viral load ≥200000 IU/ml compared with 15.38% GT carriers and 0% GG carries with such a viral load with a significant difference. At allelic level, T allele was more continual in patients than controls (60% contra 42%) with a significant variation (OR=2.07, 95%CI=1.18-3.64, p= 0.015.

Conclusions: T allele of rs10515746 considered a risk factor for HCV infection as well as for higher hepatitis C viral load in those patients, and it could predict treatment failure. However, with more aggressive antiviral therapies, viral cure could be achieved.

KEY WORDS: Hepatitis C virus, T-cell immunoglobulin and mucin domain 3, single nucleotide polymorphism

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INTRODUCTION

Hepatitis C infection is contagious disease happened due to hepatitis C virus (HCV), Which a positive single stranded RNA virus with lipoprotein envelope offers a spherical structure of approximately 55 nm in diameter and classify of the family *Flaviviridae* located within the genus Hepacivirus [1]. Susceptibility to acute HCV infection mediated by received unsafe medical procedures, used injection drugs, and lived with human immunodeficiency virus [2]. HCV main reason for acute hepatitis infection and these evolve to chronic hepatitis C. A huge number of patients disregard to reply to antiviral treatment, thus lasting risk for illness advancement. HCV progressed mechanism to avoid immune elimination in the greater number of infected persons. The consequences chronic HCV infection induces a chronic inflammatory disease process lead to liver cirrhosis, hepatocellular carcinoma and death [3-5]. The features of HCV-specific T-cell responses from both CD4+T-helper lymphocytes (Th) and CD8+ cytotoxic lymphocytes(CTLs) in patients with HCV infection play key role in development of liver injury and viral rescue [6]. Genetic series implicated in mission and organization of T lymphocytes further confirm the task of the acquired immune response that intent the history of HCV by factors have critical action in T-cell concerning genes. In addition, several genes implicated in innate NK cell response established the complicated interaction between elements of immune apparatus needful for effective response to infection [7]. T cell immunoglobulin and mucin-domain-containing molecule-3 (Tim-3) is a type I transmembrane protein, and implied important role in innate and adaptive immunity further a diverse of metabolic and immunomodulatory pathways. As a form of T cell surface restrained molecule, does as a negative regulator of Thelper1 and Tcytotoxic1 cell task by induce planning

cell death via interactivity with Galectin-9 ligand, and promote peripheral tolerance. This negative activity of TIM-3 has immediately developed to comprise its participation in promotion status of impairment T cell function or "attrition" noticed in persist viral diseases [8-9]. On addition, immune cells express TIM-3 also on their surface such as dendritic cells, macrophages and natural killer cells and the overexpression of Tim-3 on these cells lead to harm immune action of aforementioned immunocytes [10]. In human, three genes encode for members of the TIM belongings (HAVCR1, HAVCR2 and TIMD4, encoding TIM1, TIM3 and TIM4, respectively) [11]. The TIM-3polymorphism explained to modify the interplay between TIM-3 and its ligand, just like that simulating the pathway that outcome in definite immune disease [12-13] and effectively contributed in the tumors pathogenesis [14]. Polymorphism in both coding and non-coding region of HAVCR2 in humans linked with autoimmune and allergic diseases [15]. HAVCR2 have three major Polymorphism has linked with diverse certain situations:

Number of polymorphism comprises +4259T/G (rs1036199) in the coding region and −1516G/T (rs10053538) and −574G/T (rs10515746) in the promoter area related with rhinitis and of the gastrointestinal cancer [16-17], pancreatic cancer and renal cell carcinoma [18-19], non-small-cell lung cancer. Additionally, HIV-1 infection common related with γ-chain (γc) cytokines that prompt Tim-3 expression in an antigen-independent manner in HIV-1 patients, with non-Hodgkin lymphoma [20]. One report declared that the certain kind of SNP interconnection to elevation the TIM-3 expression that include PD1 (+8669AA (rs10204525) and HAVCR2 (−1516G/T) via liver-infiltrating lymphocytes in patients who have HBV infection and suffer from hepatocellular carcinoma [21]. Further the patients with osteoarthritis carrying the HAVCR2 +4259T/G allele, was found direct relation between T cell task and polymorphism of HAVCR2, who viewed elevation levels of IFNγ output by their CD4+ T cells [22]. Another study announced the expression of TIM-3 protein level was revealed by immunohistochemistry in women with breast cancer, subsequently was reported rs10053538 had a strikingly elevated risk of BC, contrasting with the wild-type genotype [23]. The polymorphism of TIM-3 is clinical significance that intervention in prediction of HBV-concerning liver disease [24]. As expected, Tim-3 was intended to serve as a significant barrier to prevent the T cells from performing their duties, In a northern Chinese Han population with myasthenia gravis (MG), the -574 site variant was examined and a significant difference was found between the GT+TT genotype and the frequency of the T allele on the Tim-3 promoter [25].

AIM

The aim of our report was to investigate the diversity of the Tim-3 gene promoter region and the association with viral infection and load.

MATERIALS AND METHODS

THE STUDY POPULATION

Hundred subjects enrolled in the control study, including 50 HCV patients and 50 healthy controls. The study carried out at Gastroenterology and liver Hospital-Medical City (Baghdad, Iraq) during period from March 2024 to August 2024. Documentary consent acquired from all groups before involvement in the study. The diagnosis HCV was performance by laboratory tests including Anti-HCV antibodies identified with enzyme-linked immunosorbent assays (ELISAs) (CAMP, Romania) and confirms these tests by viral load measurement by real time-PCR completed in laboratory of aforementioned hospital. Ethical approval to perform the research acquired from College of Medicine, University of Diyala.

DATA AND SAMPLE COLLECTION

Demographic characteristics including age, sex, body mass index (BMI) was calculated, residence, family history of HCV and comorbidities collected through direct interview with all participants in a preformed form. Clinical characteristics of patients including type of infection, treatment, almost 5 mL of venous blood obtained from each entrant, storage the blood samples at −80 °C until be used.

MOLECULAR ASSAY

DNA extraction from the genome was accomplished using a commercially available kit (GsyncTM DNA extraction kit, Geneaid, Taiwan) It follows the protocol documented at the time of its creation. The concentration of DNA extracted from the samples was measured at 260 nm/280 nm (A260/A280) using a Biospec nanospectrophotometer. Allele-specific PCR (AS-PCR) was used to examine the -574 locus (rs10515746). To achieve this, three different primers were used.

Forward 1 (F1)
5′-GGCTTATGCTGGGAGTTGCT-3′
Forward 2 (F2)
5′-GGCTTATGCTGGGAGTTGCG-3′
Reverse for the F1 and F2 (R) series.
5′-GGT GTCTGATTGCCAGTGATTC-3′[25]

The F1 and R primers were employed to amplify the T alleles, while the F2 and R primers were employed to

Table 1. Demographic features of the study population

Variables	Patients (n=50)	Controls (n=50)	p-value
Age, years			
Mean±SD	41.26±15.22	44.98±9.5	0.146
Range	9.0-73	17-62	
Gender			
Male	27(54%)	22(44%)	0.317
Female	23(46%)	28(56%)	
BMI, kg/m^2			
Mean±SD	25.94±3.56	25.26±3.6	0.225
Range	17.75-33.2	21.67-28.73	
Residence			
Rural	38(76%)	41(82%)	0.641
Urban	12(24%)	9(18%)	
Family history			
No	39(78%)	47(94%)	0.041
Yes	11(22%)	3(6%)	
Comorbidity			
No	27(54%)	36(72%)	0.062
DM	6(12%)	5(10%)	0.727
Hypertension	7(14%)	5(10%)	0.538
Renal failure	8(16%)	0(0%)	0.006
Others	4(8%)	6(12%)	0.505

SD: standard deviation, BMI: body mass index, DM: diabetes mellitus.

amplify the G alleles. Overall, the samples were subpar to AS-PCR with F1/R and F2/R, all of the expatiate fragments have a total of 539bp. Overall, the segments that were magnified were 539 bp in length, each sample had to be optimized for AS-PCR by following several steps.

As a result, the following mixture of nucleotides on reactivity expansion was generated in the 25 µl final volume, which comprised 12.5 µl of master mix (Promega, USA), 2 µl of primers (1.0 µl from the forward side and 1.0 from the reverse side) and 1.0 µl of DNA template, all of which were achieved by double distilled water. Touchdown PCR method used to amplify the target sequence, during heating applied:

A thermal cycler was used for this purpose under the following conditions; 94°C for 5 min; 94°C for 30 s; annealing temperature 60°C for 30 s; extension at 72°C for 30 s; then cycle at 72°C for 30 s for 30 s; the final extension was performed at 72°C for 7 min. For gel electrophoresis, the amplified product exposed to 1.5 g agarose in 100 ml 10x Tris-borate-EDTA (TBE) (Promega, USA) and developed with ethidium bromide to identify the product.

STATISTICAL ANALYSIS

The statistical software SPSS 25.0 (SPSS, Chicago) used to conduct the analyses. The mean and standard deviation of continuous data displayed, and the Student t-test utilized for analysis. The Chi-square test utilized to assess categorical variables, which reported as numbers and percentages. The relationship between HCV infection and rs10515746 in the TIM-3 gene's promoter region was assessed applying binary logistic regression. The odds ratio (OR) and associated 95% confidence interval (CI) were computed from this test. Any change that deemed statistically significant had a p-value of less than 0.05.

Table 2. Clinical features of the patients

Variables	Values
Type of infection	
Acute	31(62%)
Chronic	19(38%)
Treatment	
No	29(58%)
Yes	21(42%)
Viral load, IU/ml	
<200000	39(78%)
≥200000	11(22%)

RESULTS

DEMOGRAPHIC FEATURES OF THE PATIENTS

The mean age of the patients was 41.26±15.22 years (range 9.0-73 years) compared with 44.98±9.5 29 years (range 17-62 years) for controls with no significant variation. Although females were less frequent in patients than controls (46% vs. 56%), the difference was not significant. Likewise, the two groups were comparable

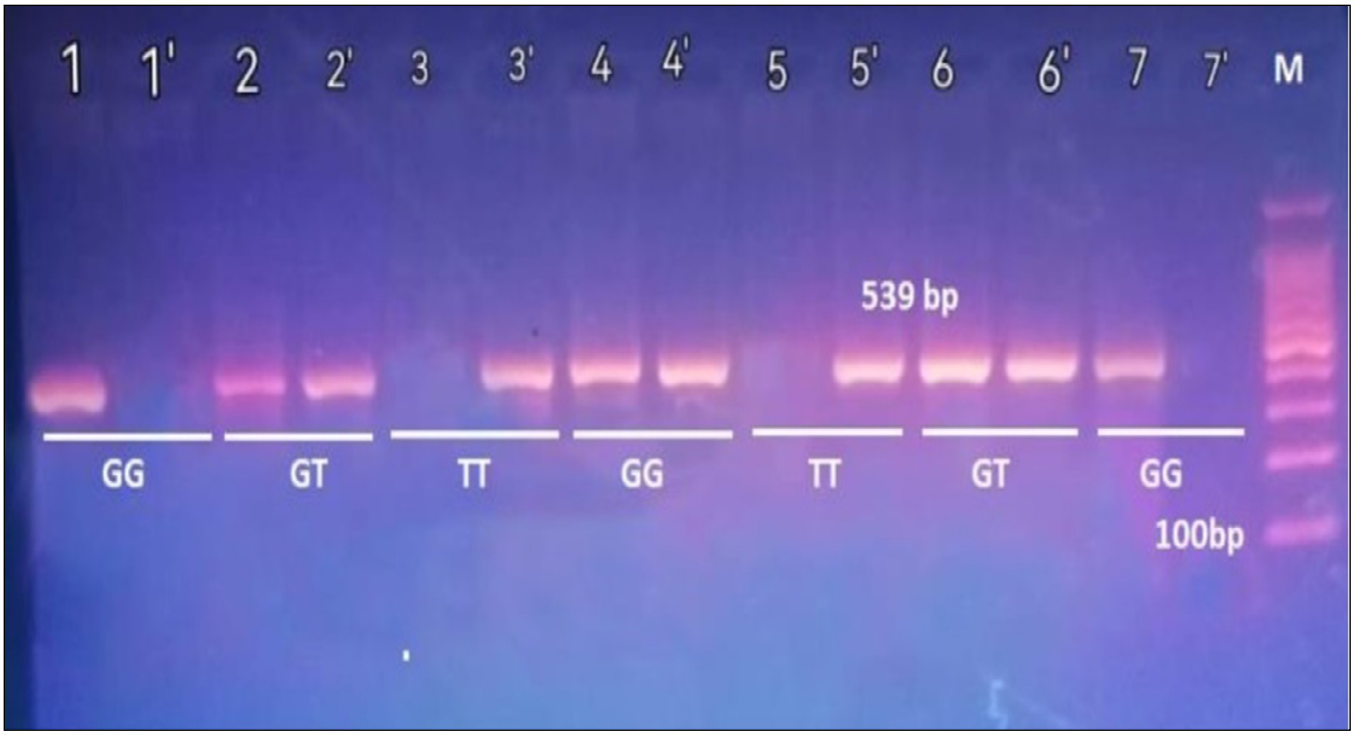

Fig. 1. Genotype patterns of rs10515746 in the promoter region of TIM-3 gene allele-PCR conceived under UV transluminator.
M: DNA marker, lanes 1, 4and 7: GG genotype, lanes 2 and 6: GT genotype, lanes 3 and 5; TT genotype.

in terms of BMI and residence with no significant variation. However, 22% of patients had a family history of HCV compared with only 6% of control with such a history, with significant difference. Furthermore, 16% of patients had renal failure comorbid disease versus none in controls with a highly significant difference, Table 1.

CLINICAL CHARACTERISTICS OF THE PATIENTS

Acute infection reported in 31 patients 62% while chronic infection found in 19 patients 38%. Only 42% of the patients were under specific treatment for HCV. Viral load was ≥200000 IU/ml in 11 patients 22% as viewed in table 2.

MOLECULAR ASSAY

In this study, the SNP rs10515746 in the promoter region of TIM-3 gene investigated in its association with HCV infection. Genotyping performed using allele specific-PCR. PCR products are shown in fig.1. The SNP appeared in three genotypes: GG, GT and TT. The distribution of these genotypes was in a good accordance with Hardy Weinberg equilibrium in patients and controls.

ASSOCIATION OF RS10515746 WITH HCV INFECTION

The GG genotype of this polymorphism was less frequent in patients than controls (14% vs. 38%) with a significant difference (p= 0.028). In contrast, the heterozygous genotype (GT) was more frequent in patients than controls (52% vs. 40%) with a important variation (OR= 4.19, 95%CI=1.32-13.47, p=0.015). Similarly, the mutant homozygous genotype (TT) was more frequent in patients than (34% vs. 22%) although the difference was not significant. In recessive model, the frequency of GT-TT genotype was significantly higher in patients than controls (86% vs. 62%) with a highly significant variation (OR= 3.76, 95%CI= 1.41-10.05, p= 0.008). At allelic level, T allele was more frequent in patients than controls (60% vs. 42%) with a significant variation (OR=2.07, 95%CI=1.18-3.64, p= 0.015) as shown in table 3.

ASSOCIATION OF DIFFERENT GENOTYPES OF RS10515746 WITH THE CLINICAL CHARACTERISTICS OF THE PATIENTS

There was no significant impact of different genotypes of rs10515746 polymorphism on the development of infection into chronic status. However, 41.81% of patients carrying TT genotype had viral load ≥200000 IU/ml compared with 15.38% GT carriers and 0% GG carries with such a viral load with a significant difference, Table 4.

DISCUSSION

Hepatitis C-virus infection is critical public health issue worldwide. A study represented the predominance of HCV in Iraq and its geographic rating is significant to

Table 3. The frequency of different genotypes and allele of rs10515746 polymorphism in HCV patients and control

Rs10515746 Polymorphism	Patients (n=50)	Controls (n=50)	P-value	OR(95%CI)
Genotypes				
GG	7(14%)	19(38%)	0.028	1.0
GT	26(52%)	20(40%)	0.015	4.19(1.32-13.27)
TT	17(34%)	11(22%)	0.723	1.19(0.45-3.09)
HWE	0.556	0.206		
Dominant model				
GG+GT	33(66%)	39(78%)	0.184	1.0
TT	17(34%)	11(22%)		1.83(0.75-4.44)
Recessive model				
GG	7(14)	19(38%)	0.008	1.0
GT+TT	43(86%)	31(62%)		3.76(1.41-10.05)
Alleles				
G	40(40%)	58(58%)	0.011	1.0
T	60(60%)	42(42%)		2.07(1.18-3.64)

Table 4. Association of different genotypes of rs10515746 with the clinical characteristics of the patients

Variables	GG (n=7)	GT(n=26)	TT(n=17)	p-value
Type of infection				
Acute	5(71.43%)	14(53.85%)	12(70.59%)	0.465
Chronic	2(28.57%)	12(46.15%)	5(29.41%)	
Viral load, IU/ml				
<200000	7(100%)	22(84.62%)	10(58.82%)	0.041
≥200000	0(0%)	4(15.38%)	7(41.81%)	

administer the increasing incidence of HCV [26]. For age, our study explained no impact variation between patients and controls. A previous study explained nearly two decades average of recent HCV infection has been declined contrast to other world range HCV epidemic, the fast evolution of this wide scale pandemic from the seventies during the nineties and the reality that it has been particularly affecting individuals in their twenties and thirties is amazing in Pakistan. By 2050, individuals older than 60 consider the age group with widest infection encumber, as the young infected cohort sensible and the incidence endure to decline HCV [27]. The history of prevalence of HCVAb and viral hepatitis in families appeared to be significant in regards to variation between groups. Previous research demonstrated the association between familial histories of viral hepatitis C and the investigation of clusters of situations within households and the documented elevated prevalence of disease in individuals with an infected family member in comparison to the general population [28-29]. Additionally, a cross sectional study of patients with HCV conducted in China demonstrated that those who had long term exposure to the disease were more likely to be infected with it [30]. A significant that a long-standing commitment to low-risk methods such as tooth brushes, accidental exposure to the infected blood of a razor, and the nail clipper could still lead to infection. However, a potential commentary that is specific to the same household could be vulnerable to the same external dangers. For instance, visiting family members to the same healthcare professional's office with a significant relationship to increase the risk of infection, such as the use of intravenous drugs, dangerous healthcare-related injections, or blood transfusions. [31]. One of most substantial outcome in our study was the significant related between renal failure and HCV infection. This result is compare with results of other local studies. Hemodialysis patients with diabetes mellitus and dental operation more expanded to hepatitis C infection [32]. Another study in 2023 considering relationship between HCV infection and transmission and dental patterns, no statistically significant difference was found between the hemodialysis patients who received a transfusion and those who did not receive any transfusion before and significantly differences showed between the hemodialysis patients who received dental treatment and did not receive any dental treatment before ($p>0.05$) [33]. This might relate to different degree of sanitation and disinfection of HD machines instruments and environmental surface to prevent nosocomial transmission [34]. Tim-3 is an effect on the immune system's regulatory functions, its location on chromosome 5q33.2 in humans is comprised of 301 amino acids that are involved in the initial structural domains, such as the cytometer domain of the phosphorylation site, the structural domain that is similar to mucin, the transmembrane area and the

signal domain. [35-37]. Two perpendicular β segments and a metal ion are involved as the ligand-binding site of Tim-3, in the immunoglobulin V domain of constant composition [38-39]. In current study, the heterozygous genotype (GT) was more frequent in patients than controls with a significant difference. Similarly, the mutant homozygous genotype (TT) was more frequent in patients. These finding specified as correlation between the -574 locus polymorphism and HCV infection in Iraq. Furthermore, patients bearing TT genotype had viral load ≥200000 IU/ml compared with 15.38% GT carriers and 0% GG carries with such a viral load with a significant difference, Table 4. Several studies have demonstrated that the immunoglobulin-and mucin domain-containing molecule-3 (Tim-3) is involved in the abnormal behavior and loss of function of CD8 + T cells in relation to hepatitis B and C. The expression of Tim-3 is increased in HBV/HCV-specific CD8 + T cells, this is of concern because of the loss of CD8 + T cells in patients with HBV/HCV infection [40-42]. Additionally, the association of Tim-3 with its ligand, galectin-9, can increase the expression of the Tim-3 protein during the stimulation of regulatory T cells (Treg), this increase in Tim-3 causes the death of helper T cells, and the decrease in Tim-3 is associated with a decrease in Th1 cells and an increase in Th2 cells. [43, 44]. Association of TIM3 SNPs with disease more predispositions in diverse autoimmune disease, such as type I diabetes and Ankylosing Spondylitis (AS) have been investigated [45]. Unfortunately, there is no previous study that addresses association between role of -574 locus genes with HCV infection. However, it can be deduced that the presence of substitution of thymine instead of guanine in -574 locus of the promoter region of TIM-3 gene could increase the transcription of this gene may be due to increase RNA polymerase to the promoter region. Upregulation of TIM-3 transcriptions with impede the T-cell activity and increase the HCV opportunity to replicate and initiate the infection, and therapeutic interventions that block or modulate TIM-3 could potentially restore effective T-cell responses and improve viral clearance. Chronic viral infections like HCV are often associated with T-cell exhaustion, partly mediated by TIM-3 overexpression. Targeting TIM-3 to reinvigorate exhausted T cells could enhance antiviral immunity.

CONCLUSIONS

T allele of rs10515746 considered a risk factor for HCV infection as well as for higher hepatitis C viral load in those patients. The association between the GT+TT genotype and higher viral loads suggests that patients with this genotype may benefit from closer monitoring and more aggressive antiviral therapies to achieve better disease control. Screening for the rs10515746 polymorphism could be useful in identifying patients at risk for higher viral loads, enabling personalized treatment approaches.

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CONFLICT OF INTEREST

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ORIGINAL ARTICLE

CONTENTS

Cytomorphological characteristics of the nasopharyngeal mucosa in children with acute respiratory infections

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ABSTRACT

Aim: This study aims to investigate the relationships between cytomorphological markers of pathological processes and the development of acute respiratory infections in children.

Materials and Methods: A microbiological study was conducted to identify pathogens based on their morphological, cultural, and biochemical properties using nasopharyngeal swabs. Pure culture isolation and subsequent colonization were performed on standard nutrient media. Cytological studies were carried out using electron microscopy.

Results: A total of 114 strains of conditionally pathogenic microorganisms were identified, including 33 strains (29.0%) of Gram-positive bacteria (*Streptococcus pyogenes, Staphylococcus aureus, Enterococcus faecalis*) and 81 strains (71.0%) of Gram-negative bacteria (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli). Escherichia coli* (37.0%) and *Staphylococcus aureus* (21.0%) were the predominant pathogens. The study identified key metabolic markers, including sucrose (n=69), maltase (n=87), and lactoperoxidase (n=89), with lactoperoxidase showing the highest levels. Aminoacids and alcohols were analyzed to assess their role in the inflammatory response. Electron microscopy confirmed bacterial localization within epithelial cells and extracellular areas, with morphological signs of epithelial cell destruction, including nuclear degradation and increased vascularization.

Conclusions: The findings highlight the significant role of nasopharyngeal microbiota in respiratory infections and their correlation with inflammation markers. The high prevalence of Gram-negative bacteria, particularly *Escherichia coli and Staphylococcus aureus*, underscores the need for targeted prevention and treatment strategies. Understanding the cytomorphological and biochemical changes in the nasopharyngeal mucosa contributes to a better comprehension of pathogen-host interactions in respiratory infections.

KEY WORDS: acute respiratory infections, cytomorphological study, children

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INTRODUCTION

The colonization of the nasopharyngeal zone is the first stage in the development of respiratory pathology. The next stage of primary colonization involves the transmission of infection within the environment. Nasopharyngeal carriage of microorganisms can play a key role in the development and spread of respiratory infections, and the so-called «healthy» carriage, under the influence of various pathological factors, may transform into an infectious process [1, 2].

During acute respiratory viral infections, there is active proliferation of microorganisms. Under the influence of infectious agents and other immunosuppressive factors, the bacterial process progresses [3].

AIM

To investigate the relationships between cytomorphological markers of the pathological process and the development of acute respiratory pathology.

MATERIALS AND METHODS

The microbiological study included the isolation of pathogens of the pathological process, their identification based on morphological, cultural, and biochemical properties through the analysis of nasopharyngeal swabs. The algorithm for isolating pure cultures and their subsequent colonization was carried out using standard nutrient media (meat-peptone agar, blood agar, meat-peptone broth, Endo medium, Sabouraud medium), as well as specialized chromogenic differential diagnostic media (Biomerieux). Cytological studies were conducted at the interfaculty laboratory of experimental research methods (electron microscopy) at Ivan Franko National University of Lviv.

RESULTS

In the analysis of the nasopharyngeal microflora of patients with acute upper respiratory tract infections, 114 strains of opportunistic microorganisms were isolated.

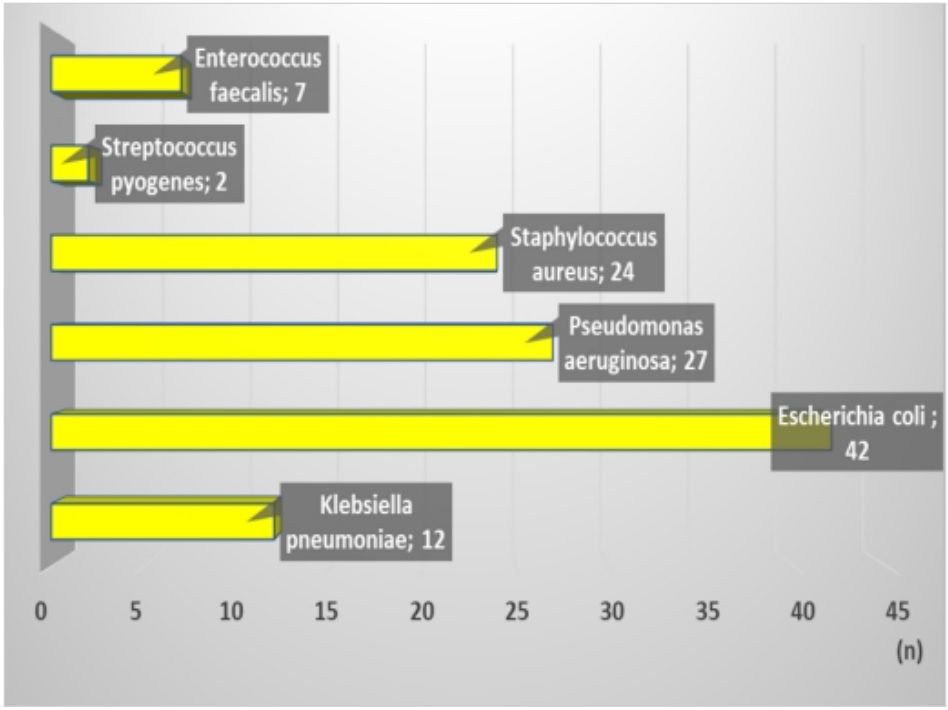

Fig. 1. Characteristics of identified pathogens in children (absolute values).

Table 1. Correlation relationships between the structural components of the microbiome and inflammatory process markers

Parameters		Correlation coefficient (r)	Statistical significance (p)
Escherichia coli	Free T_4	0,19	0,05
	TNF-α	0,20	0,04
	Cu	-0,21	0,03
Staphylococcus aureus	Lactoperoxidase	-0,20	0,04
	Acetone	-0,21	0,03
Pseudomonas aeruginosa	Free T_3	0,20	0,04
	Free T_4	-0,28	0,003
	TNF-α	-0,20	0,04
Streptococcus pyogenes	IFN-γ	0,32	0,001
Klebsiella pneumoniae	Free T_4	0,20	0,04
Enterococcus faecalis	Cortisole	0,26	0,007

Among them, 33 strains (29.0%) were Gram-positive bacteria (*Streptococcus pyogenes, Staphylococcus aureus, Enterococcus faecalis*), while 81 strains (71.0%) were Gram-negative bacteria (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli*) (Fig. 1).

According to the obtained research data, there is a predominance of Gram-negative bacterial strains—81 (71.0%), specifically *Klebsiella pneumoniae* (11%), *Pseudomonas aeruginosa* (24%), and *Escherichia coli* (37.0%). Gram-positive bacteria accounted for 33 strains (29.0%), including *Streptococcus pyogenes* (2.0%), *Staphylococcus aureus* (21.0%), and *Enterococcus faecalis* (6.0%). Among the groups, the leading pathogens were *Escherichia coli* (37.0%) and *Staphylococcus aureus* (21.0%). The composition of the nasopharyngeal microflora in healthy children depends on various factors, including age category, hormonal status, and pathological conditions [4].

The biomaterial study design included the identification of enzyme groups such as sucrase (*n*=69), maltase (*n*=87), and lactoperoxidase (*n*=89). The highest level in our research was observed for lactoperoxidase, which is a heme-containing glycoprotein. Its function involves utilizing H_2O_2 for the synthesis of hypothiocyanite (OSCN), which has the ability to inhibit bacterial replication, fungi, viruses, and parasites, as well as neutralize intestinal pathogens in infants. The enzyme maltase (α-glucosidase) breaks down the disaccharide maltose into glucose, while sucrase also hydrolyzes sucrose and maltose [5].

The obtained glucose levels in the analysis of children (*n*=84) indicate the functionality of the degradation system and monosaccharide formation. The biomaterial

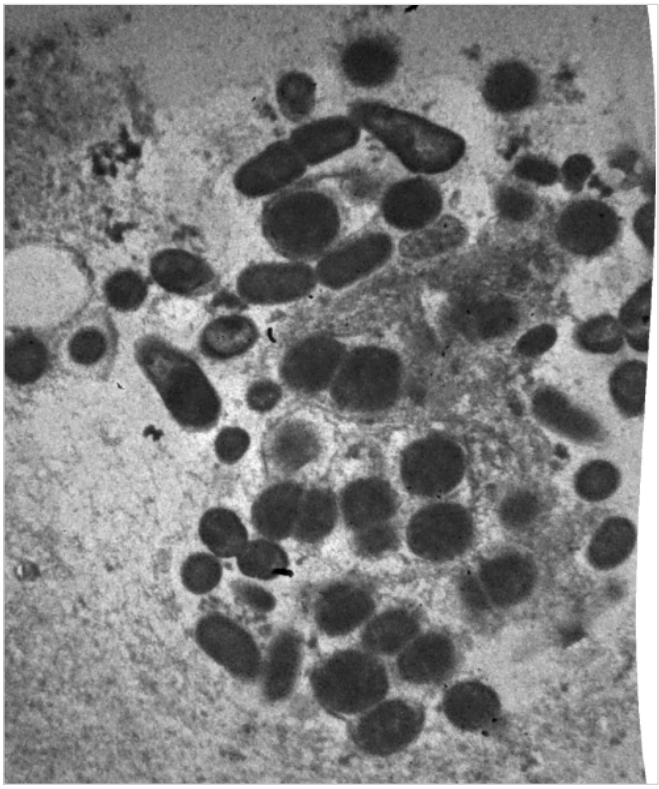

Fig. 2. Electron Micrograph (Magnification x10,000).

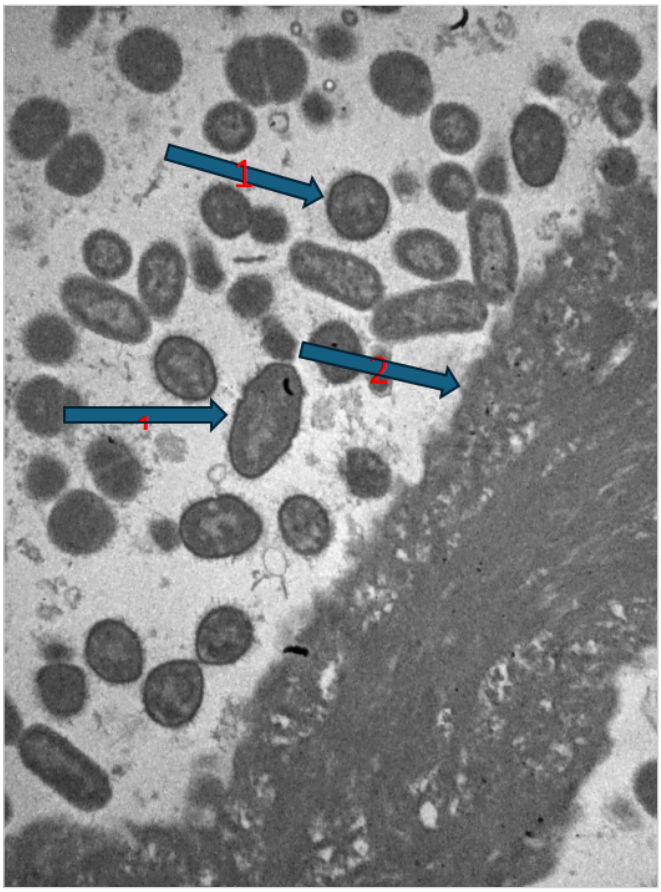

Fig. 3. Electron Micrograph (Magnification x10,000).

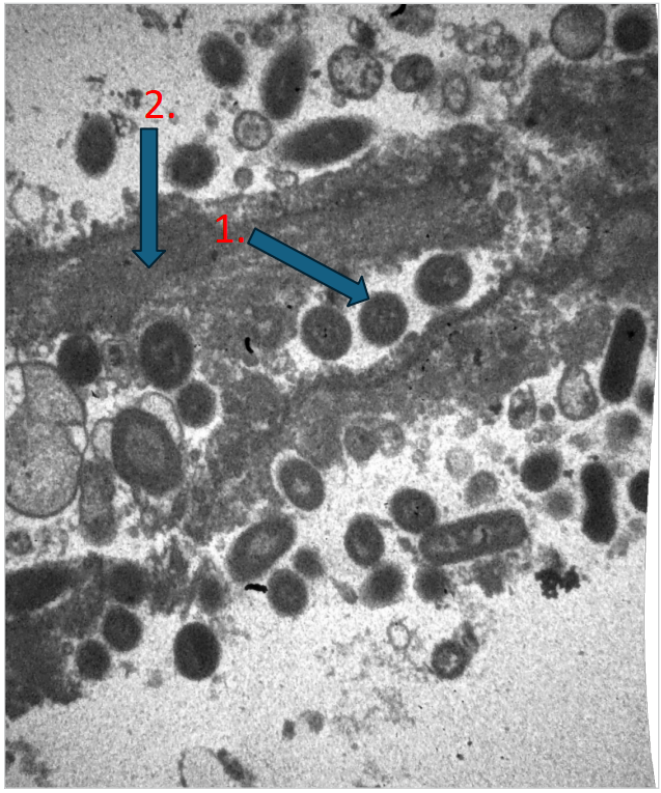

Fig. 4. Electron Micrograph (Magnification x10,000).
1. Electron-dense bacteria in the peri- and perinuclear space of the cell
2. Partial nucleus destruction

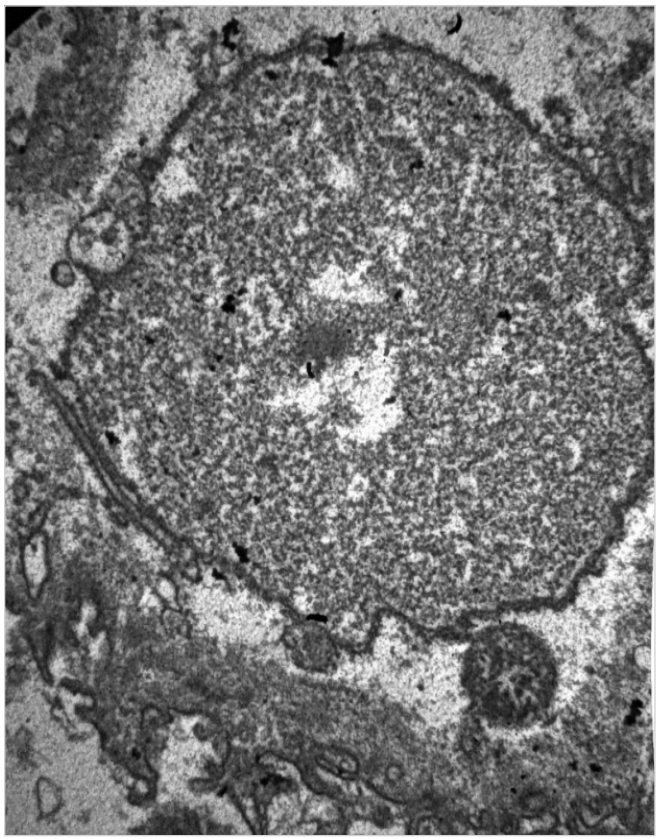

Fig. 5. Electron Micrograph (Magnification x10,000).
Macrophage in the process of phagocytosis of electron-dense bacteria.

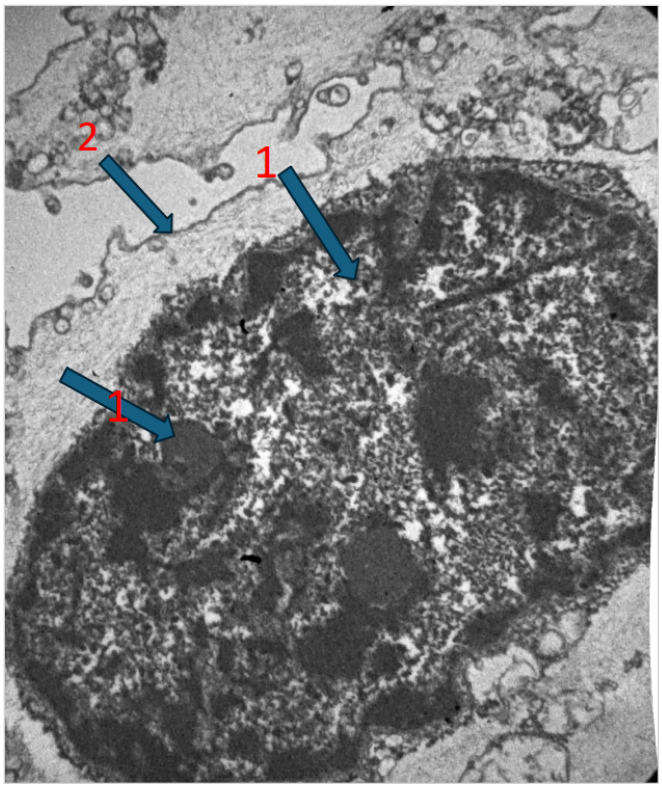

Fig. 6. Electron Micrograph (Magnification x10,000).
1. Destruction of the epithelial cell nucleus with the formation of a significant number of nucleoli and cellular debris.
2. Peripheral thinning of the basal cell membrane

also revealed the presence of amino acids: lysine (*n*=96), ornithine (*n*=62), and arginine (*n*=30). The presence of alcohols—sorbitol (a hexahydric alcohol, *n*=102), mannitol (a hexahydric alcohol, *n*=84), and xylitol (a pentahydric alcohol, *n*=86) - suggests the detoxification capabilities of the child's body. Additionally, intoxication-related factors were detected: acetone (*n*=105), which indicates excessive bacterial replication in the oral cavity. The identification of indole in 72 cases suggests its influence on the regulation of various aspects of bacterial physiology and virulence levels. Tryptophan, a derivative of indole and a precursor of the neurotransmitter serotonin, may cause vomiting and angiospasm in patients [3].

The presence of relationships between inflammation markers and microflora will be considered (Table 1).

According to Table 1, we will consider the correlation relationships between the levels of detected microorganisms and indicators of the inflammatory response in the child's body. The following significant correlations were identified. The colonization level of *Escherichia coli* increases with the positive influence of free T4 and TNF-α and decreases with the negative impact of Cu levels. *Staphylococcus aureus* has negative correlations with lactoperoxidase enzyme levels ($r = -0.20$, $p = 0.04$) and acetone ($r = -0.21$, $p = 0.03$). According to the correlation data, the replication of *Pseudomonas aeruginosa* is supported by an increase in free T3 levels, while growth inhibition occurs due to the negative impact of T4 and TNF-α levels. The level of *Streptococcus pyogenes* increases with elevated IFN-γ levels, *Klebsiella pneumoniae* is positively influenced by free T4 levels, and the growth and development of *Enterococcus faecalis* are supported by high cortisol levels.

Given the high prevalence of acute respiratory diseases in childhood, there is a need for scientific research to identify pathogenetic factors for treatment and prevention of this pathological process. The respiratory microbiome plays a key role in diseases. Microflora imbalances and bacterial carriage contribute to the clinical presentation of acute respiratory illnesses. Disturbances in balance and the presence of pathogenic microorganisms trigger an inflammatory response in the respiratory mucosa. Changes in enzymatic, detoxification, and characteristic features of the microbial landscape reflect the inflammatory response of the child's body. Positive correlations are observed between T4 levels, hormones, and TNF-α in the presence of *Escherichia coli*. *Staphylococcus aureus* disrupts enzymatic properties in the oral cavity, particularly lactoperoxidase activity, and contributes to signs of intoxication in the child's body.

Respiratory pathology is the most common issue in clinical pediatrics, and its relevance in childhood is linked to its prevalence, the potential for severe progression and complications, and the need for continued treatment. Addressing the etiopathogenetic aspects is crucial for understanding the disease and its prevention [5, 6].

The obtained data showed a predominance of Gram-negative bacterial strains—81 (71.0%), specifically *Klebsiella pneumoniae* (11%), *Pseudomonas aeruginosa* (24%), and *Escherichia coli* (37.0%). Gram-positive bacteria accounted for 33 strains (29.0%), including *Streptococcus pyogenes* (2.0%), *Staphylococcus aureus* (21.0%), and *Enterococcus faecalis* (6.0%). The predominant pathogens were *Escherichia coli* (37.0%) and *Staphylococcus aureus* (21.0%). The most representative electron microscopy images (Fig. 2, Fig.3, Fig.4, Fig.5, Fig.6) are provided based on scrapings from the nasopharyngeal mucosa.Кінець форми

Scraping of the palatine tonsil from a patient with acute respiratory infection (ARI). The cytoplasm of the epithelial cell is filled with clusters of electron-dense bacteria, forming multiple colonies.

Patient T., 12 years old. Electron-dense bacteria (1) are located in close proximity to the cell membrane (2). An

increase in blood flow in the cytoplasmic membrane of the cell is observed.

Based on the electron micrographs obtained from patient T., 12 years old, diagnosed with acute respiratory infection (ARI), and based on material from a scraping of the mucosal area of the oropharynx, primarily the palatine tonsil, the following changes were observed: the cytoplasm of the epithelial cell was filled with electron-dense bacteria, resembling grape-like clusters, and their multiple colonies. Additionally, loci of electron-dense bacteria were observed in close proximity to the cell membrane, in both the peri- and perinuclear space of the cell. An increase in blood flow in the cytoplasmic membrane, peripheral thinning of the basal cell membrane, and destruction of the epithelial cell nucleus with the formation of a significant number of nucleoli and cellular debris were noted. A representative finding was the electron microscopy documentation of the macrophage in the process of phagocytosis of electron-dense bacteria (Fig. 4).

Apoptosis of the host cell is an intrinsic immune defense mechanism in response to the invasion of infectious agents. However, bacterial pathogens utilize various strategies to manipulate host cell death and survival pathways to enhance their replication and persistence. Bacteria employ different mechanisms to evade host immunity and cause diseases in humans. The susceptibility of the organism to bacterial infection depends on the effectiveness of the immune system, overall health status, and genetic factors [7,8].

Scientific sources describe several mechanisms of infectious invasion, particularly streptococcal invasion into epithelial cells. Two mechanisms of focal adhesion and penetration through the cellular membrane have been demonstrated—the zipper-like mechanism and the membrane-ruffling mechanism. Many bacteria can also induce their own internalization into non-professional phagocytes. In this case, two main entry mechanisms are involved: the zipper and the trigger mechanism. Both rely on the activation of signaling cascades that lead to the reorganization of the actin cytoskeleton at the level of the host plasma membrane. In the "zipper" mechanism, the interaction of bacterial surface proteins with host proteins triggers cytoskeletal and membrane remodeling, leading to bacterial internalization. In the "trigger" mechanism, bacterial effectors injected into the host cell cytoplasm initiate extensive cytoskeletal rearrangements and membrane ruffling, allowing bacterial engulfment and internalization [9].

Invasive bacteria actively induce their own uptake via phagocytosis in normally non-phagocytic cells, subsequently either creating a protected niche where they survive and proliferate or spreading from cell to cell through actin-based motility. Bacterial adhesion to host surfaces is a crucial aspect of host colonization, as it prevents mechanical clearance of pathogens and provides a selective advantage to bacteria of endogenous flora. Accordingly, bacteria have developed a vast arsenal of molecular strategies that enable them to target and adhere to host cells.

DISCUSSION

Local immunity of the respiratory tract mucous membranes remains insufficiently studied [10]. At the same time, the variability in the course of respiratory infections - from mild or asymptomatic forms in the upper respiratory tract to autoimmune disorders and severe purulent infections - is determined both by direct viral or bacterial aggression and by the immune response of the body to infection [11].

Among the most important nonspecific factors of local defense are the phagocytic function of granulocytes and alveolar macrophages, mucociliary clearance of the airway mucosa, as well as a number of antibacterial and antiviral components present in the respiratory tract secretions, including lysozyme, lactoferrin, complement, interferon, and surfactant [12, 13].

During evolution, a specific lymphoid tissue - Mucosa-associated lymphoid tissue (MALT) - formed in the mucous membranes, where innate and adaptive defense reactions develop in response to pathogenic aggression [14, 15]. At all stages of immune system formation, the first barrier preventing the development of infectious processes is the local MALT defense of the respiratory tract [16]. Under normal conditions, the mechanisms of local protection have sufficient potential to prevent acute respiratory viral infections (ARVI) even in the early stages of immune system development. Viruses entering the upper respiratory tract with inhaled air encounter several local immunity factors, such as viscous secretions, ciliary epithelial movement, the antiseptic properties of lysozyme and lactoferrin, the competitive effect of natural microflora, the enzymatic activity of secretions, and the specific action of IgA, among others [17, 18].

Thus, the mucous membranes of the nasal and oropharynx serve as the primary entry points for respiratory infections. Aggressive environmental conditions, the presence of chronic infection foci, and disturbances in the microbial balance of the saprophytic microflora impair the colonization resistance of the respiratory tract MALT in children. Therefore, the activation of the body's own defense mechanisms is not only a means of treatment but also a strategy for preventing upper respiratory tract infections (rhinitis, sinusitis, nasopharyngitis, tonsillitis, pharyngitis, tonsillopharyngitis) [19].

CONCLUSIONS

A total of 114 strains of opportunistic microorganisms were isolated, including 33 strains (29.0%) of Gram-positive bacteria (*Streptococcus pyogenes, Staphylococcus aureus, Enterococcus faecalis*) and 81 strains (71.0%) of Gram-negative bacteria (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli*). The predominant pathogens were *Escherichia coli* (37.0%) and *Staphylococcus aureus* (21.0%).

The biomaterial analysis included the identification of enzyme groups such as sucrase (n=69), maltase (n=87), and lactoperoxidase (n=89). The highest level in our studies was found for lactoperoxidase. Sucrase also hydrolyzes sucrose and maltose. The detected glucose level in the children's analysis (n=84) indicates the functionality of the degradation system and monosaccharide formation.

The presence of alcohols - sorbitol (a hexahydric alcohol, n=102), mannitol (a hexahydric alcohol, n=84), and xylitol (a pentahydric alcohol, n=86) - suggests the detoxification capabilities of the child's body. Additionally, intoxication-related factors were identified: acetone (n=105), which indicates excessive bacterial replication in the oral cavity. The identification of indole in 72 cases suggests its involvement in regulating various aspects of bacterial physiology and virulence levels. Tryptophan, a derivative of indole and a precursor of the neurotransmitter serotonin, may cause vomiting and angiospasm in patients.

Most Frequently Identified Microorganisms the next: *Escherichia coli* showed significant positive correlations with Free T4 (r=0.19, p=0.05) and TNF-α (r=0.20, p=0.04), while exhibiting a negative correlation with Cu levels. *Staphylococcus aureus* demonstrated negative correlations with lactoperoxidase enzyme levels (r=-0.20, p=0.04) and acetone (r=-0.21, p=0.03).

Bacterial invasion involves effector molecules that support pathogenic adhesion to the cells of the child's body and intracellular changes induced by the pathogen [7].

The data obtained showed a predominance of Gram-negative bacterial strains—81 (71.0%), specifically *Klebsiella pneumoniae* (11%), *Pseudomonas aeruginosa* (24%), and *Escherichia coli* (37.0%). Gram-positive bacteria accounted for 33 strains (29.0%), including *Streptococcus pyogenes* (2.0%), *Staphylococcus aureus* (21.0%), and *Enterococcus faecalis* (6.0%). The predominant pathogens were *Escherichia coli* (37.0%) and *Staphylococcus aureus* (21.0%).

Electron Microscopy Findings. Electron microscopy results revealed the localization of coccal flora both inside epithelial cells and in the extracellular area. The main pathological manifestations in the epithelial cells of the nasopharyngeal zone included nuclear destruction with the formation of a significant number of nucleoli and cellular debris in the cytoplasmic membrane, increased blood flow in the cytoplasmic membrane, and peripheral thinning of the basal cell membrane.

Morphological analysis of the studied images revealed all stages of apoptosis, from initiation to degradation.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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

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ORIGINAL ARTICLE

CONTENTS

Study of the influence of medication properties and lifestyle of patients with coronary heart disease on adherence to treatment

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ABSTRACT

Aim: To investigate how lifestyle factors of patients with CHD and comorbid conditions, along with medicines properties, influence treatment adherence.
Materials and Methods: include the results of a survey conducted among patients with CHD and comorbid conditions at the Department of Cardiology of the P.L. Shupyk National Healthcare University of Ukraine from June to September 2024 (n = 101). The study employed methods of analysis, synthesis, deduction, induction, comparison, statistical and bibliographic research.
Results: The study revealed a significantly low adherence rate of 13.9% [CI 95% 13.9 ± 0.002; $p<0.0001$] to risk factor modification and prescribed pharmacotherapy in secondary prevention of CHD. It confirmed a statistically significant influence of patient preferences with CHD and comorbid conditions on effective pharmacotherapy ($\chi 2 = 3.350{,}232$, $p = 0.067$). Patients receiving consultations from both doctors and pharmacists were 22 times more likely (OR = 22.67) to adhere to pharmacotherapy compared to those consulting only doctors. The study found that only 7.8% [CI 95% 7.8 ± 0.05; $p<0.0001$] of surveyed patients with CHD and comorbid conditions such as hypertension, diabetes mellitus and chronic kidney disease utilized the "Affordable Medicines" program.
Conclusions: The decline in the socioeconomic status of the population during the war in Ukraine negatively affects adherence to clinical guidelines for risk factor modification and secondary prevention of CHD, as evidenced by observational studies EUROASPIRE IV and V. The study identified that 42.8% of respondents prefer original medicines when selecting medications, but financial constraints prevent prolonged use as part of prescribed pharmacotherapy, adversely affecting treatment adherence. The effectiveness of a multidisciplinary approach in enhancing treatment adherence among patients with CHD and comorbid conditions was confirmed, demonstrating a 22-fold higher adherence rate compared to consultations with doctors alone.

KEY WORDS: Coronary heart disease, treatment adherence, pharmaceutical care, multidisciplinary team, generic medicines, original medicines

Wiad Lek. 2025;78(4):860-875. doi: 10.36740/WLek/203897 DOI

INTRODUCTION

Cardiovascular diseases (CVD) are the leading cause of mortality worldwide, accounting for 9.7% of the global disease burden [1]. Globally, Ukraine ranks fourth in CVD mortality after Tajikistan, Azerbaijan and Uzbekistan [2]. The primary cause of CVD-related deaths in Ukraine is ischemic heart disease (CHD) [3]. The ongoing war in Ukraine has evidently worsened the socioeconomic conditions of the population [4], reducing access to medications and placing additional strain on the national healthcare system [5].

According to the National Health Service of Ukraine (NHSU), the statistics for issuing temporary disability certificates in specialized medical care for cardiologists in the "general illness or injury" category indicate nearly a twofold increase in the number of signed certificates in 2023 (0.57 million) compared to 2022 (0.29 million). In the first half of 2024 alone, this number increased by an additional 0.24 million compared to 2023 [6]. Consequently, there has been a rise in unplanned hospitalizations, along with increased prescriptions of medications, which partially contribute to the effectiveness of pharmacotherapy [7] in the treatment strategy for the CHD patients.

Clinical guidelines from the European Society of Cardiology (ESC) [8, 9], the American Heart Association (AHA) [10], and the Ukrainian Unified Clinical Protocol on Stable Coronary Heart Disease [11] emphasize the modification of risk factors and effective pharmacotherapy to achieve better clinical outcomes and improve the quality of life for patients with CHD. Observational findings from the EUROASPIRE V study (n=3562) [12] highlight suboptimal treatment adherence among CHD patients with comorbid conditions such as arterial hypertension (AH), diabetes mellitus (DM) and chronic kidney disease (CKD). Specifically,

adherence rates were reported as 64.9% for antihypertensive medicines, 61.3% for lipid-lowering medicines and 76.5% for hypoglycemic agents among CHD patients [12].

Given the above, it is considered appropriate to define the term "treatment adherence" as "the degree to which a patient's behavior corresponds with clinical recommendations (prescriptions), including the timing, dosage, and frequency of medication intake prescribed by healthcare providers over a specified period" [13]. It has been determined that adherence to pharmacotherapy is influenced by: health care providers (doctors, nurses, pharmacists, state health insurance programs); patients (socioeconomic status, knowledge of the correct use of medicines, polypharmacy, personal biases in the use of medicines, etc.) and properties with characteristics inherent in medicines (price, quality, shelf life, adverse reactions, polypharmacy, pharmacokinetic and pharmacodynamic properties, method of use, release form, etc.) [13].

Therefore, a likely reason for the lack of adherence to treatment due to the deterioration of the socioeconomic status of the population may be the use of original and generic medicines. The use of original medicines leads to the impact of high costs and additional burden on the budgets of health care systems and patients' households. In turn, the use of generic medicines, on the contrary, reduces the burden on the budgets of national health care systems and households of patients with chronic diseases. Such medicines are bioequivalent to the original ones.

According to European data on the use of medicines [14], the consumption of generic medicines in Europe is 70% by volume and 14% by value. The use of generic medicines expands and improves the population's access to medicines. And the redistribution of budget expenditures of national health systems from original to generic medicines leads to significant savings. WHO emphasizes that the average monthly burden of medicine consumption on the family budget of patients should be no more than 10-25% of the total monthly family budget [15]. Therefore, in order to address the issues of the relationship between the deterioration of the socioeconomic status of the population during the war in Ukraine with low adherence to pharmacotherapy of patients with ischemic heart disease and further obtaining better clinical outcomes, the study of a number of factors influencing the choice of medicines within the same molecule by International Nonproprietary Name (INN) in the pharmacotherapy of ischemic heart disease with comorbid conditions is being updated.

According to the current Ukrainian legislation, original (innovative) medicinal products are understood as those medicinal products that are for the first time in the world "registered on the basis of a complete registration dossier" with evidence of efficacy, safety and quality. Generic medicinal products have the same "qualitative and quantitative composition of active substances, dosage form" compared to the original medicinal product (reference medicinal product) and proven bioequivalence with the original medicinal product "as a result of relevant studies" [16]. According to the Law of Ukraine "On Medicinal Products", all medicinal products are prescribed by the INN [16].

Thus, in connection with the decline in the socioeconomic status of the population of Ukraine and the increase in unplanned hospitalizations, in particular of patients with ischemic heart disease [5], the issue of studying factors influencing adherence to pharmacotherapy in patients with ischemic heart disease with comorbid conditions, related to lifestyle and medicines properties, is becoming more relevant.

AIM

The aim of the article is to study the factors influencing the lifestyle of patients with ischemic heart disease with comorbid conditions and medicines properties on adherence to treatment.

MATERIALS AND METHODS

The materials and methods of this study were the results of a survey of patients with ischemic heart disease with comorbid conditions, conducted at the Department of Cardiology of the P.L. Shupyk National University of Health Care of Ukraine. The empirical study took place from June to September 2024 in the process of an online survey using Google Form of 101 patients (66 women, 35 men) with ischemic heart disease and concomitant hypertension, diabetes, CKD from 13 regions of Ukraine. Patients were previously given verbal consent to fill out online questionnaires. In the introduction to the questionnaire, patients were provided with the definitions of "original medicines" and "generic medicines" in accordance with current Ukrainian legislation [16]. The questionnaire contained three blocks of questions: "socioeconomic", "determination of adherence to treatment", "patient perception of original and generic medicines". In this study, we analyze the influence of "socioeconomic" factors, factors of "patient perception of medicines", determine the relationship of their influence on "adherence to pharmacotherapy" of CVD, in particular CHD with concomitant hypertension, diabetes, CKD.

The "socioeconomic" block of questions examined gender, age, education, professional employment, monthly burden on the family budget of patients (ratio of monthly income to expenses for medicines). Patients were asked to choose the level of income according to the data of the Ministry of Finance of Ukraine (subsistence minimum (2920 UAH), minimum (8000 UAH) and average salary (14577 UAH) and above) [17]. It should be noted that the USD exchange rate of the National Bank of Ukraine is 41.24 UAH [18]. This block included questions to determine the lifestyle of patients and their self-assessment of their health. The block "patients' perception of medicines" included questions to determine factors that influence the choice of medicines by INN; the number of medicines used daily; reasons for discontinuation of pharmacotherapy. It is proposed to define statements regarding the general characteristics of original and generic medicines to further identify factors influencing patients' choice of medicines by INN and awareness regarding original and generic medicines.

The "adherence to pharmacotherapy" block contained four questions determined by the standardized Morisky-4 treatment adherence scale (MMAS-4) [19]:

1) Have you ever forgotten to take medications prescribed for cardiovascular diseases (CVD) and associated conditions such as arterial hypertension (AH), diabetes mellitus (DM), or chronic kidney disease (CKD)?

2) Have you ever made mistakes in taking medications prescribed for cardiovascular diseases (CVD) and associated conditions such as arterial hypertension (AH), diabetes mellitus (DM), or chronic kidney disease (CKD)?

3) Have you ever stopped taking medications prescribed for cardiovascular diseases (CVD) and associated conditions such as arterial hypertension (AH), diabetes mellitus (DM), or chronic kidney disease (CKD) on your own if you felt better?

4) Have you ever stopped taking medications prescribed for cardiovascular diseases (CVD) and associated conditions such as arterial hypertension (AH), diabetes mellitus (DM), or chronic kidney disease (CKD) on your own if you felt worse?

Patients were asked to select "yes/no" answers. A "yes" answer was scored as 1 point, a "no" answer as 0 points. Patients were considered compliant with pharmacotherapy when they did not score points for four answers.

Patients were also asked to rate their own health on a scale from 0 to 10, where 10 is the highest health indicator, in order to further establish the relationship between adherence to pharmacotherapy and self-assessment of health status.

It was determined that the sample size required for statistical analysis should be 96 respondents. For calculations, the acceptable margin of error for marketing research (10%), the variation for the sample – 50% and the confidence coefficient (standard deviation) CI – 1.96 (probability p = 0.95). The difference in indicators was considered significant at a CI level of 95% ($p<0.0001$).

To test the hypotheses about the significance of the differences between frequencies, the $\chi 2$ criterion with a likelihood adjustment was used. The φ (phi) criterion was determined, intended for the relationships of four-way (2x2) tables. For multi-way tables, the Cramer V criterion was used. The values of both criteria vary from 0 to 1. Both criteria are based on the $\chi 2$ criterion.

The results were visualized in Microsoft Office Excel spreadsheets, where the accumulation, adjustment, systematization of the original information, etc. took place. Statistical processing was performed using the STATISTICA.13 and IBM SPSS Statistics programs.

The empirical study was conducted in accordance with the Declaration of Helsinki of the World Health Organization "Ethical Principles of Medical Research Involving Human Subjects as Research Subjects".

RESULTS

According to the results of the study, it was determined that the average age of patients was 54.22 ± 4 years, more than two-thirds of the respondents had higher education (77.2%), the rest had vocational education (22.8%). Professional employment was observed in 73.3% of respondents (working or working pensioners), other respondents (unemployed and pensioners) were 26.7%. Exclusion criteria: oncological diseases, concomitant rheumatic diseases, dyscirculatory encephalopathy of more than II degree, patients with mental disorders and myocardial infarction more than a year.

The results of the analysis of changes in the lifestyle of patients after the diagnosis and their relationship with adherence to pharmacotherapy (Fig. 1) indicate that 1.9% [CI 95% 1.9 ± 0.03; $p<0.0001$] of patients who were committed to pharmacotherapy gave up smoking. The largest proportion of respondents who were committed to pharmacotherapy "clearly followed the recommendations of doctors" and was 13.9% [CI 95% 13.9 ± 0.002; $p<0.0001$]. At the same time, 10.9% [CI 95% 1.9 ± 0.06; $p<0.0001$] of patients who were committed to pharmacotherapy started to follow a diet. Compliance with the sleep regimen was determined by 7.9% [CI 95% 7.9 ± 0.05; $p<0.0001$] and changes in the daily schedule were determined by 6.9% [CI 95% 6.9 ± 0.05; $p<0.0001$] of patients who

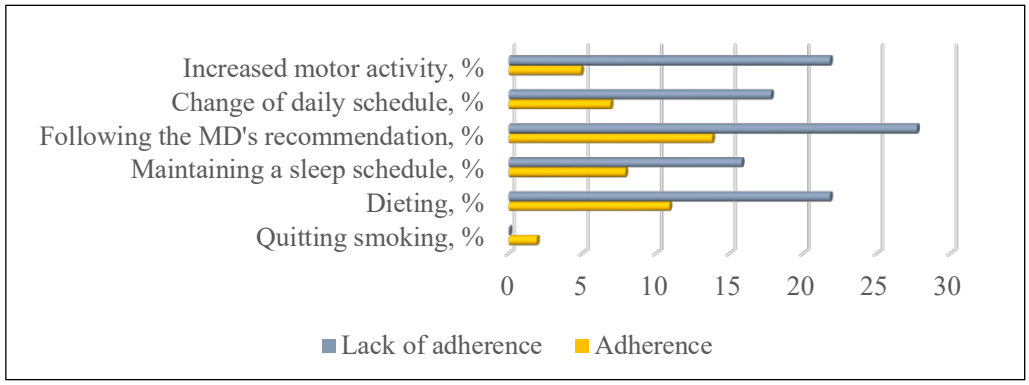

Fig. 1. Relationship between adherence to pharmacotherapy and changes in patients' lifestyle after diagnosis.

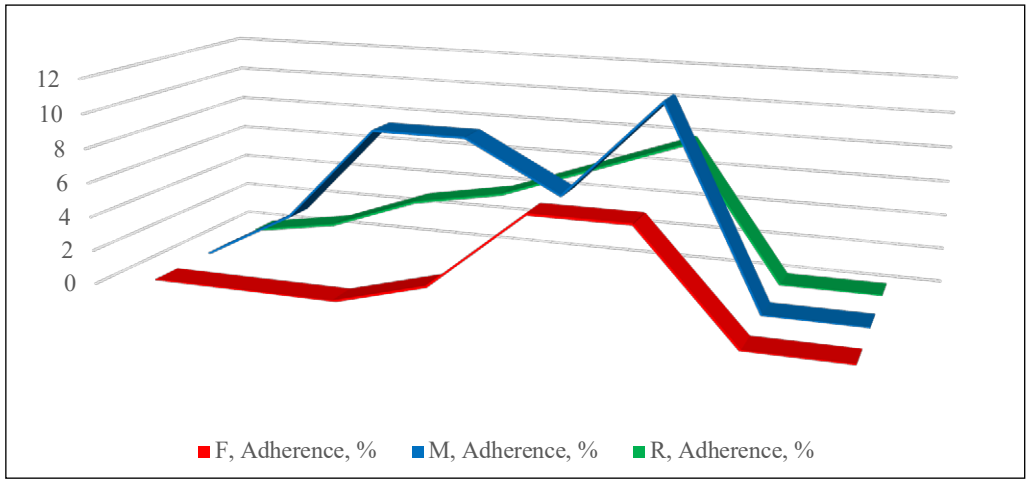

Fig. 2. Relationship between adherence to pharmacotherapy and self-assessment of health status among the surveyed respondents (R).

were committed to pharmacotherapy, respectively. 17.8% [95% CI 17.8 ± 0.07; p<0.0001] of respondents did not change their lifestyle and were not compliant with pharmacotherapy.

The relationship between adherence to pharmacotherapy and self-assessment of the health status of the surveyed respondents (Fig. 2) was analyzed on a scale from "0 – 10", where "10" is the highest health indicator. The results of the observation show that the surveyed patients assessed their own health status from 3 (3.9% [CI 95% 3.9 ± 0.04; p < 0.0001]) to 10 (1.9% [CI 95% 1.9 ± 0.03; p < 0.0001]). At the same time, the largest proportion of patients adhering to pharmacotherapy was observed in the group with a self-assessment of their own health status "8" - 7.9% [CI 95% 7.9 ± 0.05; p < 0.0001]. Adherent to pharmacotherapy patients with self-assessment of their own health status "7" were 5.9% [CI 95% 5.9 ± 0.04; p<0.0001]. There is a tendency for decreased adherence to pharmacotherapy with worsening self-assessment of health status of their own. Within the patients with self-assessment of their own health status "3", "9" and "10" there was a lack of adherence to pharmacotherapy.

We tested the hypothesis of the influence of self-assessment of health status on adherence to pharmacotherapy (Fig. 2). According to the calculations of the Pearson Chi-square criterion, this thesis had to be refuted: = 9.726, p=0.246, fd=5, α=0.211. Chi-square is not statistically significant. Therefore, there is no relationship - p=0.115 between adherence to pharmacotherapy and self-assessment of the health status of the surveyed respondents (Table 1).

We present descriptive data on the factors influencing the choice of medicines in the prescribed pharmacotherapy. It was determined that (Fig. 3) the most significant factors were those related to doctor's prescriptions (73.3%), the cost of medicines (27.68%), and the choice of original medicines in the use of pharmacotherapy (13.8%). At the same time, 12.1% of respondents trust the recommendations of friends and relatives in the use of medicines. 7.8% of respondents pay attention to the availability of the "Affordable Medicines" program. It should be noted that the largest share of patients who are committed to pharmacotherapy is observed in the group for whom the cost of medicines is important – 21.78% [CI 95% 21.78 ± 0.07; p<0.0001]. In the group that adheres to medical prescriptions, 18.8% [CI 95% 18.8 ± 0.07; p<0.0001] of respondents are committed to pharmacotherapy. Some of the respondents (3.9% [CI 95% 3.9 ± 0.04; p<0.0001]) who are committed to pharmacotherapy follow the advice of pharmacists. Other patients (1-2%) are committed to pharmacotherapy, trust data from the Internet, advice from

Table 1. Calculations of the Relationship between Adherence to Pharmacotherapy and Self-assessment of Patients' Health Status Using Pearson's Chi-square Criterion Adjusted for Likelihood

Contingency Tables					
	Absent Present		Adherence		Total
Health condition	3,00	Count	4	0	4
		Expected count	3,1	,9	4,0
	4,00	Count	7	1	8
		Expected count	6,3	1,7	8,0
	5,00	Count	14	3	17
		Expected count	13,3	3,7	17,0
	6,00	Count	14	4	18
		Expected count	14,1	3,9	18,0
	7,00	Count	21	6	27
		Expected count	21,1	5,9	27,0
	8,00	Count	10	8	18
		Expected count	14,1	3,9	18,0
	9,00	Count	7	0	7
		Expected count	5,5	1,5	7,0
	10,00	Count	2	0	2
		Expected count	1,6	,4	2,0
Total		Count	79	22	101
		Expected count	79,0	22,0	101,0

Chi-square Criteria			
	Value	fd	Asymptotic Significance (2-sided)
Pearson Chi-square	9,626[a]	7	0,211
Likelihood Ratio	11,599	7	0,115
Valid Observations Count	101		

		Value	Approximate Significance
Nominal/Nominal	Phi	0,309	0,211
	Cramér's V	0,309	0,211
	Contingency Coefficient	0,295	0,211
Valid Observations Count		101	

friends and relatives, pay attention to the originality of the medicine, the presence of the "Affordable Medicines" program, and the "manufacturer of the medicines".

The dependence of patients' adherence to prescribed pharmacotherapy on the participation of pharmacists in pharmacotherapy management was established. 80 valid questionnaires were selected to answer the question. The answers were used to determine the factors influencing the choice of medicines in prescribed pharmacotherapy (Fig. 4).

According to the results of the Chi-square, adjusted for likelihood = 28.633, $p<0.000$, the statistical significance of the difference in observed frequencies

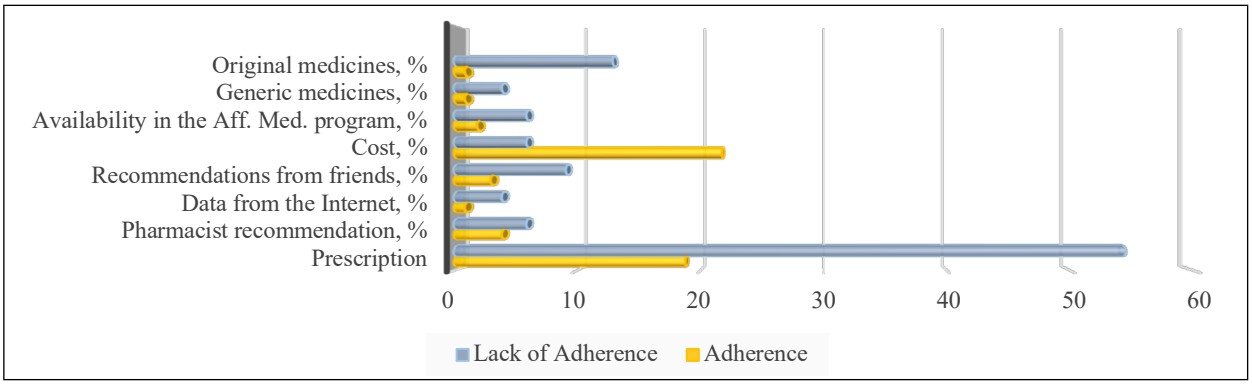

Fig. 3. Factors influencing the choice of medicines in the prescribed pharmacotherapy.

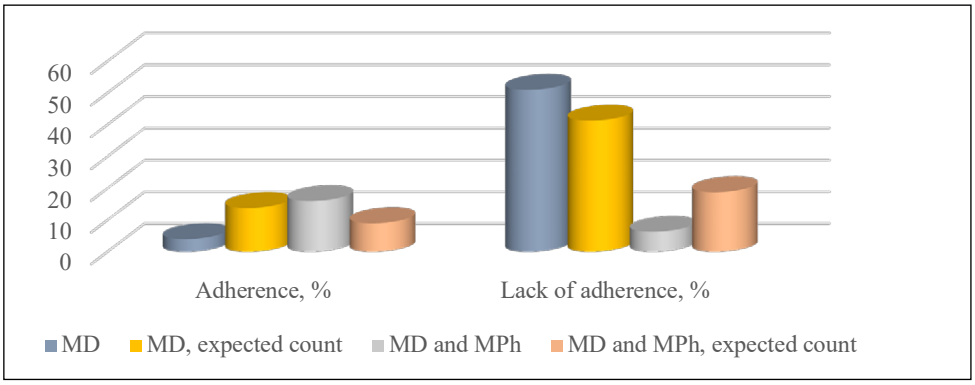

Fig. 4. Dependence of patient adherence to prescribed pharmacotherapy on pharmacists' participation in pharmacotherapy management.

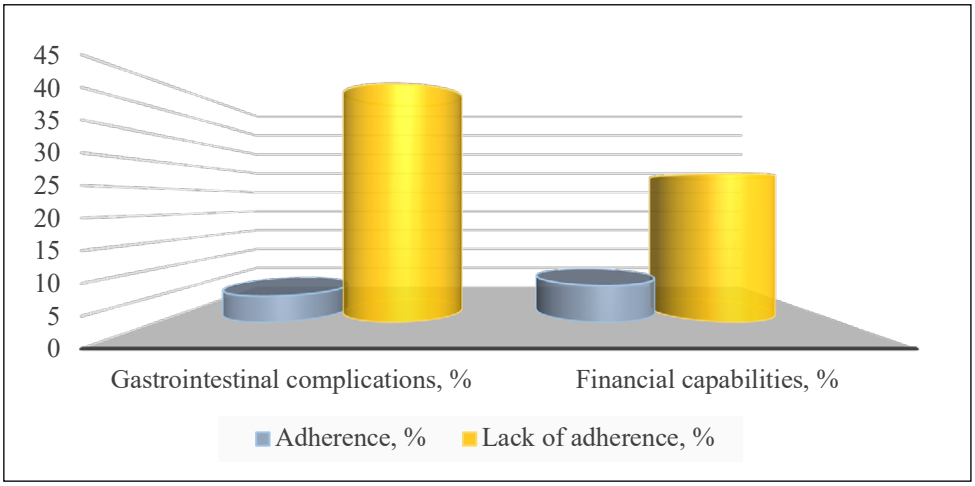

Fig. 5. Factors influencing the discontinuation of prescribed pharmacotherapy.

between patients who consulted only with doctors or doctors and pharmacists was established. The relationship is quite strong, the coefficient of association φ and Cramer = 0.607, p<0.000.

The odds ratio OR=22.67 was also determined. The data obtained indicate that patients with consultations with a doctor and a pharmacist have 22 times greater adherence to pharmacotherapy (Table 2) compared to patients who received consultations with a doctor only.

A relationship was established between adherence to pharmacotherapy and the use of medicines under the "Affordable Medicines" program. We selected 91 valid responses. It was determined that patients who use the "Affordable Medicines" program do not have adherence to pharmacotherapy compared to those patients who do not use the reimbursement program. This may be explained by the adherence of patients with ischemic heart disease and comorbid conditions to more effective, and therefore expensive, medicines that are not included in the "Affordable Medicines" program (Table 3). Chi-square adjusted for likelihood = 5.353 p = 0.021. The relationship is moderate φ and Cramer's coefficient 0.234, p =0.025.

It has been statistically proven that patients who do not use the "Affordable Medicines" program have a greater commitment to pharmacotherapy compared to those who use the reimbursement program.

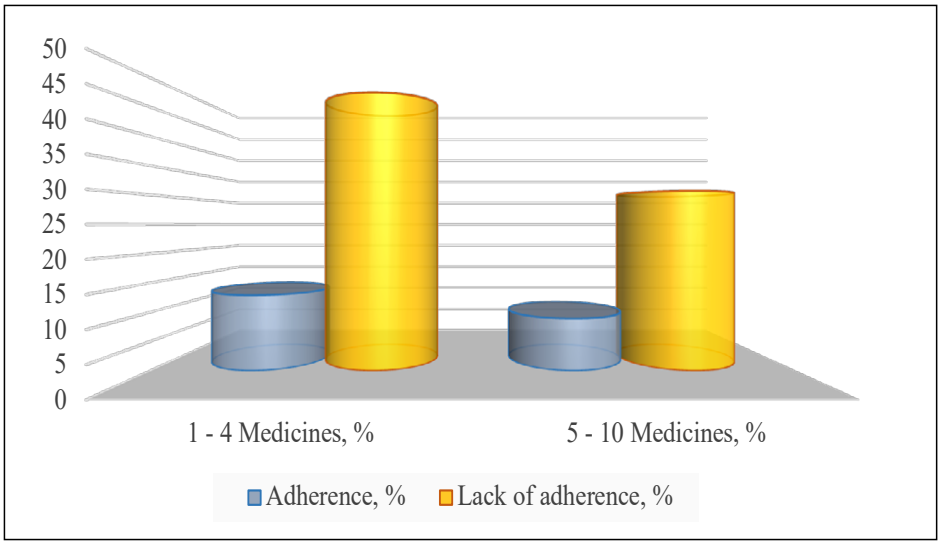

Fig. 6. Relationship between adherence to pharmacotherapy and the amount of daily medication consumption.

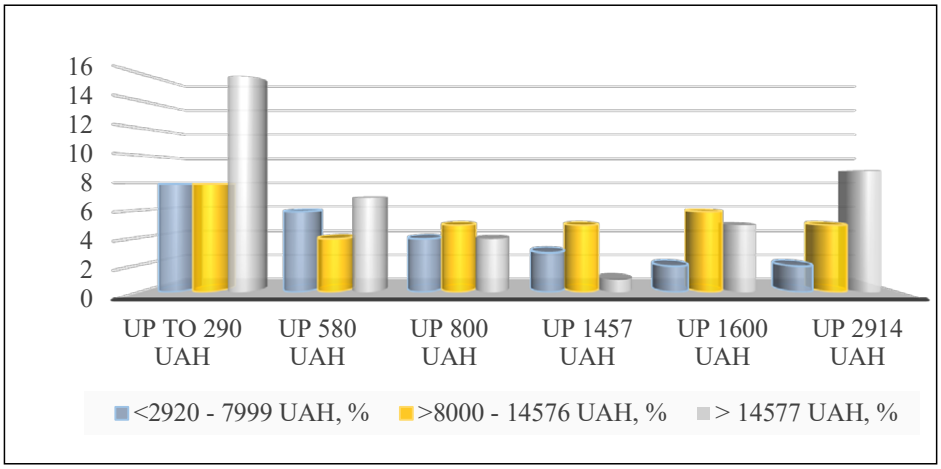

Fig. 7. Relationship between average monthly income of patients and average monthly expenses for medicines.

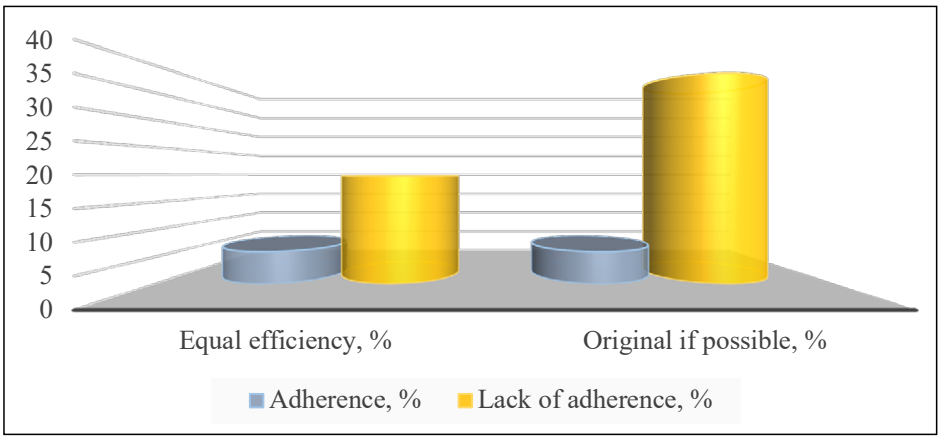

Fig. 8. Relationship between patient preferences and adherence to pharmacotherapy.

This situation is explained by the desire to use more effective medicines. In both groups, patients noted that they have a preference for original medicines. However, financial capabilities do not allow all patients to use effective medicines in pharmacotherapy all the time. For Chi-square calculations we selected 91 valid responses. $\chi 2$ adjusted for probability is not statistically significant, $\chi 2=3.350.232$, $p=0.067$. There is no relationship, $p = 0.069$. Therefore, patients who currently use the "Affordable Medicines" program do not have the opportunity to use a number of medicines and have low commitment to pharmacotherapy. The observation of adherence to original medicines due to their effectiveness, and therefore price, indicates that all patients can purchase generic medicines for long-term use if necessary (Table 4).

It was found that the main reasons for discontinuation of pharmacotherapy (Fig. 5) were gastrointestinal complications (49.4%) and financial capabilities of patients (34.6%). The proportion of respondents who

Table 2. The Dependence of Patient Adherence to Prescribed Pharmacotherapy on Pharmacist Involvement in Pharmacotherapy Management

Consolidated Observation Report						
	Observations					
	Valid		Missing		Total	
	N	Percentage	N	Percentage	N	Percentage
Pharmacotherapy Management* Adherence	80	100,0%	0	0,0%	80	100,0%

Cross-tabulation of Pharmacotherapy Management Adherence*					
			Adherence		Total
			Lack of Adherence	Presence of Adherence	
Pharmacotherapy Management	Doctor Only	Count	51	4	55
		Expected Count	41,3	13,8	55,0
	Doctor and Pharmacist	Count	9	16	25
		Expected Count	18,8	6,3	25,0
Total		Count	60	20	80
		Expected Count	60,0	20,0	80,0

Chi-square Criteria					
	Value	Dg.	Asymptotic Significance (2-sided)	Exact Significance (2-sided)	Exact Significance (1-sided)
Pearson Chi-square	29,498[a]	1	0,000		
Continuity Correction[b]	26,550	1	0,000		
Likelihood Ratio	28,633	1	0,000		
Fisher's Exact Test				0,000	0,000

Symmetric Measures[c]			
		Value	Approximate Value
Nominal/Nominal	Phi (Φ)	0,607	0,000
	Cramér's V	0,607	0,000

Risk Estimate			
	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Pharmacotherapy Management (Doctor / Doctor + Pharmacist)	22,667	6,148	83,574
Number of Valid Observations	80		

were supportive of pharmacotherapy and who noted gastrointestinal complications was 4.9% [CI 95% 4.9 ± 0.04; p<0.0001]. Another proportion of patients who were supportive of pharmacotherapy (6.9% [CI 95% 6.9 ± 0.05; p<0.0001]) focused on financial capabilities, which became a burden for the whole family. Patients noted that "other family members pay for the treatment". Some respondents (4%) with a lack of adherence to pharmacotherapy noted that they take "a large number of medications,""have other burdens," "take dietary supplements," "are worried about their liver," "clearly follow the doctor's recommendations, but forget to take medications on time."

To establish the relationship between the reasons for discontinuation of pharmacotherapy for patients and its impact on adherence (side effects, high cost),

Table 3. The Relationship between Adherence to Pharmacotherapy and the Use of Medications under the "Affordable Medicines" Program

Consolidated Observation Report						
"Affordable Medicines" Program * Adherence to Pharmacotherapy	Observations					
	Valid		Massing		Total	
	N	Percentage	N	Percentage	N	Percentage
	91	100,0%	0	0,0%	91	100,0%

Cross-tabulation of "Affordable Medicines" Program * Adherence

			Adherence		Total
			Lack of Adherence	Presence of Adherence	
"Affordable Medicines" Program	Do not apply	Count	37	16	53
		Expected Count	41,4	11,6	53,0
	Apply	Count	34	4	38
		Expected Count	29,6	8,4	38,0
Total		Count	71	20	91
		Expected Count	71,0	20,0	91,0

Chi-square Criteria

	Value	df	Asymptotic Significance (2-sided) p-value	Exact Significance (2-sided) p-value	Exact Significance (1-sided) p-value
Pearson Chi-square	4,990[a]	1	0,025		
Continuity Correction[b]	5,353	1	0,021		
Likelihood Ratio				0,039	0,022

Symmetric Measures[c]

		Value	p-value
Nominal/ Nominal	Phi (Φ)	0,234	0,025
	Cramér's V	0,234	0,025

81 valid responses were filtered (single responses "absence of symptoms", "communication with a doctor", "taking dietary supplements", etc. were excluded). All responses were divided into 4 groups – gastrointestinal problems, side effects, financial problems, responses where respondents mentioned both problems (side effects, finances) and others.

It was found that there is no significant difference between the problems experienced by people and their adherence to pharmacotherapy. In general, patients react equally to the listed problems. The indicator of the adjusted for likelihood $\chi 2$ is not statistically significant.

The relationship of adherence to pharmacotherapy between the number of daily medications used was analyzed (Fig. 6). It was determined that 60.4% of respondents take one to four medications daily, the remaining 39.6% of patients have to take five to ten medications daily. The relationship of adherence to pharmacotherapy in the group of respondents who take from 1 to 4 medications daily was established – 12.9% [CI 95% 12.9 ± 0.06; $p < 0.0001$]. Respondents who are adherent to treatment with daily intake of 5 to 10 drugs are 8.9% [CI 95% 8.9 ± 0.001; $p < 0.0001$].

It was determined that adherence to pharmacotherapy does not depend on the number of medications taken. The indicator adjusted for the probability of $\chi 2$ is not statistically significant.

It was found that 31.6% of respondents spend up to 290 UAH per month on medications – 7.9% [CI 95% 7.9 ± 0.05; $p<0.0001$] with an average monthly income from the minimum subsistence minimum (2920 UAH) to the average salary (14576 UAH) and 15.8% [CI 95% 7.9 ± 0.05; $p<0.0001$] with an average

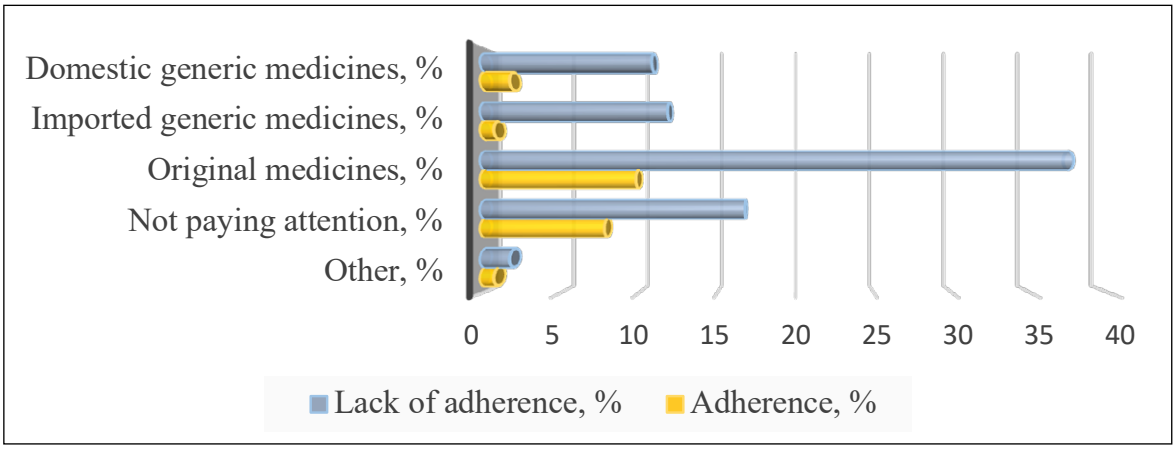

Fig. 9. Relationship between adherence to pharmacotherapy and factors influencing the choice of medicines by INN.

monthly income higher than the average salary (>14577 UAH) (Fig. 7). Another 37.1% of respondents spend from 1457 UAH to 2914 UAH per month. It was determined that 24.23% of respondents had a monthly income from the minimum subsistence minimum (2920 UAH) to the minimum wage (7999 UAH). The surveyed patients with an average monthly income from the minimum wage (7999 UAH) to the average wage (14576 UAH) accounted for 31.8%. The remaining respondents had a monthly income of more than the average wage (14576 UAH) – 43.93%. It should be noted that due to the deterioration of the socioeconomic status of the population, not all patients were able to complete the survey using modern electronic devices.

The relationship between adherence to pharmacotherapy and medicines costs has been statistically proven: the higher the price, the higher the adherence to pharmacotherapy (invested in treatment, need for taking medicines). According to the results of the calculated $\chi 2$, adjusted for plausibility = 19.476, $p = 0.002$, $fd = 5$, at a given level of hope $\alpha = 0.05$, there is. The strength of the relationship is moderate (Table 5). $\chi 2 = 0.445$, $p = 0.000$ and $\varphi = 0.445$ $p = 0.000$. However, the survey results indicate that such patients eventually cancel pharmacotherapy used in CHD with comorbid conditions due to the lack of funds.

The relationship between patient preferences and adherence to pharmacotherapy has been determined (Fig. 8). The results of the analysis show that 47.5% of the surveyed patients prefer to use original medicines, of which the proportion of those who are committed to pharmacotherapy is 9.9% [CI 95% 9.9 ± 0.06; p<0.0001]. The remaining part of the respondents (25%) does not pay attention to the manufacturer of the medicine and the proportion of those who are committed to pharmacotherapy in this group is 7.9% [CI 95% 7.9 ± 0.05; p<0.0001]. The opinions of the respondents regarding the use of domestic and generic medicines in pharmacotherapy were equally divided by 12.8%. At the same time, in the indicated groups of patients, 1.9% [CI 95% 1.9 ± 0.03; p<0.0001] and 0.9% [CI 95% 0.9 ± 0.19; p<0.0001] are inclined to use foreign medicines. The remaining 2.8% of respondents use in pharmacotherapy those medicines that are offered in pharmacies.

According to the results of the analysis of the factors influencing the choice of medicines by INN and patients' awareness of original and generic medicines, it was determined that a larger proportion of respondents have an understanding of the main differences between these medicines (Fig. 9). Patients noted that original and generic medicines have the same effectiveness (25.7%). At the same time, patients in this group who are committed to pharmacotherapy were 2.8% [CI 95% 2.8 ± 0.05; p<0.0001]. The proportion of respondents who, if possible, have a desire to receive pharmacotherapy with original medicines is 42.8%. Of these, 5.9% [CI 95% 5.9 ± 0.04; p<0.0001] are committed to pharmacotherapy. Almost all respondents noted that "original medicines are more expensive", and "generic medicines are cheaper". About 2% of respondents emphasized that "the quality of these medicines depends on the manufacturer", "generic medicines are fully controlled", "domestic medicines are not always effective".

DISCUSSION

The implementation of guidelines (clinical recommendations) for patients with ischemic heart disease for the prevention of cardiovascular diseases in clinical practice [8;9] was investigated in a series of EUROASPAIR III, IV, V surveys. In these studies, an objective assessment of the implementation of clinical recom-

Table 4. The Relationship Between Adherence to Pharmacotherapy and the Use of Generic Medicines

Consolidated Observation Report						
Observations Valid	Observations					
	Valid		Valid		Valid	
	Percentage	Percentage	Percentage	Percentage	Percentage	Percentage
Possibility * Preference	91	100,0%	0	0,0%	91	100,0%

Cross-tabulation of Generic Medicines * Preference					
			Preference		Total
			Generic	Original	
Possibility	No	Count	24	12	36
		Expected Count	19,8	16,2	36,0
	Yes	Count	26	29	55
		Expected Count	30,2	24,8	55,0
Total		Count	50	41	91
		Expected Count	50,0	41,0	91,0

Chi-square Criteria					
	Value	df	Asymptotic Significance (2-sided) p-value	Exact Significance (2-sided) p-value	Exact Significance (1-sided) p-value
Pearson Chi-square	3,306[a]	1	0,069		
Likelihood Ratio	3,350	1	0,067		
Fisher's Exact Test				0,086	0,054
Number of allowed observations	91				

Symmetric Measures[c]			
		Value	P-value
Nominal/ Nominal	Phi	0,191	0,069
	Cramér's V	0,191	0,069

mendations for patients with ischemic heart disease was carried out by surveying in EUROASPAIR IV, V (n = 16259, 27 countries) on lifestyle modification and pharmacotherapy management [20]. The data obtained as a result of the surveys indicate low rates of risk factor modification. Thus, among the surveyed patients with coronary heart disease (n = 16259) - 18.1% (n = 2950) followed a diet and 61.3% (n = 9963) led an active lifestyle after a coronary event. It was determined that out of 31% (n=4998) of surveyed smokers, 48.2% (n=2410) quit smoking after a coronary event [20].

According to the results of the analysis of the survey conducted by us (Fig. 1), in Ukraine it was found that 32.7% of the surveyed patients with ischemic heart disease follow a diet and began to move more (26.7%). 23.7% and 24.7% of patients with ischemic heart disease changed their sleep and daily schedule, respectively. There is also a very low level of patients with ischemic heart disease who have given up smoking (5.8%). These observations indicate that Ukrainian patients are more inclined to follow a diet, but continue to lead a sedentary lifestyle and cannot give up smoking. An interesting fact is that half of the respondents (50.6%) self-assessed their own health status from "5" to "7" (Fig. 2). In this survey, we did not investigate the mental state of the respondents, but according to statistics from the Ministry of Health of Ukraine, 70% of Ukrainians have mental health disorders (sleep disorders, anxiety, stress, depression, post-traumatic stress disorder, etc.) related to the war in the country [21]. It should be noted that factors of the mental state of the Ukrainian population negatively affect lifestyle changes and behavior correction and may explain the low level of lifestyle modification in patients with ischemic heart disease in Ukraine [20].

Observations of factors influencing the choice of medicines in the prescribed pharmacotherapy within

Table 5. Calculations of the Relationship between Adherence to Pharmacotherapy and Monthly Expenditures on Medicines Based on Pearson's Chi-square Adjusted for Likelihood

Contingency Table					
	Lack of Adherence Presence of Adherence		Adherence		Total
Expenditures, UAH	Up to 1457	Count	9	0	9
		Expected Count	7,0	2,0	9,0
	Up to 1600	Count	12	1	13
		Expected Count	10,1	2,9	13,0
	Up to 290	Count	26	4	30
		Expected Count	23,3	6,7	30,0
	Up to 2914	Count	7	10	17
		Expected Count	13,2	3,8	17,0
	Up to 580	Count	12	5	17
		Expected Count	13,2	3,8	17,0
	Up to 800	Count	11	2	13
		Expected Count	10,1	2,9	13,0
Total		Count	77	22	99
		Expected Count	77,0	22,0	99,0

Chi-square Criteria			
	Value	df	Asymptotic Significance (2-sided) p value
Pearson Chi-square	19,567[a]	5	0,002
χ^2 (Likelihood Ratio)	19,476	5	0,002

Measures of Association			
		Value	**p value**
Criterion	Phi	0,445	0,002
	Cramér's V	0,445	0,002

the limits of one molecule by INN indicate that the overwhelming number of surveyed patients with CHD (75.7%) trust the prescriptions and recommendations of doctors (Fig. 3). However, the decline in the socio-economic status of the population during the war in the country forces Ukrainian citizens to seek advice from pharmacists (13.6%) and acquaintances (12.1%) and to conduct independent data searches on the Internet (4.5%) to choose the cheapest medicines with proper efficacy and quality.

It should be noted that 9.1% of the surveyed respondents use the "Affordable Medicines" program for medicines that are dispensed on reimbursement. Not all surveyed respondents were aware of the existence of this program, as indicated in the respondents' comments. The significant relationship between adherence to pharmacotherapy and monthly costs probably indicates that patients prefer the effectiveness of pharmacotherapy and the convenience of using medicines. It should be noted that the "Affordable Medicines" program does not contain fixed combinations, atorvastatin and rosuvastatin with proven efficacy and safety, which are proposed by the latest ESC/AHA clinical recommendations, the unified clinical protocol "Stable ischemic heart disease" [8-11] and are included in the list of "Essential medicines" (EML list) proposed by WHO [22, 23]. Our data coincide with the data of another study (n = 208) on the preferences of patients with CHD for original medicines, which are considered essential medicines and are not covered by insurance companies in foreign countries [24].

Therefore, efforts can be directed at regulating and improving the mechanisms for providing citizens with medicines dispensed under the "Affordable Medicines" program (prescribing of medicines for the long-term use, popularization among the population and health care providers).

It should also be noted that the analyzed data obtained as a result of the survey reveal barriers between the number of prescribed medicines and possible

complications from the gastrointestinal tract (Fig. 4, 5). A proportion of the surveyed patients (56.03%) have concerns about possible adverse reactions to the prescribed medicines. Practical experience indicates that the vast majority of respondents (77.2% of respondents have higher education) carefully study the instructions for medicines and pay attention to possible adverse reactions, conduct their own data search on the Internet after which they refuse to take some of the prescribed medicines or return to the doctor's appointment to change pharmacotherapy. Such actions lead to unplanned hospitalizations and an additional burden on the healthcare system.

Therefore, given the proportion of patients who trust the recommendations of doctors and pharmacists, efforts aimed at a multidisciplinary team approach to education and additional explanations on the use of medicines (frequency of adverse reactions, method of administration, prevention of possible drug interactions, including prediction of possible drug-food interactions) would be possible. Numerous international clinical studies have shown the high effectiveness of pharmacists' influence on adherence to pharmacotherapy in patients [25-27].

Analysis of the results of the study confirms the data of monitoring companies on the decline in the socioeconomic status of the population [4]. When choosing medicines in the prescribed pharmacotherapy, 27.68% of respondents pay attention to the pricing policy of medicines (Fig. 3), 27.2% may cancel the prescribed pharmacotherapy due to the high price of medicines (Fig. 4). According to the latest clinical recommendations [9,28,30], the surveyed patients in the prescribed pharmacotherapy can use direct oral anticoagulants (from 800 UAH), NGKTH inhibitors (from 850 UAH) [30], fixed combinations, etc. for simultaneous long-term administration. These groups of medicines in Ukraine are expensive in relation to the average budget of patients (Fig. 6), therefore, patients refuse the prescribed pharmacotherapy after the respondents "felt an improvement" in their health. The listed pharmacological groups of medicines have been included in the medical guarantee programs [31], however, in accordance with domestic legislation [32], there is no necessary number of registered medicines of the listed pharmacological groups in Ukraine for their further inclusion in the "Affordable Medicines" program.

According to the results of the EUROASPAIR V observational survey of patients with high cardiovascular risk (n=2759) in primary care, it was found that less than 47% of patients are adherent to pharmacotherapy with antihypertensive and lipid-lowering medicines, and another 65% of the surveyed patients adhere to hypoglycemic pharmacotherapy. Such data indicate low adherence to pharmacotherapy in secondary prevention of patients with high cardiovascular risk [33]. The results of our survey showed low adherence of 21.8% [21.8 ± 0.07; $p<0.0001$] to pharmacotherapy in patients with ischemic heart disease and concomitant hypertension, diabetes, CKD. However, of the 42.8% of respondents who preferred original medicines (Fig. 7), 5.9% [CI 95% 5.9 ± 0.04; $p<0.0001$] showed the highest adherence to treatment. The same proportion of respondents indicated that if possible they would be treated only with original medicines (Fig. 8), but generic medicines are used in pharmacotherapy.

Thus, the analysis of awareness regarding the use of original and generic medicines and the identified factors influencing the choice of pharmacotherapy allow us to draw the following conclusions. The surveyed patients are aware of the difference between original and generic medicines. However, the financial capabilities of a significant part of the modern Ukrainian population do not allow them to use original medicines. To resolve this issue, doctors, who are trusted by a large proportion of respondents, should explain in more detail to socially unprotected segments of the population the difference in the use of generic and original medicines and redistribute the prescription of medicines from original to generic. Pharmacists could also join the interaction in the patient-doctor-pharmacist team within the framework of providing pharmaceutical care in advising patients on the benefits of medicines, their clinical effectiveness and safety in the prescribed pharmacotherapy and finally convincing patients to adhere to clinical recommendations [34,35].

CONCLUSIONS

1. It was investigated that the decrease in the socioeconomic status of the population during the war in Ukraine negatively affects compliance with clinical recommendations for the modification of risk factors and secondary prevention of CHD within the use of medicines, which is confirmed by the results of observational studies EUROASPAIR IV, V.
2. A significantly low adherence of 13.9% [CI 95% 13.9 ± 0.002; $p<0.0001$] to the modification of risk factors and prescribed pharmacotherapy in the secondary prevention of CHD was established, which is emphasized in observational studies EUROASPAIR IV, V.
3. The reliability of the influence of preferences of patients suffering from CHD with comorbid conditions to effective pharmacotherapy was confirmed ($\chi 2 = 3.350.232$, $p = 0.067$).

4. It was determined that 42.8% of respondents prefer original medicines when choosing medicines, however, the financial capabilities of patients do not allow them to be used for a long time in the prescribed pharmacotherapy, which negatively affects adherence to treatment.
5. It was found that patients who receive consultations from a doctor and a pharmacist are 22 times more likely to adhere to pharmacotherapy than patients who receive consultations only from a doctor. Odds ratio OR=22.67.
6. It was found that 7.8% [CI 95% 7.8 ± 0.05; p<0.0001] of the surveyed patients suffering from ischemic heart disease with concomitant hypertension, diabetes, CKD use the "Affordable Medicines" program and do not have the opportunity to use a number of medicines proposed by clinical recommendations.
7. The integration of pharmacists into multidisciplinary teams is recommended as well.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Dynamic morphofunctional characteristics of the respiratory tract in children with recurrent respiratory diseases depending on the method of therapy

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ABSTRACT

Aim: To investigate the data of the morphofunctional study of the respiratory tract in children with recurrent respiratory diseases in dynamics, depending on the method of therapy.

Materials and Methods: The study included 118 children of primary school age with a diagnosis of recurrent respiratory diseases and 26 healthy children identical in age, gender and anthropometric parameters. Two study groups were created, depending on the treatment method: group 1 – 62 patients (optimized therapy,OT), group 2 – 56 patients (basic therapy,BT).

Results: Significant differences after treatment were observed in following spirogram indicators: FVC (%) at $p_1<0,01$, $p_3=0,008$ in the OT group; FEV1 (%) at $p_1=0,001$, $p_3=0,01$ in the group with OT; PEF (%) at $p_1=0,02$ in the group of children of OT and $p_4=0,006$ in the group of children of BT; MEF 25 (%) with $p_1=0,03$, $p_3=0,05$ in the group of OT children and $p_4=0,01$ in the group of BT children; Tifno index (%) at $p_4=0,002$, $p_6=0,01$, $p_7=0,02$ with significant changes between the data on OT and BT groups; MEF 50 (%) at $p_7=0,01$ with a significant prevalence of OT data (by 1,2 times) against BT data.

Conclusions: The obtained data allow us to say that the addition of vitamin-mineral complex drugs to the standard treatment regimen has a positive effect on the state of the respiratory system in children with a diagnosis of recurrent respiratory diseases.

KEY WORDS: recurrent respiratory diseases, acute respiratory diseases, morphofunctional characteristic, children, therapy

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INTRODUCTION

Breathing is one of the most important vital functions of the human body and determines the gas exchange between the external environment and the child's body, due to the consumption of oxygen, the release of carbon dioxide and the generation of energy necessary for cellular metabolism. The following gradation is distinguished – external respiration, transport of gases by blood and gas exchange in tissues, or internal respiration. External breathing includes: ventilation of the lungs, diffusion of gases through the alveolar-capillary membrane and processes of blood perfusion in the pulmonary capillaries. Violation at one of the levels of this physiological process leads to changes in breathing and, as a result, the occurrence of respiratory failure. Therefore, the methods of researching the function of external breathing are of great diagnostic value.[1]

Spirometry is one of the most informative tests for analyzing and evaluating lung function. To date, when studying the function of external breathing, the volume of various phases of the respiratory cycle and the flow rate during inhalation and exhalation are measured.[2]

AIM

To investigate the data of the morphofunctional study of the respiratory tract in children with recurrent respiratory diseases in dynamics, depending on the method of therapy.

MATERIALS AND METHODS

Our study examined a group of children with acute respiratory diseases: J01 – acute sinusitis; J02, J02.9 – acute pharyngitis, unspecified; J03, J03.9 – acute tonsillitis, unspecified; J06.9 – acute upper respiratory tract infection, unspecified; J20, J20.9 – acute bronchitis, unspecified; H66.9 – otitis media, unspecified, identified more than 6 times a year. Two study groups were created, depending on the treatment method: group 1 – 62 patients (optimized therapy), group 2 – 56 patients (basic therapy). The control group consisted

of 26 healthy examined children, identical in age, sex, and anthropometric parameters, without clinical and laboratory presentations of acute respiratory syndrome. A group of children (1) received therapy with the addition of vitamin-mineral complex drugs to the standard treatment regimen for 1 month in therapeutic doses. The developed treatment scheme was aimed at promoting rapid recovery and preventing further episodes of acute respiratory diseases.

RESULTS

Many indicators are studied, in particular, the volume of air exhaled for certain periods of time during full exhalation, which is preceded by a maximum inhalation, is determined. Variables that include total expiratory volume (forced vital capacity (FVC), the volume exhaled in the first second, known as forced expiratory volume in the first second (FEV1), and their ratio (FEV1/FVC) are also determined. The results of the study, both volumes and combinations of these volumes, are called capacities, and are used as a diagnostic test to monitor patients with respiratory diseases.[3]

Let's consider the main parameters of the spirogram in the studied children when using different methods of therapy (Table 1).

A step-by-step approach to spirometry ensures ease and reliability of interpretation. Airway obstruction is suspected when there is a decrease in forced expiratory volume in first second/forced vital capacity (FEV1/FVC), but there is no conclusive evidence to clearly define what constitutes a significant decrease in this ratio. Low FVC is defined as less than 80 % of predicted in children and adolescents aged 5 to 18 years. The FEV1/FVC ratio and FVC are used together to identify obstructive defects and restrictive or mixed patterns. FEV1 is used to determine the severity of obstructive and restrictive disease, although the values were determined arbitrarily and were not based on patient outcome data.[4]

According to Table 1, positive dynamics were observed after treatment on all indicators. Significant differences after treatment were observed in the following parameters: FVC (%) at $p_1<0,01$, $p_3=0,008$ in the OT group; FEV1 (%) at $p_1=0,001$, $p_3=0,01$ in the group with OT; PEF (%) at $p_1=0,02$ in the group of children of OT and $p_4=0,006$ in the group of children of BT; MEF 25 (%) with $p_1=0,03$, $p_3=0,05$ in the group of OT children and $p_4=0,01$ in the group of BT children; Tifno index (%) at $p_4=0,002$, $p_6=0,01$, $p_7=0,02$ with significant changes between the data on OT and BT groups, which characterizes the increase of clinically useful air flow with the predominance of OT data; MEF 50 (%) at $p_7=0,01$ with a significant prevalence of OT data (by 1,2 times) against BT data. The indicator indicates a decrease in the narrowing of the respiratory tract.[5–7]

Let's consider the changes in the main indicators during the study of children with OT (Table 2).

According to Table 2, positive dynamics of spirometry indicators were observed. The level of the FVC indicator (%) reached physiological values in 84,61 % of cases, compared to the starting values – 73,08 %. Moderate, significant and drastic changes were also not observed after treatment. IVC (%) increased after treatment by 1,2 times, no significant and drastic changes were identified. The value of FEV1 (%) increased (from 80,77 % to 92,31 %) and no easy, moderate, significant, or drastic changes were detected. The level of FEV 25-75 (%) reached 100 % in all OT children. The level of PEF (%) increased after treatment by 1,2 times, and moderate, significant, drastic changes in the indicator were not observed.

Taking into account the needs of clinical assessment of respiratory function, two types of ventilation insufficiency are distinguished: obstructive and restrictive, as well as mixed-type disorders. Obstructive type is characterized by impaired passage of air to the alveoli. For restrictive – a decrease in the respiratory surface or the ability of the lung tissue to stretch.[8]

Consider the presence of obstructive and restrictive disorders in children with OT:

BEFORE TREATMENT

1. Obstructive and restrictive violations were not detected – 75,01 %
2. Restrictive violations of a light degree of severity. Poor breathing mechanics – 1,92 %
3. Restrictive violations of a light degree of severity – 19,23 %
4. Obstructive disorders of a light degree of severity. Restrictive violations of medium severity – 1,92 %
5. Obstructive disorders of a light degree of severity – 1,92 %

DURING TREATMENT

1. Obstructive and restrictive violations were not detected – 78,84 %
2. Restrictive violations of a light degree of severity – 17,31 %
3. Restrictive violations of medium severity – 3,85 %

AFTER TREATMENT

1. Obstructive and restrictive violations were not detected – 88,46 %
2. Restrictive violations of a light degree of severity – 11,54 %.

Table 1. Data of spirograms in children with the use of various methods of therapy

Parameters of spirometry	The mean value of the norm with standard deviation for our sample	1st group - OT (n=62)			2nd group - BT (n=56)		
		Before treatment	During treatment	After treatment	Before treatment	During treatment	After treatment
FVC (L) forced vital capacity	1,89±0,38	1,68 ± 0,36	1,79 ± 0,37 (p_1=0,08)	1,79 ± 0,45 (p_2=0,92; p_3=0,14)	1,71 ± 0,26	1,86 ± 0,38 (p_4=0,02)	1,87 ± 0,30 (p_5=0,97; p_6=0,005; p_7=0,23)
FVC (%)		88,11 ± 12,44	97,37 ± 15,36 (p_1<0,01)	94,68 ± 14,63 (p_2=0,32; p_3=0,008)	92,20 ± 11,63	95,64 ± 10,82 (p_4=0,11)	94,66 ± 9,36 (p_5=0,61; p_6=0,22; p_7=0,99)
IVC (L) inspiratory vital capacity	1,89±0,38	1,63 ± 0,44	1,69 ± 0,39 (p_1=0,38)	1,71 ± 0,49 (p_2=0,83; p_3=0,32)	1,76 ± 0,34	1,77 ± 0,42 (p_4=0,95)	1,86 ± 0,38 (p_5=0,22; p_6=0,16; p_7=0,07)
IVC (%)		85,98 ± 16,00	91,43 ± 14,71 (p_1=0,05)	90,11 ± 15,82 (p_2=0,63; p_3=0,15)	95,87 ± 20,51	89,97 ± 14,58 (p_4=0,08)	93,32 ± 12,10 (p_5=0,19; p_6=0,42; p_7=0,22)
FEV1 (L) forced expiratory volume in 1st second	1,66±0,31	1,53 ± 0,35	1,63 ± 0,30 (p_1=0,09)	1,63 ± 0,35 (p_2=0,99; p_3=0,11)	1,55 ± 0,25	1,69 ± 0,30 (p_4=0,006)	1,70 ± 0,24 (p_5=0,86; p_6=0,001; p_7=0,19)
FEV1 (%) forced expiratory volume 1%	90,27±1,21	92,28 ± 14,18	100,00 ± 11,64 (p_1=0,001)	98,35 ± 11,84 (p_2=0,43; p_3=0,01)	95,89 ± 15,67	98,84 ± 9,18 (p_4=0,23)	98,29 ± 8,64 (p_5=0,75; p_6=0,32; p_7=0,98)
Tifno index (%) FEV1 / IVC	90,27±1,21	95,84 ± 12,95	98,27 ± 15,02 (p_1=0,34)	97,68 ± 10,28 (p_2=0,79; p_3=0,38)	88,94 ± 8,69	99,49 ± 22,66 (p_4=0,002)	93,33 ± 9,69 (p_5=0,06; p_6=0,01; p_7=0,02)
FEF 25-75 (L/s) forced expiratory flow	2,08±0,24	2,06 ± 0,53	2,12 ± 0,42 (p_1=0,47)	2,15 ± 0,44 (p_2=0,69; p_3=0,30)	2,09 ± 0,51	2,22 ± 0,34 (p_4=0,11)	2,12 ± 0,47 (p_5=0,20; p_6=0,72; p_7=0,75)
FEF 25-75 (%)		99,05 ± 22,13	103,25 ± 18,49 (p_1=0,25)	102,99 ± 17,33 (p_2=0,93; p_3=0,27)	103,50 ± 27,52	105,19 ± 13,89 (p_4=0,68)	98,84 ± 19,02 (p_5=0,05; p_6=0,30; p_7=0,22)
FEF 75-85 (L/s) forced expiratory flow		1,01 ± 0,32	1,05 ± 0,29 (p_1=0,38)	1,06 ± 0,23 (p_2=0,84; p_3=0,44)	1,12 ± 0,30	1,09 ± 0,28 (p_4=0,49)	1,05 ± 0,29 (p_5=0,56; p_6=0,23; p_7=0,87)
PEF (L/s) peak expiratory flow	3,62±0,61	3,41 ± 0,89	3,65 ± 0,70 (p_1=0,10)	3,72 ± 0,81 (p_2=0,60; p_3=0,04)	3,32 ± 0,51	3,89 ± 0,74 (p_4<0,01)	3,80 ± 0,50 (p_5=0,43; p_6<0,01; p_7=0,51)
PEF (%)		96,46 ± 24,09	105,59 ± 20,34 (p_1=0,02)	102,08 ± 19,59 (p_2=0,33; p_3=0,16)	95,85 ± 19,86	106,26 ± 19,29 (p_4=0,006)	100,89 ± 10,75 (p_5=0,07; p_6=0,09; p_7=0,69)

Table 1. Cont.

MEF 25 (L/s) maximum expiratory flow	3,42±0,55	2,93 ± 0,84	3,09 ± 0,60 (p_1=0,20)	3,23 ± 0,71 (p_2=0,28; p_3=0,03)	2,79 ± 0,61	3,31 ± 0,65 (p_4<0,01)	3,22 ± 0,46 (p_5=0,38; p_6<0,01; p_7=0,96)
MEF 25 (%)		86,28 ± 23,22	94,69 ± 17,81 (p_1=0,03)	93,81 ± 19,32 (p_2=0,79; p_3=0,05)	85,10 ± 21,55	94,82 ± 18,25 (p_4=0,01)	90,76 ± 13,05 (p_5=0,18; p_6=0,09; p_7=0,32)
MEF 50 (L/s) maximum expiratory flow	15,6±10,99	2,20 ± 0,57	2,27 ± 0,47 (p_1=0,43)	2,29 ± 0,48 (p_2=0,86; p_3=0,35)	2,22 ± 0,49	2,39 ± 0,36 (p_4=0,04)	2,26 ± 0,56 (p_5=0,15; p_6=0,70; p_7=0,75)
MEF 50 (%)		75,14 ± 37,73	86,68 ± 31,57 (p_1=0,07)	87,33 ± 26,75 (p_2=0,90; p_3=0,04)	74,58 ± 43,59	75,62 ± 38,27 (p_4=0,89)	71,57 ± 40,91 (p_5=0,59; p_6=0,71; p_7=0,01)
MEF 75 (L/s) maximum expiratory flow	1,24±0,11	1,30 ± 0,36	1,31 ± 0,31 (p_1=0,78)	1,28 ± 0,26 (p_2=0,58; p_3=0,84)	1,33 ± 0,34	1,34 ± 0,27 (p_4=0,87)	1,28 ± 0,32 (p_5=0,27; p_6=0,41; p_7=0,92)
MEF 75 (%)		104,13 ± 25,98	107,49 ± 23,39 (p_1=0,45)	102,62 ± 18,27 (p_2=0,20; p_3=0,71)	109,61 ± 30,81	105,62 ± 17,44 (p_4=0,40)	100,02 ± 23,28 (p_5=0,15; p_6=0,07; p_7=0,49)

Notes: p_1 – statistical significance of differences in 1st group between the values of indicators before and during treatment; p_2 – statistical significacnce of the differences in 1st group between the values of indicators during and after treatment; p_3 – statistical significance of the differences in 1st group between the values of indicators before and after treatment; p_4 – statistical significance of the differences in the 2nd group between the values of indicators before and during treatment; p_5 – statistical significance of the differences in the 2nd group between the values of indicators during and after treatment; p_6 – statistical significance of the differences in the 2nd group between the values of indicators before and after treatment; p_7 – statistical significance of the differences between the values of indicators of groups 1 and 2 after treatment.

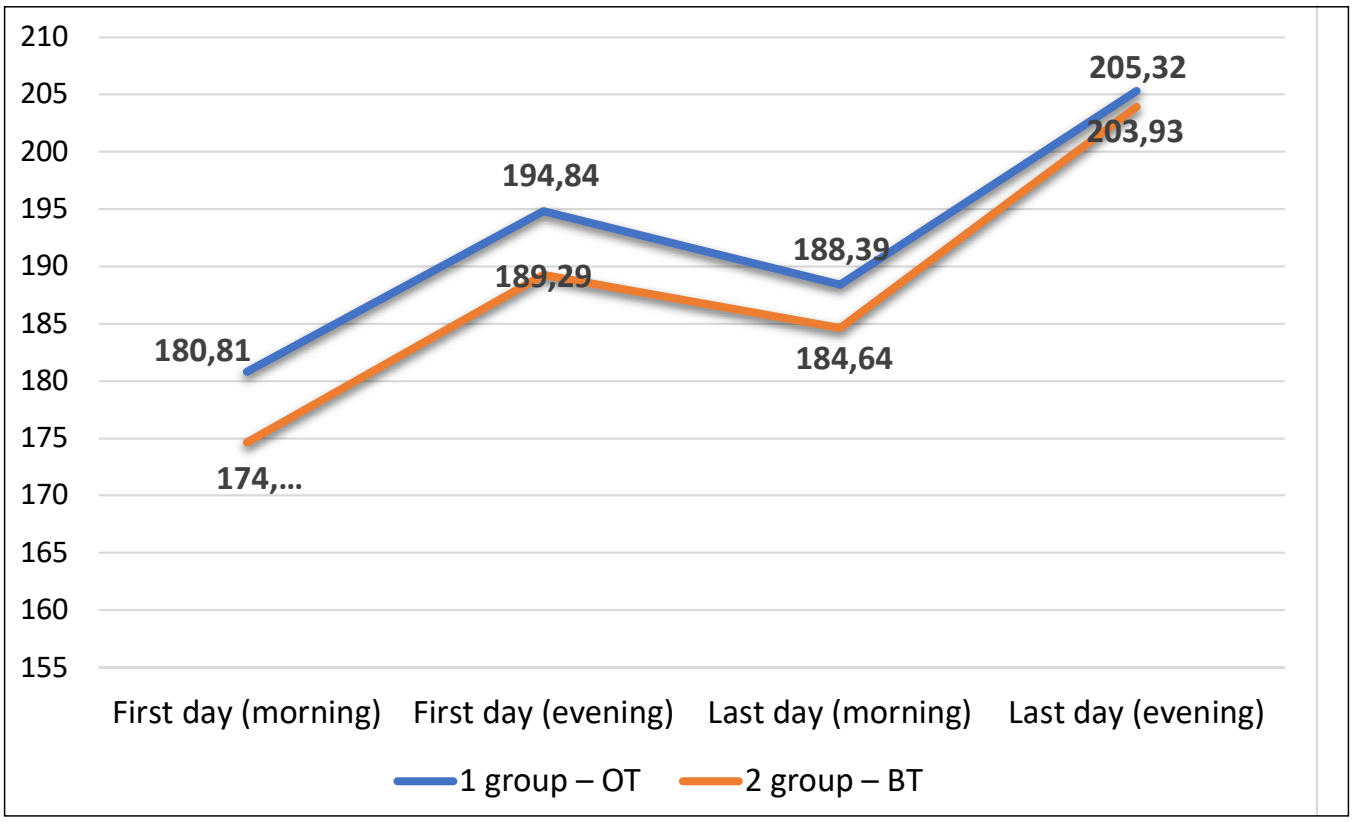

Fig. 1. Graphical representation of the results of the peak flowmetry study.

Table 2. Changes in spirometry indicators of the group of children with OT

Parameters of spirometry	1st group – OT (n=62)																	
	Before treatment						During treatment						After treatment					
	Norm	Conditional norm	Easy changes	Moderate changes	Significant changes	Drastic changes	Norm	Conditional norm	Easy changes	Moderate changes	Significant changes	Drastic changes	Norm	Conditional norm	Easy changes	Moderate changes	Significant changes	Drastic changes
FVC forced vital capacity (%)	73,08	11,54	11,54	0	1,92	1,92	78,84	11,54	9,62	0	0	0	84,61	9,62	5,77	0	0	0
IVC inspiratory vital capacity (%)	65,38 %	11,55	15,37 %	3,86	3,84	0	61,53 %	17,31 %	17,31 %	0	3,85	0	75,00 %	13,46 %	7,69	3,85	0	0
FEV1 forced expiratory volume in 1st second (%)	80,77	7,69	5,77	1,92	3,85	0	82,70	15,38	1,92	0	0	0	92,31	7,69	0	0	0	0
FEF 25-75 forced expiratory flow (%)	90,39	1,92	7,69	0	0	0	94,23	5,77	0	0	0	0	100	0	0	0	0	0
PEF peak expiratory flow (%)	76,92	11,54	7,69	0	3,85	0	88,50	9,62	1,92	0	0	0	94,30	3,85	1,92	0	0	0

There is also a positive trend in the level of violations. At the initial stage of the study, obstructive and restrictive disorders were not detected – in 75,01 % of cases, while after treatment – in 88,46 %.

Let's consider changes in spirometry indicators in children with BT (Table 3).

According to the table, positive dynamics were observed in spirometry values, but it was not possible to reach 100 % of the physiological norm. There was an increase in the final indicators of FVC (%), IVC (%), FEV1 (%), FEF 25-75 (%) by 1,1 times, PEF (%) by 1,2 times. Moderate, significant, drastic changes were not observed after treatment in terms of FVC (%), FEV1 (%) (even easy changes were not noted), FEF 25-75 (%), PEF (%), according to the IVC indicator (%) there were additionally moderate changes.

According to the characteristics of the presence of obstructive and restrictive changes in children with BT, the following results were obtained:

BEFORE TREATMENT

1. Obstructive and restrictive violations were not detected – 74,88 %
2. Restrictive violations of a light degree of severity. Poor breathing mechanics – 2,01 %

Table 3. Changes in spirometry indicators of a group of children with BT

Parameters of spirometry	2nd group – BT (n=56)																	
	Before treatment						During treatment						After treatment					
	Norm	Conditional normv	Easy changes	Moderate changes	Significant changes	Drastic changes	Norm	Conditional norm	Easy changes	Moderate changes	Significant changes	Drastic changes	Norm	Conditional norm	Easy changes	Moderate changes	Significant changes	Drastic changes
FVC forced vital capacity (%)	73,08	10,67	12,02	0	1,92 %	2,02	7 5,91	10,51	10,58	3,00	0	0	82,16	9,58	8,26	0	0	0
IVC inspiratory vital capacity (%)	64,94	11,88	14,50	5,02	3,66	0	62,48	16,31	17,29	0	3,92	0	73,46	14,21	9,37	2,96	0	0
FEV1 forced expiratory volume in 1st second (%)	80,73	7,22	6,12	1,96	3,97	0	84,17	14,01	1,82	0	0	0	90,26	9,74	0	0	0	0
FEF 25-75 forced expiratory flow (%)	89,47	3,85	6,78	0	0	0	95,18	3,27	1,55	0	0	0	97,56	1,26	1,18	0	0	0
PEF peak expiratory flow (%)	76,12	11,106	8,92	0	3,86	0	85,31	12,38	2,31	0	0	0	91,18	6,81	2,01	0	0	0

3. Restrictive violations of a light degree of severity – 18,79 %
4. Obstructive disorders of a light degree of severity. Restrictive violations of medium severity – 1,80 %
5. Obstructive disorders of a light degree of severity – 2,52 %

DURING TREATMENT

1. Obstructive and restrictive violations were not detected – 76,98 %
2. Restrictive violations of a light degree of severity – 15,26 %
3. Restrictive violations of medium severity – 8,76 %

AFTER TREATMENT

1. Obstructive and restrictive violations were not detected – 81,52 %
2. Restrictive violations of a light degree of severity – 18,48 %.

The level of obstructive and restrictive disorders decreased by 1,1 times, and breathing mechanics disorders were eliminated. Restrictive violations of a light degree of severity were registered in 18,48 % of cases.

In the studied children, the Stange's test with breath hold on inhalation and the Hench's test – breath hold on exhalation were performed before and after treatment (Table 4).

Table 4. The results of the Stange's and Hench's tests in children with the use of various methods of therapy

Parameters	1st group – OT (n=62)		2nd group – BT (n=56)	
	Before treatment	After treatment	Before treatment	After treatment
Stange's test (min 21s)	20,59 ± 5,19	21,95 ± 4,89 (p_1=0,14)	20,29 ± 4,65	21,80 ± 4,17 (p_2=0,07; p_3=0,86)
Hench's test (min 12-13 s)	13,34 ± 3,89	15,58 ± 3,77 (p_1=0,001)	12,86 ± 3,64	15,16 ± 3,47 (p_2=0,53 p_3=0,001)

Notes: p_1 – statistical significance of the differences in the 1st group between the values of indicators before and after treatment; p_2 – statistical significance of the differences in the 2nd group between the values of indicators before and after treatment; p_3 – statistical significance of the differences between the values of indicators after treatment of groups 1 and 2.

Table 5. The results of peak flowmetry in children depending on the method of treatment

Peak flowometry	1st group – OT (n=62)	2nd group – BT (n=56)	Statistical significance of differences
1st day (morning)	180,81 ± 41,82	174,64 ± 29,35	0,36
1st day (evening)	194,84 ± 40,32	189,29 ± 28,28	0,39
Last day (morning)	188,39 ± 39,84 (p_1=0,30)	184,64 ± 27,30 (p_2=0,07)	0,56
Last day (evening)	205,32 ± 40,48 (p_1=0,05)	203,93 ± 28,52 (p_2=0,01)	0,83

Notes: p_1 – statistical significance of differences in 1st group between the values of indicators before and after treatment; p_2 – statistical significance of the differences in the 2nd group between the values of indicators before and after treatment.

According to the data, there were significant differences in the Hench's test indicator, both in the OT group (p_1=0,001) and between groups (p_3=0,001), with a predominance in the OT group (15,58 ± 3,77 vs. 15,16 ± 3,47 s). The obtained data on the study of the Stange's test increased to the physiological norm, but without reliable values [9–11].

Peak flowmetry is a method of functional diagnostics to determine the peak volume velocity of exhalation. This method makes it possible to assess the speed with which a person exhales air, and thus determine the degree of obstruction (narrowing) of the respiratory tract [11].

Consider the obtained peak flowmetry data (Table 5, Fig. 1).

After the peak flowmetry study, a significant positive dynamics of the values (p_1=0,05, p_2=0,01) was observed in both groups with an absolute difference between the groups and with a predominance of values in the OT group.

DISCUSSION

Spirometry is most often performed to study and evaluate lung function. This method provides clinically useful information for making decisions about the treatment of a wide range of diseases and disorders of the respiratory tract. Being a non-invasive and accessible method, which is very important in childhood, and almost completely without any adverse consequences, it can be repeated as often as there is a need for it.[12]

Interpretation of spirometry data requires knowledge of the pathophysiology of lung diseases, it also requires a rational familiarity with statistics [13].

Thus, each person will have different «normal» or expected values, which are also not fixed or constant, and are constantly changing with growth and aging. For each lung function parameter the expected value, the normal value is calculated using «prediction» or «regression» or «reference» equations that take into account known and unknown predictors or determinants of the parameter of interest. These equations are developed by studying lung function, and a large sample of carefully selected and well-defined «normal» subjects. The criteria for normality are strict, excluding sick people. In studies such as the National Health and Nutrition Examination Survey of the United States III (NHANES III), a sample of normal subjects was selected from the entire population [14–17].

Spirometry is a valuable and informative method of research and monitoring in the process of treatment of various diseases of the respiratory tract, but it has limitations in detecting early disease and in patients with borderline disorders, and therefore provides

information only about the mechanical properties of the respiratory tract, lungs and chest wall, and gives enough information to determine a preliminary diagnosis [18,19].

CONCLUSIONS

1. Significant differences after treatment were observed in the following parameters: FVC (%) at $p_1<0,01$, $p_3=0,008$ in the OT group; FEV1 (%) at $p_1=0,001$, $p_3=0,01$ in the group with OT; PEF (%) at $p_1=0,02$ in the group of children of OT and $p_4=0,006$ in the group of children of BT; MEF 25 (%) with $p_1=0,03$, $p_3=0,05$ in the group of OT children and $p_4=0,01$ in the group of BT children; Tifno index (%) at $p_4=0,002$, $p_6=0,01$, $p_7=0,02$ with significant changes between the data on OT and BT groups, which characterizes the increase of clinically useful air flow with the predominance of OT data; MEF 50 (%) at $p_7=0,01$ with a significant prevalence of OT data (by 1,2 times) against BT data.
2. Positive dynamics of spirometry indicators were observed in children with OT. The level of the FVC indicator (%) reached physiological values in 84,61 % of cases, compared to the starting values – 73,08 %. Moderate, significant and drastic changes were also not observed after treatment. IVC (%) increased after treatment by 1,2 times, no significant and drastic changes were identified. The value of FEV1 (%) increased (from 80,77 % to 92,31 %) and no easy, moderate, significant, or drastic changes were detected. The level of FEV 25-75 (%) reached 100 % in all OT children. The level of PEF (%) increased after treatment by 1,2 times, and moderate, significant, drastic changes in the indicator were not observed.
3. At the initial stage of the study of children from the OT group, obstructive and restrictive disorders were not detected in 75,01 % of cases, while after treatment – in 88,46 %. Restrictive violations of a mild degree of severity after treatment were found in 11,54 % of cases.
4. A positive trend in spirometry values was determined in children with BT, but it was not possible to reach 100 % of the physiological norm. There was an increase in the final indicators of FVC (%), IVC (%), FEV1 (%), FEF 25-75 (%) by 1,1 times, PEF (%) by 1,2 times. According to spirometry data, no moderate, significant, drastic changes in violations were detected after treatment in terms of FVC (%), FEF 25-75 (%), PEF (%), with the exception of FEV1 (%) (when even easy changes were not noted) and according to the data of the IVC indicator (%) no significant and drastic changes were observed.
5. The level of obstructive and restrictive disorders decreased by 1,1 times in children with BT, and breathing mechanics disorders were eliminated. Restrictive violations of a light degree of severity were registered in 18,48 % of cases.
6. There were significant differences in the Hench's test indicator, both in the OT group ($p_1=0,001$) and between groups ($p_3=0,001$), with a predominance in the OT group ($15,58 \pm 3,77$ vs. $15,16 \pm 3,47$ s). The obtained data from the Stange's test indicated an increase in the level to the physiological norm, but without reliable values.
7. After the peak flowmetry study, a significant positive dynamics of the values ($p_1=0,05$, $p_2=0,01$) was observed in both groups with an absolute difference between the groups and with a predominance of values in the OT group.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Casein phosphopeptide-amorphous- calcium phosphate's effect on enamel microhardness of teeth treated with nano silver in sodium fluoride solution

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ABSTRACT

Aim: To detect the effect of an experimental 0.7% nanosilver in sodium fluoride (NSSF) and compare it to that of 5% sodium fluoride (NaF) on demineralized teeth, also observes the impact of applying 10% Casein phosphopeptide-amorphous-calcium phosphate (CPP-ACP) on those groups and compares that to CPP-ACP's lone impact on demineralized teeth.

Materials and Methods: The sample consisted of 60 sound, premolar teeth without hypo-mineralization or cracks. They were divided into three groups following the formation of caries-like lesions using a Feather Stone pH cycle. Group No.1 was treated with NSSF, group No.2 was treated with NaF, and deionized water was used for Group No.3. All groups had two minutes of application time and were then stored for 24 hours in artificial saliva. After that, they were treated with CPP-ACP, for seven days twice a day.

Microhardness measurements were carried out four times: before any intervention, after the pH cycle, after 24 hours from the application of NSSF and NaF, and a final one after seven days from the application of CPP-ACP on the previous groups.

An XRF, XRD analysis, and particle size analyzer were used to confirm the nanosilver properties of the powder.

Results: There was a statistically significant increase in microhardness values ($P<0.05$) following CPP-ACP's application on teeth previously subjected to NSSF and NaF. The group that was subjected to deionized water before CPP-ACP's application revealed a statistically significant value ($P<0.001$).

Conclusions: CPP-ACP enhances the microhardness and, hence, the mineralization of teeth previously treated with NSSF and NaF solutions.

KEY WORDS: CPP-ACP, NSSF, 5% NaF, Microhardness

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INTRODUCTION

Dental caries is a widespread disease, still considered a burden to humans affecting their everyday lives. Its prevalence can be attributed to lifestyle or socioeconomic level, which is higher for permanent teeth in high socioeconomic areas and vice versa. However, caries level is noticeably lower among educated individuals [1]. Bacterial acids lead to degrading teeth's hard tissues, making it urgent to take early preventive measures to preserve the remaining tissues [2].

Dental fluoride has been used for decades to positively alter the constituents of dental plaque and saliva, increasing the minerals within them and consequently increasing the minerals diffusing to teeth [3].

Nanoparticles have been intensively investigated because of their unique characteristics. they have improved the field of preventive dentistry in tremendous ways by acting as drug delivery systems for treating and preventing dental diseases [4], and microbial biofilms prevention [5]. As nanomaterials, silver nanoparticles have a large surface area, which helps them adhere to the bacterial outer cell membrane and change the permeability and structure of the cells. Thus, at low concentrations, silver nanoparticles efficiently destroy bacterial cells [6].

Furthermore, the remineralizing effect of nanosilver, especially when it is combined with dental fluoride, has been identified by many studies.[7-8] Primary teeth with initial caries treated with fluoride varnish containing silver nanoparticles exhibited superior hardness compared to those treated with traditional NaF varnish when examined by diagnodent (detection of caries by laser fluorescence).[8] Also, nanosilver in sodium fluoride (NSSF) mouthwashes were found to be more effective than sodium fluoride (NaF)mouthwashes [9]. The exact mechanism is not clear, it is more likely to

be due to the fluoride component of NSSF helping to promote enamel remineralization and inhibiting the breakdown of enamel minerals in addition to the ability of nanosilver particles to penetrate deep in demineralized enamel due to their size and structure, which can consequently strengthen its hardness [7].

Previous animal studies have investigated milk and cheese for their anti-cariogenic effect, and have indicated that the actions of phosphoprotein casein and calcium phosphate components in cheese have been linked to this effect [10-11]. Recently, many in vitro and in vivo studies have shown that casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) has a remineralizing effect [12-13].

Due to their tiny size and naturality ionic, CPP-ACP complexes can penetrate subsurface enamel defects. Reports stated that in early carious lesions, the hydroxyapatite is surrounded by a crystalline cavity where the nano-complexes settle after diffusing through the holes into the lesion and releasing loosely bound calcium and phosphate ions [14].

Microhardness tests are frequently used to assess the hardness of teeth as well as to investigate changes in the physical characteristics of materials, such as density and hardness, or to determine their chemical composition. This study was assessed using the Vickers microhardness test which has been used in many previous studies.

AIM

To detect the effect of adding 10% CPP-ACP to teeth previously treated with either experimental NSSF or NaF solutions. And compare those to CPP-ACP's lone effect on demineralized teeth.

MATERIALS AND METHODS

SAMPLE SELECTION

The inclusion criterion was extracted permanent first premolar teeth, caries-free, without hypoplasia or fluorosis.

SAMPLE PREPARATION

The sample size was calculated to be 30 as determined by G power 3.1.9.7 (program writing by Franz-Faul, University of Kiel, Germany), so we doubled the sample size to increase the power of the study. A sample of 83 teeth was examined using x 6 dental loops (Univit, Italy); 60 of these satisfied the inclusion criterion. 10% thymol was used to store the teeth until used, to protect them from dehydration and any microbial growth. The sample was first washed with deionized water, subsequently cleaned with an ultrasonic cleaner (in an ultrasonic bath) and finally polished with non-fluoridated pumice powder. after that sof-lex discs (3M, St Paul, Minnesota, USA) were used to eliminate the fluoride-rich layer. The teeth were then coated with modeling wax (Schuler-Dental GmbH und Co. KG, Ulm, Germany) except for a (3X3mm) window at the center of the buccal surface [15].

CARIES LIKE LESION FORMATION

The 10-day Featherstone 1986 pH cycle was used to induce white spot lesions on our study sample,[16] which included applying the samples for six hours in a demineralization solution consisting of calcium chloride, acetic acid, and phosphate chloride, of about 1.0 mM/L, 0.075 M/L, and 2.0 mM/L respectively. The pH was adjusted to 4.3 at 37 °C.

This was followed by cleaning with deionized water for 60 sec and the application of the sample for 17 h in a remineralization solution consisting of potassium phosphate, calcium nitrate, and potassium chloride of about 0.9mM/L, 105 mM/L and 150 mM/L, respectively. The pH was adjusted to 7 at 37 °C. The pH was checked every day for solutions with the use of a pH meter [17].

NANOSILVER IN SODIUM FLUORIDE SOLUTION PREPARATION

The concentration of the nanosilver to be used was determined by previously made pilot study containing varying concentrations of NSSF.

A wight dilution percent was used to prepare 0.7 % nanosilver powder (supplied by Hongu International Group. Ltd, China) in 5% sodium fluoride solution by applying 0.7 gm of nanosilver powder to a volumetric flask, then adding 5 gm of sodium fluoride powder (supplied by Thomas Baker (Chemicals) Pvt. Ltd. India) and dissolve it in deionized water of 100 ml. An ultrasonic homogenizer (model UP200Ht, Hielscher, Germany) was then used and stirred until a colloidal suspension was formed [18].

APPLICATION OF REMINERALIZATION AGENTS

60 upper premolars were used in this study as shown in fig.1. They were subjected to the pH cycle, and then, to the application of remineralizing agents which occurred in two steps as follows:

1st step: The sample was divided into three groups, 20 teeth each:

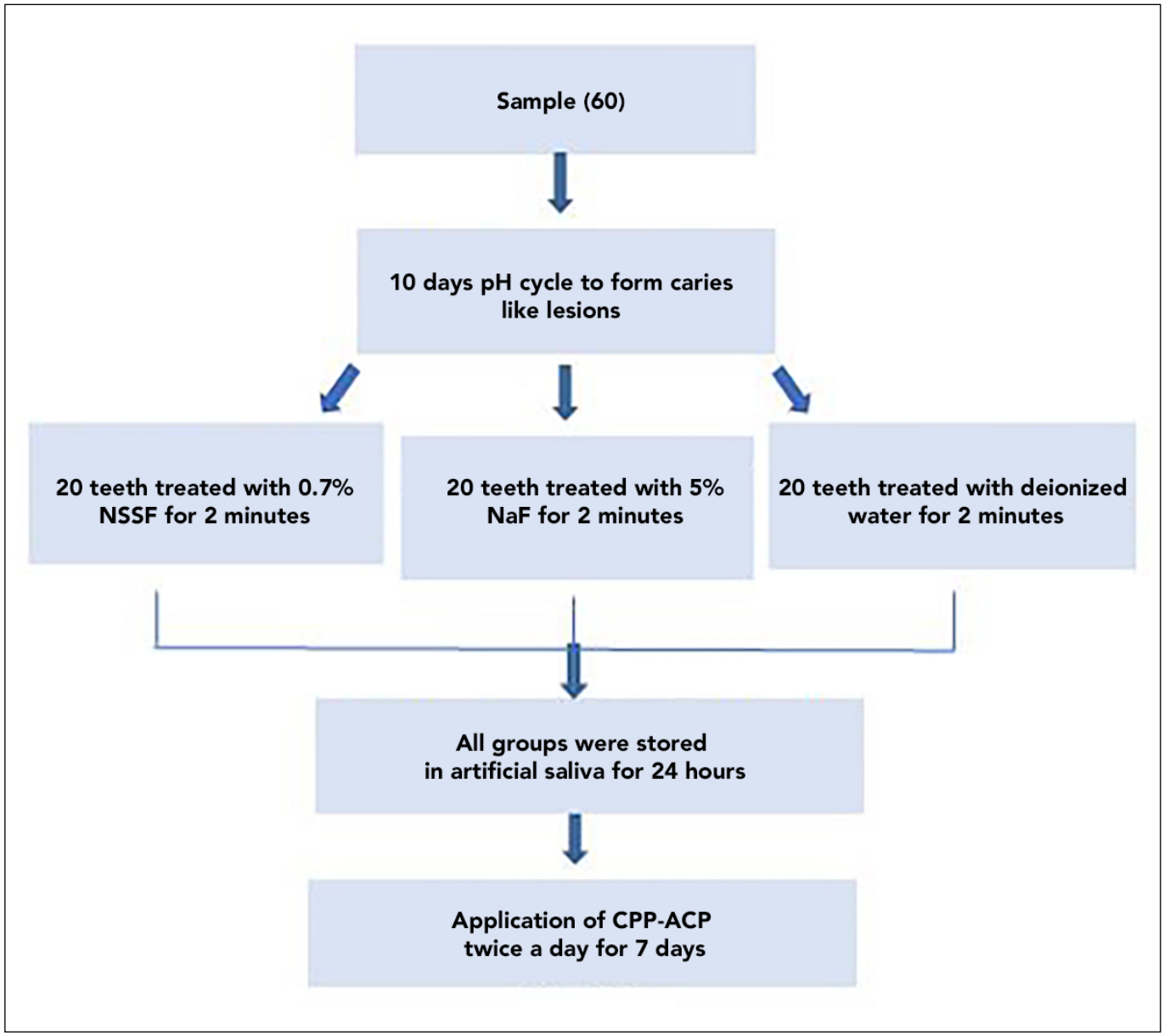

Fig. 1. Study design.

Group No.1: The NSSF group was treated with 0.7 % nanosilver in a 5% sodium fluoride solution for 2 minutes using a micro brush.

Group No.2: The NaF group was treated with 5% sodium fluoride solution for 2 minutes using a micro brush.

Group No.3: The control group was treated with deionized water for 2 minutes using a micro brush.

The teeth of all groups were washed with deionized water for 60 seconds and stored in artificial saliva for 24 hours.

2nd step: all teeth were subjected to 7 days application of 10 % CPP-ACP(GC mousse, Tokyo), twice a day with the use of an insulin syringe, by applying the paste in the syringe and disposing 0.2 ml of the paste for each tooth. The teeth were then rubbed with a micro brush, the past was left on the specimens for 3 minutes then wiped with gauze, washed with deionized water for 60 seconds, and stored in artificial saliva separately in an attempt to mimic the oral environment.[19]

ASSESSMENT OF SURFACE MICROHARDNESS (SMH):

Four microhardness readings were achieved with the use of the Vickers microhardness test by applying a load of 300 grams for 15 seconds (machine instruction); three indentations at the center of the enamel surface were made.

The measurements were as follows: one at baseline before any intervention, the second after subjecting the teeth to the pH cycle, the third after applying nanosilver in sodium fluoride and sodium fluoride and a final measurement after applying CPP-ACP, as shown in fig.2. The microhardness measurement was immediately taken at the end of each stage.

To confirm the nanosilver properties of the powder, X-ray fluorescence (XRF) and X-ray diffractometer (XRD) and particle size analyzer have been performed.

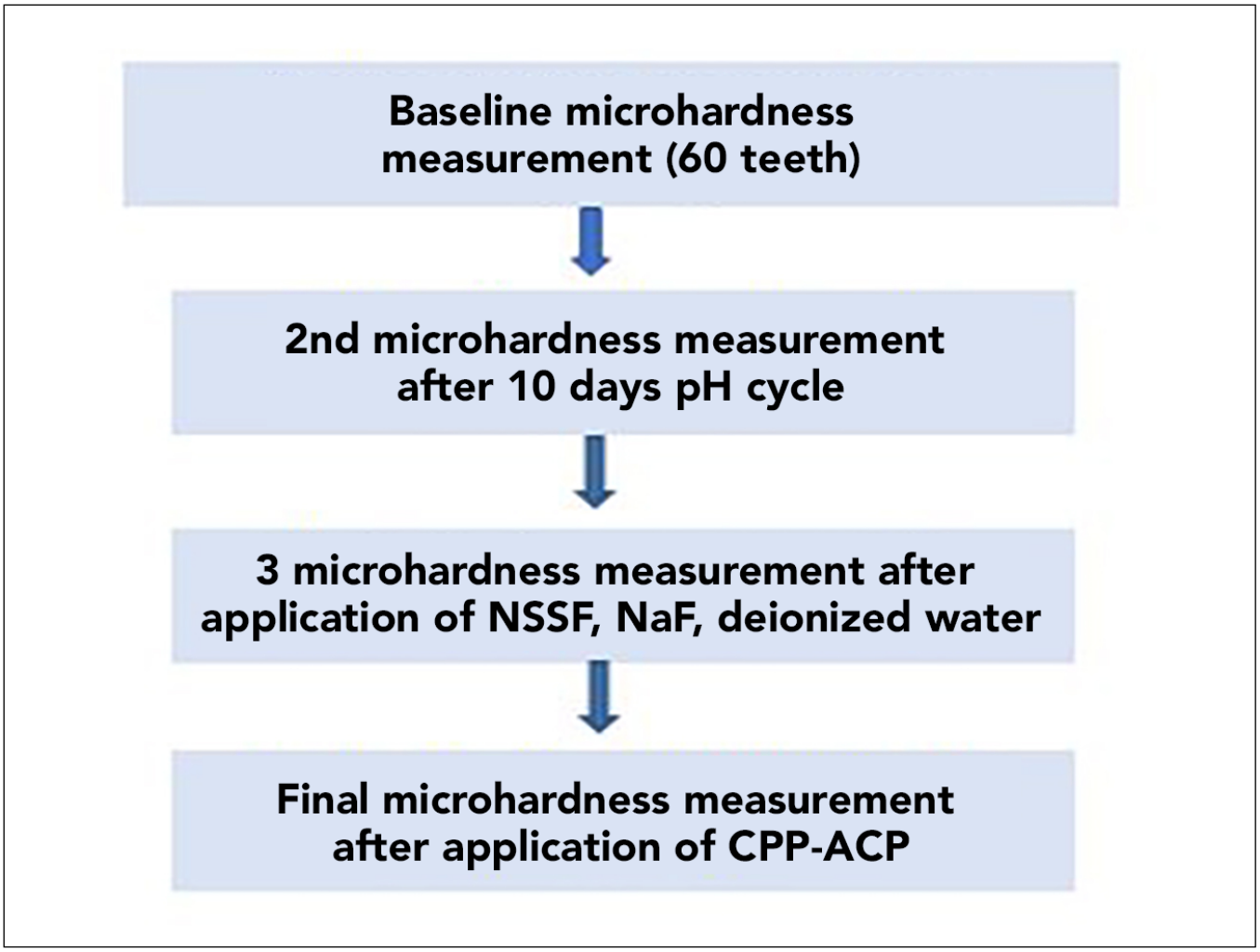

Fig. 2. Microhardness measurements sequence.

STATISTICS

The analysis of the data was performed with the use of a statistical package for the social sciences software (SPSS) the data were analyzed using;

1. Shapiro–Wilk analysis (to check the normality of the data)
2. Descriptive analysis
3. Onaway (ANOVA)
4. LSD.

ETHICS APPROVAL

Ethical approval was taken from The MUCOD Human Research Ethics Committee, study number (MUPRV005).

RESULTS

WRF AND XRD ANALYSES

A. XRF analysis showed high-purity powder containing 98.4% silver.

B. XRD analysis was used to examine composition, structure of crystals, and the orientation of the powder. The result showed that the powder had distinct diffraction peaks which conforms to the conventional power diffraction pattern established by the Joint Committee on Powder Diffraction Standards (JCPDS).

A particle size analyzer was used to confirm the nano size of the nanosilver solution showed that the effective size of the particles was (39.2 nm), polydispersity was (0.272).

ENAMEL MICROHARDNESS RESULTS

The descriptive analysis of teeth in the NSSF group (group No.1) before and after CPP-ACP application, is shown in Table 1. While that of the NaF group (group No.2) is shown in Table 2. Meanwhile, Table 3 represents the descriptive analysis of the control group (group No.3) before and after CPP-ACP application.

ANOVA test that shows the degree of freedom and significance. The LSD test that shows the mean defer-

Table 1. Descriptive analysis of the NSSF group (group No.1) before and after the application of CPP-ACP

Stage	Min	Max	Mean	Std. deviation
Baseline	282.3	383.3	326.030	28.1003
Demineralization	48.3	168.0	113.940	33.9648
NSSF	100.3	258.6	199.255	47.4818
CPP-ACP	118.0	302.6	235.210	52.1675

Table 2. Descriptive analysis of the NaF group (group No.2) before and after the application of CPP-ACP

Stage	Min	Max	Mean	Std. deviation
Baseline	295.0	385.0	327.795	22.9774
Demineralization	54.6	193.6	119.115	36.9409
NaF	74.0	302.0	195.910	51.8414
CPP-ACP	115.3	297.0	230.41	46.9890

Table 3. Descriptive analysis of the deionized water group (group No.3) before and after the application of CPP-ACP

Stage	Min	Max	Mean	Std. deviation
Baseline	281.6	378.6	327.96	27.203
Demineralization	55	190.1	113.19	36.454
Deionized	58	194	121.15	36.352
CPP-ACP	98	232	169.92	40.294

Table 4. ANOVA and LSD analysis of the NSSF group (group No.1) before and after the application of CPP-AC

Type Stage	ANOVA			LSD		
	F -value	P -value	Degree of freedom	Pairwise comparisons	Mean difference	P -value
Baseline (1)	89.864	<.001	3	1vs 2	212.090	<.001
Demineralization (2)				1vs 3	126.775	<.001
NSSF (3)				1vs 4	90.825	<.001
CPP-ACP (4)				2vs 3	85.315	<.001
				2vs4	121.256	<.001
				3 vs 4	35.950	<.05
Statistical significant (p<0.05)						

ence and significance of each step of each group are shown in Tables (4-7).

The result revealed a statistical significance increase (P<.05) in microhardness values of samples treated in the CPP-ACP step compared to that of the first remineralizing step for NSSF and NaF groups, as shown in Tables 4 and 5. Moreover, a statistical significance increase (P<0.001) in microhardness values of samples treated in the CPP-ACP step compared to that of the first remineralizing step for the control group, as shown in Table 6.

LSD analysis showed a non-significant increase (P>.05) between the CPP-ACP stage in the NSSF group and its effect in the NaF group, as shown in Table 7.

DISCUSSION

Recently, CPP-ACP has shown to be a successful non-invasive method for caries lesion prevention. Several investigations have demonstrated the effectiveness of CPP-ACP in remineralizing enamel lesions [20-21].

An inverse correlation exists between the saturation of phosphor and calcium ions and the onset of dental caries. Rajendran et al. suggested that CPP-ACPF and CPP-ACP and added fluoride varnish were more effective than CPP-ACP alone [22].

This investigation used CPP-ACP as a second step for mineralizing teeth previously treated with 0.7% NSSF or 5% NaF or deionized water to assess its efficacy on the enamel surface through the Vickers microhardness test.

Table 5. ANOVA and LSD analysis of the NaF group (group No.2) before and after the application of CPP-ACP

Type stage	ANOVA			LSD		
	F -value	P -value	Degree of freedom	Pairwise comparisons	Mean difference	P -value
Baseline (1)	88.291	<.001	3	1vs 2	208.680	<.001
Demineralization (2)				1vs 3	131.885	<.001
NaF (3)				1vs 4	97.385	<.001
CPP-ACP (4)				2vs 3	76.7950	<.001
				2vs4	111.295	<.001
				3 vs 4	34.500	<.05
Statistical significance (P< 0.05)						

Table 6. ANOVA and LSD analysis of the deionized water group (group No.3) before and after the application of CPP-ACP

Type Stage	ANOVA			LSD		
	F -value	P -value	Degree of freedom	Pairwise comparisons	Mean difference	P -value
Baseline (1)	58.921	<.001	3	1vs 2	214.760	<.001
Demineralization (2)				1vs 3	206.805	<.001
Control (3)				1vs 4	158.040	<.001
CPP-ACP (4)				2vs 3	7.955	>0.5
				2vs4	56.720	<.001
				3 vs 4	48.765	<.001
Statistical significance (P<0.05)						

The results revealed that the mean microhardness values at the baseline stage of all groups were similar, which indicates that the teeth were sound and fit for the study. The mean microhardness values of the demineralization stage of all groups were also similar, with no significant difference between groups, ensuring that the pH cycle used in this study effectively created caries-like lesions, as has been shown by other studies that have used it before [17,23]. Following the application of the first remineralization agent on each group (NSSF for group No.1, NaF for group No.2, and deionized water for group No.3), the results showed a statistically significant increase between the demineralization stage for NSSF and NaF groups (P <0.001), and remineralization stage of those group, ensuring minerals transportation to the defective enamel.

The difference between the demineralization stage and the deionized stage in the control group was without significant increase (P>.05), ensuring the non-mineralizing capacity of deionized water.

The incorporation of nanosilver compound into fluoride has recently been studied as a treatment of early carious lesions. In a study conducted on preschool children, Quritum M et al. Found that NSSF exhibited superior efficacy in combating caries among preschool children [24].

Table 7. ANOVA test and LSD between groups after CPP-ACP application

Type Stage	ANOVA			LSD		
	F -value	P -value	Degree of freedom	Pairwise comparisons	Mean difference	P -value
CPP-ACP in the NSSF group (1)				1vs 2	4.795	>.05
CPP-ACP in NaF group (2)	12.220	<0.001	2	1vs 3	65.290	<.001
CPP-ACP in control group (3)				2vs 3	60.495	<.001
Statistical significance (P<0.05)						

Contrary to our study, Nozari et al. concluded in their study that the surface microhardness of enamel was considerably greater following nano-silver fluoride treatment compared to groups treated with fluoride varnish and nano-hydroxyapatite [25] this could be due to different preparation methods for NSSF and different types of teeth used since deciduous teeth were used in their study while permanent premolars were used in the current study.

Our results revealed that the mean microhardness of teeth treated with NSSF and NaF were (199.255 and 195.910) respectively, which reflects a close resemblance in their effect. An investigation conducted by Kooshki et al. using premolar teeth agreed with our results that there was no difference in enamel microhardness between groups treated with different concentrations of NSSF and NaF varnish [26].

Following the application of CPP-ACP on each group, the results showed a significant increase (P<0.05) between the CPP-ACP stage and NSSF and NaF stages, which ensures that the CPP-ACP has performed an additive remineralization effect that led to the increase in microhardness of the teeth in these two groups.

The results also showed a high significant increase (P<0.001) between the remineralization stage and the CPP-ACP stage of the deionized group, revealing the strong effect the CPP-ACP performed alone. These results align with data from earlier studies indicating that CPP-ACP possesses a preventive capability against dental caries and enhances the remineralizing action of NaF [27-28].

Adhering to the guidelines provided by the manufacturer, the duration of CPP-ACP application in this investigation was 3 minutes. Pulido et al. proposed that extended treatment durations could be necessary to identify and monitor changes in calcium and phosphorus deposits [29]. According to AL-Mullahi et al., it was found that increasing the application period of CPP-ACP to 30 minutes might enhance the effectiveness of enamel remineralization further. Due to its ability to attach to the bacterial biofilm on the tooth surface, CPP-ACP causes circumstances of supersaturation. However, the lack of biofilm in in-vitro investigations may hinder CPP-ACP from fully exerting its impact [30].

Based on the current study results, the hypothesis declaring that CPP-ACP's effect on NSSF-treated teeth has greater microhardness than that of only NSSF treated teeth was accepted, since there was a statistically significant increase (P<0.05) in microhardness values after the application of CPP-ACP on NSSF treated teeth.

The possible limitations of this study would be the short duration of CPP-ACP application, lack of dental biofilm, individual's dietary patterns, and oral cleaning routines, which may result in different results.

Our study revealed that the final microhardness values of the groups that were treated with NSSF and NaF before being treated with CPP-ACP were closest to the baseline microhardness values. The final microhardness values of these groups were so close to each other but much higher than that of CPP-ACP alone, reflecting the extra mineralization CPP-ACP supply when used after other remineralizing materials.

CONCLUSIONS

This study confirmed the successful remineralizing potentials of CPP-ACP when used following 0.7% nanosilver in sodium fluoride and 5% sodium fluoride solutions. CPP-ACP gives an extra mineralization effect even when used for a short duration. Within the limitation of the study, we recommend the daily use of CPP-ACP, whether alone or subsequent to other types of professional remineralization, since it is not costly, easily accessible, and has a remarkable remineralizing effect. Several investigations are required to detect CPP-ACP's impact on extended duration alone and subsequent to NSSF and NaF solutions.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Practical experience in the surgical treatment of newborns with isolated gastrointestinal perforations based on scientific evidence

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ABSTRACT

Aim: To share first-hand experience in the surgical treatment of newborns with isolated gastrointestinal perforations.

Materials and Methods: This study examines 71 newborns with perforated peritonitis: 53 (74.65%) had necrotizing enterocolitis, 14 (19.72%) had isolated gastrointestinal perforations, and 4 (5.63%) had other intestinal perforations. Diagnosis involved clinical, laboratory, radiographic, ultrasound, and histological examinations of surgical and autopsy samples.

Results: The most significant risk factors for isolated perforations of the gastrointestinal tract in newborns were acute birth asphyxia and pathology of the respiratory system, which required tracheal intubation in 100% of children. Isolated perforations were localized in the stomach (n=6), jejunum (n=3), ileum (n=2), duodenum (n=1), colon (n=2). Morphological features of isolated perforations are as follows: a rapid muscle layer wasting of the wall, sometimes with the absence of muscle fragments; vascular malformations in the submucosal layer of the wall; ulcerous defect without necrotic changes; absence of pneumatosis of the intestinal wall. In isolated perforations, the operation of choice was closure of perforation in 11 children. In 2 patients direct interintestinal anastomoses were performed. Case mortality rate was 21.43% (3 newborns died).

Conclusions: 1. Clinical and pathomorphological features of gastrointestinal perforations in newborns indicate that isolated perforations are a separate nosological entity. 2. Isolated perforations of the gastrointestinal tract in newborns are characterized by such clinical differences as distress syndrome, prematurity, early onset, local lesions of a hollow organ, moderate peritonitis, favorable course and prognosis. 3. Isolated perforations are secondary to fibromuscular dysplasia of the wall of a hollow organ, indicating congenital pathology. 4. The operation of choice for isolated perforations is the excision of the edges of the perforation and closure of the perforation. 5. The prognosis for the gastrointestinal isolated perforations is favorable. Mortality was 21,43%.

KEY WORDS: newborns, perforative peritonitis, surgical treatment

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INTRODUCTION

Cases of isolated intestinal perforations (IP) account for 1-2% in newborns with very low birth weight (less than 1500 g) and 5-8% in newborns with extremely low birth weight (less than 1000 g) [1-3]. IPs are more common in infant boys but can also occur in full-term newborns. The average age of perforation occurrence is 7 days (ranging from 0 to 15 days) [4-6].

The clinical and pathomorphological characteristics of gastrointestinal perforations in newborns indicate that IP is a distinct nosological entity. It is characterized by specific clinical features such as prematurity, early onset, localized damage to a hollow organ, moderate peritoneal inflammation, and distress syndrome. IPs arise against the background of muscular and vascular dysplasia of the hollow organ wall, supporting the hypothesis of congenital pathology [7-10]. According to the literature, factors contributing to the development of IPs include various antenatal and postnatal factors (fetal hypoxia, medication effects, chorioamnionitis, etc.) [11, 12].

There is currently no unified approach to the surgical treatment of isolated (also referred to as spontaneous or localized) perforations in newborns. The literature describes a wide range of interventions, from primary peritoneal drainage to suturing of the perforation site and resection procedures [13-15].

It is estimated that 7% of newborns in intensive care units have gastrointestinal perforations. Among them, 53% are perforations associated with NEC, while 27% are intestinal perforations [16-18].

However, the issue of IP in newborns remains controversial and insufficiently studied. The relevance of this problem is due to the increasing prevalence of the

Table 1. Clinical Differences in Gastrointestinal Perforations in Newborns

No.	Clinical Features	NEC (n=53;100%)	IP (n=14;100%)
1.	Age of the child at the time of perforation (days)	7.2 ±0.6	2.5±0.3
2.	Respiratory distress syndrome and other respiratory pathologies	3 (5.66%)	11 (78.57%)
3.	Endotracheal intubation	46 (86.79%)	14 (100%)
4.	Perinatal encephalopathy	53 (100%)	5 (35.71%)
5.	Congenital cardiopathy	19 (35.85%)	5 (35.71%)
6.	Location of perforations in the lower GI tract and multiple perforations	51 (96.23%)	4 (28.57%)
7.	Location of perforations in the upper GI tract	2 (3.77%)	10 (71.43%)

pathology [19, 20], the risk of severe pre- and postoperative complications [21, 22], high mortality rates [23-26], and the lack of effective treatment strategies.

AIM

to share first-hand experience in the surgical treatment of newborns with isolated gastrointestinal perforations.

MATERIALS AND METHODS

The study is based on the examination and treatment results of 71 newborns with perforative peritonitis. Among them, 53 (74.65%) patients had necrotizing enterocolitis (NEC), 14 (19.72%) had spontaneous gastrointestinal perforations, and 4 (5.63%) had other types of perforations. Male newborns were twice as prevalent as females. There were 14 (19.72%) full-term newborns and 57 (80.28%) preterm newborns. Among 14 newborns with IP, 2 were full-term, and 12 were preterm. The average gestational age was 30 weeks for NEC patients and 31 weeks for those with IP. The average birth weight of newborns with NEC was 1850 g, while for those with IP, it was 1710 g.

Comprehensive diagnostics of perforative peritonitis in newborns included clinical and laboratory examinations, instrumental studies (radiography, ultrasound), and histological examination of biopsy samples from surgical and autopsy materials. All patients underwent general blood and urine tests, biochemical blood analysis, bacteriological tests, and monitoring of lactate levels, C-reactive protein, and procalcitonin tests.

The somatic and obstetric status of the mothers of these newborns was also analyzed.

The study utilized widely accepted statistical methods for analyzing medical and biological research data. Nonparametric statistical methods were applied due to the small sample size and the predominance of qualitative rather than quantitative characteristics. Numerical data are presented in absolute values (n) and percentages (%).

This research was approved by the Ethics and Bioethics Committees of Shupyk National Healthcare University of Ukraine and Lesya Ukrainka Volyn National University.

RESULTS

The study showed that all 14 newborns (100%) with IPs presented with a high-risk perinatal history:

- 10 (71.43%) were born from complicated pregnancies
- 11 (78.57%) were preterm
- 4 (28.57%) had extremely low birth weight.

Significant risk factors for IP development were as follows:

- chronic fetoplacental insufficiency (n=10; 71.43%)
- intrauterine fetal hypoxia (n= 8; 57.14%)
- low gestational age (n= 11; 78.57%)
- severe respiratory distress syndrome requiring prolonged mechanical ventilation (n=11; 78.57%)
- congenital cardiopathy (n=5; 35.71%)
- inadequate early postnatal nutrition (12 newborns, 85.71%, were formula-fed with a higher osmolarity than breast milk).

All newborns with gastrointestinal IP developed severe or critical conditions in the first days of life (see Table 1).

The typical clinical presentation of perforative peritonitis did not pose diagnostic challenges. However, identifying the type of perforation responsible for peritonitis was crucial. Laboratory tests revealed the following:

- anemia (n=9; 64.29%)
- thrombocytopenia (n=11; 78.57%)
- metabolic acidosis and electrolyte imbalances (n=12; 85.71%)
- elevated lactate and C-reactive protein levels (n=12; 85.71%).

Based on the clinical examination of patients, key differences between gastrointestinal IP and perforations associated with NEC were identified (Table 1).

The most reliable risk factors for IP of the gastrointestinal tract in newborns were acute asphyxia during

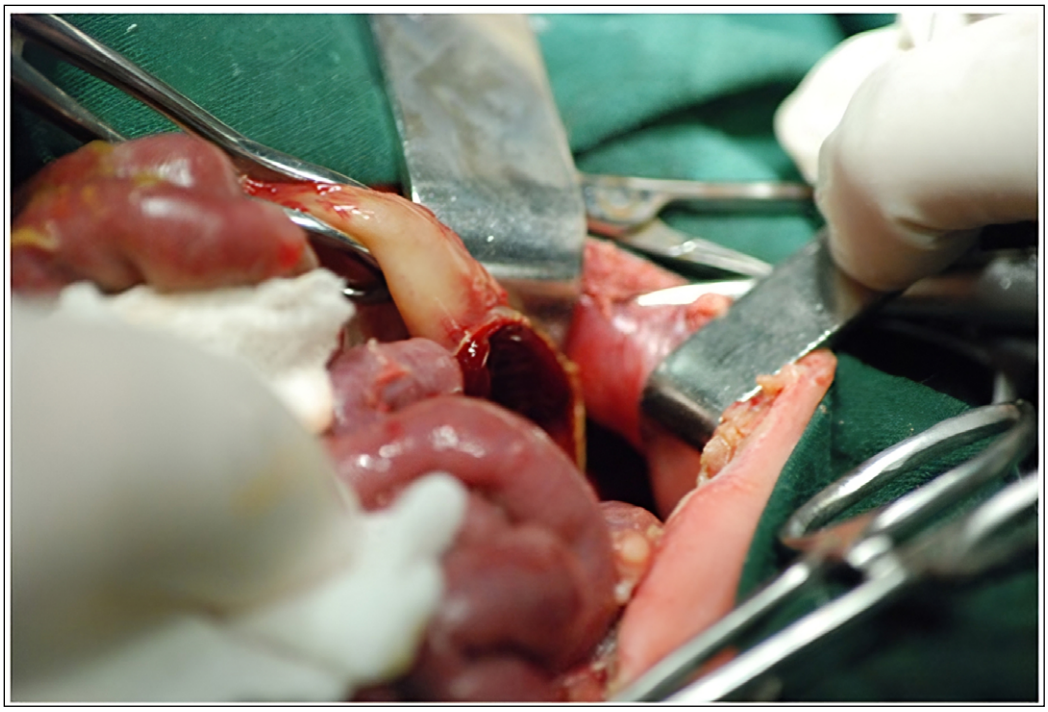

Fig. 1. Intraoperative Appearance of Isolated Gastrointestinal Perforation.

childbirth and respiratory pathology requiring endotracheal intubation in 100% of cases. In IP, respiratory distress syndrome served as the leading risk factor.

Intestinal perforations in NEC were predominantly located in the ileum (n=16), the large intestine (n=18), and only in 2 patients in the jejunum. Multiple perforative lesions of the gastrointestinal tract in NEC were observed in 17 newborns, accounting for 32.07%.

IPs, in most clinical cases, were localized in the jejunum (n=3), stomach (n=6), duodenum (n=1), and ileum (n=2). In the large intestine, 2 cases of IP were detected. Extensive damage to the gastrointestinal tract was not observed in newborns with IPs.

For diagnostic purposes, newborns with perforative peritonitis underwent radiological examinations, including plain radiography and contrast radiography when indicated, as well as abdominal ultrasound. To confirm the diagnosis, a morphological examination of biopsy material (both surgical and autopsy specimens) was performed.

The effectiveness of radiological methods in our observations was high, with false results obtained in only 3 cases. These occurred when the perforation sites were covered by an adjacent intestinal wall loop or a band of the greater omentum. A large amount of free gas in the abdominal cavity (pneumoperitoneum) led to severe respiratory and cardiac disturbances.

Abdominal ultrasound revealed free fluid between intestinal loops, decreased pneumatosis intestinalis, and static bowel loops with pendulum-like movement of the contents.

The morphological characteristics of IPs in newborns included: severe thinning of the muscular layer of the hollow organ wall, sometimes with the absence of individual muscle fragments; vascular aneurysms or vascular malformations in the submucosal layer of the hollow organ wall; an ulcerative defect with extensive hemorrhages in the perifocal tissues without necrotic changes; and the absence of intestinal wall pneumatosis.

All newborns with IP of the gastrointestinal tract underwent surgery. In five infants with extremely low birth weight (950–973 g), primary peritoneal drainage was used as preoperative preparation. Two infants with extremely low birth weight and concomitant congenital cardiopathies died. The other nine infants with IP underwent surgery. The surgical approach was determined based on the location of the perforation site, the extent of the pathological process in the wall of the hollow organ, and the overall condition of the infant. Macroscopically, the intestines and stomach appeared normal, with moderate peritoneal inflammation. Intraoperatively, IP presented as a localized perforation in the hollow organ wall with a localized pathological process, without extensive spread (Fig. 1).

All infants required preoperative preparation aimed at stabilizing hemodynamics, infusion and antibacterial therapy, correction of electrolyte imbalances and acidosis, and maintaining normal body temperature. Upon admission to the intensive care unit with a diagnosis of perforative peritonitis, the newborn was placed on enteral rest, a nasogastric tube was inserted for continuous aspiration of gastric contents, and intestinal

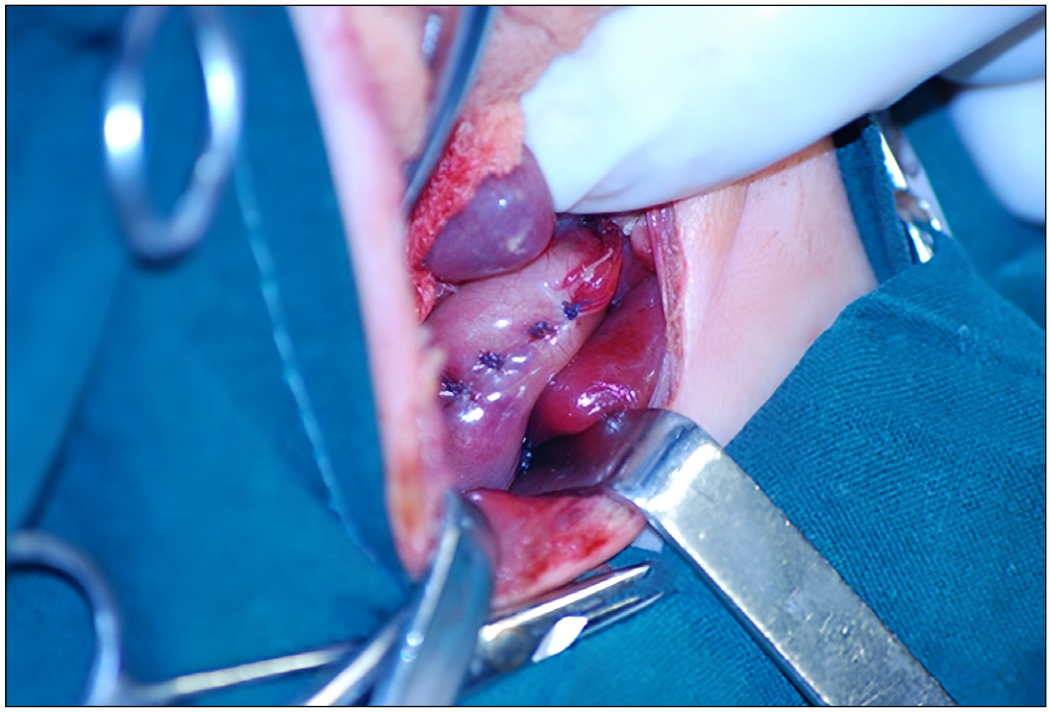

Fig. 2. Suturing of Gastrointestinal Perforation.

decompression was performed using a rectal tube. Additionally, bladder catheterization was carried out to monitor hourly urine output, and central venous catheterization was performed.

During NEC, the procedure of choice was suturing the perforation in 8 newborns. Surgical treatment involved excision of the defect edges and closure of the opening using Vicryl 5/0-6/0 sutures. In 4 patients who were in a stable condition, with large intestinal wall defects in the presence of a localized inflammatory process and absence of widespread peritonitis, resection procedures were performed with the creation of direct intestinal anastomoses. (Fig. 2).

In the postoperative period, prolonged infusion therapy, parenteral nutrition, as well as antibacterial and antifungal treatment were administered.

Enteral feeding was successfully initiated in 3 children starting from the 7th postoperative day. The initial formula used for enteral nutrition was Alfare, followed by a gradual transition to breast milk.

Three children with IP died, resulting in a postoperative mortality rate of 21.43%. All deceased patients were preterm infants with extremely low birth weight and congenital cardiopathies or heart defects, which led to severe hemodynamic disturbances and low systemic blood flow.

DISCUSSION

IPs of the gastrointestinal tract are among the most severe pathological conditions in the neonatal period. Cases of intestinal isolated perforations account for 1-2% in newborns with very low birth weight and 5-8% in those with extremely low birth weight. According to numerous literature sources and our own observations, significant factors contributing to IP include prematurity, low birth weight, respiratory distress syndrome on the background of an unfavorable premorbid condition, and inadequate nutrition in the early postnatal period [4].

The pathophysiology of neonatal IP remains a subject of discussion. Recently, IP of the gastrointestinal tract in newborns has been recognized as a distinct nosological entity, supported by clinical and scientific research. Pathomorphological examination of biopsy samples in IP cases reveals dysplasia or even the absence of the muscular layer of the hollow organ wall, indicating a congenital origin of the pathology.

IP is associated with a high mortality rate. Timely diagnosis and urgent surgical consultation are crucial, as they facilitate early diagnosis and prompt surgical intervention. The diagnosis of neonatal IP is based on premorbid background assessment, clinical, laboratory, and instrumental examination data, as well as mandatory morphological verification. Our observations indicate that the preferred surgical approach for IP of the gastrointestinal tract is perforation site suturing, which aligns with literature data. In stable patients with large intestinal wall defects, in the presence of a localized inflammatory process and the absence of widespread peritonitis, resection procedures with direct intestinal anastomoses are reasonable [6].

Future research on neonatal IP of the gastrointestinal tract should focus on developing preventive measures aimed at eliminating the etiopathogenetic factors that lead to intestinal blood circulation disorders and impaired barrier functions of the intestinal wall.

CONCLUSIONS

1. Clinical and pathomorphological features of gastrointestinal perforations in newborns indicate that IP is a distinct nosological entity.
2. IPs of the gastrointestinal tract in newborns are characterized by clinical features such as prematurity, early onset, localized damage to the hollow organ wall, moderate peritoneal inflammation, and distress syndrome, which is considered a leading risk factor for IP development.
3. IPs occur in the context of muscular-vascular dysplasia of the hollow organ wall, supporting its congenital origin.
4. The operation of choice for IPs in newborns is excision of the perforation edges and suturing of the perforation site.
5. The postoperative mortality rate among the studied group of neonates with IP of the gastrointestinal tract was 21.43%.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

Surgical treatment strategy for Barrett's esophagus as a complication of hiatal hernia

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ABSTRACT

Aim: To assess current approaches to treatment of Barret`s esophagus in patient`s with hiatal hernia.

Materials and Methods: English language search restriction was used. The PubMed, Scopus, Embase, Cochrane, and Google Scholar databases were searched using syntaxes consisting of keywords ("Barret`s esophagus" OR "Hiatal Hernia" OR "Esophageal Adenocarcinoma" OR "Esophagitis").

Conclusions: Barrett's esophagus is a precancerous condition, prone to causing esophageal adenocarcinoma. Currently, approaches to treatment and monitoring in different European countries and worldwide vary for Barrett's Esophagus. Nevertheless, most current guidelines favour endoscopic therapy as a first line of treatment of Barrett`s esophagus in patients with hiatal hernia.

KEY WORDS: Barrett`s esophagus, hiatal hernia, endoscopy

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INTRODUCTION

Barrett's esophagus (BE) is a precancerous condition characterized by the replacement of normal squamous epithelium in the esophagus with specialized intestinal metaplasia. This transformation significantly increases the risk of developing esophageal adenocarcinoma. One of the key factors contributing to the development and progression of Barrett's esophagus is gastroesophageal reflux disease (GERD), which is often associated with hiatal hernia. Hiatal hernia can exacerbate acid reflux, leading to chronic esophageal irritation and an increased likelihood of Barrett's esophagus.

AIM

Evaluation of current approaches to the treatment of Barrett's esophagus in patients with hiatal hernia.

MATERIALS AND METHODS

The PubMed, Scopus, Embase, Cochrane, and Google Scholar databases were searched using syntaxes consisting of keywords ("Barret`s esophagus" OR "Hiatal Hernia" OR "Esophageal Adenocarcinoma" OR "Esophagitis"). English language search restriction was used.

REVIEW AND DISCUSSION

Barrett's Esophagus (BE) is a precancerous condition, prone to causing esophageal adenocarcinoma. Currently, approaches to treatment and monitoring in different European countries and worldwide vary for Barrett's Esophagus.

The diagnosis of Barrett's Esophagus is established when the distal part of the esophagus, which is normally lined with stratified squamous non-keratinized epithelium, is lined with columnar epithelium (CLE) more than 1 cm above the esophagogastric junction and may contain specialized intestinal metaplasia upon histological examination [1].

Barrett's Esophagus is named after the British surgeon Norman Barrett, who in 1950 published his foundational article 'Chronic Peptic Ulcer Disease of the Esophagus and Esophagitis', describing an esophagus with metaplasia of columnar cells. However, the first description of this pathology was made by Wilder Tileston, who reported three cases of "peptic ulcer disease of the esophagus" in 1906, where he described the histological structure of the esophageal ulcer and the adjacent epithelium resembling a gastric ulcer with adjacent columnar epithelium.

Over the next four decades, there were widespread discrepancies regarding the histological structure of

the mucosa of the distal third of the esophagus. Some authors argued that ulcers in the distal esophagus were gastric ulcers located intrathoracically in patients with a congenitally short esophagus. Barrett supported this theory in his work in 1950.

In 1953, Ellison and Johnston published a convincing article rejecting Barrett's hypothesis and disputing the possibility of intrathoracic gastric placement, since the latter:

1. Lacked an external serous coat;
2. The muscular structure was identical to the esophagus;
3. Its mucosa was composed of columnar epithelium with areas of squamous epithelium;
4. The epithelium did not include parietal cells characteristic of the stomach.
5. There were mucosal glands characteristic of the esophagus.

This paper led N. Barrett to revise his previous statements and publish an article in 1957, in which he described this area as "The lower esophagus lined with columnar epithelium." [2].

Between 1960 and the mid-1970s, there were various histological descriptions of subtypes of columnar tissue in the distal esophagus, including transitional epithelium cardio-gastric type, gastric-fundal type, and intestinal epithelium with goblet cells.

This histological issue was resolved in 1976 by Paul and co-authors, who performed biopsies on 11 patients with BE and identified a histological spectrum that could include: columnar epithelium containing villi and goblet cells which is now known as intestinal metaplasia, sometimes referred to as specialized intestinal metaplasia, with subsequent transitional epithelium, and atrophic fundic gastric epithelium with basal and parietal cells [2].

In the 1980s, it was established that gastroesophageal reflux disease (GERD) and the presence of a hiatal hernia were risk factors for BE.

To avoid errors, diagnostic criteria for BE were established by Skinner and co-authors, who proposed a diagnostic criterion—the presence of a metaplastic area of at least 3 cm in length.

By the mid-1980s, the link between BE and esophageal adenocarcinoma was firmly established, and it was proven that intestinal metaplasia has a mosaic distribution and a significant predisposition to the development of dysplasia, which led to the recognition of intestinal metaplasia as a defining feature of BE.

In the mid-1990s, Spechler and co-authors challenged the widely accepted practice of performing biopsies in BE over 3 cm, as they demonstrated that in 18% of patients with endoscopic signs of BE, with metaplasia less than 3 cm in length, goblet cells were still present in the mucosa. Moreover, reports of esophageal adenocarcinoma developing on the background of BE with segment sizes less than 3 cm were also noted. Currently, the classification of BE into short and long segments has proven important for diagnostic criteria and treatment strategy [2]. Histological criteria for the diagnosis of BE remain a contentious issue. The American College of Gastroenterology (ACG) considers biopsy to confirm intestinal metaplasia as a necessary condition for diagnosing Barrett's Esophagus [3]. However, the British Society of Gastroenterology (BSG) in its guidelines states that the diagnosis of Barrett's Esophagus can be made with the presence of visible columnar epithelium (gastric type) and confirmation with biopsy as an intestinal metaplasia is not a mandatory condition for the diagnosis of BE [4].

Japanese scientists consider the diagnosis of Barrett's Esophagus confirmed with the presence of gastric-type columnar epithelium, based on studies confirming the possibility of developing esophageal adenocarcinoma against the background of BE without intestinal metaplasia [5,6].

The International Group on BE and Esophageal Cancer (BOBCAT) defines BE as the presence of columnar epithelium in the lower third of the esophagus but specifies that it must be noted whether intestinal metaplasia is present above the esophagogastric junction [7].

The differences in recommendations depend on the differential risk of malignant transformation of columnar epithelium with and without intestinal metaplasia. Emphasis on intestinal metaplasia as a defining feature of Barrett's esophagus (BE) is based on an increasing number of studies that have shown a stronger association between BE with intestinal metaplasia and adenocarcinoma than BE without intestinal metaplasia.

A study of 8522 patients showed that the risk of malignant progression of intestinal metaplasia was higher compared to columnar cell metaplasia of the stomach (0.38% per year vs. 0.07% per year) [8]. A recent detailed genomic analysis comparing BE with intestinal metaplasia and BE without intestinal metaplasia in 45 patients reported higher mutation frequencies in cancer-related genes such as CDKN2A, WWOX, c-MYC, and GATA6 in patients with BE featuring intestinal metaplasia [9]. However, other studies did not confirm these findings. A retrospective analysis of 688 patients showed no significant difference in cancer risk between patients with BE and intestinal metaplasia and BE without intestinal metaplasia [10]. Several studies have also highlighted the detection of esophageal cancer in the context of columnar cell epithelium without the presence of goblet cells [11].

The biopsy collection protocol developed by Seattle, which includes 4-quadrant biopsies every 2 cm along the length of BE, is a reliable method for obtaining appropriate material for diagnosing BE. However, this protocol is not always strictly followed in clinical practice.

In a comparative study designed to determine the optimal number of biopsies for detecting intestinal metaplasia, researchers showed that the diagnostic value of detecting intestinal metaplasia increases with the number of biopsies. When the number of biopsies increased from 4 to 8 to 16, the diagnostic accuracy for intestinal metaplasia increased from 34.7% to 67.9% and to 100%, respectively [12]. These findings led to the latest recommendations from the American College of Gastroenterology (ACG) to obtain at least 8 random biopsies when BE is suspected during diagnostic endoscopy. Obtaining 16 biopsy samples achieves high accuracy, but this would not only take a lot of time for the procedure but could also increase the risk of bleeding after the biopsy and raise the cost of biopsy processing [13].

Dysplasia is a biomarker of cancer risk in BE, classified according to the Vienna classification [14]. Since the assessment of dysplasia affects treatment strategies, most recommendations require that the diagnosis of dysplasia be confirmed histologically by two pathologists.

The use of alternative biomarkers, particularly the expression of p53 protein, has become a valuable addition for improving BE risk stratification. Sikkema and colleagues showed that overexpression of p53 was a stronger predictor of progression to high-grade dysplasia (HGD) or esophageal adenocarcinoma, regardless of histology, compared to diagnosing low-grade dysplasia (LGD) [15].

In an analysis of over 12,000 biopsies in 635 BE patients, it was shown that abnormal p53 expression, either overexpression or loss of expression - increased the risk of cancer, and the risk was higher for BE with loss of p53 expression (14.0) compared to BE with overexpression of p53 (5.6) [16]. Furthermore, immunohistochemical studies for p53 detection have shown good interobserver reliability. Although immunohistochemistry for p53 has not yet been widely implemented in clinical practice, its use could allow for a more accurate risk group assessment for more intensive monitoring of these patients [13].

Barrett's esophagus is diagnosed in 7% to 10% of individuals with chronic gastroesophageal reflux disease (GERD), and it is estimated to be present in 1% to 2% of the general adult population [3, 4]. In chronic GERD, 5-15% of the esophageal mucosa may transform normal squamous epithelium to columnar mucous epithelium. The development of Barrett's esophagus leads to a tenfold increase in the risk of esophageal adenocarcinoma compared to the general population [17].

The incidence of esophageal adenocarcinoma in non-dysplastic Barrett's esophagus is approximately 1 case per 300 patients per year [18].

Esophageal adenocarcinoma continues to be one of the fastest-growing cancers in Western populations, and this correlates with the rising mortality from this disease [19, 20].

Survival of patients with esophageal adenocarcinoma correlates with the disease stage. The 5-year survival rate is around 20% in patients with locally advanced disease and less than 5% for those with distant involvement. The low survival rate for patients with advanced esophageal adenocarcinoma highlights the necessity of early detection. Endoscopic surveillance for BE has become the cornerstone for preventing esophageal adenocarcinoma, especially in Western countries, and this trend has accelerated with the advent of visualization technologies and endoscopic treatment methods [21, 22].

BE can be classified as short-segment or long-segment depending on the extent of metaplastic changes in the esophagus observed during endoscopic examination. If intestinal metaplasia extends less than 3 cm above the gastroesophageal junction, it is considered short-segment BE, while more than 3 cm indicates long-segment BE [23].

Risk factors for BE include white race, male gender, age over 50 years, obesity, and persistent gastroesophageal reflux [3]. The presence of a hiatal hernia is also associated with the development of BE [4].

Hiatal hernia (HH) is a common condition characterized by the displacement of the esophagogastric junction, stomach, or other abdominal organs through an enlarged diaphragmatic hiatus into the chest cavity. According to the widely accepted anatomical classification, HH is divided into four types. Type I hernias are the most common, with a frequency of up to 90% of all HH cases. Types II - IV are classified as paraesophageal hernias, with type III being the most common (about 90%) [24, 25, 26]. The prevalence of HH in the population can range from 3% to 30%, and in individuals over 50 years of age, it may reach up to 50%, according to Mittal's data.

The frequency of HH diagnosis depends on the quality of diagnostics, geographic features, and the ethnic composition of the population. The frequency of symptomatic cases of HH is linked to the diagnosis of GERD, as these conditions are closely correlated. GERD, which most commonly manifests as heartburn and acid regurgitation, affects 18-28% of the population. In Ukraine, statistical registration of GERD started in 2009,

Table 1. Endoscopic classifications for the diagnosis of lesions in patient`s with Barret`s esophagus

	BING classification	JES classification for BE
Non – dysplasia	Mucosal pattern: regular Vascular pattern: regular	Mucosal pattern: regular Vascular pattern: regular flat pattern
Dysplasia	Mucosal pattern: absent or irregular Vascular pattern: irregular	Mucosal pattern: irregular Vascular pattern: irregular
Diagnostic accuracy	Sensitivity 80% Specificity 88%	Sensitivity 87% Specificity 97%
Reproducibility	k = 0.68	k = 0.77

with the primary incidence rate being 10 cases per 1000 population and a tendency to increase. The prevalence of GERD is approximately 30% (25.1% in men and 39.1% in women) [4]. More than 80% of patients with HH have endoscopic signs of esophagitis.

Among patients who underwent endoscopy for various indications, the connection between HH and reflux esophagitis is significant in different countries, regardless of the prevalence of HH. HH statistics are not maintained in Ukraine, and data are aggregated with the total number of abdominal wall hernias. Untimely detection and treatment of HH can lead to chronic anemia, acute gastric bleeding, esophageal strictures, perforation, and subsequent development of ulcers and erosions of the gastric mucosa, acute gastric incarceration, and its necrosis [17]. The presence of HH leads to anatomical and functional disturbances at the esophagogastric junction, causing the reflux of gastric contents into the esophagus. This includes gastric secretions such as hydrochloric acid and pepsin, as well as pancreatic enzymes and bile. Chronic exposure to these substances is considered a factor in the development of BE [27,28]. Observational and experimental studies have demonstrated that acid and duodenogastroesophageal reflux have a synergistic effect and increase the risk of BE development [29,30]. A meta-analysis showed a strong association between HH and BE, which is linked to dysplastic changes. It is plausible that HH increases the risk of BE by increasing exposure of the esophageal mucosa to gastric contents such as acid and bile, making BE more common in individuals with HH. The role of gastric contents or gastric contents and bile in damaging the esophageal mucosa, leading to metaplasia, is supported by animal models [23].

In a study involving 118,750 BE patients, 24,030 of whom had HH, a connection was found between the size of the HH and its complications. Larger hernias were proportionally associated with an increased risk of BE development, its extent, and the occurrence of dysplasia and adenocarcinoma [31].

Endoscopic surveillance for Barrett's esophagus has become the foundation for the prevention and early detection of esophageal adenocarcinoma. Surveillance typically includes periodic endoscopy of the upper gastrointestinal tract with biopsies of suspicious areas and random biopsies from four quadrants. However, targeted biopsies using narrow-band imaging can detect more dysplastic areas, thereby reducing the number of necessary biopsies. Several specific pit structures and vascular patterns characteristic of Barrett's esophagus have been described, but the proposed criteria are complex and varied. Recently, simpler classifications have been developed focusing on differentiating between dysplasia and the absence of dysplasia. One such classification is the Japanese Society of Esophagus Classification, which identifies correct and incorrect structures in terms of mucosal and vascular patterns (Table 1).

The depth of cancer invasion is diagnosed using endoscopic ultrasound (EUS); however, a meta-analysis of EUS diagnostics for superficial esophageal adenocarcinoma showed favorable combined data for mucosal-stage cancer but unsatisfactory results for adenocarcinoma at the gastroesophageal junction. Endoscopic resection has recently been proposed as a more accurate method for assessing the depth of invasion compared to EUS. European guidelines describe endoscopic resection as therapeutic for well- or moderately differentiated mucosal cancer without lymphovascular invasion, and these criteria can be extended to lesions invading the submucosa (≤ 500 μm) and tumors smaller than 3 cm. These criteria were confirmed by a recent study in Japan [32].

The European Association of Endoscopists recommends changing surveillance intervals based on the length of the Barrett's esophagus (BE). For patients with an irregular Z-line or the presence of columnar metaplasia without dysplasia in the esophagus less than 1 cm, routine biopsies or endoscopic surveillance are not recommended. For BE ≥ 1 cm and <3 cm, surveillance should be conducted at intervals of 5 years. For BE ≥ 3 cm and <10 cm, the interval for endoscopic surveillance should be 3 years. Patients with BE of a maximum size ≥ 10 cm should be referred to a specialized BE center [33].

Endoscopic screening for BE is not recommended. However, screening can be considered for patients with

a family history of BE and prolonged GERD symptoms with risk factors**.**

High-definition endoscopy (endoscope, processor, and screen) is recommended for endoscopic surveillance of BE. It is recommended to use virtual chromoendoscopy (NBI mode) and dye spraying - acetic acid is the only dye-based chromoendoscopy method that meets ASGE PIVI thresholds [34].

Endoscopic examination of patients with BE should include:

1. The degree of BE using Prague criteria: circumferential extent (C), maximal extent (M), and any individual areas proximal to the maximal extent.
2. A description of the location: in cm from the teeth and clock face orientation of any visible abnormalities within the metaplasia.
3. Presence or absence of erosive esophagitis according to the Los Angeles classification.
4. The location of biopsies taken from the Barrett's segment: number of biopsies and location in cm from the teeth.

Biopsy sampling is performed according to the Seattle protocol and includes 4 points around the circumference of the esophagus every 2 cm, as well as a biopsy from the proximal point of metaplasia and material sampling from suspicious areas of the mucosa with disrupted pit and vascular patterns. If Barrett's esophagus with low-grade dysplasia (LGD) is found, endoscopic ablation should be proposed. In the presence of visible lesions (nodular lesions, suspicion of adenocarcinoma), the area should be removed by endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD) with further histological confirmation.

If high-grade dysplasia (HGD) is found during random biopsy, endoscopic ablation of BE or resection/dissection of the pathological area is recommended [35].

If drug control of gastroesophageal reflux disease (GERD) is not possible in patients, an anti-reflux surgery should be considered for patients with BE to prevent further neoplastic progression [3,33].

The American Society for Gastrointestinal Endoscopy (ASGE), the British Society of Gastroenterology (BSG), and the European Society of Gastrointestinal Endoscopy (ESGE) guidelines do not comment on the presence of GERD in patients with BE. [22,31,45] When it comes to choosing the optimal treatment strategy for patients with GERD, conservative treatment, particularly proton pump inhibitors (PPIs), provides only temporary symptomatic relief and cannot fully prevent complications. Clinical studies show a significant advantage of laparoscopic surgery over conservative treatment when compared for symptoms and quality of life after treatment.[17] Laparoscopic anti-reflux surgery is safe option with a low complication rate. The main aim of GERD treatment is symptom control, which improves quality of life. Results from laparoscopic Nissen fundoplication have proven effective in relieving typical GERD symptoms. According to a study of patients who underwent laparoscopic Nissen fundoplication (37% of them had BE) 87% considered themselves completely healthy, and another 11% reported noticeable improvement in symptoms with an average follow-up of 2 years. For patients with BE undergoing anti-reflux surgery, symptom control was similar. In a cohort of 85 patients with a follow-up period of 5 years, 77% considered themselves completely healthy, and another 22% showed significant improvement in symptoms. A third study with 215 patients followed for 8 years after fundoplication showed that 86% of patients had fully controlled heartburn and regurgitation. These studies show that laparoscopic anti-reflux surgery leads to effective symptom control of GERD in most patients with BE [37].

Using proton pump inhibitors (PPIs) in comparison to no therapy reduces the progression of BE to dysplasia or esophageal adenocarcinoma. Although there is no evidence in highly selective prospective studies, there is scientific plausibility to this claim; preventing injury is the main preventive factor for mutations and neoplasms. Cohort studies demonstrate that PPI use reduced the development of neoplasia. Systematic reviews report a strong negative correlation between PPI use and the risk of severe dysplasia or esophageal adenocarcinoma in patients with BE [7].

Anti-reflux surgical interventions offer an alternative to PPIs in the treatment of GERD, as abnormal gastroesophageal and duodeno-gastroesophageal reflux are prevented.

Performing anti-reflux surgery has advantages over PPI use, as it prevents the entry of duodenal content as well as non-acid gastric content such as pepsin into the esophagus, which are irritants not alleviated by PPIs[37].

The risk of dysplasia or adenocarcinoma progression in BE is similar when comparing medical therapy with fundoplication.

Surgical treatment of reflux in patients with GERD, with or without BE, can provide long-term symptom control and esophageal pH control. [38] Some cohort studies suggest that effective anti-reflux surgery may reduce the risk of BE progression. [39,40] However, a study comparing treatment and monitoring of 101 patients found no significant difference in the development of severe dysplasia in BE when comparing medical therapy and fundoplication after a median follow-up of 5 and 6 years, respectively [41]. A meta-analysis comparing anti-reflux surgery with PPI use in patients

with BE showed similar results in terms of progression to dysplasia or cancer [42]. Thus, performing anti-reflux surgery is not an anti-cancer measure.

In several studies, it has been proven that only surgical anti-reflux intervention without endoscopic ablation of BE does not prevent BE progression but eliminates the need for antisecretory drugs [7,43,44].

CONCLUSIONS

The approach to BE treatment has significantly evolved over the last twenty years. Esophagectomy and esophageal resection were the only options for the surgical treatment of high-grade dysplasia and esophageal adenocarcinoma; however, with significant technical advancements, endoscopic therapy has become the main method of BE treatment.

In patients with dysplastic BE or intramucosal carcinoma, endoscopic surgical interventions such as ablation and mucosal resection have become the standard of care, replacing esophagectomy as the best treatment option in most cases. These endoscopic methods are associated with reduced morbidity and mortality, fewer complications, and improved long-term quality of life compared to esophageal resection.

The choice of ablation method is also subject to discussion in patients with GERD, as the lower third of the esophagus is usually dilated, and the diameter of the esophagus may vary. Radiofrequency balloon ablation may produce poorer results and increase the risk of recurrence, requiring more sessions and increasing the risk of dysplasia.

Argon plasma ablation (APA) is a well-known technique in gastrointestinal endoscopy with various indications, such as thermal ablation of the mucosa in BE, treatment of vascular malformations, removal of BE segments after endoscopic resection, endoscopic hemostasis, and others.

A relatively newer technology, radiofrequency ablation (RFA), is now often the method of choice compared to traditional APA, especially for long-segment BE. However, there are not enough studies comparing APA and RFA for an accurate comparison of techniques.

Recent studies show several advantages of hybrid radiofrequency ablation. When performing ablation in BE using the traditional APA method, the risk of stricture formation may reach 12-15%, while with RFA, it is 5%. Therefore, there is still a need for technical improvements in BE ablation techniques. The ideal technique would result in complete ablation of BE while minimizing the risk of complications. One possible approach to reduce the number of strictures is submucosal injection of fluid before thermal ablation (hybrid ablation). This prevents damage to the deeper layers of the esophageal wall. This technique combines submucosal injection of isotonic saline with standard APA in one procedure. However, the thermal impact on the esophageal wall, uniformity of ablation, and penetration depth, depending on the solutions used, require further research. Preliminary data from studies using hybrid APA show significant advantages compared to APA and are comparable in effectiveness and safety to RFA.

The issue of treating Barrett's esophagus combined with a diaphragmatic hiatal hernia remains unresolved and requires further study. Based on current global literature, hybrid argon plasma coagulation is an effective method for treating Barrett's esophagus with low complication rates and good long-term outcomes.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

Features of clinical flow of traumatic illness on a background chronic stress

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ABSTRACT

Aim: To focus on the dependence of the severity of the clinical features of traumatic illness on stress components and chronic stress in particular.
Materials and Methods: Analysis of data on the results of existing studies assessing the impact of chronic stress on the course of traumatic illness.
Conclusions: The introduction of a therapeutic complex of immunotherapy and sedatives for traumatic illness with a pronounced stress component, with the possible development of severe complications, will make it possible to improve the results of treatment and rehabilitation of patients with traumatic illness.

KEY WORDS: traumatic illness, chronic stress, clinical course, stress mechanisms, immunity, recovery

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INTRODUCTION

Traumatic illness is a complex process that includes not only mechanical damage to tissues, but also secondary disorders that arise as a result of stress reactions of the body. In this case, there is a general response of the body to injury, which is manifested by a number of adaptive and pathological reactions, as well as local and general symptoms that are characteristic of a particular injury. Traumatic illness greatly affects the outcome of the injury, determines the prognosis for the course of the pathological process, the results of treatment and performance.

Of great importance are changes in the psycho-emotional sphere, metabolic processes and hemostasis, the state of the immune system, the work of the heart, lungs, digestive organs and the central nervous system.

AIM

The aim of our study was to focus on the dependence of the severity of clinical features of traumatic illness on stressful components and chronic stress in particular. Pay attention to the importance of analyzing the functioning of the immune, nervous and endocrine systems in this category of patients. To emphasize the importance of psychological support and immunocorrection in the treatment process of patients with traumatic illness against the background of stressful manifestations.

MATERIALS AND METHODS

Analysis of data on the results of existing studies assessing the impact of chronic stress on the course of traumatic illness.

REVIEW AND DISCUSSION

CLINICAL FEATURES OF THE COURSE OF TRAUMATIC ILLNESS AGAINST THE BACKGROUND OF CHRONIC STRESS

The clinical picture in traumatic illness depends on the severity of the injury. In patients with mild injuries, the disease is erased, with the absence of characteristic periods and complications. In patients with a severe clinical course and, especially, with a traumatic illness, the stages and symptoms are more pronounced, since in this case there is a mutual encumbrance syndrome - a situation in which pathological factors not only «add up» with each other, but also mutually burden each other.

One of the main components of a traumatic illness is a wound. A wound is a mechanical injury accompanied by a violation of the integrity of the outer integumentary tissues, primarily the skin. The wound process is the body's reaction to trauma, characterized by a sequence of stages that have their own anatomical, pathohisological, biochemical, clinical features. The essence of the wound process

is to mobilize general and local protective reactions aimed at wound healing [1-4].

Acute stress disorder is an intense, unpleasant reaction to a severe traumatic incident that begins shortly after it and lasts less than a month. The symptoms of acute stress disorder are similar to post-traumatic stress disorder and can include feelings of detachment from reality or feelings of disconnection from themselves and their experiences. After a traumatic event in a person's life, psychological changes first occur in his body, but in the later stages this is very strongly reflected in physical health, and this can manifest itself even decades after the injury [5-8].

From the standpoint of immune distress syndrome, the course of a traumatic disease is characterized by a stage in which four periods are distinguished.

The first period of traumatic illness begins immediately after the traumatic injury and lasts 6-12 hours. This period is characterized by neuroendocrine reactions with activation of the sympathoadrenal system, impaired vascular tone, signs of traumatic shock or a terminal condition. The main cause of death of patients during this period is life-threatening injuries, IV degree shock and massive blood loss with the development of DIC syndrome.

The second period of traumatic illness, or immunotoxicosis with the possible development of immunoparalysis, lasts 12-48 hours. Patients are in the intensive care unit of the intensive care unit, where they are given intensive anti-shock therapy. According to vital or absolute indications, urgent surgical interventions are carried out during this period: operations on the brain and spinal cord, main vessels, abdominal and chest organs, spine, bones.

The treatment plan, volume and method of surgical interventions are determined individually, taking into account the nature of injuries, the likelihood of developing dangerous complications, the age and general condition of the patient, the presence of acute and chronic diseases. The main cause of mortality of victims during this period is multiple organ failure.

The third period of traumatic illness is characterized by late manifestations of dystrophic and sclerotic processes. This period is characterized by the development of complications. Complications occur at a certain frequency. So, lung disorders in traumatic illness are usually detected on the 3-4th day, on the part of the abdominal cavity, as well as local infectious complications - on the 6-10th day. Treatment tactics are determined individually. Intensive detoxification therapy and antibiotic therapy are carried out, immunotherapy in some cases there are indications for surgical interventions.

The duration of the fourth period of traumatic illness - the rehabilitation period, depends on the location and severity of the injury, as well as the presence and nature of complications. The condition of the body gradually improves, all vital functions are normalized. During this period, planned operations are carried out to restore damaged organs and tissues, conservative treatment is prescribed, and rehabilitation measures are carried out.

Along with traditional medical measures, at this stage, work on normalizing the psychological state of patients is of great importance. Since the stressful component of a traumatic illness persistently does not lose its relevance. Acute stress disorder is an intense, unpleasant reaction to a severe traumatic incident that begins after it and lasts up to thirty days. The symptoms of acute stress disorder are similar to post-traumatic stress disorder and include a feeling of detachment from reality, a feeling of disconnection from oneself and one's experiences. After a traumatic event in a person's life, psychological changes occur in the body, which later significantly affects physical health, and this can manifest itself even decades after the injury. Of course, stress can be caused by the presence of a wound surface on the body. Wounds can cause stress for a variety of reasons. Thus, trauma, accompanied by a loss of the ability to self-care, a change in living conditions and social status, is a severe stress not only for the body, but also for the psyche. And long-term treatment further aggravates these changes. There may be a loss of motivation, increased aggressiveness, emotional lability, depression, the presence of a secondary benefit effect, etc. Stress is a strong tension of the body that does not go away without a trace. The negative impact of stress on health is very large and has the worst consequences. It is the stressful situation that causes many diseases that will manifest themselves later - both physical and mental [9-13].

Chronic or «long-term traumatic» stress, in turn, can cause disorders in many physiological systems, including nervous, endocrine and immune. This worsens the results of the treatment of traumatic injuries, contributing to the development of inflammatory processes, deterioration of wound healing, and even an increase in the likelihood of infectious complications. Chronic stress has a complex pathophysiological mechanism that includes the activation of the sympathetic-adrenal system and the release of stress hormones such as cortisol and adrenaline. They are able to change microcirculation in tissues, impairing the supply of oxygen to damaged areas and increasing the inflammatory process. The term «long-term traumatic stress» was coined in the context of political violence and social conflict, and refers to both social and individual conditions.

There are three levels of response of body systems to stress, and it depends on their reactivity: 1) cognitive-emotional level (involved cortical and limbic structures located above the hypothalamus), which is responsible for individual psychological differences in the cognitive-emotional response; 2) autonomous-endocrine level (involved anatomical structures localized in the hypothalamus and brain stem), which connects information received from the upper centers with endocrine organs and the autonomic system; 3) the peripheral level, which is responsible for the individual reactivity of the periphery (for example, structural changes in the arteries can alter the response to information coming from the central nervous system) [9,10].

Chronic stress leads to an imbalance in the immune response, reduces the activity of cells responsible for protecting the body from infections and contributes to the chronicity of inflammatory processes. In the context of traumatic illness, this leads to a slowdown in healing and an increased risk of developing infectious and inflammatory complications such as sepsis or osteomyelitis. Statistics show that 30-40% of patients who experience severe injuries due to chronic stress have a higher risk of developing infectious and inflammatory complications, namely wound infections, impaired healing or even sepsis [1].

Sensitivity of patients to immunotherapy, the effectiveness of different methods of which is not the same in different periods of the disease. Immunotherapy is able to solve both independent tasks - the prevention of purulent complications and generalization of infection, and be a component of the treatment complex, improving the transfer of surgical interventions, the effectiveness of sanitation of destructive foci, the eradication of pathogens and others.

The nervous system, under the influence of chronic stress, can lead to changes in central nervous regulation, which affects the pain threshold, the level of anxiety and depression. Patients with chronic stress often suffer from more severe pain, which can make it difficult for them to recover from an injury. In addition, stress can increase fatigue levels and reduce the overall endurance of the body. Studies show that patients with high levels of stress often need 15-20% more time for rehabilitation compared to patients without stress [3,7,15,16].

Stress affects the release of cortisol, which is the main stress hormone. Chronically elevated cortisol levels slow down the healing process, as cortisol has an anti-catabolic effect, which interferes with tissue regeneration. In addition, under stress, the levels of other important hormones such as testosterone and insulin decrease, which also slows down the regeneration and healing processes. Studies show that patients with elevated cortisol levels have 25-30% slower healing after injuries [17,18].

Patients with chronic stress have a number of clinical features that can complicate the diagnosis and treatment of injuries. Among them are increased soreness, protracted inflammatory processes, slow wound healing, a tendency to develop depressive and anxiety disorders. Also, chronic stress can cause a shift in the pain threshold, which complicates rehabilitation measures, especially in the postoperative period. According to the results of numerous studies, patients with chronic stress against the background of traumatic injuries have a 20-25% higher level of depression and anxiety, which complicates their post-traumatic psycho-emotional state [19]. Stress and increased cortisol levels lead to an increase in the incidence of opportunistic infections, a decrease in the activity of human growth hormone, which means a slowdown in wound healing, the occurrence of hypertrophic, keloid scars and long-term non-healing (trophic) wounds. Gouin et al. compared the healing rate of a small perforated biopsy wound in a group of dental students. They found that the participants' healing was on average three days slower when they were under the stress of exams, compared to those who were on vacation. This means that the healing time of a small standard wound in young and healthy people has increased by 40%. Older people with numerous comorbidities are also at risk of slowing down wound healing, and stress further increases this risk. People who are stressed are more prone to bad habits that can slow down wound healing, such as drinking alcohol, tobacco or drugs, little physical activity, sleep disturbances, poor diet and not following a medication regimen [11,20-22].

Against the background of all the drama of the impact of stress on the human body, and health in particular, one should not forget about the adaptive capabilities of the body in order to maintain the constancy of functioning. Adaptation of the human body to the action of stress factors occurs as a result of changes in physiological constants with the preservation of the level of metabolic processes – physiological adaptation, which is provided by simultaneous changes in biochemical, namely energy supply of tissues. Regardless of the nature of the stress-inducing effect, this mechanism is universal. The body reacts to stress with a stereotyped set of biochemical and physiological processes, which in turn provide nonspecific or cortical adaptation. Environmental factors, to which the body adapts, acting in different ways, lead to a monotonous general complex of disorders - a deficit of energy supply,

which means the mobilization of energy resources. As a result of these events, the chain of metabolic reactions leads to the expression of marker genes that determine the sensitivity of cells to stressors. Chronic psycho-emotional stress is a consequence of continuous or periodic prolonged exposure to emotionally negative factors [11,16,23,24].

TREATMENT AND REHABILITATION STRATEGIES

Treatment of patients with traumatic illness against the background of chronic stress requires an integrated approach, which includes not only traditional methods of medical care, but also psychological support. An important part is the correction of stress through psychotherapy, in particular cognitive behavioral therapy, which helps to reduce anxiety and depression. Statistics show that patients receiving psychotherapeutic care recover their psycho-emotional state faster, as well as improve the healing process of trauma [10,25].

In the acute phase of stress, the hypothalamic-pituitary-adrenal system is activated, mediated by cortisol. According to the results of preclinical studies, it is known that according to the type of response to stress, respondents are divided into two categories: whose cortisol levels will be increased or decreased and whose anxiety levels will be high or reduced. An adequate response of the hypothalamic-pituitary-adrenal axis and, as a result, a high level of cortisol is a general adaptation syndrome; It is the body's natural way of experiencing stress. Conversely, low cortisol levels are a phenomenon that leads to a pathological stress response; It is the result of a defect in the hypothalamus. This hypothesis was confirmed in preclinical studies: the injection of high doses of hydrocortisone was effective only if it was used in the first 6 hours («golden hours») after the traumatic event. Taking 100-140 mg of the drug can reduce the incidence of post-traumatic stress disorder from 60% to 16%. Benzodiazepines, enhancing the effect of gammaaminobutyric acid, cause sedative, hypnotic and anxiolytic effects, and actually block the work of the hypothalamic-pituitary-adrenal axis. The medical community opposes the early administration of benzodiazepines and believes that they interfere with the body's natural adaptive response. The fact that early administration of benzodiazepines increases the risk of developing post-traumatic stress disorders is also evidenced by the data of studies. However, the results of a study in rodents, on the contrary, showed that a number of drugs prescribed immediately after exposure to stress helped to reduce the symptoms of post-traumatic stress disorders. According to the mechanism of action, groups of drugs are distinguished that block the consolidation process (anisomycin), supplement the lack of cortisol (hydrocortisone in high doses, oxytocin) and antidepressants (agomelatine, escitalopram, certralin). The opposite effect was cortisol in small doses, alprazolam, ketamine and alcohol consumption [18,24-26].

It is also necessary to take into account pharmacological correction - the use of anti-stress drugs, adaptogens and means to improve microcirculation, which will contribute to better healing of injuries. Given the negative effects of cortisol, medications are used to correct the level of this hormone in the body, which is an integral part of immunotherapy.

One alternative treatment for anxiety disorders is the use of oxytocin, which mainly affects social behavior. For a long time it was considered a «maternal hormone», but in the 1990s. according to the results of the study, it was found that it is able to reduce anxiety and regulate social behavior (caring for offspring, sexual instinct, social memory). According to the results of preclinical studies, it was found that the administration of oxytocin was as effective in reducing socially determined fear and social withdrawal as the administration of benzodiazepine, or long-term administration of paroxetine. But unlike new psychoactive substances, it did not affect the severity of anxiety and fear caused by sound stimuli; New psychoactive substances were effective in both situations. It should be noted that oxytocin at low doses did not affect either behavioral or physiological manifestations. It demonstrates its effectiveness only when administered in high doses. Reviews on long-term prescription of the drug show that its anxiolytic effect differs depending on gender. Yes, in women, the effect is more pronounced, but further research is needed in this direction. Oxytocin, administered before the onset of stress, slows down the intracellular increase in the production of corticotropin-releasing factor and cortisol. That is, in the future, it reduces the intensity of the activation response, orientation, anxiety, fear, restlessness and tension. Thus, oxytocin and new psychotropic substances are drugs that have a very good prospect in the treatment of anxiety disorders [9,26-28].

The composition of the therapeutic complex of immunotherapy of traumatic illness with a pronounced stress component, with the possible development of severe complications, includes 4 main components in individual combinations:

1. Anticytokine therapy (the goal is the elimination of excess cytokines, the current clinical situation is high cytokineemia, the threat of septic shock).

Options: hemofiltration with sorption of tumor necrosis factor; Plasmapheresis; introduction of monoclonal anticytokine antibodies; therapy with corticosteroids, hydroxystarch derivatives, aprotinin.
2. Prosthetic therapy: immunoglobulins for intravenous use (the goal is to bind microbial antigens and toxins, enhance opsonization and phagocytosis; the maximum recorded effect is in septic shock); transfusions of hyperimmune plasma; extracorporeal hemosorption, which provides a combination of detoxifying and immunological effects.
3. Cytokine therapy (the goal is to correct the key link in the pathogenesis of sepsis and cytokine imbalance; the drug of choice is roncoleukin, the indications are the fact of sepsis, the dosage and frequency are determined by the nature and individual severity of immunodeficiency).
4. Metabolic support of the immune system: enteral and parenteral nutritional support; systemic antihypoxia (hyperbaric oxygenation, indirect electrochemical oxidation of blood with sodium hypochlorite); systemic antioxidant.

The above immunometabolic complex is a powerful means of pathogenetic therapy, which not only complements, but also optimizes standard etiotropic antibiotic therapy, which is aimed only at passive eradication of pathogens.

When studying the problems of traumatic illness, little attention is paid to the integration of psychosomatic and physiological approaches, which makes it possible to answer a significant part of the questions posed, and instead molecular and cellular mechanisms that do not express emotions are studied. Chronic stress load is one of the main factors in the progression of the most common somatic diseases, which certainly worsens the course of traumatic illness. Undoubtedly, in order to prevent this, it is necessary to apply improved approaches to the problem of the influence of stress factors, which would cover a complex of preventive, therapeutic and rehabilitation measures, taking into account the impact of chronic stress on the development of metabolic disorders.

Stress, both psycho-emotional and physiological, causes a wave-like activation of free radical oxidation in the blood and tissues, in the brain tissues in particular. A short-term oxidative rise is observed already in the first minutes of stress, then it decreases and disappears, due to the reactive activation of antioxidant systems, and a secondary rise occurs at the 2-4th week of severe chronic stress and is characterized by exhaustion phenomena. In the limbic-reticular structures of the brain, local blood flow slows down and structural damage occurs in the hippocampus, hypothalamus and cerebral cortex. Under the influence of intense and prolonged stress, the anxiety response, the resistance phase and the exhaustion phase quickly replace each other. Numerous chemical processes necessary for the body to adapt to new conditions of existence lead to the formation of a certain amount of insoluble toxins, which provoke a loss of tissue elasticity, which means a slowdown in all regenerative processes. The degree of resistance of the body to the action of a stress factor is determined by the functional state of the hypothalamic-pituitary-adrenal system. The activation of this system provides adaptive restructuring in the body and acts as an indicator of the manifestation of a stress reaction. With age, the level of its activity decreases and, accordingly, the adaptive capabilities of the body decrease. With emotional stress, the neurochemical properties of neurons are restructured, the sensitivity of membrane receptors to neurotransmitters and neuropeptides decreases, as a result of which postsynaptic processes slow down. As a result of the above changes, stagnant arousal is formed in the emotional zones of the brain, which leads to functional disorders. It has been established that disorders in the hypothalamolimbic-reticular structures of the brain form a general nonspecific reaction, subsequently spreading to the cortex of the cerebral hemispheres and further to peripheral organs through the autonomic nervous system, providing adaptive restructuring in the body. This is what triggers pathogenetic factors that are responsible for the next chain of pathophysiological disorders, in chronic stress, which directly affect the course of traumatic illness [29,30-32].

CONCLUSIONS

Chronic stress is an important factor that affects the clinical course of traumatic illness through the development of neuropsychiatric disorders due to the impact of stressful events on brain and body functions. The impact of stress occurs in several dimensions simultaneously (anatomy, electrophysiology, behavior, social interaction). Thus, chronic stress promotes the development of inflammatory processes, slows down healing and increases the risk of infectious complications, through an imbalance of the nervous, immune and humoral systems of the body. In this regard, the treatment of such patients should be comprehensive, including not only pathogenetic eradication of pathogens, but also immunocorrection, hormone control and psychological support. The introduction of a therapeutic complex of immunotherapy of traumatic disease with a pronounced stress component, with the possible development of severe complications, will make it possible to improve the results of treatment and rehabilitation of patients with traumatic illness.

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REVIEW ARTICLE

CONTENTS

Certain ethical issues that arise when using 3D bioprinting technology

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ABSTRACT

Aim: Theoretical and applied study of the ethical issues that arise when using three-dimensional printed bioproducts and their significance for the development of principles for the application of additive manufacturing technologies in medicine.

Materials and Methods: Various methods of scientific knowledge make up the methodological basis of on interdisciplinary approach, which includes a set of methods that allow us to investigate the technological, legal and social aspects of the application of 3D bioprinting, its potential, limitations and ethical challenges.

Conclusions: Ethical principles are one of the foundations for ensuring the provision of proper medical care and medical services with the use of three-dimensional printed bioproduct technologies at an appropriately high level, and are directly related to strict adherence to the principles and standards of additive manufacturing and bioethics, which are imposed on all participants (be it a doctor a 3D designer, a manufacturer of a 3D bioprinter, etc).

KEY WORDS: bioprinting, bioethics, informed consent, bio-ink, three-dimensional printed bioproducts, additive manufacturing

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INTRODUCTION

Among modern breakthrough technologies, three-dimensional printed bioproduct technologies occupy a significant area. In addition to widespread research on intellectual property rights, risks and professional responsibility, morality and religion, bioethics issues play an equally significant role, the urgent solution of which may affect the further application of additive technologies in medicine. In general, problematic issues of the application of innovative technologies and bioethics have already been the subject of our scientific research [1, 2]. At the same time, the relevance of the study lies in identifying ethical issues when using three-dimensional bioprinting technologies. This is primarily due to the fact that ensuring and guaranteeing the right to life and proper medical care, society expects their proper, professional and responsible implementation, which is impossible without ensuring high standards of bioethics in additive manufacturing.

The issue of determining the essence of ethical aspects that arise when using three-dimensional printed bioproducts has been the subject of research by many foreign scientists. Among the scientists who have investigated individual aspects of this issue, it is appropriate to single out the works of F. Gilbert, Z. Jin, J. Kim, L. Lategan, P. li, M. Munsie, A. Recum, M. Rizzo, E. Salvaterra, A. Siddique, Q. Yan, R. Veeravalli, N. Vermeulen, D. Williams, and others. At the same time, a comprehensive study of of the ethical issues that arise when using three-dimensional printed bioproducts and methods of their resolution was virtually disregarded by the scientists.

AIM

The aim of the article was a theoretical and applied study of the ethical issues that arise when using three-dimensional printed bioproducts and their significance for the development of principles for the application of additive manufacturing technologies in medicine.

MATERIALS AND METHODS

Various methods of scientific knowledge make up the methodological basis of on interdisciplinary approach, which includes a set of methods that allow us to investigate the technological, legal and social aspects of the application of 3D bioprinting, its potential, limitations and ethical challenges. Thus, the comparative legal method was used to compare the legislative norms and approaches to regulating 3D bioprinting in different countries. The system-complex method gave us the opportunity to

analyze the essence of informed consent, sources of biomaterials and availability of technologies, as well as the impact of these technologies on social perceptions of medicine and scientific progress. The following other methods were used in the study, in particular: dialectical, analysis and synthesis, formal-logical etc.

REVIEW AND DISCUSSION

INTRODUCTION TO THE CONCEPT OF ADDITIVE TECHNOLOGIES

3D printing is one of the most innovative technologies of our time, and 3D bioprinting is revolutionizing the medical technology industry, the essence of which has already been the subject of our scientific research. The technology is even called the megatrend of the fourth industrial revolution [3].

3D bioprinting is an advanced application of additive manufacturing, which involves the layer-by-layer creation of a tissue or organ using a bioprinter using instructions from computer graphics software [4]. It is defined as the process of applying biocompatible materials layer by layer to create tissues that can mimic the properties of living cells. The creation of tissue constructs is carried out by combining computer-aided design with computer-aided manufacturing to carefully transform the corresponding biomaterials and bio-inks into tissue substitutes, which at the same time provides significant control over their structure, reproducibility, and functional accuracy. This technology offers the simultaneous printing of different cell types in specific spatial locations, making it prime for use in regenerative medicine [5].

This technology is one of the most promising technologies being implemented in tissue engineering and regenerative medicine. As a widespread and fundamental biomanufacturing technology that uses various biological components (such as cells, growth factors, proteins, and biomaterials), this technology can create 3D models, replacement organs, and other therapeutic products. Bioprinting has already shown incredible growth and has become a technology that can overcome the current limitations of tissue engineering and regenerative medicine. It also has the potential to develop personalized implants that can be a solution to the organ shortage crisis. 3D bioprinted tissue models can also be a platform for high-throughput toxicological screening and drug discovery [6].

Thus, the first human organ transplant obtained using 3D printing was a trachea, which was implanted in an infant with a congenital defect [7]. And an implanted bionic ear printed on a 3D printer had better hearing sensitivity than the human ear [8].

Unlike the traditional use of 3D printing to create acellular scaffolds, 3D bioprinting requires different technical methods, such as biomicroscopy, autonomous self-assembly, and mini-tissue building blocks, to create 3D structures with mechanical and biological properties suitable for the deposition of living cells and the restoration of tissue and organ functions. Cells, bio-inks, and bioprinters are all necessary components of the bioprinting process, and each of them has biological, technological, ethical, and other challenges related to cost and clinical effectiveness. As a result, a number of difficulties arise in integrating 3D bioprinting into widespread clinical practice [9].

ETHICAL ISSUES OF INFORMED CONSENT AND SOURCES OF BIOINKS IN THE APPLICATION OF 3D PRINTED BIOPRODUCTS TECHNOLOGIES

Research and commercialization are advancing at such a rapid pace that issues related to the technology, in terms of ethics, policy, regulation, and public acceptance, are not being adequately addressed. Although identifying the ethical, legal, and social aspects of this technology at an early stage is not only part of our social responsibility but also a benefit for the future of the technology itself [10].

Thus, 3D bioprinting technology raises a multitude of ethical issues, among which, in this study, we will consider such as informed consent and sources of bio-inks.

A fully informed consent process will minimize the risk of harm and possible ethical violations [11]. Informed consent is a legal doctrine based on the fundamental ethical principle of the patient's autonomy to make free and informed decisions about medical treatment or research involving their body. Although informed consent for medical treatment is a consolidated practice worldwide and is characterized by different processes and forms of decision-making depending on the purpose of the clinical intervention or research, there is currently no standard procedure for obtaining informed consent for 3D procedures. This is mainly due to the current lack of specific regulations regarding this technology and opens up several avenues for developing informed consent models for bioprinting that are based on respect for the autonomy of the donor and/or the patient involved in the process [12].

E. Salvaterra identifies certain ethical issues that informed consent for medical 3D printing faces. First, the unknown behavior of materials incorporated into the recipient's body requires that the patient be informed of the potential risks of developing teratoma or other diseases not foreseen at the time of transplantation.

Furthermore, it is necessary that the patient or their legal representatives be informed of the difficulty of terminating participation in current protocols by requesting the removal of bioconstructs after their transplantation. Specific information should also be provided on the methods used to ensure the protection of the confidentiality of all subjects involved in biomanufacturing during the collection, storage, and use of personal data collected during the bioprinting process [12].

3D bioprinting using appropriate bio-inks has become a major tool for fabricating 3D biomimetic complex structures that mimic physiological functions [13]. Bio-inks are a combination of living cells and a compatible scaffold, such as collagen, gelatin, silk, alginate, or nanocellulose. The exact material depends on the patient and the function [14]. The bio-inks themselves are used in the printing process to create 3D structures and consist of a mixture of living cells and biomaterials that provide support for the cells after printing [15]. Bio-inks can be defined as any natural or synthetic materials used in bioprinting and designed to interact with a biological system [16]. Bio-inks, which are the most important component, refer to cell aggregates deposited on or within scaffolds or cell constructs that can consist of bioactive components and biomaterials [5].

Currently, sources of cells for bioprinting include adult stem cells and human embryonic stem cells. The use of the latter cells is particularly controversial because it involves the destruction of human embryos, which raises moral and ethical questions about the value and sanctity of human life. Conversely, the use of adult stem cells and induced pluripotent stem cells may be considered more ethically acceptable because it does not involve the destruction of embryos. Some scientists view the embryo as a being with the same moral rights as an adult or a child, arguing from religious and moral perspectives that life begins at the moment of conception, making the embryo a person with rights and interests that need to be protected. Therefore, removing cells from a blastocyst to create an embryonic stem cell line is tantamount to committing murder. However, until proven otherwise, unless the blastula attaches to the uterine wall, it cannot develop into a child. Moreover, it is quite reasonable to argue that the embryo acquires a true "moral person" at the stage of development after fertilization. This is an eternal debate, the resolution of which is unlikely to be achieved [17]. However, according to domestic legislation, a person has a civil legal capacity at the moment of his birth (part 2 of article 25 [18]).

It should also be remembered that the commercial use of embryonic cells is taboo. The protection of human rights in the field of biomedical research is based on two principles, namely: informed consent and confidentiality. The use of bioprinting may endanger health (e.g. organs) or quality of life (e.g. reproductive organs). The possible commercialization of bioprinting may also raise ethical issues. The point is the safety, quality, and effectiveness of bioprinting technologies that respect human rights and dignity [19].

One of the ethical issues related, in particular, to the use of stem cells in 3D bioprinting is the origin of these biomaterials and focuses on the distinction between autologous and allogeneic stem cells. While the use of autologous stem cells raises well-known issues related to patient safety (e.g., the risk of oncogenicity), the processing of allogeneic stem cells raises additional questions regarding the perception of a new identity (or personhood) by the recipient of the engineered cells (development of consent procedures that clarify the complex stage of biomanufacturing, protection of confidentiality and intellectual property rights arising from biomanufacturing) [12].

It is difficult to foresee in advance the side effects of implantable devices printed on a 3D printer since it is only possible to analyze how they react in the body after they have been implanted. This impasse can be overcome by using autologous cells, which are specifically adapted to the patient and cannot be tested on any other patient. However, even with the use of autologous cells, the risk of side effects will not be completely eliminated, and there will still be a need for standardization of the materials for manufacturing. In addition, it would be impossible to conduct clinical trials, since it would be unethical to first test this patient-adapted material on another population of non-specific subjects if these treatments are not life-saving. Moreover, great attention should be paid to the long-term outcome of the implants [7].

There are other issues that may affect the moral acceptability of using bio-inks from non-autologous cells. For example, the use of stem cell bio-inks obtained from donors who have been coerced into donating their cells, or donors who are unable to give informed consent to the use of their cells (e.g., unconscious patients in intensive care units). In this regard, a trusted person, such as a family member, may be able to make arrangements on behalf of such a person. In any case, potential donors and their families should fully understand the risks and have sufficient information to justify their expectations. The use of bio-inks from autologous cells can often be considered ethically understandable because they are derived from the patient's body [20]. Equally important, as autologous induced pluripotent stem cell lines may outlive their donors and potentially be used for proj-

ects not planned at the time of tissue/cell collection, it is important to routinely seek permission for research or other use throughout the life of the donor and/or project, avoiding the need to re-contact the donor for consent at a later date [21].

Bioprinting using stem cells poses a risk of abnormal cell growth, potentially exposing the recipient to the risk of developing cancer or other adverse effects, such as zoonotic diseases from non-human stem cells [22]. Furthermore, when using xenogeneic cells, patients should be fully informed about the source of the cells in the informed consent, as they may not agree to the use of cells from certain animal species (e.g., porcine) for religious reasons.

It is also essential that bio-inks demonstrate biocompatibility and, where appropriate, biodegradability by reproducing the natural microenvironment of tissues. Bio-inks should be chemically modified to meet the specific requirements of different tissue types. Finally, they should have the potential for large-scale production, minimizing batch-to-batch variations [23]. Biocompatibility of bio-inks for 3D bioprinting refers to the ability to perform the desired function that will support appropriate cellular activity, including cell viability, adhesion, proliferation, and differentiation, to promote tissue regeneration without causing any systems [24].

ETHICS IN BUSINESS: ADDITIVE TECHNOLOGIES ON THE LINE OF ECONOMIC STRATIFICATION

Another important ethical issue that arises when applying 3D bioprinting technologies is affordability. The cost of 3D bioprinters and starting materials is high, often making the technology unaffordable for many and potentially exacerbating social inequalities. Most available 3D bioprinters are built on modified 3D deposition modeling frameworks that are adapted to apply biocompatible materials and their price ranges from US$13,000 to US$300,000. This makes the biomaterials expensive and creates a barrier to the affordability of bioprinting, given that high manufacturing costs translate into high costs for patients. In an attempt to democratize the technology, prototypes of a cost-effective 3D bioprinter built from recycled materials and off-the-shelf electronics have been reported. This approach, which uses open-source methodology and affordable materials, could make bioprinting more accessible, potentially bringing its benefits to low- and middle-income countries and narrowing the economic gap in healthcare [17].

However, social stratification is still possible in this area. These are expensive scientific and technological solutions that are unlikely to benefit everyone. 3D bioprinting is another game-changer that will not be available to everyone, and certainly not to most in its immediate application. Despite the promise of organs printed "on demand" for everyone, it is likely that the specter of a "social stratification of biofabrication" will arise with those who can afford to pay for their own organs. A tiered system of therapeutic organ replacement is likely intended for those who can afford to pay for their own organs, who live longer; perhaps enjoying a significantly higher quality of life, avoiding the negative physical consequences of taking immunosuppressants. While others will wait until a human organ donor becomes available, they will then be forced to take a punitive drug regimen for the rest of their lives to prevent episodes of rejection of the transplanted organ. Others who cannot afford to pay will make do with "used" organs from another living or deceased donor when they become available (as is done in the current system) [25].

Tissue-engineered medical devices and 3D bioprinting are biomedical applications of additive manufacturing processes for the artificial production of biological tissues. Their goal is to replace damaged tissues and organs. The process of 3D bioprinting is the spatial structuring of biological cells by combining them using a computerized layer-by-layer method. This is necessary for growing living tissues and organs for further use in biological research, in particular, such as regenerative medicine, tissue engineering, and pharmacokinetics [26].

Ethics should become part of the human potential development program. When using technologies in healthcare, both the capabilities of the technologies and the impact and consequences of the technologies on healthcare should be taken into account [19]. The attention of ethicists is not so much on the technology itself as on its application, since it affects people and the environment [19].

The advent of 3D bioprinting technology represents a significant leap forward in the field of medical science, opening up unprecedented opportunities for organ transplantation, regenerative medicine, drug testing and development, and disease modeling. However, the rapid growth and development of this technology has outpaced existing regulatory, legal, and ethical frameworks, resulting in a multitude of bioethical and legal implications that require careful consideration. Safety remains a top priority. As with any medical innovation, the potential risks and adverse effects associated with 3D bioprinting of organs and tissues must be carefully assessed and mitigated [17]. However, in the blind pursuit of innovation, safety cannot be neglected [27].

CONCLUSIONS

The possibility of providing medical care and medical services when using three-dimensional printed bioproduct technologies at an appropriately high level is directly related to the strict adherence to the principles and standards of additive manufacturing and bioethics by all participants. This is due to the fact that, taking into account the specifics of new technologies in the field of 3D bioprinting, we can conclude that not only a doctor who does not have the appropriate experience in the application of such innovative technologies but also one who does not adhere to ethical principles is unable to properly ensure the realization of a person's right to life and health, which is the highest social value in society.

Among the important ethical principles, we can highlight those that are necessary at the pre-preparation stage, namely: informed consent and the suitability of the source of bio-ink as printing materials containing living cells for the creation of living tissues, bones, blood vessels, and even organs. Providing full informed consent by the patient will allow us to avoid further problems that may potentially arise when using any innovative technologies that are at the stage of implementation, including 3D bioprinting technology. Patient awareness of the sources of bio-ink is necessary, since, despite the legislation of the state, there may be moral and religious obstacles for the patient in their use. Thus, an appropriate delineation of ethical standards for 3D bioprinting technologies is useful and necessary for the future of the technology itself.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

Comparative analysis of the clinical application of modern adhesive protocols for ceramic restorations (literature review)

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ABSTRACT

Aim: To analyze the well-known physicomechanical and clinical properties of modern adhesive systems, explore the latest trends and approaches to their improvement, and assess the prospects for the further development of restorative dentistry.

Materials and Methods: A comprehensive search for relevant publications related to the topic was conducted using scientific databases such as Scopus, PubMed, BVS, and Scielo.

Conclusions: The standardization of knowledge regarding tooth tissue preparation, adhesive application techniques, as well as potential errors and complications, will contribute to enhancing the quality of dental care and elevating it to a new level.

KEY WORDS: tooth hard tissues, ceramic restorations, dental adhesive system, «ethanol wet bonding,» dental adhesives

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INTRODUCTION

The true revolution in global aesthetic dentistry is associated with the development and implementation of adhesive restorative materials in practice [1-4]. Achieving effective fixation through micromechanical retention has made it possible to perform minimally invasive preparation while maximizing the preservation of the tooth's hard tissues [3,5]. The capabilities of adhesive techniques have allowed for the creation of ceramic structures with a range of advantages that, according to both domestic and international specialized literature, positively affect the quality of treatment, ensuring a longer service life [2,5,6]. The widespread application of this technology has revealed complications in adhesive treatments due to insufficient theoretical knowledge and the unjustified expansion of indications for their use [5,7]. Mistakes during adhesive fixation reduce the strength of the bond between ceramics and the tooth's hard tissues, which often leads to complications such as debonding, cracking of the structure, margin displacement, recurrence of caries, and other unfavorable outcomes [4,6]. According to several authors, this is linked to a lack of theoretical understanding regarding the mechanisms of adhesion and the impact of enamel, dentin, and ceramic surface preparation processes on adhesive fixation [1,2,5,8,9].

A search of scientific works by foreign authors in the «US National Library of Medicine National Institutes of Health» revealed 699 papers on the query «dental adhesive system» and 186 papers on «ethanol wet bonding.» To date, adhesion to enamel and the interaction of existing adhesive systems with dentin have been studied in detail. Specialized literature provides data confirming the degradation of the adhesive bond, the role of chemical components, the main application techniques, and the influence of the dentin's enzymatic system on the degradation speed of the hybrid layer. A number of studies have been conducted to optimize the adhesive bond using inhibitors of metalloproteinases and ethanol solution. In the field of ceramic preparation, questions regarding the permissibility of sandblasting, as well as the clarification of etching and cleaning algorithms for ceramic surfaces, remain relevant [2,4].

Currently, there is no unified methodological approach to the adhesive fixation protocol and its algorithm [1,9-11]. The lack of knowledge about the factors that contribute to the weakening of the adhesive bond and its mechanisms requires not only theoretical justification but also experimental research [7,8,11]. Therefore, this issue is not only an important scientific direction but also a practical challenge, making it both timely and necessary.

AIM

Analysis of the known physicochemical and clinical properties of modern adhesive systems, emerging trends and approaches to their improvement, and prospects for the further development of restorative dentistry.

MATERIALS AND METHODS

The search for relevant publications related to the objective topic was conducted using scientific databases such as Scopus, PubMed, BVS, and Scielo. The following keywords were used: dental hard tissues, ceramic restorations, dental adhesive system, «ethanol wet bonding,» and dental adhesives. The review included original articles, research studies, and official recommendations from medical associations. All collected articles were processed following the principles of content analysis, with subsequent systematization and categorization of the obtained data using CADIMA software.

REVIEW AND DISCUSSION

The evolution of adhesive systems has progressed through the gradual simplification of procedures and the reduction of the number of application steps [2,3]. Self-etch adhesives were developed to eliminate the need for separate dentin etching; however, their effectiveness in removing the enamel smear layer and etching prismatic enamel was found to be insufficient [3]. Consequently, a combined technique emerged, integrating acid etching of enamel with dentin conditioning using a self-etching primer [7,11].

Contemporary research in the field of adhesion focuses on studying a new method of dentin adhesion—ethanol wet bonding. Recent experimental data indicate that the use of ethanol promotes deeper infiltration of dimethacrylates into inter- and intraprismatic spaces, prevents phase separation between hydrophilic and hydrophobic monomers, and strengthens the enamel-adhesive interface [1,11].

According to studies by Ayar M. K. et al. (2019), the application of Single Bond 2 3M ESPE in wet and ethanol bonding techniques on enamel demonstrated a significant difference in bond strength, ranging from 17.4 MPa to 28.7 MPa, respectively [4,8,11]. The traditional dry bonding method involved drying both enamel and dentin, causing collagen fiber collapse and reducing adhesive strength to 5 MPa, which, in turn, did not allow the material to withstand polymerization stress of 24 MPa [2,5,10,11].

Despite the high effectiveness of the wet bonding method, its clinical application remains complex and requires a high level of professional training [9,11]. Further development of adhesive technologies and research into dentin adhesion have led to the simplification of the adhesive application process and the emergence of self-etching systems, which contribute to increased efficiency and predictability of dental treatment.

In modern dental practice, most clinicians successfully apply the dentin self-etching method in combination with enamel etching [4]. An important discovery for the further development of adhesive technologies was the fact of hybrid layer degradation and destruction over time [5].

Further improvement of dentin adhesion has two goals:

I — slowing down the degradation of the adhesive bond by introducing new application techniques and modifying the structure of adhesive systems [4].

II — creating universal adhesives that are less dependent on operator skills and easier to use [4].

The first classification of adhesive systems is based on their order of development and is divided into generations [4,6,8,11-13]. The earliest clinically relevant generation in dentistry is the fourth, which introduced the total-etch technique for enamel and dentin, where orthophosphoric acid, primer, and adhesive were contained in separate containers [5,6,8,13]. The next generation combined the hydrophilic primer and hydrophobic adhesive in one container, which negatively affected their ability to wet the dentin and enamel surfaces. The sixth generation of adhesive systems introduced the concept of self-etching for the first time [10,11,13,14]. The acidic monomers in the primer etch and infiltrate the smear layer of dentin and enamel, followed by the application of the adhesive [2,8,9].

The seventh generation was developed to maximize ease of use for dentists. A single container combining functional and structural monomers can be used in total-etch, selective-etch, and self-etch concepts [1,2,5,9,11,14]. Although combining components with different properties in one container may lead to phase separation of hydrophilic and hydrophobic phases, weakening the adhesive layer's strength, and some researchers indicate the possibility of worsening adhesion in the long term, the seventh generation has gained popularity due to its simplicity and versatility [14].

Selective adhesive systems of the eighth generation are one-step complexes that combine a conditioner, primer, and self-etch adhesive. These formulations also contain nanoparticles that can deeply penetrate dentinal tubules into the formed hybrid layer, preventing its expansion. This mechanism allows for a faster tooth restoration procedure and ensures excellent adhesion of the restorative material to dental tissues [8,9].

In specialized international literature, adhesive systems are classified based on the etching concept and the number of steps in the adhesive protocol [3,4,6,8,10,14,15]. Based on the first criterion, systems are divided into total-etch, selective-etch, and self-etch systems [7,9,11,14-16]. Based on the second criterion, adhesives can be three-step, two-step, and one-step [7].

In three-step adhesives of the fourth and sixth generations: etching, priming, bonding; in two-step adhesives of the fifth and sixth generations: etching, primer and bond combined, or primer bond.

One-step adhesives include those of the seventh and eighth generations, provided they are used in the self-etching technique [5,7,11,12,15,16].

The chemical composition of adhesive systems consists of an acidic component, primer, bond, initiators, stabilizers, fillers, and a solvent [3]. Monomers are organic molecules capable of polymerization. During polymerization, they form a single structure that ensures the strength of the adhesive layer, its retention, and connection with tooth tissues.

There are two main types of monomers:

- Functional monomers contain a functional group and a polymerization group. They are primarily responsible for etching and are used to prime the hydrophilic dentin surface [4,5].
- Structural monomers are generally hydrophobic and form a polymer network during polymerization, significantly improving the adhesive's strength properties [8,12,14,16,17].

Over time, more specialized monomers have been developed that perform functions such as fluoride ion release, antibacterial activity, or enhanced polymerization [16,17]. In adhesive systems of the fifth, seventh, and eighth generations, functional and structural monomers are contained in a single container, while in the fourth and sixth generations, hydrophilic functional monomers are included in the primer, and hydrophobic monomers are in the adhesive [3,5,8,9,11,17,18].

Initiators are substances that activate the polymerization reaction in the adhesive system. The choice between initiation types depends on the intended application of the adhesive system. Systems with chemical initiation of polymerization are used for bonding indirect restorations, while systems with photo-initiators are applied for direct restorations [3,13].

Solvents are included in adhesive systems to improve the wetting of the tooth surface and the diffusion of monomers into the microporous structure of enamel and dentin. According to several studies, partial retention of the solvent deteriorates the mechanical properties of the hybrid layer and increases its degradation rate [3,7,11,14]. Ethanol is a polar solvent, and its low dielectric constant enhances its ability to dissolve less polar substances, such as adhesive system monomers [6]. Another property of ethanol is its ability to form hydrogen bonds with water, facilitating water evaporation from the tooth surface [2,4,6,8,11,18]. Acetone evaporates four times more easily than ethanol, which contributes to improving the structure of the polymerized hybrid layer. However, the evaporation of the solvent from the adhesive container can reduce the material's shelf life [10,11,13,15].

Inorganic fillers are rarely included in adhesive systems due to the increased viscosity of the adhesive caused by filler addition [15,17]. The film thickness increases, which limits the application of adhesive for pre-polymerization before fixing ceramic adhesive restorations [7,8,11,17]. However, greater resistance to polymerization stress due to increased elasticity, higher resistance to degradation, and better load distribution have made such adhesive systems among the most effective [16,18,19].

The surface layer of enamel, composed of randomly arranged hydroxyapatite crystals, is called aprismatic enamel. Aprismatic enamel is significantly more resistant to etching, making it a poor substrate for adhesion. This must be considered when fabricating no-prep ceramic or composite restorations [7,11,15,18-20]. The presence of an aprismatic enamel layer necessitates longer etching, pre-treatment of the tooth surface with rotating diamond instruments, or air abrasion. The enamel smear layer is easily removed using 38% orthophosphoric acid but may act as a barrier to certain self-etch adhesives [4,5,8,9,11].

Most dentists limit tooth tissue preparation to diamond burs of high and low abrasiveness. However, according to specialized national and international literature, the best method for final preparation of enamel and dentin is air abrasion, which achieves a uniformly rough surface while preserving the integrity of enamel prisms [11,12,17,19]. Scanning electron microscopy has revealed the absence of cup-shaped depressions and fractured hydroxyapatite crystals. Furthermore, in cases of delayed cavity filling or bonding of laboratory-fabricated restorations, air-abrasive treatment most reliably removes biofilm and residues of temporary filling materials from tooth tissues [2,6,8,11,15]. Healthy dentin consists of 50% mineral phase, 30% collagen fibers, and 20% water.

As a rule, dentists work with teeth that have previously undergone a carious process and must consider that the structure of caries-infected dentin, caries-demineralized dentin, and sclerotic dentin differs significantly. A minimally invasive approach to cavity preparation requires the preservation of as much of the natural

tooth tissue as possible; therefore, specialists must be knowledgeable about the effectiveness of adhesion to each type of altered dentin [2,4,8,10,19,21].

Infected dentin has undergone destruction of both mineral components and the organic matrix, and the cohesive forces between layers of infected carious dentin are too weak for it to serve as a reliable substrate for adhesion [18,20]. In the structure of demineralized dentin, partial destruction of the organic matrix and disruption of the crystalline structure of the mineral phase occur, leading to the formation of a deeper hybrid layer [2,4,8]. However, the altered structure and depth of demineralization prevent adhesive monomers from properly distributing within the adhesive interface [5,6,11,12,18,19]. Monomers only partially fill the free spaces, making the dentin bond prone to degradation and less durable [6,7,11].

Adhesion to sclerotic dentin is compromised due to dentin's response to external factors, resulting in the formation of acid-resistant tricalcium phosphate crystals in the dentinal tubules. These impede the penetration of adhesive monomers into the dentinal tubules and the depth of the demineralized zone [12,14,16,21,22]. Thus, all caries-affected tissues serve as inferior substrates for adhesion compared to healthy dentin. This is attributed to the exposure of fresh acid molecules to the surface and the washing away of weakened crystals from the aprismatic layer.

Dynamic enamel etching may also have a positive effect when applied to prepared enamel; however, this process requires further investigation. The use of self-etch adhesives on enamel still lacks a consensus within the scientific community. E. Can Say, E. Özel, H. Yurdagüven et al. (2020) reported in their studies that one-step self-etch adhesives exhibit lower bond strength to prepared enamel than two-step and three-step adhesives used in the phosphoric acid etching technique [17,19,26,27]. Conversely, several national and international authors report identical adhesion strength between self-etching and separate etching techniques [24,25,27].

Beyond the immediate adhesion strength following fixation, an important factor is the bond's resistance to masticatory forces. The hybrid layer formed by a self-etch adhesive is significantly thinner and more linear in structure, and its durability under chewing forces requires further investigation [8,14,16,19,20,26].

Two main approaches exist for dentin etching: the first involves using orthophosphoric acid gel [20,25-27]. In this case, complete dissolution of the smear layer occurs, regardless of its thickness (ranging from 1 to 2.4 microns), along with the dissolution of smear layer fragments filling dentinal tubule entrances to a depth of up to 10 microns, as well as complete dissolution of 50% of the inorganic dentin components to a depth of 3–5 microns [17,23,24,27]. Interfibrillar spaces contain a negatively charged proteoglycan hydrogel, which, under prolonged exposure to the enzyme chondroitinase ABC, increases the adhesion strength of Scotch Bond Multi-Purpose and Prime&Bond NT by 49% and 63%, respectively [18].

The second approach involves the use of self-etch adhesives. In this case, the acidic monomers in the adhesive simultaneously etch and prime the dentin surface, and since there is no rinsing phase, the etching by-products remain on the surface [15,25]. Modern self-etch adhesives are classified as weak-acid (pH ≥ 2), medium-acid (pH = 1.5), and strong-acid (pH ≤ 1). Weak-acid adhesives superficially etch dentin, leaving hydroxyapatite crystals between collagen fibers, so dentinal tubules are only partially freed from the smear layer, forming a hybrid layer thinner than one micron [14,15,19,22,25]. Medium-acid adhesives cause deeper demineralization of dentin and partially penetrate the dentinal tubules, while strong-acid adhesives penetrate dentinal tubules entirely, forming a hybrid layer comparable to that of three-step adhesives in the total-etch technique.

Aside from the chemical composition of the adhesive, the etching technique significantly influences its effectiveness. Static etching involves applying acid for a set time without additional actions, whereas dynamic etching involves acid application followed by activation with a brush inside the cavity. According to Meerbeek BV et al. (2020), etching activation can increase the aggressiveness of the self-etching primer, positively affecting adhesion to dentin with a thick smear layer [17,21].

The original enamel bonding technique, proposed by Buonocore, was dry bonding [27]. This technique continued to be used even after the introduction of fourth- and fifth-generation adhesive systems in the total-etch technique. Researchers initially assumed that a dry cavity was necessary for effective adhesion, and etched enamel was expected to have a characteristic chalky appearance. At the time, it was not yet known that drying etched dentin led to collagen fiber collapse and a reduction in interfibrillar spaces, which are essential channels for adhesive monomer infiltration [10,24,26-28].

The adhesion strength to collapsed dentin is only 5 MPa and cannot withstand polymerization stress of 24 MPa, leading to debonding of one of the cavity walls, microleakage, and various complications such as postoperative sensitivity, secondary caries, and restoration failure [24,28,29].

As a solution to dentin adhesion issues, J. Kanca (1992) proposed the wet bonding technique [13,20,25]. The liquid remaining in the cavity is gently dispersed or dried using an aspirator, sponge, or paper points, maintaining the dentin and enamel in a slightly moist condition [5,7,11].

Differences in drying methods were analyzed by Magne P. et al. (2008), who found no statistically significant difference between cavity drying with an air stream or an aspirator when using the fourth-generation adhesive system OptiBond FL [11].

Thus, collagen fibers released during etching remain extended, maximizing the dentin surface area and opening access to dentinal tubules. The hydrophobic monomers of older adhesive systems cannot penetrate deep into moist demineralized dentin; therefore, more hydrophilic monomers, which are part of fourth-generation primers and fifth- and seventh-generation adhesives, are used for this purpose [18,22,29,30]. The wet bonding technique significantly increases the hybridization area of dentin; however, moisture control remains one of the most technique-sensitive processes in adhesive dentistry.

The primer (fourth generation) or adhesives (fifth and seventh generations) contain a solvent that must fully evaporate, as residual solvent creates voids and water channels in the polymerized hybrid layer. This phenomenon reduces the adhesive's mechanical properties and resistance to hydrolysis [24,25,30]. According to research by M. Toledano et al. (2022), the immediate tensile strength of fourth- and sixth-generation adhesives significantly decreases under conditions of incomplete solvent evaporation [18,22,26,29]. When using a fourth-generation adhesive, the next step is applying a bond consisting of large hydrophobic molecules without a solvent, which is then air-blown to form a uniform thin film [8,11,21,26,29].

Diego Spreafico et al. (2018) studied the effect of strong (0.68 MPa) and weak (0.12 MPa) air-blowing on self-etch adhesives and found minimal impact on the adhesion strength of Clearfill SE and G-Bond adhesives. However, they noted a thinner adhesive layer when weak air-blowing was used.

In a study of samples prepared with one-step adhesives G-Bond and Prompt L-Pop, where strong air-blowing was used, scanning electron microscopy revealed areas of dentin with exposed collagen but without a hybrid layer. This phenomenon is likely associated with excessive removal of the adhesive layer from the dentin surface [25,26,29,30]. The observed effects are explained by monomer separation from water as the solvent evaporates, leaving water droplets trapped in the polymerized adhesive layer, significantly weakening its mechanical properties [11,19,21,25].

These issues were not observed when using the two-step adhesive Clearfill SE, which features separate application of hydrophilic and hydrophobic components, leading to better compatibility with the dentin surface primed by the primer. The final stage of adhesive preparation—polymerization—depends on the functional and structural monomers in the adhesive system, the photo-initiator, environmental moisture, and the type and intensity of the curing light [26,27,30,31].

Apart from potential errors in applying adhesive systems, the quality of bonding is also affected by enzyme-induced hydrolysis and degradation of the polymer and collagen fibers in the hybrid layer [13,16,17,22,26,31]. Matrix metalloproteinases (MMPs) play a crucial role in both physiological and pathological metabolism of collagen-based tissues. They participate in tooth tissue formation, but after mineralization, the enzyme becomes covered with a layer of hydroxyapatite crystals, which prevents its movement and renders it inactive [17,19,22,26,31].

According to C. Sabatini et al. (2022), acid etching followed by adhesive application increases MMP activity and triggers the degradation process of demineralized and weakly hybridized collagen [8,13,19,25,31]. The degradation rate can be reduced by using proteolytic activity inhibitors such as chlorhexidine, benzalkonium chloride, ethanol, and other substances, as well as by ensuring more complete infiltration of demineralized dentin with polymer [5,6,16,22,29,31].

One of the modern methods for improving dentin hybridization is the use of a 95% ethanol solution for treatment before primer application [12,14,18,22,26,30,31]. D. Pashley et al. (2017) demonstrated the positive effect of ethanol in their studies, describing the process of breaking down the proteoglycan gel between collagen fibers, which facilitates deeper penetration of hydrophobic monomers [29,31]. However, several other studies found no significant differences compared to conventional bonding techniques, and the ethanol-based adhesive protocol still requires further investigation [23,25,28,30].

Recent studies on traditional methods of dentin preparation using diamond and carbide burs have shown differences in smear layer thickness, ranging from 2.4 microns when using coarse-grain diamond burs to 1 micron with fine-grain diamond burs and carbide burs [22]. The method of preparation plays a significant role in the use of self-etch adhesive systems, as a thinner smear layer dissolves more easily under the influence of acidic monomers [1,3,7,15,18,21,23].

Currently, laser preparation techniques exist, with the undeniable advantage of virtually eliminating the

smear layer. However, damage to the microstructure of dentin, associated with the destruction of organic molecules and the formation of microcracks, actually weakens adhesion to the laser-treated surface [19,20-24]. Air abrasion, ultrasonic, and sonic preparation methods create a thinner smear layer on the dentin surface compared to treatment with medium-grit burs and leave the surface more intact and uniform. The margins of cavities formed using air-abrasion and sonic instruments demonstrate better long-term adhesion stability [17,19].

For indirect adhesive ceramic restorations, adhesion to both the hard dental tissues and the ceramic surface is equally crucial [16-18,27]. Today, three primary methods are used for ceramic surface preparation before bonding: sandblasting, etching, and silanization [18,26].

Air abrasion with aluminum oxide particles under controlled pressure is used in traditional ceramic restoration techniques, such as pressed ceramics and refractory die fabrication, to remove investment residues [24,25,27,28]. Several scientific studies have confirmed the effectiveness of air abrasion in enhancing adhesive bond strength. However, some ceramic manufacturers still prohibit sandblasting in their material usage instructions [6,14,16,26].

Another method, etching ceramics with hydrofluoric acid of varying concentrations, is a widely accepted technique for preparing surfaces for adhesive bonding. Among clinicians, there is ongoing debate regarding dynamic etching, where hydrofluoric acid gel is actively distributed across the internal surface of the restoration throughout the exposure time. However, no studies have yet demonstrated a significant advantage of this technique [7,28].

The third method, silanization, involves applying a substance that bonds to ceramic on one side and to dental polymers on the other. This technique has evolved with the introduction of a new material, Monobond Etch & Prime, which combines etching and silanization. The specific features of its application require further study [22,24,26,28].

Analysis of publication trends indicates a sustained interest in the problem of bonding zirconia restorations, which remains an active area of research. In addition to strengthening the bond between polymers and ceramics, studies have also focused on the longevity of this adhesion. The durability of the bond between zirconia-based ceramics and polymer has been investigated in numerous studies, with findings indicating that it largely depends on the surface treatment method of zirconia.

All chemical methods for improving zirconia adhesion can be broadly classified into two groups:

1. Application of a silicate coating using various techniques (selective infiltration etching, pyrochemical methods, and magnetron sputtering deposition).
2. Application of chemical cross-linking agents, such as bicon – methacryloxydecyl dihydrogen phosphate (MDP) and other monomers or silanes [7,11,17,22,24,25].

According to the literature, adhesive systems containing phosphate monomers provide more reliable adhesion than silica-based or silane coatings on zirconia. Studies have confirmed that the MDP monomer enhances the adhesion strength of polymer cement to zirconia due to the formation of chemical bonds (P=O, OH=Zr), and even ionic bonds. MDP monomer is considered the most effective agent for reliable fixation of zirconia-based prostheses.

Despite extensive research, the challenge of improving the adhesion of polymer cement to zirconia and extending the longevity of bonded restorations remains unresolved. New methods for zirconia ceramic surface preparation offer the potential to enhance the bond strength between polymers and zirconia. However, these techniques remain expensive and inaccessible to most practitioners. None of the innovative bonding techniques function effectively without the use of an MDP primer [8,9,11,13,20,22].

One type of etching of zirconium ceramics is laser impregnation with a high-energy laser to melt and re-harden the surface by creating small holes to increase the mechanical strength of the zirconium. and resin. Lasers that are often tested are the Er:YAG laser, the Nd:YAG laser, and the carbon dioxide (CO2) laser [17,18].

Ma Yonggang and other studies confirmed that the performance value of the three laser-treated ceramics was significantly lower than that of the control group, and there was no difference between the three statistically significant. Laser etching has a significant impact on improving the bond between ceramic and resin. However, this technique does not interfere with the flow of energy to enhance the value of communication. The adhesion of laser-etched zirconium dioxide ceramics and resin-based paints is significantly reduced after staining for 6 months. Also, I'm dreaming technology for processing the surface of NobelBond ceramics, which was used to bond the surface with zirconium at the end of the day [25,26]. The principle is that the surface of the previously sintered or the surface of the sintered zirconium frame, after cutting, is coated with a suspension, where the zirconium dioxide powder is placed and cured, and after sintering, The solution then unfolds, creating pores on the surface with zirconium. Phark et al. The value of zirconium dioxide after NobelBond and sand blasting was determined. The results show that the first one has a high vitality immediately after the old

one and the rest, and the last one has a high value after the one-piece thermal cycle of the old one. At the same time, the surface of the zirconium porcelain is coated with NobelBond and does not require sandblasting. Since the technology is still new, the assessment of effects will require further verification [22,25].

According to current research, 15 seconds of perforation of the prepared enamel surface produces adhesive fixation similar to that of 60 seconds. F. R. Tay and D. H. Pashley et al., 2019, provide evidence that activation of the mordant gel with a pencil leads to more uniform and uniform penetration of aprismatic enamel [11,18,20,22,25].

Clinical adhesion studies are carried out on the basis of previously established light practice criteria, the United States Public Health Service (USPHS) sees five evaluation criteria: 1) Retention of the restoration. (indicated by the presence or loss of restoration; 2) Damage to the marginal fit, which is indicated by the use of a dental probe and a probe; 3) Maybe change the color between the restorations; 4) Visible carious boundary defect; 5) post-operative sensitivity (visible with a spray from a distance of 2-3 cm while the other teeth are covered with cotton rolls) [13,26,30].

Nathaniel C. Lawson et al., 2019, examined the effectiveness of restorations in non-carious lesions according to Black class 5, with the use of Scotch Bond Multi-Purpose adhesives, Scotch Bond Universal self-protrusion, Scotch Bond Universal total protrusion2 136]. After 24 months, the final restoration was assessed according to the criteria of Cvar and Ryge 1) Regional jurisdiction. 2) Farbuvannya cordon. 3) Secondary caries. 4) Sensitivity, and a statistically significant difference was found between the three adhesive techniques [18,26,29,31].

Van Meerbeek B. et al., 2020, indicate that laboratory studies of the adhesive interface give us the ability to accurately measure the strength of the bond, however, the weakest adhesive bonding forces can clinically effectively eliminate the function of a worn-out restoration in an empty tooth in patients with low risk [9,11,18,30,32,33]. The clinical effectiveness of adhesive restoration lies not only in the solidification of the correct adhesive and adhesive protocol, but also in the mechanical preparation of tooth tissue and the surface of ceramics, empty form, Isolation of the working field from the line, forms of restoration and occlusal interactions with antagonist teeth [29,32,34,35]. An equally important factor is the choice of dental cement and the method of preparing the restoration material before fixation [28,30,36].

CONCLUSIONS

The content analysis of scientific publications conducted in this study highlights the presence of numerous unresolved issues in the field of adhesive dental technologies, necessitating further scientific inquiry and experimental research. Current investigations focus on optimizing the composition and structural properties of adhesive systems, integrating experimental functional monomers with antibacterial activity and enhanced chemical interactions with dental tissues, as well as developing substances capable of prolonging the inhibition of hybrid layer degradation.

An important area of ongoing research involves the refinement of adhesive protocols to facilitate deeper and more effective infiltration of demineralized dentin. This aspect is considered a critical determinant of adhesive bond longevity and, therefore, warrants prioritized study.

The systematization and standardization of knowledge regarding tooth tissue preparation, adhesive application techniques, and potential procedural errors and complications are essential for advancing the quality of dental care. Within the scope of the findings obtained and in accordance with the objectives of departmental research, it is feasible to establish optimal parameters for air-abrasive treatment of ceramic surfaces and dental hard tissues, assess their impact on adhesive bond strength, and investigate the influence of etching gel application methods and exposure duration on the micro-roughness of ceramic and dental tissue surfaces.

Furthermore, the study aims to evaluate the effects of various factors on adhesive bond strength within the ceramic/composite material system and to refine adhesive surface preparation protocols for both dental and ceramic substrates. The implementation of these advancements is expected to substantially reduce the risk of treatment-related complications, enhance the reliability of adhesive bonding, and improve the long-term success of ceramic restorations for the rehabilitation of dental hard tissue defects.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

Fourth generation rights in healthcare: Theoretical and practical aspects of implementation

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ABSTRACT

Aim: To comprehensively analyze the theoretical and practical aspects of the implementation of fourth-generation rights in the field of healthcare in Ukraine.
Materials and Methods: This study examines Ukraine's Constitution, codes, laws, and regulations, along with international treaties, European Court of Human Rights decisions, and scholarly works on fourth-generation human rights.
Conclusions: Fourth-generation healthcare rights represent a novel legal phenomenon emerging from the biomedical revolution, encompassing legal opportunities for individuals to utilize modern medical technologies for personal needs. In Ukraine, the implementation system remains underdeveloped in both regulatory and institutional mechanisms. Effective implementation requires balancing private rights with public interests, aligning national legislation with international standards, and establishing functional institutional mechanisms—particularly crucial in today's global challenges.

KEY WORDS: human rights, fourth generation of rights, health care, biomedical technologies, mechanisms for the implementation of rights

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INTRODUCTION

In times of rapid development of biomedical technologies and global challenges in the field of health care, the concept of fourth-generation human rights is gaining particular relevance. These rights focus on the ethical aspects of the use of new technologies that directly affect the human body, genetic structure and the very definition of the boundaries of life. They cover a wide range of issues: from the right to organ transplantation and access to advanced treatment methods to the right to euthanasia and protection from genetic discrimination.

Fourth-generation rights in the field of health care are formed at the intersection of bioethics, legislation and medical practice, creating a new dimension of human legal protection in the context of the biotechnological revolution. Unlike previous generations of human rights, these rights arise in response to the opportunities and risks associated with genetic engineering, reproductive technologies, neuroscience and other fields that allow modifying human nature. They require a rethinking of traditional ethical paradigms and the creation of new regulatory mechanisms.

The peculiarity of fourth-generation human rights in the field of health care lies in their global nature and inevitable impact on future generations. The issues of access to genetic data, control over one's own body, the right to genetic integrity and information privacy in the medical field are becoming central in the international discourse on human rights. These rights go beyond national borders and require a coordinated international approach that takes into account cultural and ethical diversity, but at the same time protects universal human values in the era of revolutionary changes in medicine and biotechnology.

AIM

The purpose of the study is to comprehensively analyze the theoretical and practical aspects of the implementation of fourth-generation rights in the field of health care in Ukraine.

MATERIALS AND METHODS

To achieve the goal, a complex of general scientific and special research methods was used in the work: the

dialectical method allowed us to consider fourth-generation rights in their development and interconnection; the formal-legal method was used in the analysis of regulatory legal acts at the national and international levels; the system-structural method helped to identify the relationships between the elements of the system for implementing fourth-generation rights; the comparative legal method was used to compare approaches to regulating fourth-generation rights in different legal systems; the historical-legal method allowed us to trace the evolution of the concept of human rights and the formation of fourth-generation rights.

REVIEW AND DISCUSSION

First of all, we note that the fourth generation of human rights in the field of health care include such rights as: gender transformation; the use of artificial insemination methods; receiving transplantation services; the use of cloning technologies; freedom of choice of gender self-identification; autonomy in sexual life; free choice of a partner regardless of sex; reproductive choice, including abortion; modification of one's own body; participation in organ and tissue donation programs; posthumous donation; participation in surrogacy or paternity programs; the right to a dignified end to life through euthanasia; access to genetic modifications; the use of artificial intelligence technologies in medicine; unhindered access to environmental information; the right to obtain data on the sanitary and epidemiological state.

The Constitution of Ukraine enshrines that our state is a legal, social and democratic state. This constitutional norm establishes a fundamental principle according to which the key characteristic of a democratic and legal state is the presence of effective and practically operating mechanisms for ensuring the rights and freedoms of man and citizen. However, it is worth noting that some legal experts hold the opinion that the mechanisms for ensuring the rights and freedoms of citizens remain underdeveloped. This applies to both the sphere of preventing and combating corruption and other spheres of public life. In their opinion, these mechanisms are still at the initial stage of formation, which leads to an unacceptably low level of guaranteeing the implementation of the relevant rights of citizens. This situation creates a gap between the constitutional ideal and the real state of human rights protection in Ukraine, which requires further improvement of legislation and law enforcement practice [1].

The mechanism of legal regulation in the sphere of ensuring fourth-generation rights in the healthcare system should be understood as a complex system of legal instruments that influence relations in the sphere of fourth-generation rights and organize them in accordance with the goals and objectives defined by society and the state. At the same time, when studying the mechanism of legal regulation in this area, it is important to distinguish two key components that are crucial for the proper functioning of this mechanism: ensuring the rights and legitimate interests of individuals as subjects of fourth-generation rights in the field of health care; ensuring the public interest of the state and society as a whole in the implementation and protection of these rights. It is the balance and proper implementation of these two components that allow us to objectively assess the effectiveness or ineffectiveness of the relevant mechanism of legal regulation. When both components function harmoniously, we can talk about the effectiveness of legal regulation in the field of ensuring fourth-generation rights in the health care system. The object of legal regulation in the field of ensuring fourth-generation rights covers a complex of relations and phenomena that arise in the process of implementing fourth-generation rights, as well as legal norms that regulate these relations. Such relations, according to S. Boldizhar, in particular, we can include those that have been formed regarding the implementation of: the right to cloning, the right to sex change, the right to artificial insemination, the right to transplantation, the right to euthanasia, the right to gender identity, the right to sexual orientation. Each of these categories of rights forms a separate cluster of legal relations that requires specific legal regulation, taking into account both the private interests of the subjects of these rights and the public interests of the state and society as a whole [2].

In accordance with Article 6 of the Law of Ukraine "On the Application of Transplantation of Anatomical Materials to Humans" dated May 17, 2018, the subjects of the administrative and legal regulation mechanism in the field of ensuring fourth-generation rights regarding the human right to transplantation include: the Cabinet of Ministers of Ukraine, the Central Executive Body that ensures the formation and implementation of state policy in the field of healthcare, the central executive body that implements state policy in the field of providing medical care using transplantation and carrying out activities related to transplantation, healthcare institutions that have a license to conduct economic activities in medical practice, which provides for the right to provide medical care using transplantation and/or carrying out activities related to transplantation, according to the list approved by the central executive body that ensures the formation and implementation of state policy in the field of healthcare, the Bureau of Forensic

Medical Examination and other business entities that carry out activities related to transplantation, transplant coordinators. At the same time, this list defines the key institutional entities that ensure the functioning of the system of transplantation of anatomical materials in Ukraine and the implementation of the corresponding human right [3].

However, the specified list needs to be supplemented, because it lacks two key entities: recipients (individuals who receive transplants in the form of cells, tissues, organs or their parts) and donors (individuals who voluntarily provide their cells, tissues, organs or their parts for transplantation to recipients). The importance of including these entities in the list is due to the fact that ensuring their fundamental rights to life and health is the main goal and purpose of the entire mechanism of administrative and legal regulation in the field of fourth generation rights [2].

Thus, the term "fourth generation of human rights in the field of health care" can be interpreted as modern rights of the individual in the field of medicine and health care, which have been reflected in national legislation and/or international legal acts. These rights are aimed at creating appropriate conditions for the implementation of the relevant legal opportunity provided for by legal norms, by citizens of a particular state, foreign citizens, stateless persons - both directly and through organizations founded by them, as well as by other authorized entities. The main purpose of these rights is to ensure an adequate level of health care, an environmentally safe environment, sanitary and epidemiological well-being, as well as the realization of the needs and interests of the individual in this important area of public relations.

Based on the above definition of the concept of "fourth generation of human rights in the field of health care", we can distinguish the following characteristic features of this legal phenomenon: these are innovative rights of the individual in the medical field, which have received legal support both in national legal systems and/or at the level of international law; the implementation of these rights is available to a specifically defined circle of subjects - citizens of the relevant country, foreign citizens, stateless persons (independently or through organizations formed by them), as well as other subjects authorized in accordance with the norms of law that establish the parameters of possible behaviour; a prerequisite for the practical implementation of fourth-generation rights in the medical field is the presence of current legal norms that determine the boundaries and procedure for exercising the relevant rights; the implementation of the opportunities provided for by legal norms is carried out by authorized subjects in order to protect health, ensure an environmentally safe environment, proper sanitary and epidemiological condition, as well as to meet their own medical needs and interests.

Within the framework of the study of the concept of the "fourth generation of human rights in the sphere of health care", it is important to consider the essence of the term "realization of law". Legal science demonstrates a variety of approaches to understanding this concept. In particular, L. Vasylchuk and Yu. Bysaga interpret the realization of law as the practical implementation of the provisions of current legal norms in the activities of citizens, legal entities, state authorities, officials and other subjects of legal relations [4]. This definition emphasizes the dynamic aspect of law, when regulatory provisions move from a state of potential possibility to a state of real action through specific actions and decisions of subjects of law. In the context of the fourth generation of human rights in the sphere of health care, this is of particular importance, since the practical implementation of such new rights often requires not only legal consolidation, but also the development of special implementation mechanisms, taking into account their innovative nature and ethical complexity. A. Kolodiy and A. Oliynyk offer a slightly different approach to understanding the implementation of rights and freedoms. They define this phenomenon as a special form of the existence of rights, which consists in the transformation of social benefits enshrined in legal norms into a state of their potential and actual use by a specific person or group of persons to satisfy various individual needs and interests [5]. This approach, in our opinion, emphasizes the important transition from the formal consolidation of the right to its real implementation in life through the use of the corresponding social benefits. In the context of the fourth generation of rights in the field of health care, such an understanding of the implementation of the right emphasizes the need to create not only legal, but also practical mechanisms for access to the latest medical technologies, procedures and opportunities that constitute the content of these rights. Human rights, in particular in the field of health care as their integral component, are most fully reflected through the construction of subjective rights of the individual. A subjective right is a measure of possible behavior of a person (citizen or organization) guaranteed by law, which is aimed at achieving certain goals directly related to the satisfaction of his legitimate interests [6]. This concept of subjective right is particularly important for understanding fourth-generation rights in the field of health care, as it emphasizes their individual nature and connection with the personal interests of a person. Subjective right provides a person with the legal oppor-

tunity to act in a certain way to realize their interests in the field of using the latest medical technologies, methods of treatment or body modification, which constitute the essence of fourth-generation rights.

The implementation of human rights means the practical implementation of the opportunities provided by legal norms in order to meet the individual needs and interests of the relevant subjects. When considering the concept of "implementation of human rights", it is worth focusing on the practical aspect of using legal opportunities enshrined in regulatory acts. Accordingly, the implementation of fourth-generation rights in the health care system is a process of practical application of opportunities established by legal norms, aimed at ensuring an adequate level of health care and meeting personal needs and interests in the medical field.

The implementation of fourth-generation rights in the field of health care is an integral element of the general system of implementation of human rights. In the framework of our study, it is especially important to determine the forms of implementation of fourth-generation rights in the field of health care. In our opinion, the form of implementation of fourth-generation rights in the field of health care can be interpreted as an external manifestation of the practical implementation of the latest human rights in the field of health care, enshrined in national and/or international legislation, which are aimed at creating conditions for the implementation of relevant legal opportunities established by legal norms, in order to ensure an adequate level of health care, an environmentally safe environment, sanitary and epidemiological well-being, as well as meeting individual needs and interests in the medical field [7].

Within the framework of studying the specified topic, it is also appropriate to consider the system of implementation of fourth generation rights in the sphere of health care, which is an integral element of the comprehensive system of ensuring human rights in the state. In our opinion, the system of implementation of fourth generation rights in the health care system as an integral component of the integral system of implementation of human rights is an internally organized set of regulatory and institutional elements aimed at effectively ensuring the implementation of opportunities defined by the norms of law in order to ensure health care, safe for life and healthy environment. Based on the above definition of the concept of "system of implementation of fourth generation rights in the sphere of health care", the following key elements of the system of implementation of fourth generation rights in the sphere of health care can be distinguished: normative component - a set of legal norms that enshrine fourth generation rights and regulate the procedure for their implementation; institutional component - a network of state bodies, institutions and health care institutions that ensure the practical implementation of rights; procedural component - established mechanisms and algorithms for the implementation of relevant rights; guarantee component - a system of legal and organizational guarantees that ensure the implementation of fourth-generation rights; control component - mechanisms for supervision and control over the observance of rights; protective component - a system of legal remedies in the event of a violation of fourth-generation rights in the field of health care; regulatory system for the implementation of fourth-generation rights in the field of health care; institutional system for the implementation of fourth-generation rights in the field of health care [8].

Below we will analyze the main provisions of each of these components of the system for the implementation of fourth-generation rights in the field of health care.

The regulatory system for the implementation of fourth-generation human rights in the field of health care consists of two main components: a regulatory subsystem for ensuring fourth-generation human rights in the field of health care in accordance with domestic law; a regulatory subsystem for ensuring fourth-generation human rights in the field of health care in accordance with international law. At the same time, the regulatory subsystem for ensuring fourth-generation rights in the field of health care under domestic law includes the following components:

Constitution of Ukraine of June 28, 1996;

codified acts of Ukraine, in particular: the Civil Code of Ukraine of January 16, 2003, the Criminal Code of Ukraine of April 5, 2001 and others;

Laws of Ukraine regulating the implementation of fourth-generation rights in the field of health care, in particular: the Law of Ukraine "On the Commissioner of the Verkhovna Rada of Ukraine for Human Rights" of December 23, 1997, the Law of Ukraine "On the Application of Transplantation of Anatomical Materials to Humans" of May 17, 2018;

subordinate regulatory legal acts in the field of ensuring fourth-generation human rights in the field of health care, in particular: the Resolution of the Verkhovna Rada of Ukraine "On the Principles of State Policy of Ukraine in the Field of Human Rights" of June 17, 1999, the Resolution of the Cabinet of Ministers of Ukraine "On the Implementation of Article 281 of the Civil Code of Ukraine" of February 15, 2006 No. 144.

The regulatory system for the implementation of fourth-generation rights in the field of health care in Ukraine under international law includes the following

components:

UN international treaties ratified by Ukraine that regulate fourth-generation rights in the field of health care, including: the Universal Declaration of Human Rights of December 10, 1948, the International Covenant on Civil and Political Rights of December 16, 1966, the International Covenant on Economic, Social and Cultural Rights of December 16, 1966, the Aarhus Convention on Access to Information, Public Participation in Decision-Making and Access to Justice in Environmental Matters of June 25, 1998;

international treaties of the Council of Europe, to which Ukraine has acceded, on ensuring the rights of the fourth generation in the field of health care, in particular: the Convention for the Protection of Human Rights and Fundamental Freedoms of November 4, 1950, the revised European Social Charter of May 3, 1996, the Convention on Human Rights and Biomedicine of April 4, 1997;

international documents of the OSCE, to which Ukraine is a party, on the implementation of the rights of the fourth generation in the field of health care: the Helsinki Final Act of August 1, 1975, the Charter of Paris for a New Europe of November 21, 1990, the Budapest Declaration of December 6, 1994;

bilateral international agreements of Ukraine on the implementation of fourth-generation rights in the medical sphere: Agreement between the Ministry of Health of Ukraine and the Ministry of Health of Azerbaijan on medical cooperation dated March 24, 1997, Agreement on cooperation in the field of health care between the Ministry of Health of Ukraine and the relevant ministry of Georgia dated June 25, 2013;

international treaties within the CIS, to which Ukraine is a party, relating to ensuring the right to a safe environment: Agreement on cooperation in the field of public health care dated June 26, 1992, Agreement on ecological interaction dated February 8, 1992;

practice of the European Court of Human Rights, in particular decisions in the cases of "Grymkovskaya v. Ukraine", "Gray v. Germany", "Petrova v. Latvia", "Women on Waves and others v. Portugal".

Therefore, the regulatory system for the implementation of fourth-generation rights in the field of health care represents a mutually agreed upon holistic set of norms and principles of law enshrined in relevant documents of domestic and international law, which is aimed at effectively ensuring the proper practical implementation of the opportunities established by legal norms to ensure health care, create an environment safe for life and health, achieve proper sanitary and epidemiological well-being, as well as meet the own needs and interests of citizens in this important area.

The institutional system for the implementation of fourth-generation rights in the field of health care is structurally divided into two interconnected blocks: the national institutional system for ensuring fourth-generation rights in the field of health care; the international institutional system for ensuring fourth-generation rights in the field of health care. The institutional system for the implementation of fourth-generation rights in the field of health care unites authorized entities whose activities are aimed at effectively ensuring the proper practical implementation of the opportunities enshrined in legal norms in order to guarantee health care, create an environmentally safe environment, maintain proper sanitary and epidemiological conditions, as well as meet individual needs and interests in the medical field. The current stage of Ukraine's development in the context of establishing the principles of democracy and the rule of law necessitates a significant improvement in the mechanism for ensuring the implementation of human rights, including fourth-generation rights in the field of health care. We believe that proper implementation of fourth-generation rights in the field of health care is an integral component of the mechanism for the effective functioning of the entire system of natural human rights in Ukraine [9].

The issue of implementation and protection of these new rights is becoming particularly relevant in the context of the rapid development of biomedical technologies and global challenges in the field of health care. Effective implementation of fourth-generation rights requires not only proper regulatory consolidation, but also the creation of effective institutional mechanisms that will ensure the practical implementation of these rights in life.

State and international policy on ensuring the proper implementation of fourth-generation rights in the field of health care serves as a fundamental basis not only for the comfortable existence of people, the development of civil society, the establishment of the principles of tolerance and respect for human rights, but also as a guarantee of further sustainable development of humanity in difficult modern conditions.

In the era of globalization and digitalization, rapid scientific and technological progress, and potential risks of losing control over artificial intelligence, proper provision of these rights is of critical importance. The effective implementation of fourth-generation rights in the field of health care is directly related to the preservation of a favorable living environment both in individual regions and countries and on planet Earth as a whole.

This issue is particularly relevant in the context of the existing common environmental and biological challenges facing humanity - in particular, the problems of

global warming and the coronavirus pandemic, which demonstrate the interdependence of the right to health care, environmental rights, and new human rights in the biomedical field.

The fourth generation of human rights in the field of health care represents the new rights of the individual in the medical field, which have found their consolidation in domestic and/or international law. These rights are aimed at creating appropriate conditions for the practical implementation of the relevant legal opportunities defined by the norms of law in order to ensure human well-being, as well as to meet their individual needs and interests in the field of health care.

CONCLUSIONS

Summing up the study of fourth-generation rights in the field of health care, it should be noted that they represent the latest human rights that have emerged in response to the rapid development of biomedical technologies and the global challenges of our time. These rights cover a wide range of opportunities: from organ transplantation, artificial insemination and gender transformation to euthanasia, cloning and the use of artificial intelligence technologies in medicine. A feature of fourth-generation rights is their global nature and direct impact on future generations, which requires a rethinking of traditional ethical paradigms and the creation of new regulatory mechanisms at the national and international levels.

The system for implementing fourth-generation rights in the field of health care is a complex internal structure that includes a regulatory subsystem (national legislation and international treaties) and an institutional subsystem (national and international bodies and organizations). The effective functioning of this system depends on the balance of two key components: ensuring the rights and legitimate interests of subjects of fourth-generation rights and ensuring the public interest of the state and society. It is important to note that despite the constitutional guarantees, the mechanisms for ensuring these rights are still at the stage of formation, which creates a gap between the legal ideal and the actual state of human rights protection.

The proper implementation of fourth-generation rights in the field of health care is an integral part of the general system of human rights and is of great importance not only for the comfortable existence of individuals, but also for the sustainable development of humanity as a whole. In the context of globalization, digitalization, and rapid scientific and technological progress, the effective provision of these rights is becoming critical for preserving a favorable living environment both in individual regions and countries and on the planet as a whole. This is especially relevant in the context of environmental and biological challenges, which clearly demonstrate the interdependence of the right to health, environmental rights, and emerging human rights in the biomedical sphere.

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CONFLICT OF INTEREST
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REVIEW ARTICLE

CONTENTS

The clinical and pathogenetic manifestations of gastroesophageal reflux disease and obesity and approaches to their diagnosis, treatment, and prevention: current state of the problem (literature review)

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ABSTRACT

Aim: The purpose of this study is to review the literature to determine the common pathogenetic mechanisms (PM) between gastroesophageal reflux disease (GERD) and obesity, as well as to analyze the impact of one disease on the other, followed by a review of the basic principles of diagnosis and treatment of patients with a combination of these comorbidities.

Materials and Methods: The literature review included an analysis of articles from the Scopus and Web of Science databases, with a focus on pathogenesis, clinical data, diagnosis and treatment of GERD and obesity. The following keywords were used to find relevant materials on the research topic: "GERD", "obesity", "pathogenesis", "treatment", "clinical trials".

Conclusions: A series of studies have shown that there are risk factors that increase the development of GERD: stress, bad habits, excessive body mass index (BMI), advanced age and lifestyle. At the same time, obesity has similar risk factors to GERD, which in turn prompts the search for correction of common PM, new ways of diagnosis and comprehensive personalized treatment.

KEY WORDS: gastroesophageal reflux disease, obesity, pathogenesis, diagnostics, treatment

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INTRODUCTION

According to the World Gastroenterology Organization (WGO), gastroesophageal reflux disease (GERD) is defined as symptoms of reflux, erosive esophagitis, which contribute to a person's quality of life (QOL) (work performance, sleep quality), as well as complications lasting one or more days a week, resulting from retrograde backflow of gastric contents into the esophagus, oropharynx, and/or airways [1].

GERD is a "disease of the XXI century," as it is characterized by the WGO, based on epidemiological data that show that it affects 20 to 50% of the population of different countries [1]. GERD is one of the leading causes of decreased QOL, disability, and the development of a number of complications [2]. The Ukrainian Association of Gastroenterologists began statistical registration of GERD in Ukraine in 2009, and as of 2017, the prevalence was 190 cases per 100,000 people [3].

Obesity is a chronic disease characterized by excessive body fat, which can lead to an increased risk of lipid and carbohydrate metabolism changes and can contribute to the development of type 2 diabetes, cardiovascular disease, and increase the risk of certain cancers and other health problems [4].

According to the World Health Organization, in 2022, one in eight people in the world was obese (about 890 million were obese), while 43% of adults aged 18 years and older were overweight (2.5 billion adults) [4].

Thus, GERD and obesity are one of the most common chronic diseases in the modern world, which can aggravate each other's course through pathogenic links [1, 4].

AIM

The purpose of this study is to review the literature to determine the common pathogenetic mechanisms (PM) between GERD and obesity, as well as to analyze the impact of one disease on the other, and to consider the basic principles of diagnosis and treatment of patients with a combination of this comorbid pathology.

Table 1. Common and distinctive RF for obesity and GERD

RF[1]	Obesity	GERD[2]
I. FRs that cannot be modified		
Age [7]	+	+
Gender [8]	+	+
Genetic predisposition [9]	+	+
II. FRs subject to modification		
Physical inactivity [7]	+	+
Unbalanced diet [11]	+	+
High body mass index [10]	+	+
Abdominal obesity [12]	+	+
Smoking [13]	+	+
Alcohol consumption [14]	+	+
Stress [15, 16]	+	+
Influence of internal abdominal pressure [17]	-	+
Use of non-steroidal anti-inflammatory drugs [18]	-	+
Living conditions (socio-economic and environmental reasons) [7]	+	+

Note: [1] – RF – risk factor, [2] – GERD - gastroesophageal reflux disease.

MATERIALS AND METHODS

The literature review includes the analysis of articles from the scientometric databases Scopus and Web of Science, with a focus on the pathogenesis, diagnosis and treatment of GERD and obesity. The following keywords were used to find relevant materials on the research topic: "GERD", "obesity", "overweight", "pathogenesis", "treatment". All selected sources were carefully analyzed for data on common mechanisms of development of these diseases, as well as methods of their diagnosis and treatment. This study was conducted in compliance with the ethical standards approved by the Ethics Committee of Uzhhorod National University.

REVIEW AND DISCUSSION

According to the literature, GERD and obesity are considered in terms of modifiable and non-modifiable risk factors (RF) [5, 6], pathogenetic features, lifestyle, physiological features, etc.

The most significant gaps in research related to GERD and obesity are not related to the study of the number of RFs and their quality of influence, but to insufficient information about their interaction with each other and their joint impact on the development of certain diseases [6-8, 10, 11].

A number of studies have shown that overweight, in particular abdominal obesity (AO), leads to an increase in intra-abdominal pressure (IAP) [12]. In addition, obese patients are often diagnosed with a hiatal hernia, which further disrupts anti-reflux mechanisms and aggravates GERD [14]. At the same time, an increase in IAP leads to mechanical distortion of the esophageal orifice of the diaphragm and the formation of a partial hernia of the esophageal orifice of the diaphragm, which in turn increases the ingress of acid into the lower esophageal sphincter (LES), contributing to the development of GERD and the risk of esophageal cancer [4, 7, 10, 14].

In addition, an increase in IAP, in turn, leads to a deterioration in the function of the LES and promotes retrograde throwing of gastric contents into the esophagus, increases the frequency of reflex episodes, which contributes to the development of GERD and related complications [14, 15].

Obesity is associated with impaired GERD function, including increased transient GERD relaxation and acid reflux (AR), especially after meals, indicating that impaired GERD function may be an early sign of obesity-related GERD [14, 15].

Obese patients have changes in motility and acid exposure (AE), as well as an increase in the frequency of transient relaxation of the LES, which contributes to a longer exposure to acid in the esophagus [15, 16]. These data are confirmed by increased AE and a significantly higher DeMeester index compared to people with normal body weight [16].

Overweight and obesity, as well as increased waist circumference, correlate with increased IAP and a gastroesophageal pressure gradient that induces reflux [17].

A number of studies have shown that AO is an important factor in the development of GERD due to mechanical pressure on the diaphragm and changes in the function of the LES [18].

Obesity contributes to the displacement and discoordination and imbalance of the structures of the esophageal opening of the diaphragm, which further weakens the anti-reflux barrier [14, 16, 17].

Obesity is clinically and physiologically characterized by excessive accumulation of metabolically active adipose tissue [10, 19]. Adipose tissue secretes a number of pro-inflammatory cytokines, such as interleukin-6, tumor necrosis factor α (TNF-α) and leptin, which contribute to the stimulation of inflammatory processes in the esophagus, worsening the clinical picture of GERD, leading to chronic inflammation in the esophagus, increasing the risk of developing GERD complications such as erosive esophagitis and esophageal adenocarcinoma [10, 20].

However, a study of nearly half a million adults with GERD in Scandinavian countries found that the risk of developing esophageal cancer (EC) in people with non-erosive GERD (about 60-70% of patients with GERD [21, 22]) was not increased compared to the general population. Instead, patients with an existing erosive form of GERD, in which inflammation of the esophageal mucosa occurs, had an approximately 2.4 times higher risk of EC compared to those patients with non-erosive GERD [21, 22].

Obesity is associated with metabolic changes such as an imbalance of hormones, in particular ghrelin and leptin. In the case of reduced ghrelin levels, which are associated with decreased motility of the upper gastrointestinal tract, food stagnation occurs and increases the risk of reflux [5, 10, 11, 19]. On the other hand, elevated leptin levels are associated with the development of leptin resistance, which in turn further worsens the course of GERD [5, 10, 11, 19].

Chronic elevation of IAP in obese individuals is associated with various comorbidities: GERD, hypertension, and other conditions, suggesting that elevated IAP plays a significant role in the pathogenesis of these disorders [23].

On the other hand, GERD can cause weight gain due to reduced physical activity (PA) and the use of certain medications. Long-term use of proton pump inhibitors (PPIs) may be associated with weight gain due to the effect on metabolism and possible impaired appetite regulation [8, 24, 25].

The use of anesthesia, in particular in obese patients, decreases the LES pressure and barrier pressure, is more pronounced in obese patients, which increases the risk of regurgitation and aspiration [26].

Patients with GERD can also change their eating habits by eating more food to temporarily relieve symptoms or by choosing high-calorie foods that do not cause esophageal irritation [27].

In addition to eating habits, patients with GERD may often avoid PA due to discomfort or pain during movement, especially when bending over or doing strenuous activities [13].

Modern methods of diagnosing GERD include endoscopic examination, esophageal manometry, and pH metering, and in the case of overweight or obesity, an assessment of BMI and body composition is also added [15, 17, 28, 29].

The prevention of both GERD and obesity is based on lifestyle changes, dietary correction, dosed PA, and, in the presence of bad habits, quitting them. Therefore, only a comprehensive positive impact on the lifestyle of patients can reduce IAP and improve the function of the LES, which is important in preventing the progression of GERD [29-31].

In obese patients, in addition to pharmacotherapy, the treatment plan may also include the use of surgical methods such as bariatric surgery (BS) to achieve weight loss (WL) [32]. Gastric bypass (GB), or sleeve gastrectomy, has been shown to be very effective in reducing the symptoms of GERD [32]. However, some studies show that BS can both improve and worsen patients' condition [32]. There is evidence that patients after sleeve gastrectomy may experience intrathoracic gastric migration due to changes in the anatomy of the esophageal opening of the diaphragm, which causes new episodes of clinical manifestations of GERD, such as heartburn and regurgitation, requiring additional treatment and, accordingly, cost and time with a simultaneous deterioration in QOL [32].

Studies confirm a close and complex multifactorial relationship between obesity, overweight and the development and clinical course of GERD [33-34]. However, despite the progress in understanding the PM, some aspects remain insufficiently investigated, including the prevention and optimal therapy of these conditions [33-34]. Literature data indicate the need for an integrated and personalized approach to patient treatment, combining lifestyle changes, regular dosed physical activity, drug therapy and, in severe cases, surgical methods [33-34].

PPIs are still the main method of medical control of AR, despite the emergence of a new class of drugs, potassium-competitive blockers (PCBs) of hydrochloric acid secretion [34, 35]. In overweight patients, the efficacy of PPIs may be lower due to changes in pharmacokinetics and increased levels of proinflammatory cytokines that affect esophageal sphincter function [33, 34, 35]. In contrast,

PCBs drugs are acid-resistant, lead to reversible inhibition, and can be dosed regardless of meal times [34, 35].

Surgical treatments, such as laparoscopic gastrectomy (LG) or GB, have been shown to be effective in reducing GERD symptoms in obese and overweight patients, although some cases have been reported to persist or worsen after surgery [32, 36].

GB surgery, in particular Roux-en-Y, is often associated with a reduction in GERD symptoms due to the bypass mechanism for stomach acid and a decrease in IAP [37].

A number of studies show that some patients may develop de novo GERD or even Barrett's esophagus after LG [38]. Having a confirmed diagnosis of GERD before BS may be a risk predictor of a higher likelihood of future reoperation [36-38]. Some studies indicate that the effect of surgery may be temporary, for example, in a few years after BS, patients may experience symptoms, especially in the case of LG [39].

Therefore, the question remains as to the long-term effects of preventing possible complications from this type of BS [40].

CONCLUSIONS

In recent decades, the number of obese and overweight people has increased significantly. In turn, GERD affects a large number of people around the world. Therefore, these data are of increased interest in finding and analyzing the close links and development of these diseases.

The literature review confirms that obesity, in particular AO and overweight, negatively affect the functioning of the esophagus, increasing the GERD, which contributes to reflux.

Various risk factors are involved in the mechanisms of GERD induction, each of which plays a role in the pathogenesis of GERD, including muscle abnormalities such as impaired esophageal motility and impaired tone of the LES, while anatomical factors such as hiatal hernia or AO, in particular by increasing the IAP, also significantly affect this condition. This creates a vicious cycle where one disease worsens or induces another.

The combination of GERD and obesity is a complex medical problem that requires a multidisciplinary and comprehensive approach to the treatment and prevention of comorbidities. WL is a key component of therapy and an important step in the prevention of GERD and obesity complications. The main principles of GERD treatment aimed at reducing the IAP and are crucial for reducing the risk of GERD episodes and include WL, diet and food control, PA and the use of antisecretory drugs, BS.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

Ethical and legal principles of biomedical research

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ABSTRACT

Aim: To analyze the regulatory and legal foundations of biomedical research by examining key ethical principles, including respect for individual autonomy, human dignity, voluntary informed consent, and benefit, with a particular focus on their implementation in both global and Northern European contexts, while identifying challenges to their practical application in modern healthcare systems.

Materials and Methods: This research employed a comprehensive analytical approach utilizing comparative legal analysis and systematic review of international and national regulatory frameworks governing biomedical research. The methodology included examination of constitutional provisions, specialized legislation, and bioethical guidelines from European jurisdictions, with particular attention to Northern European regulatory models established since the late 1980s. The study incorporated analysis of scholarly literature addressing theoretical foundations and practical implementation of key bioethical principles, focusing on works by established authorities in the field such as J. Hans, O. Pasternak, and S. Shevchuk.

Conclusions: Ethical regulation of biomedical research requires strict adherence to the fundamental principles of respect for autonomy, human dignity, voluntary informed consent, and benefit, which collectively provide a comprehensive framework for protecting research participants while enabling scientific progress.

KEY WORDS: biomedical research, human rights, informed consent, personal autonomy, ethical principles, bioethics, human dignity, informed consent

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INTRODUCTION

The rapid development of biomedical technologies and research in the 21st century is accompanied by the emergence of unprecedented legal, ethical and social challenges that require an adequate response from the legislation. Revolutionary achievements in the field of genetic engineering, cell technologies, bioinformatics, personalized medicine and biobanking open up prospects for significant progress in the treatment and prevention of diseases, but at the same time give rise to complex legal issues regarding patients' rights, data confidentiality, intellectual property and bioethics. Insufficient certainty of the regulatory framework or its inconsistency with modern realities can restrain the innovative potential of biomedical research, jeopardize the rights of participants in experiments and create obstacles to international scientific cooperation.

The implementation of legal norms in the field of biomedical research is characterized by a set of problems, including: inconsistency of national legislation with international standards; variability of interpretation of bioethical principles in different jurisdictions; lagging behind legal regulation from the pace of technological progress; the difficulty of balancing the interests of scientific progress and the protection of fundamental human rights. The problem of implementing adequate mechanisms for monitoring and supervising compliance with ethical and legal norms in biomedical research is of particular relevance. The development of effective models for implementing legal norms is a necessary prerequisite for minimizing the risks of abuse and unethical practices in this area.

Transformational processes in the global health system and medical science, intensified by the COVID-19 pandemic, have revealed a critical need to rethink existing legal approaches to regulating biomedical research. The acceleration of the pace of development of vaccines and diagnostic methods, increased attention to clinical trials in emergency situations, as well as the intensification of international exchange of biomedical data have exacerbated the issues of adequacy and effectiveness of existing legal mechanisms. The study of contemporary problems of implementing legal norms in the field of biomedical research has not only theoretical, but also significant practical significance for the formation of a balanced regulatory policy that will simultaneously promote scientific progress and ensure proper protection of human rights and dignity.

AIM

The aim of this study is to analyze the regulatory and legal foundations of biomedical research by examining key ethical principles, including respect for individual autonomy, human dignity, voluntary informed consent, and benefit, with a particular focus on their implementation in both global and Northern European contexts, while identifying challenges to their practical application in modern healthcare systems.

MATERIALS AND METHODS

This research employed a comprehensive analytical approach utilizing comparative legal analysis and systematic review of international and national regulatory frameworks governing biomedical research. The methodology included examination of constitutional provisions, specialized legislation, and bioethical guidelines from European jurisdictions, with particular attention to Northern European regulatory models established since the late 1980s. The study incorporated analysis of scholarly literature addressing theoretical foundations and practical implementation of key bioethical principles, focusing on works by established authorities in the field such as J. Hans, O. Pasternak, and S. Shevchuk. Primary sources included constitutional texts, decisions of constitutional courts, and international legal instruments that establish normative frameworks for biomedical research involving human subjects.

REVIEW AND DISCUSSION

The tradition of cooperation between the countries of Northern Europe in the field of regulation of biomedical technologies dates back to the late 1980s. In 1989, the Nordic Committee on Bioethics was founded, one of the tasks of which was to monitor legislative developments in the field of biomedical technologies both worldwide and in the countries of Northern Europe in particular, as technologies, research and services in the field under consideration are developing very rapidly and cross national borders [1].

The regulatory and legal framework of biomedical research is based on a number of key practical principles. Let us consider one of the most important among them: the principle of respect for the autonomy of the individual; the principle of respect for human dignity; the principle of voluntary informed consent; the principle of benefit in biomedical research.

The principle of respect for the autonomy of the individual is the fundamental basis of modern approaches to biomedical research. According to J. Hans, this principle symbolizes a significant transformation of the status of patients and research participants in the modern biomedical field. The basis of this principle is the recognition of the ability of a person to autonomous thinking and independent decision-making regarding their participation in research and awareness of their possible results. A crucial element of the implementation of this principle is the formation of such conditions that guarantee the research participant protection from all types of psychological influence or manipulative techniques, including hidden ones. In particular, the formation of artificial interest or the use of other methods of indirect influence on the expression of a person's will is considered unacceptable. Guaranteeing true freedom of choice acts not only as an ethical standard, but also as a prerequisite for the legitimacy and reliability of biomedical experiments conducted with human participation [2-16].

O. Pasternak notes that the autonomy of the individual is considered the moral basis of medicine and implies the need to avoid harm that a doctor can cause to a patient, and the principle itself is based on the recognition of a person as an unconditional value and provides for the free choice of an individual regarding his life and health (choice of a medical institution, doctor, consent or refusal of treatment, etc.), except in cases where this choice may pose a threat to other persons [17]. For bioethics, it is important that an autonomous individual has the right to decide whether other people can do something with his body, when a person can exercise this right by refusing treatment that, in the opinion of the doctor, will bring benefit [18].

The traditionally paternalistic approach in domestic medical practice was based on the assumption that the exclusive right to make decisions belonged to medical professionals, while the patient's position was considered insufficiently qualified and often ignored. This pattern continues to exist in the Ukrainian healthcare system, where there is some resistance to the active integration of patients into decision-making processes regarding their therapy or involvement in medical experiments.

However, it is necessary to understand that such approaches, which ignore the principle of personal autonomy, not only raise ethical concerns, but also pose a potential threat to the basic interests of the patient or research participant. When we put a person in a dependent position, we not only disregard their moral rights, but also create conditions under which their life priorities can be neglected or distortedly interpreted.

However, it is worth recognizing that such methods, which reject the principle of individual autonomy, are

not only ethically problematic, but also carry potential risks for the fundamental interests of the patient or research participant. By placing the individual in a subordinate position, we not only violate his moral rights, but also create the prerequisites under which his key vital interests may be ignored or inadequately treated [3].

The principle of respect for human dignity is the cornerstone of the ethical system that regulates public relations. This complex concept covers various moral and ethical aspects of interpersonal relations in society. It finds its expression through several basic behavioral patterns: expression of benevolence in communication and actions; showing respect for the diversity of thoughts and actions of others; following the norms of correct interaction; the development of politeness as a fundamental form of public communication.

The category of «human dignity» is an integral and important element of building a social state based on respect for human rights, guaranteeing their protection and defense. In a way, it is the quintessence of all other legal values, their reference point and direction. The principle of ensuring respect for human dignity is embodied in almost all world standards of human rights, acts as a determining criterion for the effectiveness of social policy, its reference point. Modern world legal doctrine considers human rights and freedoms as a higher legal value, and their provision is the primary duty of the state. This was a consequence of the recognition of human dignity as the determining basis of human rights and freedoms. The state is obliged to enshrine human rights and freedoms in the form of legal norms in constitutions or laws, in the legislative order to determine the mechanism for ensuring and implementing these rights and freedoms, as well as guaranteeing their protection in accordance with the procedure established by law [14].

This principle is a key guideline for creating a prosperous society in which each person receives recognition and respect regardless of their position or views. In the medical field in a broad sense, and especially in the field of biomedical research involving people, the principle of respect for human dignity acquires exceptional importance. It forms the essence of the relationship between a medical worker (or researcher) and a patient (or research participant), preserving all the above characteristics. It should be noted that following this principle is not only recommended, but absolutely mandatory: without its observance, it is impossible to conduct ethically permissible biomedical research, as well as to create an effective health care system in general [6].

However, despite the undisputed importance of this principle, its implementation in modern society often encounters significant obstacles. Particularly alarming is the tendency to reify the human body, when it or its components are perceived as a commodity or a material object. Vivid illustrations of this are the debates about the commercialization of organ and tissue donation, as well as the issue of prostitution. Such phenomena contribute to the emergence of a dangerous shift in collective consciousness, in which the human body begins to be perceived on an equal footing with other objects of the material world, which runs counter to the fundamental principle of respect for human dignity [5].

When conducting biomedical research involving humans, it is extremely important to understand that it is not only biological material that is being studied, but also a whole human being with his or her life and health. This concept is affirmed as the highest value in the Constitution of Ukraine and fundamental international legal documents. The principle of respect for human dignity requires a special, respectful attitude towards the individual and his or her physical integrity. It is important to note the universality of this principle: human dignity is an integral characteristic of every person, regardless of his or her individual traits or social status. Such universality implies that respect for human dignity cannot be made dependent or limited by factors such as ethnicity, skin colour, religion, socio-economic status, health status or any other external features. Therefore, within the framework of biomedical research, this principle guarantees a fair and ethical approach to all participants, regardless of their individual characteristics [6].

Note that instead of definitions of the concept of «human dignity» in European constitutions and practice of constitutional courts, there are general and abstract characteristics of individual aspects of the concept of human dignity. In European constitutions, dignity is characterized as inviolable, inalienable, supreme, the source of human rights and freedoms, the foundation of political order and social peace [15]. S. Shevchuk rightly emphasizes that human dignity plays a decisive role in the system of constitutional values. Being the core of every constitutional right, it forms the idea of a person as a unique self-determined being who is not under the power of the state. The right to respect for human dignity corresponds to the recognition of a person as «the highest social value, which makes it impossible to interpret a person instrumentally, only as an object of state will» [16].

The principle of benefit in biomedical research is a comprehensive concept that encompasses potential

benefits for both direct research participants and society at large. A central aspect of this principle is a careful analysis of the balance between possible risks and expected benefits from the research. Assessing the acceptability of such a balance requires a comprehensive approach that includes: a comprehensive analysis of all aspects of the research; a systematic consideration of alternative methods and approaches; and a detailed study of all available information related to the research. It is fundamentally important that when assessing potential harm, account is taken not only of the obvious physical and psychological risks to participants, but also of 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 at large. The principle of benefit thus requires a multifactorial analysis and a balanced approach to assessing the ethical acceptability of biomedical research [5].

The principle of voluntary informed consent is the fundamental basis of modern bioethics and medical practice. This principle not only serves as a protection against "medical dictate" and guarantees personal freedom, but also helps the research participant to make an informed decision with a full understanding of the possible consequences of medical intervention or its absence. The concept of informed consent is a relatively new phenomenon in medical ethics. In the past, many doctors adhered to a paternalistic model, considering it advisable to hide from patients comprehensive information about their health status and treatment features. This practice was based on the belief that such information could harm the patient or complicate the therapeutic process. However, modern medical science and healthcare practice recognize the principle of informed consent as fundamental. It has become one of the defining criteria for respecting the rights of both research participants and patients in general. This reflects a significant transformation in medical ethics from paternalism to a model that emphasizes patient autonomy and their right to comprehensive information and active participation in decisions about their own health and treatment [9].

In the context of biomedical research, the principle of informed consent requires that a potential participant be provided with comprehensive information to make an informed decision about their participation. Such information should include a detailed description of the purpose, objectives, and methods of the study, a clear explanation of the possible risks and expected benefits, an outline of alternative options (especially in therapeutic trials), and an explanation of the participant's right to ask questions and to withdraw from the study at any stage. The information should be provided in a standardized, understandable format and be as complete as possible. Exceptions that allow for partial disclosure of information are only possible when necessary to achieve the research purpose, provided that the undisclosed risks are minimal and that the participants are guaranteed to be fully informed afterwards. Such exceptions should be applied with particular care so as not to violate ethical standards and the rights of the research participants [9].

CONCLUSIONS

The analysis of the regulatory and legal foundations of biomedical research indicates the formation of a comprehensive system of ethical principles that ensure the protection of participants and the legitimacy of research activities. The fundamental principles among them are the principles of respect for the autonomy of the person, respect for human dignity, voluntary informed consent and benefit. These principles reflect the transformation of medical ethics from a paternalistic model to an approach that recognizes the right of a person to independently make decisions regarding their own health and participate in research.

The principle of respect for human dignity acquires special importance in the context of biomedical research, since it is the cornerstone of the entire system of legal values and serves as a determining criterion for assessing the ethical acceptability of research procedures. The universality of this principle guarantees a fair approach to all participants regardless of their individual characteristics, social status or other external characteristics. However, the objectification of the human body remains a worrying trend, which contradicts the principle of respect for human dignity and requires special attention from regulatory authorities.

The principles of beneficence and voluntary informed consent complement the ethical framework of biomedical research, ensuring a balance between scientific progress and the protection of the rights of participants. They require a comprehensive analysis of the balance of risks and benefits, as well as providing participants with comprehensive information to make informed decisions. The Nordic experience in regulating biomedical technologies demonstrates the importance of international cooperation and harmonization of legislation in this area, especially given the rapid development of technologies that cross national borders.

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CONFLICT OF INTEREST

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REVIEW ARTICLE

CONTENTS

Personal data protection in mHealth apps: international experience and prospects for legislative changes in Ukraine

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ABSTRACT

Aim: This study aims to analyze the legal aspects of mHealth apps in Ukraine, focusing on personal data protection and the effectiveness of the current legislation. The paper also zeroes in on examining international personal data protection standards and offers recommendations for improving the respective Ukrainian legislation.

Matherials and Methods: we employed method such as Overview to study Ukrainian and foreign legislation on personal data protection.

Conclusions: The study highlights the shortcomings in the legal regulation of mHealth apps in Ukraine, which creates risks to the privacy of users' personal data. To ensure the safe use of mHealth apps, it is necessary to implement international standards for protecting personal data, taking into account the experience of the United States, Canada, and the EU. Improving the legislation will help increase user confidence in mHealth apps and favour the interaction between patients and healthcare providers.

KEY WORDS: e-health, human rights, digital technologies, Ukraine

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INTRODUCTION

The term «mobile health» (mHealth) typically refers to the utilization of mobile telecommunication technologies essential for delivering healthcare services and promoting overall health maintenance [1].

It has to be emphasized that mHealth apps, like other digital products, should properly handle users' personal data. In this context, important are studies which estimate handling personal data of users of mHealth apps through the prism of different regulatory acts, such as General Data Protection Regulation (GDPR) [2-3] or Policy for Device Software Functions and Mobile Medical Applications [4]. There are also studies that analyze regulatory policies regarding mHealth apps in the USA, the European Union, France [5], Canada [6], Ireland [7], etc. Despite this, it is not clear whether the norms of Ukrainian law sufficiently address the use of mHealth apps. That is why we examine mHealth apps with the Ukrainian-language interface. On the other hand, we also explore the norms of Ukrainian law in the context of their possible implementation to regulate relations arising in connection with the use of mHealth apps.

AIM

This study aims to analyze the legal aspects of mHealth apps in Ukraine, focusing on personal data protection and the effectiveness of the current legislation. The paper also zeroes in on examining international personal data protection standards and offers recommendations for improving the respective Ukrainian legislation.

MATERIALS AND METHODS

In the course of our study overwiev method was employed, in order: to examine legal aspects which concern the use of mHealth apps in Ukraine.

We overviewed Ukrainian legislation in order to determine the legal norms that apply for the regulating of mHealth apps in Ukraine. To look for legislative acts, we referred to the «Legislation of Ukraine» platform on the website of the Supreme Council of Ukraine; we searched for normative-legal acts using the search engine on the website of the Cabinet of Ministers of Ukraine, as well as for orders issued by the Ministry of Health of Ukraine with the help of the search engine on the website of the Ministry of Health of Ukraine.

The main aim of our searchings was to find legal norms that regulate issues related to mHealth, electronic health care system, data privacy and personal data protection. It is due to this that we were supposed to determine the standards for the use of mHealth apps and handling confidential data of users of such apps.

REVIEW AND DISCUSSION

There has been determined the list of laws and legal acts which regulate relations arising from the use of mHealth apps in Ukraine.

THE LAW OF UKRAINE «ON PROTECTION OF PERSONAL DATA»

First of all, this law states that personal data are the object of protection. Additionally, the Law determines that personal data can be processed no longer than is necessary for legitimate purposes, and also determines the grounds for deleting personal data [8].

Article 7 of the Law states that for the purposes of healthcare, personal data are processed, in particular, in the case of:

- «establishing a medical diagnosis to ensure care or treatment or provision of medical services, monitoring of compliance with the set conditions for providing such services (including the terms of contracts on medical service to the population and contracts on reimbursement under the program of medical guarantees), the functioning of the electronic health care system, provided that such data are processed by a health practitioner, a rehabilitation expert or another person from a health care institution, a rehabilitation institution or an individual entrepreneur who received a license to carry out economic activities in medical practices, and its employees who are entrusted with the responsibility of ensuring the protection of personal data and are subject to medical privacy law, employees of the central executive body that implements state policy in the field of state financial guarantees of medical care for the population, employees of an institution that carries out state sanitary and epidemiological supervision and activities in the field of public health, which received a license to carry out economic activities in medical practices, who are entrusted with the duties of ensuring the protection of personal data» [8].

Instead, in accordance with Article 8 of the Law, to the rights of subjects of personal data belong: 1) to know about the sources of collection, the location of their personal data, the purpose of their processing, the location or place of residence (stay) of the owner or manager of personal data; 2) to receive information about the conditions for providing access to personal data, in particular information about third parties to whom their personal data are transferred; 3) to withdraw consent for personal data processing [8].

It is obvious that these provisions apply to any field of activity. Mobile apps are no exception and must meet the established requirements, in particular, we are talking about the provisions of Articles 7 and 8 of the Law mentioned above.

THE LAW OF UKRAINE «ON INFORMATION»

The law of Ukraine «On Information» equates personal data with information about an individual. This law also defines information about an individual, which means «data or an aggregate of data on an individual, who is identifiable or can be specifically identified» [9]. Additionally, the law states the need for state and public control over compliance with information legislation. The types of legal liability for violation of information legislation are defined as follws: disciplinary, civil, administrative or criminal ones.

THE LAW OF UKRAINE «ON ACCESS TO PUBLIC INFORMATION»

This law defines confidential information: «confidential information is information, access to which is limited by an individual or legal entity, except for government entities, and which can be distributed in the order determined by them at their will in accordance with the conditions stipulated thereby» [10]. This is important due to the fact that personal data actually belong to the category of confidential information.

THE LAW OF UKRAINE «ON MEDICINAL PRODUCTS»

This law, in particular, regulates issues related to distance trade of medicinal products [11]. The law obliges economic entities that have the right to carry out electronic retail trade of medicinal products to ensure the confidentiality of consumers' personal data. It is not determined though whether such electronic retail trade can be carried out using mobile apps.

ON THE FUNDAMENTAL PRINCIPLES OF THE UKRAINIAN HEALTH LEGISLATION

This law determines the possibility of the functioning of the electronic health care system in Ukraine. Article 24^2 of this

law specifies that: «Access to information about the patient located in the electronic health care system is possible only in the case of obtaining the consent of such a patient (his / her legal representative) in a written form or the form which enables drawing a conclusion that the consent has been given. Access to the patient's information is possible only:
– if there are signs of a direct threat to the patient's life;
– in case it is impossible to obtain the consent of such a patient or his / her legal representative (until the time when obtaining the consent becomes possible);
– by a court decision» [12].

Notably, there is no mentioning in the Fundamental Principles of the Ukrainian Health Legislation about the possibility to use mobile apps for ensuring the operation of the electronic health care system, as well as the standards for handling personal data when using the electronic health care system.

ON THE APPROVAL OF THE LICENSING CONDITIONS FOR CARRYING OUT BUSINESS ACTIVITIES IN MEDICAL PRACTICE

This regulation states that each health care institution is obliged in each case to process personal data in accordance with the terms of the Law of Ukraine «On Protection of Personal Data». In addition, it is stressed that such processing should also be carried out while working in the electronic health care system [13].

SOME ISSUES OF THE ELECTRONIC HEALTH CARE SYSTEM

This regulation determines the features of the functioning of the electronic health care system. In particular, it refers to the right of the subject of personal data to receive any information about himself / herself, as well as to change them based on a motivated request [14].

This regulation also enshrines the patient's right to apply for withdrawal of the application for processing personal data which is included in the central database [14].

The most significant thing is that this regulation establishes the possibility of the functioning of patients' electronic offices also through mobile apps of authorized state bodies or enterprises belonging to the sphere of their management. The consent to the processing of personal data is necessary in this case [14].

SOME ISSUES RELATED TO FORMING MEDICAL CONCLUSIONS ABOUT TEMPORARY INCAPACITY

According to this order, it is allowed to send medical documents or their copies to the doctor using «technical means of electronic communication» [15], among which are mobile apps. However, there are two nuances that should be taken into account. Firstly, the provisions of this order apply exclusively to the formation of medical conclusions about temporary incapacity. Secondly, it is possible to send medical documents or their copies to a doctor using mobile apps only during the period of martial law in Ukraine and within three months from the date of its termination or cancellation.

In the US, the use of mHealth apps is regulated by Policy for Device Software Functions and Mobile Medical Applications issued by FDA.

This act specifies that: «mobile medical app» is a mobile app that incorporates device software functionality that meets the definition of a device in section 201(h) of the FD&C Act; 19 and either is intended:
- to be used as an accessory to a regulated medical device; or
- to transform a mobile platform into a regulated medical device» [16].

A positive aspect of this act is that it provides a detailed list of functions of software: (1) that are not Medical Devices, (2) for which FDA intends to exercise enforcement discretion, (3) that are the focus of FDA's regulatory oversight [16].

In Canada, instead, there was adopted Guidance Document: Software as a Medical Device (SaMD): Definition and Classification that determines the notion of Software as a Medical Device (SaMD) which is defined as software intended to be used for one or more medical purposes that perform these purposes without being part of a hardware medical device [17].

This act also states that Mobile apps that meet the following definition are considered SaMD:
- SaMD is a medical device and includes in-vitro diagnostic (IVD) medical devices;
- SaMD is capable of running on general purpose (non-medical purpose) computing platforms;
- «without being part of» means software not necessary for a hardware medical device to achieve its intended medical purpose,
- Software does not meet the definition of SaMD if its intended purpose is to drive a hardware medical device,
- SaMD may be used in combination (e.g., as a module) with other products including medical devices,
- SaMD may be interfaced with other medical devices, including hardware medical devices and other SaMD software, as well as general purpose software [17].

Exclusion criteria are also clearly outlined in this act, they include cases when the following types of software do not meet the definition of a medical device and are

therefore not subject to the Regulations:

- Software intended for administrative support of a healthcare facility;
- Software that enables clinical communication and workflow including patient registration, scheduling visits, voice calling, video calling;
- Software intended for maintaining or encouraging a healthy lifestyle, such as general wellness apps;
- Software intended to serve as electronic patient records or tools to allow a patient to access their personal health information [17].

In the European Union, an important document that regulates the use of mHealth apps is Council Directive 93/42/EEC of 14 June 1993 concerning medical devices, which determines that software also belongs to «medical devices» if it is used with the purpose of:

- diagnosis, prevention, monitoring, treatment or alleviation of disease;
- diagnosis, monitoring, treatment, alleviation of or compensation for an injury or handicap;
- investigation, replacement or modification of the anatomy or of a physiological process;
- control of conception [18].

Attention should be paid to the fact that this Directive defines four classes of devices: I, IIa, IIb,III. It must be admitted that «only manufacturers of medical devices with risk II and higher are audited by NB's» [19].

The studied norms of the Ukrainian law, as well as the above-mentioned provisions of foreign legislation regarding the regulation of mHelath apps, indicate the need to introduce changes to Ukrainian legislation in the context of the examined issue.

First of all, it is necessary to legally define what «a medical device» is and to equate software with such a device, as specified in the Council Directive 93/42/EEC of 14 June 1993 concerning medical devices. That is why we support the need to adopt the Law of Ukraine «On Medical Devices». Currently, the respective draft law has been submitted to the Supreme Council of Ukraine [20]. Notwithstanding the fact that it, to an extent, duplicates the provisions of Council Directive 93/42/EEC of 14 June 1993 concerning medical devices, there is a need for its revision.

This revision implies the need for determining criteria which allow classifying a mobile app as an mHealth app and thus carrying out an audit of such an application in accordance with the practice that has developed in the USA, Canada and the European Union. This will also enable one to figure out «…whether every mobile app needs a comprehensive approval, or as international agencies do, whether each kind of risk requires its own form of approval» [21].

Additionally, the legislation on protection of personal data needs amending, in particular in the context of handling sensitive personal data, which definitely includes information about a person's state of health. In this regard we are also taking steps towards the convergence of Ukrainian legislation and European legislation, the proof of which is the appearance of the draft law «On Protection of Personal Data» [22], which takes into account the General Data Protection Regulation.

Our opinion is that mHealth apps should necessarily contain privacy policies. The presence of privacy policy is mandatory in order to place any application on App Store platform: «All apps must include a link to their privacy policy in the App Store Connect metadata field and within the app in an easily accessible manner» [23]. The respective provisions should also be reflected in Ukrainian legislation. In particular, attention has to be paid to legal liability of the owners of personal data in case the subject of personal data has no possibility to familiarize himself / herself with privacy policy due to the fact that it is absent.

CONCLUSIONS

The development of mHealth apps leads to an increase in their popularity among users. Along with this, there are certain problems associated with ensuring the confidentiality of personal data of users of such apps. The reason for this is the imperfection of Ukrainian legislation. That is why there is a need to introduce the necessary amendments, based on the standards of handling personal data developed in the USA, Canada and the EU. The point is that the use of mHealth apps should enable such app users to more easily access medical care and these apps also have to be safe. In this case, health personnel will be certain that they can communicate with patients using an mHealth app which has the appropriate level of privacy, and that no one else will gain access to medical information. Ultimately, the development of mHealth apps enables positive changes in health care relationships, and quality legislation will allow speeding up these changes розвиток mHealth. To summarize, it can be noted that in this research attention is drawn to the imprefection of the legal regulation of mHealth apps and we express the hope that further research will help to solve the existing issues.

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CONFLICT OF INTEREST

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The main aim of the conference is to present trends and results of the latest scientific research in the field of complementary disciplines of physiotherapy, emphasizing their practical dimension/application in strengthening the professional competences of physiotherapists. We cordially invite representatives of medical, social, natural, technical and other sciences who, through their activities, can participate in the comprehensive rehabilitation process and strengthen patients' functional reserves.

Conference Topics:

- Biological sciences in the development of physiotherapy
- Social activation of people with disabilities
- Animal-assisted therapy and physiotherapy of animals
- Psychotherapy and occupational therapy in physiotherapy
- Professional profile of a physiotherapist – professional profile vs. social expectations
- Technical/engineering support in physiotherapy
- Varia – other topics related to new challenges of physiotherapy in clinical medicine of humans and animals

Workshops

The conference will include the following workshops:

- Low Pressure Fitness (a form of core muscle training using specific breathing techniques);
- Augmentative and Alternative Communication (AAC).

The project is co-financed by the government budget, allocated by the Minister of Science under the Excellent Science II Program. Grant amount: 58,410.00 PLN, total project value: 65,010.00 PLN – in accordance with the contract number KONF/SP/0105/2024/02.

The organizer will provide all registered participants with conference materials, the opportunity to take part in workshops, two lunches and coffee breaks; additionally for active participants (speech, poster): dinner on 22nd May 2025, overnight stay on 22nd-23rd May 2025, breakfast on 23rd May 2025.

Research and review works will be presented in Polish during the thematic sessions and the poster session. The poster session, addressed to young scientists (students and PhD students), will include a competition with prizes – the award for the best work.

The summary written in Polish should not exceed 150-250 words. In the case of research works, the summary should include the following sections: aim of the research, material and methods, results, conclusions and keywords. In the case of review works: description and keywords.

Registration* with an abstract should be sent electronically by 11th April 2025. The Scientific Committee will qualify the works for presentations at the Conference.

It is planned to prepare a reviewed post-conference monograph in 2025 published by the Publishing House of John Paul II University in Biała Podlaska. The deadline for submitting works for review, as well as the guidelines related to the layout and formatting of the works will be provided in the 2nd Announcement.

The publication fee of 100.00 PLN (applies only to active participants who will submit their work as a chapter in a post-conference monograph) must be paid after the work has been approved for printing (no later than 14 days from the date of receiving the decision).

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**Limited number of participants. We will inform you about your qualification via e-mail*

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