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ORIGINAL ARTICLE

Statin use and muscle strength in older patients with heart failure

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ABSTRACT

Aim: To evaluate the association between statin use and muscle strength in older adults with heart failure (HF).

Materials and Methods: Hospitalized, older adults with HF were divided into two subgroups: use statin (US) and not use statin (N-US). Muscle strength was measured using a hand-held dynamometer and HF was assessed based on the New York Heart Association NYHA functional classification.

Results: 194 patients (median age 82 [78-86] years), 90 patients in US and 104 in N-US group. Muscle strength didn't differ significantly in US *vs* N-US group, 19.74kg (15.48-27.18) *vs* 17.58kg (12.73-25.66), p=0.098. In a multivariate analysis age, sex, NYHA class, hemoglobin concentration, presence of atrial fibrillation, and hypothyroidism were found to be independent factors of muscle strength (p<0.001, p<0.001, p<0.01, p<0.001, p=0.02, p=0.04 respectively).

Conclusions: statin use is not related to decreased muscle strength in older adults with HF

KEY WORDS: statins, muscle strength, heart failure, older adults

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INTRODUCTION

The prevalence of heart failure (HF) increases with age, so most patients with chronic HF are older people. The incidence of HF in older patients is estimated to be more than 10% [1]. HF is associated with poor health-related quality of life, a significant burden on the healthcare system, and increased mortality, largely related to frequent hospital admissions [2]. The main symptom in patients with HF is reduced exercise tolerance, which manifests as rapidly increasing fatigue or exertional dyspnea. In clinical practice, the basis for assessing the severity of heart failure is the patient's history and assessment of the intensity of exertion causing dyspnea or fatigue (New York Heart Association – NYHA - classification of heart failure). Restriction of physical activity is often associated with muscle weakness reported by patients. These complaints could be caused by statin therapy [3].

Through specific and reversible inhibition of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase, statins cause a decrease in hepatic cholesterol synthesis and a secondary increase in the expression of the receptor for LDL, elimination of LDL cholesterol from plasma and a decrease in the production of LDL and VLDL by the liver [4]. In addition to their hypolipemic effect, statins have pleiotropic effects resulting from their influence on the increased nitric oxide (NO) synthesis, decreasing endothelin 1 production, lowering C-reactive protein levels, decreasing metalloproteinase synthesis and inhibiting platelet adhesion [5]. In some patients, an autoantibody response against 3-hydroxy-3-methylglutaryl-coenzyme A reductase may be induced through increased expression of this autoantigen leading to structural muscle damage [6].

These medicines are widely used in the treatment of many conditions, from hypercholesterolemia to primary and secondary prevention of cardiovascular diseases, including ischemic heart disease and ischemic strokes [4, 7]. Moreover, statins are also wildly used in HF patients with reduced ejection fraction, and this therapy may be associated with a 12% reduction in the risk of hospitalization for HF when used in specific subgroups of HF patients [8].

The incidence of adverse effects of statins 9.1% on average and is significantly higher in patients aged at least 65 years [9]. The most common side effect of therapy is myalgia, with the incidence reaching 9%, another significant effect is an elevation of liver aminotransferases occurring in 1-3% of subjects, and rhabdomyolysis occurring in 0.02% of subjects was found to be a rather rare symptom [10]. Other muscular symptoms manifesting as weakness or deterioration of muscle strength were rarely reported and the number of reports was not recorded.

Reduced muscle strength leading to reduced physical activity is one of the complaints reported by older people. These patients, often burdened by multimorbidity, including cardiovascular disease, are taking some medications, including statins. Reduced muscle strength, weakness, and reduced physical function are typical symptoms of heart failure. We

therefore set out to assess the association between statin use and muscle strength in older people with heart failure.

AIM

The aim of this study was to evaluate the association between statin use and muscle strength in older patients with heart failure.

MATERIALS AND METHODS

STUDY GROUP

Consecutive patients aged at least 65 years and hospitalized for exacerbation of chronic diseases or for a diagnostic stay in an internal medicine department and geriatric cardiology Independent Public Clinical Hospital Name of Professor Witold Orłowski, Centre of Postgraduate Medical Education of Warsaw from August 2022 to May 2024. Patients who did not consent to participate in the study and patients with advanced cognitive function decline who were unable to give informed consent were excluded from the study. The group was divided into two subgroups of statin taking - "use statin" and no statin taking - "not use statin".

VARIABLE DEFINITIONS

A group of patients defined as taking statins had been taking them for at least one month before hospitalization. Heart failure and co-morbidities were diagnosed according to the current guidelines.

METHODS USED

Muscle strength measurements and question collection were performed after optimization of the patient's condition, before the day of discharge from the hospital. Muscle strength was measured using the DHD-1 SAEHAN (Digital hand dynamometer) twice on each upper limb, and the mean of the measurements for each limb was calculated and was used in the subsequent analyses. Obesity was diagnosed at a BMI ≥30 kg/m2. Patients answered questions using a standardized face-to-face questionnaire. Information on chronic diseases, laboratory tests, and medications taken was obtained from the patient interview and medical records.

All patients gave informed consent to participate in the study. The study was approved by theBioethics Committee at the Centre of Postgraduate Medical Education of Warsaw (resolution no. 73/2022 of 8 June 2022).

STATISTICAL ANALYSIS

Statistical analysis was performed using the STATISTICA 13.3 package. Categorized variables are presented as percentages and continuous variables as medians with first and third quartiles or mean with standard deviation. The Shapiro-Wolf test was used in order to assess the normality of a the variable distribution. In order to compare the continuous variables the Student's T-test or Mann-Whitney U-test were used as appropriately. The χ^2 test was used to compare categorical variables. Multiple linear regression analysis was used in order to find variables independently related to the muscle strength. A p-value below 0.05 was considered statistically significant.

RESULTS

The study included 194 patients from 65 to 97 years old with median age 82 (78-86), including 126 women and 68 men. The characteristics of the study group in terms of gender, age, chronic diseases, medications taken, and laboratory parameters are shown in Table 1. Statins were taken by 90 patients, including: atorvastatin in 49 (54%) patients, rosuvastatin in 36 (40%) patients, and simvastatin in 5 (6%) patients. Doses of statins were from 10 mg to 80 mg (median 20 [10-20]), from 5 mg to 40 mg (median 10 [10-20]), and from10 mg to 20 mg (median: 20 [20-20]), respectively. Patients using and not using statins differed in respect of prevalence of hypertension and hyperthyroidism as well as in respect of the median level of LDL cholesterol, creatinine, glucose, and Hb_{A1c}. In addition patients using statins were prescribed ezetimibe, insulin, metformin, and GLP-1 agonists more often (Table 1).

The value of muscle strength in the statin-taking and non-statin-taking groups by limb is shown in Figure 1. Table 2 presents the relation between the use od statins and the muscle strength. We did not find a significant difference in the muscle strength when the whole study group was analysed. The muscle strength was significantly lower in the study participants not using a statin as compared to users among patients with severe heart failure, in patients with hypertension, without diabetes and without obesity (Table 2).

The variables independently related to the muscle strength are presented in the Table 3: age, sex, NYHA class, hemoglobin level, atrial fibrillation, and hypothyroidism were independently related to the muscle strength. Addiationally, we tested the relation between the use of statins and the muscle strength using various multivariable models. In none of them we found a significant association between the use of a statin and the muscle strength (Table 4).

DISCUSSION

Muscle mass and muscle strength depend on age, gender, health status, and training. Muscle characteristics change

Table 1. The characteristics of the study group

Variables	Participants using a statin	Participants not using a statin	p	All
Age	82.00 [77.00-85.00]	83.50 [78.00-87.00]	0.243	82.00 [78.00-86.00]
Gender				
126 women (64.95%)	55 (61.11%)	71 (68.27%)	0.297	126
68 men (35.05%)	35 (38.89%)	33 (31.73%)		68

Table 1. cd.

Medical history				
Hypertension	82 (91.12%)	80 (76.92%)	0.029	162.00
Obesity	44 (48.89%	35 (33.65%)	0.098	79.00
Type 2 diabetes	44 (48.89%)	34 (32.69%)	0.072	78.00
Atrial fibrillation	39 (37.5%)	38 (42.22%)	0.799	77.00
Coronary heart disease	35 (38.89%)	26 (25.0%)	0.116	61.00
Chronic kidney disease	33 (36.67%)	26 (25.0%)	0.078	59.00
Current or past cancer	20 (22.22%)	28 (26.92%)	0.751	48.00
Chronic obstructive pulmonary disease	7 (7.87%)	16 (15.38%)	0.263	23.00
Hypothyroidism	20 (19.23%)	21 (23.33%)	0.485	41.00
Hyperthyroidism	0 (0.00%)	6 (5.77%)	0.031	6.00
NYHA class	1.5 [1.00-3.00]	2.0 [1.00-4.00]	0.463	2.0 [1.00-3.00]
Laboratory parameters				
Total cholesterol	137.00 [115.00-167.00]	164.00 [130.90-195.00]	0.000	152.00 [124.00-183.00]
LDL	71.30 [56.60-95.00]	97.40 [74.40- 117.80]	0.000	83.40 [62.00-108.00]
HDL	43.00 [35.00-54.00]	46.00 [37.00-61.00]	0.178	45.00 [36.00-58.00]
Non-HDL	93.00 [74.00-118.00]	116.00 [87.00-135.00]	0.000	99.50 [81.20-132.00]
Triglycerides	101.50 [76.00-135.00]	90.0 [66.0-115.00]	0.069	95.00 [73.00-121.00]
TSH	1.349 [0.808-1.889]	1.366 [0.842-2.002]	0.941	1.355 [0.835-1.995]
Hemoglobin	11.90 [10.80-13.10]	12.5 [10.60-13.20]	0.603	12.40 [10.8-13.20]
Hematocrit	37.25 [33.60-41.10]	37.8 [32.20-40.50]	0.964	37.60 [33.40-40.50]
Creatinine	1.09 [0.85-1.31]	0.94 [0.76-1.15]	0.005	0.98 [0.82-1.28]
Sodium	140.0 [137.80-142.00]	139.00 [135.30-141.00]	0.053	140.00 [136.60 -142.00]
Kalium	4.30 [4.00- 4.60]	4.40 [4.01-4.60]	0.990	4.38 [4.01-4.60]
Glucose	102.50 [94.00-133.50]	99.00 [90.50 -110.50]	0.022	99.50 [92.50-118.50]
Glycated hemoglobin	6.47 [6.00-7.60]	5.94 [5.62-6.20]	0.000	6.18 [5.75-6.70]
Pharmacology treatment				
Acetylsalicylic acid	29.0 (32.22%)	28.0 (26.92%)	0.419	57.00
Antiplatelet drugs	4.0 (4.44%)	5.0 (4.81%)	1.000	9.00
Beta-blockers	73.0 (81.11%)	73.0 (70.19%)	0.078	146.00
ACE-i	48.0 (53.33%)	45.0 (43.27%)	0.161	93.00
ARB	14.0 (15.56%)	17.0 (16.35%)	0.880	31.00
Calcium blockers	30.0 (33.33%)	39.0 (37.50%)	0.545	69.00
Diuretics	68.0 (75.55%)	74.0 (71.15%)	0.490	142.00
ARNI	1.0 (1.11%)	2.0 (1.92%)	1.000	3.00
Fibrates	2.0 (2.22%)	1.0 (0.96%)	0.597	3.00
Ezetimibe	7.0 (7.77%)	1.0 (0.96%)	0.025	8.00
PCSK-9 inhibitors	0 (0.00%)	0 (0.00%)	-	0.00
Insulin	12.0 (13.33%)	2.0 (1.92%)	0.030	14.00
Metformin	26.0 (28.88%)	17.0 (16.35%)	0.035	43.00
Sulfonylurea derivatives	3.0 (3.33%)	4.0 (3.85%)	1.0	7.00
Gliptins and GLP-1 agonists	10.0 (11.11%)	0 (0.00%)	0.000	10.00
SGLT2-inhibitors	24.0 (26.66%)	23.0 (22.11%)	0.460	47.00
Glucosidase inhibitors	0 (0.00%)	0 (0.00%)	-	0.00
Anticoagulants	43 (47.77%)	41 (39.42%)	0.295	84.00
Antidepressants	36 (40.00%)	34 (32.69%)	0.772	70.00

ACEI – angiotensin-converting-enzyme inhibitors, ARB – Angiotensin II receptor blockers, ARNI – Angiotensin Receptor-Neprilysin Inhibitor, SGLT2-inhibitors – Sodium-glucose co-transporter-2 (SGLT2) inhibitors

Source: compiled by the authors of this study

Table 2. The muscle strength by the use of statins

Variables 1st and 3rd quartile or ± standard deviation	Participants using a statin	Participants not using a statin	p -value
All n=194	19.74 [15.48-27.18]	17.58 [12.73- 25.66]	0.098
Age (65-80) n= 79	26.62 ± 11.41	24.42 ± 10.14	0.366
Age (81-96) n=115	17.43 [13.75-22.65]	15.63 [11.58-19.13]	0.135
Woman n= 126	17.35 [11.33-20.28]	15.60 [11.58-19.06]	0.238
Men n=68	27.58 [22.65-34.05]	28.63 [19.13-34.53]	0.873
I, II NYHA, n= 137	20.73 [16.15-27.18]	18.36 [15.60-28.00]	0.473
III, IV NYHA, n=57	18.95 [13.75-27.63]	12.23 [9.50-19.65]	0.029
Hypertension n=162	19.74 [15.48-27.18]	17.20 [12.68-25.66]	0.033
No Hypertension n=32	17.48±9.33	20.78±8.76	0.378
Obesity n= 79	18.62 [13.58-25.70]	18.45 [14.85-28.40]	0.474
No Obesity n=115	21.54 [17.35-28.45]	16.83 [12.10-24.70]	0.004
Type 2 diabetes n= 78	21.04±10.11	21.78±9.44	0.767
No Type 2 diabetes n=116	20.10 [16.15-27.58]	16.59 [12.68-21.20]	0.025
Atrial fibrillation n= 77	17.79 [13.75-25.43]	16.43 [11.58-19.65]	0.424
No Atrial fibrillation n=117	21.05 [17.35-28.32]	18.20 [14.05-28.75]	0.115
Coronary heart disease n=61	18.25 [14.28-27.20]	17.80 [11.38-28.63]	0.589
No Coronary heart disease n=133	20.68 [16.85-27.18]	17.59 [13.23-24.93]	0.075
Chronic kidney disease n= 59	20.67±8.97	19.21±10.15	0.561
No Chronic kidney disease n= 135	21.23 [14.90-27.20]	17.59 [14.00-27.4]	0.149
Current or past cancer n= 48	19.43 [13.31-27.38]	16.38 [14.09-28.83]	0.497
No Current or past cancer n= 146	19.74 [15.48-27.18]	18.09 [12.63-25.00]	0.133
Chronic obstructive pulmonary disease n=23	25.99±11.60	17.95±8.27	0.071
No Chronic obstructive pulmonary disease n=171	19.48 [15.48-26.75]	17.70 [12.79-26.79]	0.214
Hypothyroidism n=41	17.28 [11.33-19.48]	15.64 [12.38-19.68]	0.557
No Hypothyroidism n=153	21.23 [16.53-27.20]	17.89 [12.79-28.52]	0.102
Hyperthyroidism n= 6	0.00 [0.00-0.00]	24.14 [17.8-29.03]	1.000
No Hyperthyroidism n= 188	19.74 [15.48-27.18]	17.20 [12.68-24.93]	0.060

Source: compiled by the authors of this study

throughout a person's life and the highest values are reached by people before 30 years of age [11]. After 50 years of age, the number of muscle fibers decreases and strength in old age is reduced [12]. The higher muscle strength that men achieve during adulthood may explain the differences in muscle strength between the sexes in older age.

Handgrip strength is a simple tool to provide information about muscle strength. It is also one of the components that assess frailty syndrome [13]. This parameter can be considered as a predictor of physical disability and mobility limitation [14]. In a study by Norman et al. it was shown that low grip strength is associated with loss of independence [15]. Similarly, Snih et al. concluded that grip strength has a predictive value for impairment in basic activities of daily living (ADLs) [16]. Moreover, Sayer et al. showed that men and women with lower grip strength rated their health status lower [17].

The results of our study showed that in patients with heart failure, muscle strength is slightly higher in the group taking these lipid-lowering drugs compared to those not taking them, without statistical difference. Overall muscle strength values are low in the entire study group, which may affect the assessment of individual parameters in our study. The results of our study are consistent with other observational studies. Ashfield et al. showed that grip strength was not associated with statin use in both men and women [18]. A similar lack of association between statin use and muscle strength in a younger age group was observed by Parker [3]. Cárdenas et all, on the other hand, compared muscle strength values before and after a period of statin use in HIV-infected individuals over 18 years of age [19]. The study did not show a decrease in muscle strength after the statin treatment. Moreover, an increase in muscle strength was observed in the male study group [19].

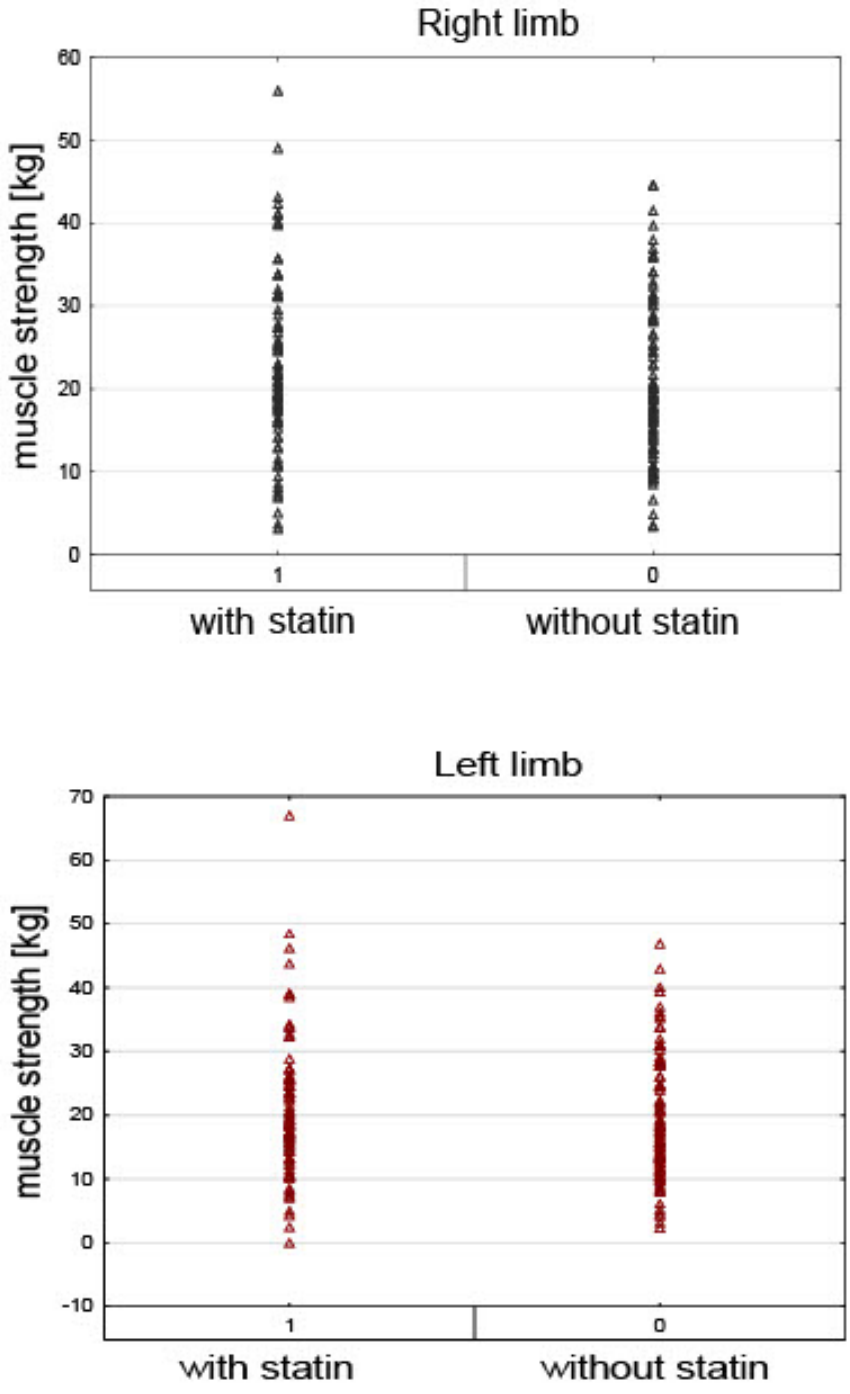

Fig. 1. Handgrip strength in right and left limb
Source: compiled by the authors of this study

Table 3. Variables independently related to the muscle strength

	Regression coefficient ± standard error	p
Age	-0.3409±0.0521	< 0.001
Male sex	0.5589±0.0519	< 0.001
NYHA class	-0.1404±0.0518	0.007
Hemoglobin	0.3779±0.0673	< 0.001
Atrial Fibrillation	-0.1698± 0.0706	0.017
Hypothyroidism	-0.1470± 0.0706	0.039

Source: compiled by the authors of this study

Table 4. The association between statin use and muscle strength in univariable and multivariable analyses

	Regression coefficient ± standard error	p
Univariable	0.1107±0.0717	0.124
Adjusted for age and sex	0.0400±0.0529	0.450
Additionally adjusted for atrial fibrillation, hemoglobin level, hypothyroidism, and NYHA class	0.0421±0.0474	0.376
Additionally adjusted for creatinine level, glycated hemoglobin level, ezetimibe, insulin, metformin, gliptins, and GLP-1 use	0.0529±0.0519	0.308

Source: compiled by the authors of this study

Similar to our observation, higher handgrip strength values in statin users compared to the group not taking the drug were observed by Veddeng et al. [20]. The study group was similar in size, gender, and age to the group assessed in our study. Such results may be due to the smaller effect statins have on upper limb muscle strength compared to lower limb muscle strength [21]. Probably patients with better muscle function and in better health had statin treatment than those in poorer health.

In the division we used between patients with NYHA class I and II and patients with NYHA class III and IV, statistical significance emerged in the group of patients classified as NYHA class III and IV, who mostly perform very limited physical activity, which is noticeable through reduced muscle strength. A similar phenomenon was shown in the study by Run-Min Li et all., authors highlighted a negative correlation between muscle strength and heart failure in a group over 45 years of age as well and similar relationships may be in the older adults population [22]. There was a statistically significant difference in muscle strength in hypertensive patients and those without obesity and without type 2 diabetes treated with and without statin treatment. In the Jeoung B et. al. analysis, muscle strength was associated with the presence of hypertension [23].

The multivariate analysis took into account individual factors that were included in the characteristics of the study group: multimorbidity, laboratory parameters, medications taken, and risk factors. After the analysis, it was shown that age, sex, hemoglobin concentration, reduced thyroid function, atrial fibrillation, and NYHA class had an impact on handgrip strength. Chen ZQ et al. had similar results in terms of sex and age in muscle strength [24]. Our study suggests that in clinical practice measuring muscle strength in patients, we can expect reduced values depending on sex, age, and chronic diseases including cardiovascular diseases.

We are aware of the limitations of our study. This is a single-center study conducted on a small group of patients. The study included patients hospitalized for a variety of reasons, which may have influenced the measurements obtained. The positive association between statin use and muscle strength may be due to the better medical care experienced by people with HF, more frequent follow-up visits, and lifestyle modifications. However, there is still a lack of studies assessing the relationship between statin use and muscle strength in the oldest patients with multimorbidity. Our analysis provides preliminary information in such geriatric population in clinical practice.

CONCLUSIONS

Statin use is not related to the decreased muscle strength in older adults with heart failure.

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Conflict of interest
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Interactions between Covid-19 and chronic disease: A multilevel analysis of treatment strategies and outcomes

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ABSTRACT

Aim: The purpose of study was to analyse the interaction between COVID-19 and chronic diseases, with a focus on evaluating the effectiveness of treatment strategies.

Materials and Methods: The study, conducted at the Infectious Disease Centre of the Regional Clinical Hospital of Karaganda, aimed to determine factors influencing therapy effectiveness in 236 COVID-19 patients. It assessed the dynamics of laboratory indicators and their relationship with chronic diseases like diabetes, hypertension, cardiovascular conditions, chronic respiratory diseases, and cancer. Demographic data, including age and gender, were also considered.

Results: The study revealed no significant age difference between patients with moderate and severe COVID-19 courses, with both groups averaging a similar age of 66 years. The duration of hospitalization was significantly longer for patients with severe disease, who required an average of 15 days, compared to 11 days for patients with moderate disease ($p<0.001$). Comorbidities, especially arterial hypertension and coronary artery disease, were common in patients with moderate disease but had less impact on outcomes compared to severe cases, where multiple chronic conditions were linked to higher mortality. Some treatments had a more pronounced effect in this group of patients, while others may have been less effective or had negative effects.

Conclusions: The study highlights the critical impact of chronic diseases on the severity and outcomes of COVID-19, emphasizing the need for personalized, multidisciplinary treatment strategies that consider comorbidities to optimize patient outcomes.

KEY WORDS: COVID-19, severe forms of infection, optimisation of therapeutic approaches, comorbid conditions, diagnostic methods, laboratory parameters, risk factors

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INTRODUCTION

Since the onset of the COVID-19 global pandemic, it has become clear that certain populations, especially those with chronic diseases, are more susceptible to severe infection and complications. In the context of this problem, conducting a multilevel analysis of the effectiveness of treatment strategies for COVID-19 in individuals with chronic diseases is a relevant scientific task. This approach allows identifying the best treatment options and factors that influence the effectiveness of therapy in patients with comorbid conditions.

Many studies have been conducted in Asia considering the interaction between COVID-19 and chronic diseases. In a retrospective study, L. Sun et al. [1] investigated the influence of diabetes on the severity and prognosis of COVID-19. The study provides further insight into how the presence of diabetes worsens outcomes in coronavirus infection, which has significant implications for clinical practice and the development of treatment strategies. M. Abu-Farha et al. [2] emphasises the role of diabetes in patients diagnosed with COVID-19. The study analyses how diabetes can affect disease severity and patient outcomes, providing valuable data for the medical community. F.A. Javid et al. [3] conducted a systematic review of the 2020 literature on the association of COVID-19 and diabetes. The review summarises the scientific evidence to help clarify the risks and mechanisms of COVID-19 exposure in patients with diabetes. The findings showed that patients with diabetes had a higher risk of developing severe COVID-19 and a worse prognosis.

In the context of multicentre studies highlighting complex aspects of the interaction between COVID-19 and chronic obstructive pulmonary disease (COPD), the value of the findings is invaluable for global healthcare. Thus, D. Singh et al. [4] studied the relationship between COPD and COVID-19, analysing how pre-existing chronic lung disease can exacerbate the course of coronavirus infection and worsen health prognosis. R.D.G. Jamora et al. [5] investigated clinical outcomes of COVID-19 infection among COPD patients based on data from the Philippine CORONA study. The researchers identified the level of risk and the need for a special approach to these patients. D.A. Puebla Neira et al. [6] analysed outcomes for COPD patients hospitalised due to COVID-19, which helps to understand how the disease affects vulnerable populations. The findings of these studies showed that the presence

of COPD increased the risk of complications and death from COVID-19.

M. Haji Aghajani et al. [7] analysed the effects of cardiovascular disease on COVID-19 prognosis in patients in different Asian countries. The findings indicated that the presence of cardiovascular disease increased the risk of severe complications and death from COVID-19. The study used data from health facilities in these countries to collect information on COVID-19 patients who also had cardiovascular disease. The presence of cardiovascular disease considerably increased the risk of developing severe complications from COVID-19. This could include worsening heart health, increased risk of thrombosis, and accelerated progression of other comorbid conditions. Patients with cardiovascular disease had a higher mortality rate compared to patients without such disease.

Within the framework of a multidisciplinary study conducted by I. Sabirov et al. [8], an in-depth analysis of medical cases demonstrating the relationship between severe and moderate forms of COVID-19, and cerebrovascular and renal complications was performed. The study focused on patients with comorbid conditions including acute respiratory distress syndrome (ARDS), which considerably exacerbated the course of the disease. Based on analyses of clinical data, the researchers found that cerebrovascular lesions are common in patients suffering from moderate to severe COVID-19. These disorders can manifest as cerebral infarction and intracerebral haemorrhage, as well as other types of cerebrovascular insufficiency, worsening the prognosis and treatment outcome.

K. Singh et al. [9] emphasised that the COVID-19 pandemic has had a marked impact on the care of patients with chronic diseases in different countries. Patients with chronic diseases, which are common in the elderly, are thought to have faced particular difficulties during the pandemic because their usual care and treatment were disrupted. A scientific analysis by E. Han et al. [10] indicates that the elderly and persons with chronic infections are most vulnerable when infection control measures are weakened, as these populations are already at increased risk of severe disease. Chronic infections may impair the immune system's response to new pathogens and reduce the effectiveness of the response to infectious diseases such as COVID-19. Older age is itself a risk factor for the development of severe COVID-19 due to the natural decline in immune reactivity and the presence of comorbidities.

All the studies described seek to identify the impact of chronic disease on the course and outcomes of COVID-19, and to evaluate the efficacy of different treatment approaches in the context of comorbidity. However, each of these studies focuses on concrete chronic diseases or patient populations, which sets them apart from each other.

AIM

The purpose of this study was to analyse the impact of multicomorbidity on the course and outcomes of COVID-19. The objectives were to evaluate the effectiveness of existing approaches to the treatment of patients with multiple chronic diseases, to analyse the results of treatment for the subsequent development of recommendations for clinical practice.

MATERIALS AND METHODS

This retrospective study was conducted at the Infectious Disease Centre of the Regional Clinical Hospital between March 2021 and February 2022 inclusive. The study examined the data from medical records of 236 patients with COVID-19 who had comorbid chronic diseases. The inclusion criteria of the patients in the sample were clearly defined and were based on available medical records and diagnoses made during the study period. All data were anonymised and used solely for research purposes following the principles of ethics and patient confidentiality. Careful data collection was performed, which included sex, age, period of hospitalisation and its duration, saturation level, the fact of transfer of the patient to the ICU, symptoms during hospitalisation, results of lung CT scan, presence of comorbidities: arterial hypertension, coronary artery disease, diabetes mellitus, obesity, oncology, presence of liver and kidney diseases, chronic obstructive pulmonary diseases, cerebrovascular diseases. The fact of respiratory support and its type was also considered, and information was collected on the severity of the course of the disease, as well as the treatment options and outcome of the disease.

A total of 236 patients' case histories were analysed in the study, of which 148 were women and 88 were men. The mean age of the patients was 66 years (minimum 19 years, maximum 96 years). Clinical symptoms considered were body temperature, presence of cough and dyspnoea, presence of intoxication symptoms, and saturation level. Computed tomography (CT) scans were also considered and categorised into CT1, CT2, CT3, CT4, which means the degree of lung tissue lesion. Computed tomography of the lung is a valuable tool to diagnose and assess the extent of lung tissue damage in patients with COVID-19. CT findings are generally categorised according to the following degrees of lesion:

- CT1: minimal lesion – presence of single small foci of inflammation affecting less than 25% of lung tissue;
- CT2: moderate lesion – multiple foci of inflammation affecting 25-50% of lung tissue;
- CT3: significant lesion – inflammatory changes affecting 50-75% of lung tissue;
- CT4: critical lesion – over 75% of lung tissue is affected, indicating severe progressive inflammation.

This study employed various statistical techniques to process and analyse the data. The data including gender, age of patients, and other clinical and demographic parameters were subjected to the following statistical treatment methods: Student's t-test was used to compare the mean values of two samples.

In this study, various statistical methods were used to process and analyse the data. Fisher's exact test was used to compare proportions in contingent tables when sample sizes were insufficient to apply the chi-square test. This test assessed whether there were statistically significant

differences between groups on various parameters such as gender and the presence of symptoms such as dyspnoea and cough. The Mann-Whitney U-test is a non-parametric test used to compare two independent samples, especially when the distribution of data deviates from normal. In the study, this method was employed to analyse variables such as duration of hospitalisation and indicators related to the severity of the course of the disease. Chi-square test was used to analyse categorical data and to assess the statistical significance of differences between groups. Specifically, this method was used to analyse chest CT scan data and other clinical indicators. The application of these statistical methods increased the reliability and validity of the study findings. This approach helped to identify real correlations between chronic diseases and the course of COVID-19, minimising the risk of random error and increasing the validity of the findings.

RESULTS

In a retrospective study conducted at the Infection Centre of the Regional Clinical Hospital, the purpose was to investigate the clinical, laboratory, and therapeutic aspects of COVID-19. The study covered the period from March 2021 to February 2022. A total of 236 patient histories were analysed in the study, of which 148 were women and 88 were men. The mean age of the patients was 66 years (19-96 years). Clinical information included the date of hospitalisation, which helped to establish the time frame of disease progression, as well as symptoms and clinical manifestations such as fever, dyspnoea, cough, signs of intoxication, and oxygen saturation levels needed to assess the severity of the patients' condition. The need for transfer to the intensive care unit indicated the critical condition of the patients, while CT scanning was used to assess the degree of lung damage. To analyse the data in general terms and to make correlations between different variables (sex, age, symptoms, oxygen saturation, vaccination, CT scores, and disease outcome), the study considered two groups of patients with moderate and severe disease.

The median age in patients with moderately severe disease was 68 years [61.7-72], whereas in patients with severe disease the median age was 66 years [57-72.8]. p=0.331, indicating that there was no statistically significant difference in age between the two groups (Table 1). In both groups, patients were hospitalised for approximately 7 days from the onset of illness. Median values were 7 days [5-10] in patients with a moderate course and 7 days [5-9] in patients with a severe course. The p-value is 0.453, indicating that there is no statistically significant difference between groups for this parameter. Patients with moderate severity had moderate symptoms such as cough, fever, dyspnoea, intoxication, and saturation above 80. 28 people (25.9%) had CT1, 49 people (45.4%) had CT2, 30 people (27.8%) had CT3, and 1 patient had CT4 (0.9%). Patients were hospitalised for an average of 11 days [10-13]. All 108 patients (100%) with moderate severity of the disease course were discharged with improvement.

Patients with severe course of the disease were hospitalised on average on the 7th day of illness with severe onset of symptoms such as cough, fever, dyspnoea, intoxication, and saturation within the values of 99-40. 2 patients had CT1 (1.6%), 20 patients had CT2 (15.6%), 46 patients had CT3 (35.9%), and 60 patients had CT4 (46.9%). 80 patients (62.5%) were discharged after a mean of 15 [12-18.8] days with improvement. 48 patients (37.5%) with a severe course of the disease had a fatal outcome. The duration of hospitalisation in patients with a moderate course was a median of 11 days [10-13], whereas in patients with a severe course the median was 15 days [12-18.8]. The p<0.001 value, indicating a statistically significant difference between these groups, pointed to a longer hospital stay in patients with a severe course of the disease (Table 2).

Table 1. Correlation of patients with moderate to severe disease course

Indicators	Course		p*
	Moderate severity, Me [Q1–Q3]	Severe, Me [Q1–Q3]	
Age	68 [61.3-72]	66 [57-72.8]	0.331
Day of hospitalisation from onset of illness	7 [5-10]	7 [5-9]	0.453
Duration of hospitalisation (bed days)	11 [10-13]	15 [12-18.8]	<0.001

Source: compiled by the authors of this study

Table 2. Correlation of patients with moderate and severe disease according to sex difference

Indicator		Disease course			
		Moderately severe		Severe	
		N	%	N	%
Sex	Male	39	36.1	49	38.3
	Female	69	63.9	79	61.7

Source: compiled by the authors based on [11]

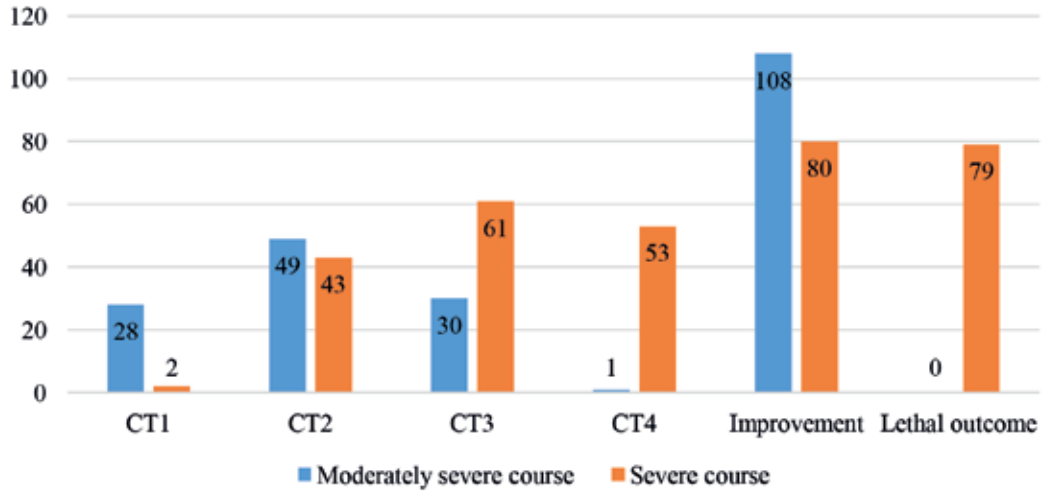

Fig. 1. Correlation of pulmonary tissue lesion index with the severity of disease course and outcome
Source: compiled by the authors based on [11]

The analysis shows that women, regardless of age group, are more likely to experience more severe forms of the disease. Vaccination considerably reduces the risk of developing severe forms, emphasising its significance as a preventative measure. Symptoms and oxygen saturation levels are essential indicators of disease severity, while CT scans can serve as additional diagnostic markers to assess patient status and predict outcome, as presented in Fig. 1.

An important part of the analysis was data on comorbidities such as arterial hypertension, coronary artery disease, diabetes mellitus, obesity, cancer, chronic kidney and liver disease, cerebrovascular disease, and chronic obstructive pulmonary disease, which influenced prognosis and outcome, as presented in Table 3.

AH is most common in patients with moderate disease severity and improvement. AH is also present in a considerable proportion of patients with a severe course of disease but with improvement. This may suggest that arterial hypertension is a common comorbidity but does not necessarily correlate with an increased risk of mortality. Coronary artery disease is the second most frequent pathology among patients with moderate severity, and less represented among patients with severe disease, regardless of outcome. Diabetes mellitus is comorbid in fewer patients but occurs in all three groups, with no significant difference between the severe and moderate groups. Obesity is a comorbid pathology in a small proportion of patients, regardless of group and outcome. Oncology is also the least common comorbidity in all three groups, as were liver diseases, kidney diseases, cerebrovascular diseases, and COPD. The study analysed the impact of chronic diseases on the course and outcome in COVID-19 disease. Correlations between certain chronic diseases (e.g., diabetes mellitus, cardiovascular disease, COPD) and worse prognosis in COVID-19 were established by analysing statistics on mortality, hospitalisations, and complications among patients with chronic pathologies infected with SARS-CoV-2. A study of comorbid pathology in patients who underwent severe clinical conditions revealed considerable variation in the number and combination of chronic conditions affecting patient outcomes. Observations have shown that patients who die usually have a higher burden of chronic diseases compared to those who are discharged with improvement after treatment.

The analysis of data of patients with COVID-19 mortality showed that multiple chronic pathologies significantly worsened the prognosis of the disease; a significant correlation between the number of diseases and the severity of the disease course was found. These data emphasise the importance of an integrated approach to the management of patients with multiple pathologies. An in-depth understanding of the interrelationships between different chronic conditions and their impact on clinical outcomes is becoming a key element in health management strategies in a multidisciplinary hospital setting. The study examined the effects of anticoagulants, glucocorticoids, antiviral agents, and monoclonal antibodies, and assessed the need for supplemental oxygen support or mechanical ventilation. Disease outcomes were recorded, including recovery, length of hospitalisation, transfer to other wards or death. Researchers considered the effectiveness of therapy in the presence of coexisting internal organ pathologies and COVID-19 disease. Treatment of COVID-19 in patients with chronic conditions requires special attention, as some medicines used to treat these conditions may interact with COVID-19 treatment or exacerbate its course [12].

During the COVID-19 pandemic, physicians were particularly careful about the use of medications in patients with chronic diseases. ACE inhibitors such as enalapril and lisinopril have been used to treat hypertension and have been found to be safe despite initial concerns; similarly, angiotensin II receptor blockers (e.g., losartan, valsartan) also did not increase the risks of worsening in COVID-19. Patients with coronary artery disease had to be careful with thiazide diuretics because of the risk of electrolyte imbalance and beta-

Table 3. Correlation of comorbidities with severity of course and outcome

Parameter	Statistics	Moderately severe course	Severe course (improvement)	Severe course (fatal)
AH				
yes	n (%)	95 (88%)	64 (80%)	42 (87.5%)
no		13 (12%)	16 (20%)	6 (12.5%)
CAD				
yes	n (%)	43 (39.8%)	31 (38.8%)	31 (64.6%)
no		65 (60.2%)	49 (61.2%)	17 (35.4%)
DM				
yes	n (%)	50 (46.3%)	42 (52.5%)	32 (66.7%)
no		58 (53.7%)	38 (47.5%)	16 (33.3%)
Obesity				
yes	n (%)	14 (13%)	19 (23.8%)	17 (35.4%)
no		94 (87%)	61 (76.2%)	31 (64.6%)
Cancer				
yes	n (%)	1 (0.9%)	2 (2.5%)	3 (6.2%)
no		107 (99.1%)	78 (97.5%)	45 (93.8%)
Kidney diseases				
yes	n (%)	25 (23.1%)	16 (20%)	9 (18.8%)
no		83 (76.9%)	64 (80%)	39 (81.2%)
Liver diseases				
yes	n (%)	1 (0.9%)	2 (2.5%)	0 (0%)
no		107 (99.1%)	78 (97.5%)	48 (100%)
CVD				
yes	n (%)	30 (27.8%)	15 (18.8%)	14 (29.2%)
no		78 (72.2%)	65 (81.2%)	34 (70.8%)
COPD				
yes	n (%)	8 (7.4%)	3 (3.8%)	1 (2.1%)
no		100 (92.6%)	77 (96.2%)	47 (97.9%)

Note: AH – arterial hypertension, CAD – coronary artery disease, DM – diabetes mellitus, CVD – cardiovascular disease, COPD – chronic obstructive pulmonary disease.

Source: compiled by the authors based on [11]

blockers, which could exacerbate respiratory symptoms. In the context of diabetes mellitus, metformin could potentially cause lactate acidosis, especially in severe infections, while SGLT2 inhibitors such as empagliflozin increased the risk of ketoacidosis. Medications metabolised in the liver may have placed additional burden on the organ in patients with liver disease and COVID-19. The use of NSAIDs, such as ibuprofen, may have impaired renal function, which was critical in patients with renal impairment. In the case of COPD, systemic corticosteroids have sometimes been used, although they may reduce immunity. Finally, cerebrovascular disease required special monitoring of anticoagulant use, considering the possible COVID-19-induced changes in the blood coagulation system.

COVID-19 treatment varies depending on the severity of the disease, the presence of comorbidities, and other individual patient characteristics [13]. There is no one-size-fits-all treatment regimen, but there are general principles and guidelines that have been developed based on clinical research and practice. For patients with moderately severe disease course who have trouble in breathing or other severe symptoms, oxygen therapy to maintain normal blood oxygen levels, antiviral medication such as, e.g., remdesivir, antibiotics may be required if there is evidence of bacterial infection. Severe and critical COVID-19 is treated in the intensive care unit with intensive oxygen therapy or mechanical ventilation to maintain vital functions, corticosteroids such as dexamethasone to reduce inflammation, blood pressure medications, and other intensive care medicines, and medications to prevent thrombosis such as low molecular weight heparins. In addition, monoclonal antibodies for patients at elevated risk of developing the severe form and immunomodulators may be used within the framework of clinical trials. COVID-19 treatment should be tailored to each individual case and any treatment should be carried out under strict medical supervision.

Comorbidities such as arterial hypertension, coronary artery disease, and diabetes mellitus were quite common among patients discharged with improvement, but they did not seem to affect the outcome as much as in the fatal group. The presence of multiple chronic conditions was also noted, but their presence did not always lead to severe outcomes, which may indicate better treatment or

lesser severity of these comorbid conditions. Treatment included anticoagulants (enoxaparin sodium), corticosteroids (prednisolone, dexamethasone), antiviral medication (remdesivir), and monoclonal antibodies (tocilizumab). Interestingly, tocilizumab was used in only three patients, which may reflect the limited use of this medication in therapeutic practice or its prescription only in particularly severe cases. Despite the milder overall nature of the disease, most patients still required oxygen support. This emphasises the significance of the availability of oxygen therapy and relevant equipment to successfully treat COVID-19, even if most symptoms are not severe. Overall, the data suggest that successful treatment of COVID-19 requires consideration of multiple factors, including early detection, adequate treatment of comorbidities, availability of respiratory support, and the ability to provide individualised therapy. Understanding these factors in the context of long-term patient improvement is key to developing more effective treatment and disease management strategies.

A closer analysis of the therapy of patients with different outcomes revealed that 5 patients with fatal outcome did not receive anticoagulant, which may have increased the risk of thromboembolic complications, especially in critically ill patients. Clexane in combination with prednisolone was administered to 11 patients who also received dexamethasone and remdesivir and may have had an increased risk of infections due to the immunosuppressive effects of glucocorticoids. Dexamethasone with clexane and remdesivir was given to many fatal patients, indicating the use of a standardised treatment protocol for the severe form of COVID-19. The presence of antiviral treatment in most patients may indicate attempts to control viral replication, but in critically ill patients, the efficacy of remdesivir may be limited. Standards of therapy for COVID-19 depend on the severity of the disease, and they are constantly evolving as new research data become available. A comprehensive treatment approach includes the use of antiviral, anti-inflammatory, anticoagulant agents, and supportive lifestyle functions.

It is important to assess each patient individually, considering disease severity, risk of thrombosis, immune status, and comorbidities. Early initiation of anticoagulant and anti-inflammatory therapy can prevent the development of severe complications. The use of remdesivir should be considered for hospitalised patients, especially those who require oxygen support but are not on ventilator. The use of glucocorticoids (prednisolone and dexamethasone) should be limited to patients with severe inflammation and need for oxygen support. Thrombosis prophylaxis should be part of standard therapy for patients at increased risk of thrombosis. Therapy should be adjusted based on laboratory data and the patient's clinical condition. This approach may help to improve outcomes for patients with COVID-19 of varying severity. The findings of this study may considerably improve the understanding of the interaction between COVID-19 and chronic diseases and contribute to the development of more effective treatment and prevention strategies.

Given the significance of statistical parameters such as hazard ratio and Kaplan-Meier score, the authors recognize that these methods may be useful for improving the accuracy of outcome prediction in the treatment of patients with COVID-19, especially among individuals with chronic diseases. Given that the use of these statistical methods in future studies will not only improve the accuracy of prognosis, but also provide more detailed information about the duration of the disease and the likelihood of complications.

DISCUSSION

Analysis of the findings of a study conducted at the Infectious Disease Centre of the Regional Clinical Hospital in Karaganda to evaluate the clinical, laboratory, and therapeutic aspects of COVID-19 highlights several key points. The study included 236 patients, with more women than men. Differences in median age between patients with moderate and severe disease were statistically insignificant, as the p-value was 0.331. This means that age alone is not a decisive risk factor for worsening of the condition, but it should be considered together with other parameters. The mean age of the patients was 66 years, indicating a high vulnerability of the older generation to COVID-19, considering that older age groups are more likely to experience severe forms of the disease. The median values of the hospitalisation period were 7 days for both groups of patients, indicating that there was no statistically significant difference ($p=0.453$). This demonstrates the significance of seeking early medical attention to initiate treatment promptly and prevent complications. Patients with a moderate course had a median length of hospitalisation of 11 days, while patients with a severe course had a median length of hospitalisation of 15 days. This difference is statistically significant ($p<0.001$), confirming a longer hospital stay in patients with a severe course of the disease.

Among the patients analysed, those with severe manifestations were more likely to have low oxygen saturation and more severe lung lesions on CT scan. Such patients required more intensive therapy and were more likely to die. Importantly, the presence of chronic diseases worsened COVID-19 prognosis. Specifically, conditions such as arterial hypertension, coronary artery disease, diabetes mellitus, obesity, and other chronic diseases increased the risk of severe outcomes. Understanding the correlation between chronic disease and COVID-19 outcomes may facilitate better risk management and treatment. Various treatments including the use of anticoagulants, anti-inflammatory agents, and antiviral medications have been shown to be effective depending on the stage and severity of the disease. Particular attention should be paid to the prompt initiation of therapy, adaptation of treatment to concrete clinical cases, and monitoring of the dynamics of laboratory parameters. The findings of the study emphasise the significance of an individualised approach in the treatment of patients with COVID-19, especially among patients at high risk due to age or chronic disease. Chronic disease and elevated levels of inflammatory markers are risk factors

for a severe course of new coronavirus infection. Earlier initiation of anticoagulant and anti-inflammatory therapy may lead to better clinical outcomes in patients at high risk of complications. Individual approach to each patient in combination with monitoring of laboratory parameters in dynamics and treatment adjustment can contribute to more effective therapy and prevention of lethal outcomes.

In the medical literature, many studies have focused on the interaction of COVID-19 with chronic diseases and their management patterns in pandemic settings. The analysis of publications creates a synthesised scientific review that identifies key trends and scientific findings. T. Kendzerska et al. [14] analyse how healthcare systems in different countries responded to the pandemic, what strategic and operational changes were implemented, and how this affected chronic disease management. Special attention is being paid to the adaptation of healthcare facilities, changing treatment protocols and the use of digital technologies. A. Darrudi et al. [15] emphasise the problems of achieving universal coverage of health services. The study examines systemic barriers and policy obstacles that became particularly prominent during the pandemic and suggests ways to optimise healthcare systems to improve their efficiency and accessibility. R. Moynihan et al. [16] focus on analysing the impact of the pandemic on the use of health services. The survey shows how the use of health services has changed, including primary care, specialised care, and emergencies, highlighting shifts in priorities and access to healthcare. Thus, the reviewed studies complement each other, providing both micro-level and macro-level perspectives on the problem of healthcare and chronic disease management in the COVID-19 pandemic, emphasising the need for an integrated approach in adapting medical and management strategies.

H. Liu et al. [17] conducted a systematic review and meta-analysis that centres around the study of the correlation between comorbid chronic diseases and the severity of COVID-19. A systematic review and meta-analysis by the researchers cover many studies conducted in different countries, allowing the researchers to collect a considerable amount of data and conduct a generalised analysis of the impact of chronic diseases on the severity of COVID-19. This enables a broader picture to be obtained and conclusions to be drawn that can be applied internationally, accommodating differences in healthcare systems and population characteristics. In contrast, the present study has a narrower geographical focus and analyses data collected directly from a single health facility. This study details the clinical outcomes, treatment, and dynamics of COVID-19 in patients with chronic diseases, providing further insight into the specific interactions between chronic and infectious diseases in a particular region. Despite its more limited scope, this approach provides valuable insights into the specifics of managing COVID-19 patients with comorbid backgrounds.

A cohort study conducted by F. Alammari et al. [18] investigated the impact of COVID-19 infection on patients with chronic diseases admitted to the intensive care unit. F. Alammari et al. [18] focused on patients with chronic diseases who were hospitalised in intensive care units. This study identifies which factors may have contributed to patients' transition to critical care and which treatment strategies were most effective in critical care. Of particular significance are findings on how the management of these patients in intensive care may improve outcomes or, conversely, have no meaningful positive impact. The researchers performed an in-depth analysis of the situation of patients in the extreme environment of intensive care units, making its findings particularly valuable for understanding critical aspects of the management of the most severe cases.

A cross-sectional study by W. He et al. [19] provide different perspectives on the effects of COVID-19 on people with chronic diseases. The results of our study showed that the presence of chronic diseases significantly worsens the outcomes of COVID-19, increases the likelihood of hospitalization in intensive care units and increases the risk of death. These findings underscore the seriousness of physical health risks for patients with chronic diseases when infected with coronavirus, indicating the need for more careful medical surveillance and individualised treatment strategies. In contrast, the researchers focused on the psychological and behavioural aspects of the impact of the pandemic on people with chronic diseases, revealing significant psychological deterioration and lifestyle changes during the lockdown. The findings showed increased levels of depressive symptoms and decreased physical activity, emphasising the significance of psychological support and social coping strategies for this group of patients. Thus, the urgent need for medical intervention and monitoring on the one hand, and psychological well-being and social support on the other hand, together form the basis for an integrated approach to caring for these patients during the global health crisis.

H. Ismail et al. [20] analyse how the pandemic affected medical conditions and adherence to pharmacological therapy. The findings indicate worse physical health and medication adherence in patients with chronic diseases, which is like the findings of this study that chronic diseases worsen COVID-19 outcomes and require more intensive medical follow-up. K. Wikström et al. [21] assessed changes in the epidemiology of chronic diseases in Finland during the pandemic. This study has identified new trends and needs in healthcare, emphasising the necessity for healthcare systems to adapt to changing conditions. The current study also emphasises the significance of monitoring and adapting to pandemic conditions to improve care for patients with chronic diseases. M. Altuntas et al. [22] focused on assessing comorbid conditions in patients with COVID-19 and identifying those that may exacerbate the outcomes of infection. These data support the findings of the present study that chronic disease can considerably worsen prognosis in COVID-19 and highlight the need for an individualised approach to treatment. S.K. Alyammahi et al. [23] present an analysis of the association between COVID-19 and chronic disorders, updating data on the prevalence and mechanisms of interaction as well as therapeutic

approaches. This study improves understanding of the relationships between infection and long-term medical conditions, which is essential for the development of effective treatment strategies.

S.H. Mboweni and P.R. Risenga [24] analysed the experiences of patients with chronic diseases during the COVID-19 pandemic in the Northwest Province of South Africa, where the researchers employed qualitative methods to explore personal experiences. H. Salah et al. [25] focused on the psychological impact of COVID-19 on patients with chronic diseases who sought medical care. This study also moves away from retrospective analyses, focusing on assessing immediate emotional reactions and stress levels among patients, which adds valuable data on psychological health in pandemic settings. V. Mauro et al. [26] emphasised the need to provide treatment for all patients with COVID-19, with special attention to those who were not infected but had chronic diseases. This study offers a population health management perspective that expands the understanding of how healthcare should adapt in crisis, again moving away from standard retrospective data analysis.

Research by scientists from around the world raises important questions about chronic disease management in the COVID-19 pandemic and provides a variety of approaches to analysing emerging issues. A. Parkinson et al. [27] examines the impact of the COVID-19 pandemic on chronic disease management in primary care in Australia, comparing this with international experience. The focus is on adapting healthcare systems to crisis conditions and identifying lessons that can be used to optimise care for patients with chronic conditions. Unlike the conducted research, the Australian project focuses more on system-wide changes and does not go into detail about concrete treatment strategies or their effectiveness. L. Balasuriya et al. [28], N.V. Drutsul-Melnyk & L.A. Ivanova [29] investigated the impact of COVID-19 on chronic disease prevention and health promotion activities at the national level. This study does not have a clear research methodology and is more of a review article or commentary. This study does not specifically analyse treatment interventions or their effectiveness. E. Barasa et al. [30] examined the level and inequalities in health insurance coverage in 36 countries in sub-Saharan Africa. The study highlighted the socioeconomic aspects of healthcare that may indirectly influence chronic disease management during a pandemic. M.E. Charlson et al. [31] is a methodological work that develops a new method for classifying prognostic comorbidity in longitudinal studies. F. Deslauriers et al. [32] focused on the impact of COVID-19 on the lives of Canadians with and without chronic non-communicable diseases, analysing psychosocial and behavioural changes.

The present study also highlights the impact of chronic diseases like arterial hypertension, diabetes mellitus, coronary artery disease, and obesity on COVID-19 outcomes, showing a higher risk of severe outcomes among this category of patients. The current study also notes the effectiveness of different treatments depending on the stage and severity of the disease, including the use of anticoagulants and antiviral medications, highlighting the significance of an individualised approach to treatment and the importance of prompt initiation of therapy.

CONCLUSIONS

In the context of the COVID-19 pandemic, the relevance of medical research has increased markedly, especially in the context of investigating the interaction between viral infection and chronic disease. Particular attention is paid to analysing the efficacy of treatment strategies for patients with chronic pathologies, as they are at higher risk of developing severe COVID-19 and complications. The findings of the present study showed that the presence of chronic diseases considerably exacerbates the course of COVID-19, often leading to the development of severe forms and increased length of hospitalisation. Some treatments have shown improved efficacy, while others may be less successful or even harmful for this patient population. Thus, the study has enriched the academic community with data on key risk factors for the development of severe COVID-19 in individuals with comorbidities.

Chronic diseases including arterial hypertension, coronary artery disease, diabetes mellitus, obesity, chronic kidney and lung diseases were associated with worsening prognosis in COVID-19 patients. Patients with multiple chronic conditions often experienced a more severe course of infection and a higher risk of mortality. The treatment approach for COVID-19 included anticoagulants (enoxaparin sodium), anti-inflammatory medications (glucocorticoids like dexamethasone and prednisolone), antiviral medications (remdesivir) and, in some cases, the use of monoclonal antibodies (tocilizumab). The decision to use each of these agents was based on the individual clinical picture and the presence of comorbidities. Anticoagulant therapy was particularly important to prevent COVID-19-induced thrombosis. It was noted that patients who did not receive enoxaparin sodium had a higher mortality rate, emphasising the significance of anticoagulant prophylaxis. Anti-inflammatory therapy with dexamethasone demonstrated a positive effect in severe patients; however, in patients with lethal outcome there was no decrease in inflammatory parameters in dynamics, indicating a possible need for early initiation of treatment and/or more intensive anti-inflammatory therapy. Remdesivir was used in many cases but did not affect mortality, possibly because of late initiation of therapy or because of disease severity.

Individual assessment of each patient is required to optimise the therapeutic approach, considering their personal risks and the potential benefits of each preparation. A multidisciplinary approach to the management of comorbidities and the underlying disease can improve outcomes through integrated treatment and monitoring. Early and active intervention with anticoagulant and anti-inflammatory therapy may contribute to better outcomes, especially in patients at high risk of complications. Continuous monitoring and flexible adaptation of treatment based on dynamic laboratory data and changes in the patient's condition are necessary. A comprehensive, personalised, and dynamic approach to

the treatment of patients with COVID-19 is recommended, considering their individual characteristics and comorbidities to achieve the best clinical outcomes.

Limitations of this study included the possible presence of bias due to the retrospective nature of the analysis, and the lack of data on the long-term effects of COVID-19 in patients with chronic diseases. Furthermore, heterogeneity in clinical protocols between different treatment facilities may have influenced treatment outcomes. Prospects for further investigation may include multicentre, prospective studies to improve representativeness and reduce potential bias. It is also important to focus on studying the long-term effects of COVID-19 on patients with multicomorbidity, which will help in the development of more effective treatment and rehabilitation strategies. Moreover, further development of individualised therapeutic approaches that are tailored to each patient is required to optimise treatment and minimise the risk of complications.

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CONFLICT OF INTEREST
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ORIGINAL ARTICLE

Investigation of stress levels based on the type of behaviour in stressful situation among internally displaced persons after relocation from areas of active hostilities

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ABSTRACT

Aim: To compare indicators of the level of stress and the tendency to be exposed to stressogenic factors in internally displaced persons who moved from regions of active hostilities in the first three months after the full-scale Russian invasion.

Materials and Methods: The following diagnostic tools were used in the study: 1. „Test for determining the level of stress" (according to V. Shcherbatykh, adapted by H. Kharko), the essence of which is to determine the level of stress of a person. The test contains several sections that are responsible for a certain sign of stress – intellectual, behavioral signs, emotional and physiological symptoms. 2. „Questionnaire for self-diagnosis of the type of behavior in a stressful situation" (according to V. Boyko), which allows you to identify reactions to stress and the tendency to be influenced by stressogenic factors depending on the type of behavior in a stressful situation. Analysis, synthesis, generalization, concretization, systematization, comparison, forecasting, and mathematical statistics were used to process, compare, evaluate, and interpret the obtained research results. Data processing was carried out using Jamovi 2.2.5 and RStudio 2024.04.1.

Results: The relevance of the topic deals with the importance of the need to preserve, develop and strengthen the health of the individual, in particular in the conditions of war, which appears as a serious stress factor that tests a person. A special category that requires research on the level of stress and the tendency to be influenced by stress factors are internally displaced persons who have moved from regions of active hostilities and have a whole set of problems related to the lack of housing, work, adaptation in a new place, establishing communication, finding new resources of existence, etc. Long-term stress has a negative effect on the human body, in particular on intellectual, behavioral, emotional, and physiological manifestations. Among 134 respondents, 92 women, 29 men and 13 respondents did not indicate their gender participated in the study. The average age of the respondents was: Me=36, Q 1=32, Q 3=44 (N =118); 16 respondents did not indicate their age. The distribution of respondents' age indicators was not subject to a normal distribution according to the Gaussian curve.

Conclusions: During the study of groups of internally displaced persons (hereinafter IDPs), which were formed according to time, gender and age criteria, moderate direct correlations were found between indicators of the level of stress and indicators of the tendency to be influenced by stressogenic factors, depending on the type of behavior in a stressful situation. Correlation is observed both for general indicators and component blocks of stress manifestations (intellectual, behavioral, emotional and physiological), which confirms the relationship between psychophysiological indicators of stress and the type of stress behavior. Our study among internally displaced persons aged 18-81 years who moved from regions of active hostilities proved the absence of a difference between women and men in the level of stress, and differences in the manifestations of intellectual, behavioral signs, emotional and physiological symptoms.

KEY WORDS: war, an internally displaced person, stress, susceptibility to the influence of stressful factors, type of behavior in stressfull situation

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INTODUCTION

The problem of determining the level and manifestations of stress is an important condition for preserving, developing and strengthening the health of an individual, in particular, in conditions of war among such a category of the population as internally displaced persons, who, unlike other categories of the population, are forced to leave their homes, find a new place of residence and work To this set of problems are added others related to adaptation in a new place, communication, search for new resources of existence, etc. [1]. The main aspects of experiencing the situation of forced migration as stressful in itself are experiencing negative life events (losses, rapid changes that occur before and during resettlement, etc.); experiencing changes in the social environment, including mental differences; experiencing partial isolation and possible deprivations [2]. The war appears as a serious stressful factor that tests a person. The personality during the war experiences a sharp increase in the level of anxiety, fear, aggression and other negative emotions. Long-term stress has a negative

effect on the human body, in particular on intellectual, behavioral, emotional, physiological manifestations [1, 3].

According to International Organization for Migration (hereinafter IOM) in August 2022, the number of internally displaced persons (IDPs) in Ukraine amounted to 6,975,000 [4]. Meanwhile, in December 2022, their numberwas 5.9 million; asof January 2023 – 5.4 million [5]. According to the consolidated data of the Ministry of Foreign Affairs of Ukraine, the Ministry of Social Policy and International Organizations, as of March 2023, 4,843,633 IDPs were officially registered in Ukraine [6]. Similar data are provided by the CHESNO Movement, according to which, as of the corresponding period, 4,845,761 IDPs were registered in Ukraine, while in May 2021, the number of citizens of this category was 1,466,077. In general, the estimated number of IDPs in Ukraine remains three times higher than before the start of the full-scale Russian invasion [7].

In the sources of scientific literature, we observe studies related to the identification of factors of traumatization, psychological characteristics, stress level and stress resistance of IDPs in Ukraine, who were forced to leave their homes due to Russian aggression. Yes, N. Maruta, G. Kalenskoy, T. Panko was proved that IDPs are distinguished by a decrease in vitality, a low level of resistance to stress, and the actualization of maladaptive ones coping strategies , moderate severity of depression and high indicators of anxiety [8], which collectively determines a high level of their vulnerability and tendency to maladaptation .

Z. Sprynska, studying some psychological properties of IDPs, which are important for adaptation and regulation of social behavior, came to the conclusion that IDPs are prone to stressful reactions to usual life situations [9]. This indicates a low level of stress resistance of IDPs and the presence of maladaptation effects .

The confirmation of this thesis is the research of O. Lushchak, M. Velykodna, S. Bolman, O. Strilbytska, V. Berezovskyi& K. Storey where the authors investigated the inner well-being, prevalence of stress, anxiety and symptoms of post-traumatic stress disorder among IDPs and refugees abroad after the first year of the full-scale Russian invasion and found high levels of stress in the subjects. Thus, the results of an online survey conducted by these authors showed that 64.4% of IDPs and 64.7% of refugees abroad from the number of citizens of Ukraine indicated the presence of moderate stress in them, and the indicators of the presence of severe stress in the groups amounted to 21.6% and 25.2%, respectively [10].

Moreover, the high level of stress development is confirmed by the results of an online survey conducted on September 22-26, 2022. Gradus Reserch Company among men and women aged 18–60 who lived in cities with a population of 50K+ on the territory of Ukraine. The results of the study showed that 71% of the surveyed respondents felt stress or severe nervousness. In addition, this study reveals some priority causes of the development of stress. Thus, among the causes of stress, the respondents named the war in the first place (72%), financial difficulties (44%), the socio-political situation in the country (30%) in the second place [11].

In some studies, the peculiarities of reactions to stress factors of certain categories of the population have been demonstrated. Thus, S. Kondratyuk considered the gender aspect of the population's reactions to stress factors. There sults of anempirical study demonstrated the level of stress experienced by women aged 25-45 in war conditions. The study found that 35.5% of respondents are in a state of high stress level, and 14.5% of women were found to have a very high level of stress, which indicates a low stress resistance of the individual and can lead to deterioration of well-being and the development of certain diseases. Stress at an average level was detected in 18% of respondents, which requires the use of simple techniques of emotional self-regulation. 25% of women were in a state of moderate stress. And only 7.5% of women show low levels of stress, they are able to resist stressful factors and be effective in stressful situations [1].

Age-specific features of the reaction to the stress factors of war were demonstrated in the studies of O. Semikina, V. Fedchenko, I. Yavdak, O. Cherednyakova& S. Volkova . The authors conducted a survey of IDPs, among whom the majority were young and middle-aged persons. In the course of the study, they noted that despite the denial of any emotional problems by internally displaced persons in the majority of cases, a detailed examination revealed a predominance of negative affect in 43% of respondents. A high prevalence of accompanying somato-neurological pathology has also been determined, which in combination with organ pathology, long-term stressful circumstances (unexpected change in living conditions, uncertainty about the present and future) is a significant factor in the development of mental disorders and severe somatic pathology [12].

Studies of stress reactions of individual groups of IDPs in conditions of war with certain types of pathologies or special physiological conditions deserve special attention. Thus, N. Maruta, T. Panko, G. Kalenska, S. Kolyadko, I. Yavdak& S. Volkov studied the dynamics of factors of mental traumatization of IDPs over time, their impact on the state ofmentalhealth, stress resistance and social adaptation depending on the presence and level of mental disorders.

The researchers divided the IDPs into groups:

- from the general population, in which no mental disorders were detected (conditionally healthy);
- with individual mental symptoms that did not reach the clinical level (risk group);
- with diagnosed mental disorders.

The results obtained in these groups showed that in the group of healthy IDPs, indicators of high and medium levels of resistance to stressful events prevailed, in the risk group – medium level, and in those examined with mental disorders – low level of resistance to stressful events [8].

In another study, I. Zhabchenko, N. Korniets, S. Tertychnyu-Telyuk& T. Kovalenko studied the peculiarities of the course of pregnancy against the background of psycho-emotional stress of pregnant women among IDPs. During the study, it was found that all pregnant women were under the negative influence of persistent stress, which play sone of the leading roles in the manifestation of gestational complications [13].

AIM

To compare indicators of the level of stress and the tendency to be exposed to stressogenic factors in internally displaced persons who moved from regions of active hostilities in the first three months after the full-scale Russian invasion.

MATERIALS AND METHODS

The study was conducted during March – May 2022. It was attended by 134 respondents aged 18 years and older who moved from regions of active hostilities, at the time of the survey lived in the city of Ternopil, had the status of IDPs, received assistance under the project „Ensuring Protection rights and legal assistance to internally displaced persons and those affected by the conflict in eastern Ukraine" and expressed a desire to participate in the experiment. The validity of the choice of the location of the study is confirmed by IOM data – compared to other macro-regions of Ukraine, in 2022, the largest number of IDPs was recorded intheWesternmacro-region, which was 16 March – 2,586,000; April 1 – 2,927,000; April 17 – 2,850,000; May 3 – 2,900,000; May 23 – 1,838,000 people [4]. Ternopil region also has the highest percentage of IDPs suffering from food shortages – 12% [14].

The following diagnostic tools were used in the study: 1. „Test for determining the level of stress" (according to V. Shcherbatykh, adapted by H. Kharko), the essence of which is to determine the level of stress of a person. The test contains several sections that are responsible for a certain sign of stress – intellectual, behavioral signs, emotional and physiological symptoms [15]. 2. „Questionnaireforself-diagnosisofthe type of behavior in a stressfulsituation" (accordingto V. Boyko) [16: 12-14], which allows you to identify reactions to stress and the tendency to be influenced by stressogenic factors depending on the type of behavior in a stressful situation.

Analysis, synthesis, generalization, concretization, systematization, comparison, forecasting, and mathematical statistics were used to process, compare, evaluate, and interpret the obtained research results. Data processing was carried out using Jamovi 2.2.5 and RStudio 2024.04.1.

The research is based on the ethical principles of organizing and conducting scientific research with human participation. All respondents gave consent to participate in the survey and data processing.

RESULTS

Among 134 respondents, 92 women, 29 men and 13 respondents did not indicate their gender participated in the study. The average age of the respondents was: Me=36, Q 1=32, Q 3=44 (N =118); 16 respondents did not indicate their age. The distribution of respondents' age indicators was not subject to a normal distribution according to the Gaussian curve.

The age range of the respondents was: women aged 19 to 81 years, the average indicators are Me=36, Q 1=32, Q 3=44 (N = 91); men Me=38.5, Q 1=31, Q 3=54 (N = 26). Based on the evaluation by the U- criterion Mann-Whitney age difference of the average indicators in the female and male groups was not found (U =1095, Z = -0.573, p =0.565). It is worth noting the fact that, as a rule, respondents who did not indicate their gender did not indicate their age either, which may indirectly indicate the infallible nature of the absence of data by age and gender.

All respondents were divided into three groups according to the time of data collection: March 2022 – group №1, April 2022 – group №2, May 2022 – group №3. „Average indicators of the stress level determined according to the Test for determining the stress level" (according to V. Shcherbatykh, adapted by H. Kharko), were Me=26, Q 1=15.5, Q 3=37 (N = 134). The distribution of respondents' stress development indicators was not subject to a normal Gaussian distribution.

On the basis of the U-Mann-Whitney test, there was no difference between women and men in the level of stress (U = 5742, Z = 0.8189, p = 0.4128). Also, the analysis of the dependence of the stress level on the age of the respondents using Spearman's correlation calculations showed the absence of such a dependence (r=0.08 at p >0.05). No dependence of the stress manifestations (intellectual, behavioral signs, emotional and physiological symptoms) on the age of the respondents was found. Similarly, there was no correlation of the stress level with age and separately in women and men (women r = 0.06 at p > 0.05, men r = 0.12 at p > 0.05). In Table 1 presents the most frequent symptoms of stress, which were noted by the respondents during the testing.

It is necessary to consider the interesting fact about the gender features that manifested themselves in the following:

- in the block of intellectual signs of stress, both women and men unanimously most often insisted on the presence of „Predominance of negative thoughts" (women Mo =49, N =92, men Mo =15, N =29);
- in the blockofbehavioral signs of stress, women mainly reported the presence of „Sleep disturbances or insomnia" (Mo =59, N =92), while men paid attention to the presence of „Increased conflict situations at work and in the family" (Mo =19, N =29);
- in the block of emotional symptoms of stress, thepresenceof „Worry, increased anxiety" became the same problem for both women and men (women Mo =77, N =92, men Mo =18, N =29);
- in the block of physiological symptoms of stress, the presence of „Pains in various parts of the body of an unspecified nature, headaches" was characteristic of more women (Mo =55, N =92), while men emphasized the problem of „Rapid or irregular pulse" (Mo =17, N =29).

The indicators of the central tendency of the Test for determining the level of stress (according to V. Shcherbatykh, adapted by H. Kharko) in the groups that were selected based on the time of information collection of respondents from March to May gave the following results:

1 group (March) Me = 24, Q1=13, Q3=31 (N=59);

2nd group (April) Me = 20.25, Q1=12.5, Q3=30 (N=50),

3rd group (May) Me = 56, Q1=50, Q3=59 (N=25).

The distribution of respondents' stress development indicators was not subject to a normal Gaussian distribution.

Also, as a result of statistical data processing, some gender features in the groups were revealed. Thus, in group No. 2

Table 1. The most frequent symptoms of stress identified by IDP respondents

Structural blocks	Symptoms of stress	Number of positive responses
Intellectual symptoms of stress	„Predominance of negative thoughts“	Mo=70
	„Constant and fruitless rotation of thoughts around one problem“	Mo = 64
	„Difficulties in making decisions, long hesitations when choosing“	Mo = 64
Behavioral symptoms of stress	„Sleep disturbance or insomnia“	Mo=82
	„Increasing conflict situations at work or in the family“	Mo = 73
	„Loss of appetite or overeating“	Mo = 69
Emotional symptoms of stress	„Worry , increased anxiety“	Mo = 108
	„Gloomy mood“	Mo = 88
	„Irritability, fits of anger“	Mo = 76
Physiological symptoms of stress	„Pains in various parts of the body of an undefined nature, headaches“	Mo = 74
	„Increased fatigue“	Mo = 73
	„Rapid or irregular pulse” and “Increased or decreased blood pressure“	Mo = 62

Source: compiled by the authors of this study

(April) in the block of intellectual signs of stress, according to the calculated indicators of the Mann-Whitney test (U = 76; Z = -3.17492, p = 0.001499), men had lower indicators of stress manifestations (Me = 1 ; Q1 = 0, Q3 = 2; N = 11) than in women (Me = 4; Q1=2, Q3=7; N=38). The same tendency was observed in the block of emotional signs of stress: in men (Me = 3; Q1=1.5 Q3=7.5; N=11), in women (Me = 9; Q1=6, Q3=12; N =38), Mann-Whitney test (MU: U= 122.5; Z = -2.06071; p = 0.039332).

Descriptive statistics for the structural blocks of the Test for determining the level of stress (according to V. Shcherbatykh, adapted by H. Kharko) are shown in Table 2.

During data processing, some changes in indicators over time were detected (groups 1-March, 2-April, 3-May). Thus, with the help of the Kruskel-Wallis criterion calculation , a significant difference was found between the indicators of three groups of respondents according to the Test for determining the level of stress (according to V. Shcherbatykh, adapted by H. Kharko). Indicators between groups 1, 2 and 3 (March, April, May) had a reliable level of differences both overall between groups and separately for women and men. Such a tendency persisted not only in the general indicators of the test, but also in its structural parts, the results of which are shown in Table 3 .

Further a posteriori analysis of the pairwise comparison was performed using the criterion calculation Mana-Whitney. The results are presented in Table 4 .

It is worth noting that significant differences in test scores are found among respondents only between groups 1-3 (March – May) and 2-3 (April – May). No differences were found between the test results of the respondents of groups 1 – 2 (March – April). This trend of indicators is preserved at the level of the entire test and its blocks. The gender characteristics of the dynamics of the test indicators have the same trends: the values have no significant difference between groups 1 and 2 (March and April) for both men and women. On the contrary, significant differences in indicators of group 3 (May) from 1 (March) and 2 (April) were found in both women and men.

The exception to this pattern is only the indicators of the Block of physiological signs of stress in women, where reliable differences between 1 group (March) (Me = 10, Q 1=4, Q 3=12, N = 38) and group 2 (April) Me = 6, Q 1=2, Q 3=8, N = 38).

It is interesting to note the fact that when the indicators of the subjective assessment of the stress development by the respondents according to the Stress Level Determination Test (according to V. Shcherbatykh, adapted by H. Kharko) were consistent, it was not possible to identify the priority and predominance of any of the blocks of stress manifestations. All four types of stress manifestations: intellectual, behavioral, emotional and physiological signs had very low unanimity in the respondents' self-assessment, which indicates the impossibility of distinguishing the main, predominant sign in the stress manifestations.

Concordance of scores was calculated using Kendall's concordance test. As already mentioned, the respondents' assessment of all four types of stress manifestations had a low concordance, while this tendency was preserved both in the entire sample of respondents and in selected subgroups: gender, age, and groups separated based on the time of the test (groups 1-3, March – May). The exception was only the women of the 3rd group (May), where the concordance coefficient was K = 0.40869, p < 0.00020, and the most pronounced manifestations of stress according to the subjective feelings of the respondents were the intellectual block of stress signs.

Thus, the obtained data of the statistical analysis indicate the complexity of stress manifestations and the equivalence of these stress manifestations, i.e. it is impossible to single out any priority direction of stress manifestations when planning assistance to victims (or prevention).

Table 2. Test for determining the level of stress

Structural blocks	1 group	2 group	3 group
General indicator	Me = 24, Q1=13, Q3=31 N=59	Me = 20,25 Q1=12,5, Q3=30 N=50	Me = 56, Q1=50, Q3=59 N=25
Women	Me = 25, Q1=14, Q3=34 N=38	Me = 24, Q1=16,5, Q3=30 N=38	Me = 54,5 Q1=50, Q3=58 N=16
Men	Me = 7,5 Q1=4,5, Q3=28,5 N=9	Me = 12,5 Q1=4, Q3=20,5 N=11	Me = 59 Q1=51, Q3=61 N=9
Block of intellectual signs of stress	Me = 4, Q1=1, Q3=6 N=59	Me = 3,5, Q1=2, Q3=6 N=50	Me = 8, Q1= 6, Q3= 9 N=25
Women	Me = 4, Q1=1, Q3=6 N=38	Me = 4, Q1=2, Q3=7 N=38	Me = 6,5 Q1=5,5, Q3=8,5 N=16
Men	Me = 2 Q1=1, Q3=5 N=9	Me = 1 Q1=0, Q3=2 N=11	Me = 8 Q1=7, Q3=9 N=9
Block of behavioral signs of stress	Me = 4, Q1=2, Q3=5 N=59	Me = 4, Q1=2, Q3=5 N=50	Me = 10, Q1= 7, Q3= 12 N=25
Women	Me = 4, Q1=2, Q3=5 N=38	Me = 4, Q1=3, Q3=5 N=38	Me = 9 Q1=7, Q3=11,5 N=16
Men	Me = 2 Q1=1, Q3=5 N=9	Me = 2 Q1=1, Q3=5 N=11	Me = 11 Q1=10, Q3=12 N=9
Block of emotional signs of stress	Me = 7,5, Q1=3, Q3=10,5 N=59	Me = 7,5, Q1=3, Q3=10,5 N=50	Me = 15, Q1= 10,5, Q3= 18 N=25
Women	Me = 8,25, Q1=3, Q3=12 N=38	Me = 9, Q1=6, Q3=12 N=38	Me = 14,25 Q1=9,75, Q3=18 N=16
Men	Me = 1,5 Q1=1,5, Q3=7,5 N=9	Me = 3 Q1=1,5, Q3=7,5 N=11	Me = 15 Q1=10,5 Q3=18 N=9
Block of physiological signs of stress	Me = 8, Q1=2, Q3=12 N=59	Me = 6, Q1=2, Q3=8 N=50	Me = 24, Q1= 20, Q3= 24 N=25
Women	Me = 10, Q1=4, Q3=12 N=38	Me = 6, Q1=2, Q3=8 N=38	Me = 24 Q1=22, Q3=24 N=16
Men	Me = 4 Q1= 0 Q3= 10 N=9	Me = 4 Q1= 0 Q3=8 N=11	Me = 24 Q1= 20 Q3=24 N=9

Source: compiled by the authors of this study

The indicators of the central tendency of the„Questionnaire of self-diagnosis of the type of behavior in a stressful situation" (according to V. Boyko) were equal to 86.6±19.22 (M±σ). The distribution of the test scores was subject to a normal Gaussian distribution. Normality was calculated using the Kolmogorov-Smirnovtest (d=0.05957, p > 0.20) and theShapiro-Wilk (W=0.98804, p=0.31973). It should be noted that there is a statistically significant difference between the test scores of women 87.74±19.04 (M±σ) and men 85.32±20.19 (M±σ) using the criterion calculation Student for independent groups was not found (t= 0.5796 ; p = 0.5 632, N = 118). Homogeneity of variances was determined by Levene's test p = 0.938219.

Also, no correlations were found between the test scores and the age of the respondents, both in general and by gender groups (Spearman correlations r = from 0.01 to 0.13; p >0.05).

Table 3. The difference between the indicators of three groups (Kruskel-Wallis criterion) of respondents from the number of IDPs according to the Stress Level Determination Test (according to V. Shcherbatykh, adapted by H. Kharko)

Structural blocks	Total	Women	Men
General indicator	KW: H (2,N = 134) =55.14334 p < 0.001	KW: H (2, N= 92) =35.56935 p < 0.001	KW: H (2, N = 29) =16.83752 p = 0.0002
Block of intellectual signs of stress	KW: H (2, N= 134) =24.22494 p < 0.001	KW: H (2, N= 92) =11.21652 p = 0.0037	KW: H (2, N = 29) =16.63789 p = 0.0002
Block of behavioral signs of stress	KW: H (2, N= 134) =50.78009 p < 0.001	KW: H (2, N= 92) =31.48963 p < 0.001	KW: H (2, N = 29) =16.92265 p = 0.0002
Block of emotional signs of stress	KW: H (2, N= 134) =26.76858 p < 0.001	KW: H (2, N= 92) =14.42118 p = 0.0007	KW: H (2, N = 29) =13.31661 p = 0.0013
Block of physiological signs of stress	KW: H (2, N= 134) =59.48456 p < 0.001	KW: H (2, N= 92) =42.00575 p < 0.001	KW: H (2, N = 29) =15.90998 p = 0.0004

Source: compiled by the authors of this study

The numerical indicators of the test (from 30 to 150) made it possible to divide respondents into so-called behavioral groups: group A – respondents who scored from 100 to 150 points; group B – respondents who scored from 30 to 75 points;group AB – from 76 to 99 points.

Group A consisted of 24% of respondents. Among women, this figure was 27%, among men 21%. 51% of the respondents made up the AB group. Among women, this indicator was equal to 49%, among men – 54%. Group B consisted of 25% of respondents. Among women, this indicator was 24%, among men – 25%.

It should be noted that the resultsofthe„Questionnaire for self-diagnosis of the type of behavior in a stressful situation" test (according to V. Boyko) had moderate direct correlations with the indicators of the Test for determining the level of stress (according to V. Shcherbatykh, adapted by H. Kharko) (Table 5).

Moreover, both the general indicators of the test and its component blocks of stress manifestations had a correlation: intellectual, behavioral, emotional, and physiological. Such correlations confirm the hypothesis of a relationship between psychophysiological indicators of stress and the type of stress behavior.

DISCUSSION

Important are the established facts of the dynamics of the stress impact on the population in different periods of the war. In a study by the Kyiv International Institute of Sociology (KIIS), conducted in December 2020 using the CATI method among the population aged 18 and older, it was stated that 67% of respondents experienced some kind of stressful situation during 2020. This study also compared the results with the previous years of measurements (2013-2019) and concluded that the situation was mostly better in 2013 before the conflict in eastern Ukraine – 48% of respondents did not experience any stressful situations. But in 2014 and 2015, the situation was the worst – two-thirds of the population experienced at least one type of stress or (severe illness, death of loved ones, loss of job, forced unemployment, loss of faith in people, lack of livelihood, etc.) [17].

Directly the authors of this publication (both independently and in co-authorship with other researchers) revealed certain aspects of the research problem that relate to the organization of work with IDPs. In particular, the peculiarities of the regulation of legal problems of social protection of IDPs in Ukraine have been determined [18]; the response strategy in social work with internally displaced families and the community is substantiated [19]; the socio-legal issues and features of providing social services to IDPs under martial law are disclosed [20]. In addition, T . Kulchytskyi participated in the project „Ensuring rights protection and legal assistance to internally displaced persons and those affected by the conflict in eastern Ukraine» under an agreement with the Office of the United Nations High Commissioner for Refugees in Ukraine" [18].

However, despite the relatively large number of studies that reveal the negative consequences of maladaptation in IDPs, the very mechanisms of its development in the conditions of war are imperfectly defined and carry mostly ascertaining nature. Therefore, in the presented work, an attempt was made to detail some mechanisms, conditions and trends in the development of the stress reaction and subsequent maladaptation in IDPs in war conditions.

In particular, the aim of this study was to establish the relationship between indicators of the level of stress and the tendency to be exposed to stressogenic factors in internally displaced persons who moved from regions of active hostilities in the first three months after the full-scale Russian invasion.

Table 4. A posteriori analysis of a pairwise comparison of groups of respondents from among IDPs according to the Stress Level Determination Test (according to V. Shcherbatykh, adapted by H. Kharko)

Structural blocks	Groups 1, 2	Groups 1,3	Groups 2,3
General indicator	U =1404 Z = 0,428719 p = 0,668128	U = 35,5 Z = -6,86298 p < 0,001	U = 28,5 Z = -6,69846 p < 0,001
Women	U = 662 Z = 0,618128 p = 0,536491	U = 18,0000 Z = -5,40834 p < 0,001	U = 13,5 Z = -5,49358 p < 0,001
Men	U = 49,5 Z = 0,037987 p = 0,969698	U = 0 Z = -3,53209 p = 0,000412	U = 3 Z = -3,49479 p = 0,000474
Block of intellectual signs of stress	U = 1415,5 Z = -0,358786 p = 0,719756	U = 261 Z = -4,65685 p = 0,000003	U = 246 Z = -4,25397 p = 0,000021
Women	U = 644 Z = -0,805125 p = 0,420748	U = 136,5 Z = -3,16354 p = 0,001559	U = 156,5 Z = -2,78468 p = 0,005358
Men	U = 34,5 Z = 1,101619 p = 0,270628	U = 3,5 Z = -3,22303 p = 0,001269	U = 2,5 Z = -3,53278 p = 0,000411
Block of behavioral signs of stress	U = 1457 Z = -0,106420 p =0,915250	U = 67 Z = -6,55481 p < 0,001	U = 58 Z = -6,36691 p = p < 0,001
Women	U = 709,5 Z = -0,124665 p =0,900789	U =37 Z =-5,04841 p < 0,001	U = 32 Z = -5,14313 p < 0,001
Men	U = 47 Z = -0,151947 p = 0,879228	U = 2 Z = -3,35548 p = 0,000792	U = 1,5 Z = -3,60875 p = 0,000308
Block of emotional signs of stress	U = 1424,5 Z = -0,304056 p = 0,761086	U = 237 Z = -4,89165 p = 0,000001	U = 225,5 Z = -4,48437 p = 0,000007
Women	U = 682 Z = -0,410354 p = 0,681546	U =116 Z = -3,55188 p = 0,000383	U = 127 Z = -3,34351 p = 0,000827
Men	U = 49 Z =0 p =1	U = 5,5 Z = -3,04642 p = 0,002316	U = 8 Z = -3,11492 p = 0,001840
Block of physiological signs of stress	U = 1213,000 Z = 1,590212 p = 0,111788	U = 27 Z = -6,94614 p < 0,001	U = 13,5000 Z = -6,86705 p < 0,001
Women	U = 502 Z = 2,280322 p = 0,022589	U = 9,5000 Z = -5,56935 p < 0,001	U = 2,5000 Z = -5,70196 p < 0,001
Men	U = 49 Z =0 p =1	U = 1 Z = -3,44378 p = 0,000574	U =5,5 Z = -3,30486 p =0,000950

Source: compiled by the authors of this study

Tasks of the research:

1) to determine indicators of the level of stress in internally displaced persons who moved from regions of active hostilities;

2) to compare the indicated indicators in groups of IDPs formed by time (IDPs who were relocated in March, April and May 2022), gender and age criteria;

3) to identify relationships between indicators of the level of stress and indicators of the propensity of internally displaced persons to be exposed to stressogenic factors depending on the type of behavior in a stressful situation.

CONCLUSIONS

1. Our study among internally displaced persons aged 18-81 years who moved from regions of active hostilities proved the absence of a difference between women and men in the level of stress, and differences in the manifestations of intellectual, behavioral

signs, emotional and physiological symptoms. Also, no dependence of stress level on age was found. However, there are certain gender specifics that relate to subjective manifestations of stress. In the intellectual and emotional blocks of signs of stress, both women and men most often paid attention to the prevalence of negative thoughts and increased anxiety. In the block of behavioral signs, women paid attention to the presence of sleep disorders or insomnia, men – to an increase in conflict situations at work and in the family. In the block of physiological symptoms of stress, feelings of pain invarious parts of the body of an undefined nature, headaches were characteristic of more women, and men had problems with an accelerated or non-rhythmic pulse.

2. A comparison of stress level indicators in groups of IDPs, which were formed according to time, gender and age criteria, showed no difference between the results of the above blocks of stress symptoms in respondents of groups 1 and 2. However, significant differences were found when comparing indicators of 1 and 3 and 2 and 3 groups. That is, the signs of stress do not appear immediately, at the same time, but grow gradually over several months. The exception to this pattern was only the indicators of the block of physiological signs of stress in women, where there were significant differences between 1 and 2 groups. The study did not reveal the predominance of any of the blocks of stress manifestations, which indicates the impossibility of distinguishing the main, predominant feature in stress manifestations. The exception was only the women of the 3rd group, in whom the most pronounced manifestations of stress were the signs presented in the intellectual block. Therefore, the gradual increase in the signs of stress indicates the urgency of organizing primary prevention in the early stages of the development of stress, however, in most cases, it is impossible to single out the priority areas of prevention of its manifestations when planning assistance to victims, since such manifestations are complex and equivalent.
3. The type of behavior in a stressful situation correlates with the level of indicators of stress manifestation, but it is not the only and significant factor of the level of stress, as indicated by their moderate correlations. Correlation is observed both in terms of general indicators and component blocks of stress manifestations (intellectual, behavioral, emotional and physiological).

We see further exploration in the formation of resilience and resistance of internally displaced persons to the influence of stressogenic factors, research of the level of their adaptation to new living conditions in the host community.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Comparative assessment of the risks of soil and groundwater pollution by triazine herbicides in Ukraine and European Union countries

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ABSTRACT

Aim: Assessment of the risk of soil and groundwater contamination by triazine herbicides to reduce anthropogenic environment pollution and decrease the potential negative impact on public health in Ukraine and the EU.

Materials and Methods: 14 preparations containing 5 active substances of the triazine class and its environmental fate in soil and water considering Ukraine and EU climatic conditions. Methods of in-field hygienic experiment, gas-liquid chromatography, analytical and statistical methods, hygienic modelling using Experimental Leaching Index (ELI), (GUS), (RLPI), (LIX), (DT50) and (KOC), SCI-GROW, LEACHmod.

Results: Analysis of the physicochemical properties of studied substances showed that they were classified as mobile or moderately mobile by migration ability. Calculated T50 for studied substances allowed us to determine the hazard classes for them. Substances pertain to 2-3 classes (promethrin and terbuthylazine - class 2, moderately persistent (30-100 days), tribenuron-methyl, thifensulfuron-methyl and metribuzin - class 3, weak persistency (less than 30 days)). The determination coefficient (R2) values ranged from 0.704 to 0.995.

Conclusions: According to the GUS, most of the studied substances have a moderate possibility of leaching into groundwater, except for thifensulfuron-methyl. While in EU soils the index for promethrin is very low, tribenuron-methyl is low, terbuthylazine and metribuzin are moderate, and thifensulfuron-methyl is high. The SCI-GROW assessment showed that most of them have significantly lower values in Ukraine than international values (except for promethrin), which can be explained by significant dissimilarities in maximum application rates, number of treatments, and in some cases - different resistance of substances in Ukrainian and EU soils.

KEY WORDS: Public Health, herbicides, risk assessment, water contamination

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INTRODUCTION

Groundwater is one of the most important sources for drinking purposes and the operation of industrial enterprises and agriculture [1]. Groundwater, like surface water, can be contaminated. Pollution can be point (spill of chemicals from industrial facilities) and diffuse (pollution with pesticides from agriculture) [2, 3].

Degradation processes of chemical pollutants in surface water systems occur within days to weeks, in contrast to groundwater, which can last for decades [2, 4].

An analysis of the number of pesticide preparations registered for use in Ukraine for 2022 showed that herbicides (1,178 preparations) occupy the first place in terms of quantity, of which 108 are desiccant herbicides. Triazine-class pesticides account for 18% of the total amount of herbicides [5]. Due to the widespread use of herbicides at industrial facilities, forest clearings, airfields, railways and highways, drainage canals, under high-voltage power lines, ponds and lakes, and especially in the agro-industrial sector of our country, to protect cultivated plants from weeds [5], the issue of assessing the risk of potential contamination of soils and groundwater with pesticide residues and their possible impact on public health is relevant [6, 7].

Particularly relevant is the issue of assessing the risk of triazine herbicide application, which is widespread in agricultural production, and, as a result, many residues of these herbicides can get into water sources, migrating from surface water bodies and soil [8, 9].

AIM

Aim of our research is to assess the risk of contamination of soils and groundwater with herbicides of the triazine class. This assessment could be considered in reducing anthropogenic pollution of environmental objects and, most importantly, to mitigate the potential negative impact on the population's health in Ukraine and the EU.

MATERIALS AND METHODS

We selected 14 formulations for our research: Gezagard, Triathlon Prime, MNT-Star, Sturnus, Parus, Harpoon, Novante, Bergen, Barrier, Deluge Extra, Trigger, Floracid Active, Propizozide, Starlent, containing 5 active substances of the triazine class mostly used for various purposes at Ukraine territory: promethrin, tribenuron-methyl, terbuthylazine, thifensulfuron-methyl, metribuzin. Physico-chemical properties of the studied active substances are given in Table 1, Fig. 1.

Table 1. Physicochemical properties of the studied triazine class active substances. PPDB [11-15]

Trade name	Chemical name (IUPAC)	Log P K_{ow}	Solubility in water, mg/l	Vapor pressure, mm Hg
Metribuzin	4-amino-6-tert-butyl-4,5-dihydro-3-methylthio -1,2,4-triazin-5-one	1.75	10700	0.121
Promethrin	N2,N4-diisopropyl-6-methylthio-1,3,5-triazine -2,4-diamine	3.34	33	0.13
Terbuthylazine	N2-tert-butyl-6-chloro-N4-ethyl-1,3,5-triazine -2,4-diamine	3,4	6.6	0.152
Thifensulfuron-methyl	methyl 3-(4-methoxy-6-methyl-1,3,5-triazin -2-ylcarbamoylsulfamoyl)thiophene-2-carboxylate	-1.65	54.1	$5.19 * 10^{-06}$
Tribenuron-methyl	methyl 2-[4-methoxy-6-methyl-1,3,5-triazin -2-yl(methyl)carbamoylsulfamoyl]benzoate	0.38	2483	$5.99 * 10^{-06}$

Source: compiled by the authors based on [11-15]

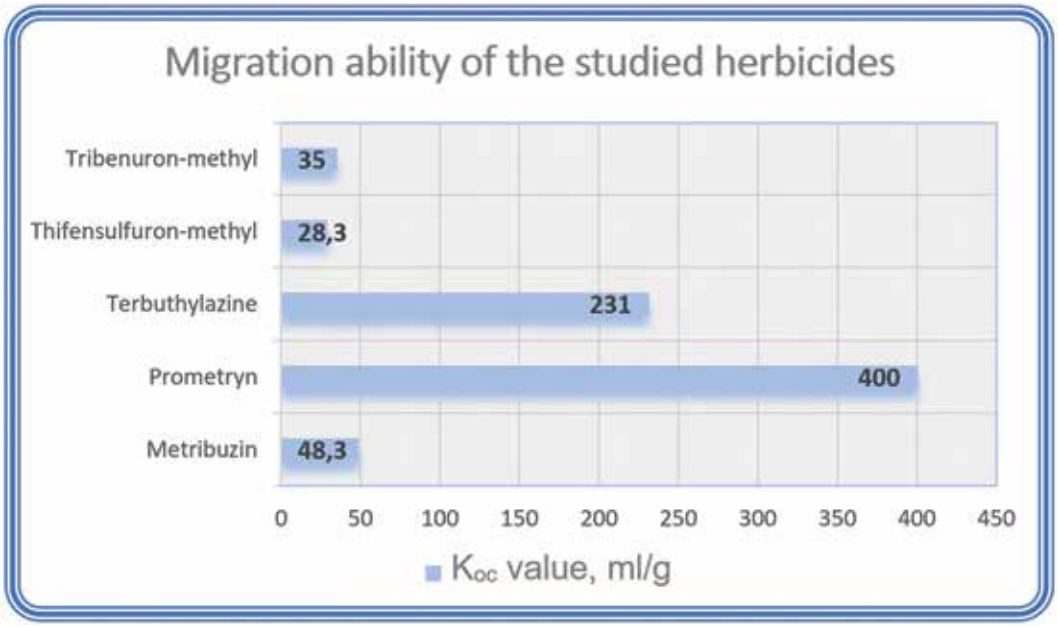

Fig. 1. Migration ability of the studied herbicides according to the Koc index [11-15]

Over the past 10 years, specialists from the Institute of Hygiene and Ecology of Bogomolets National Medical University have conducted field hygienic experiments to study the dynamics of triazine class active substances residual amounts in environmental objects, in particular in soils applying herbicides in different climatic zones of Ukraine, more specific Polissya and Forest-Steppe. These regions cover half of Ukraine's agricultural lands and are characterized by sod-podzolic, gray forest, chornozem, and dark gray soil types. Analysis of pesticide residues in soil was carried out by gas-liquid chromatography (GLC) according to the previously developed methodological guidelines MG No. 6092-91, 6076-91, 2371-81, approved by Ministry of Environmental Protection and Natural Resources of Ukraine.

The hazard of the studied class of herbicides by persistence in soil was determined in accordance with State Sanitary Rules and Norms 8.8.1.002-98 [10].

The analysis of the physical and chemical properties of the active substances was performed according to PPDB: Pesticide Properties DataBase data [11-15].

In most EU countries, a number of indicators are used to assess the migration ability of chemicals, in particular the Experimental Leaching Index (ELI), which takes into account the average annual amount of precipitation in a certain place. This index is compared with others, such as the potential leaching index (GUS - Groundwater Ubiquity Score), the relative leaching potential index (RLPI) and the leaching index (LIX), which include only degradation (DT_{50}) and sorption (KOC) parameters. Considering these data,

according to the ELI, metribuzin is highly mobile in all cases, while terbuthylazine shows high leaching capacity only in natural soils, reducing their leaching potential in laboratory soils [16].

SCI-GROW indicator was used to calculate the possible migration of herbicides into groundwater [17, 18]. Statistical data processing was carried out by the program SG version 2.3 [19, 20].

To calculate the possible contamination of groundwater with herbicides and their possible negative impact on human health, we used a point system for evaluating the studied indices and the principle of establishing an integral vector of hazard (IIHW - an integral indicator of hazard when getting into the water), which provides for 4 gradations and takes into account such indicators as LEACH, T_{50} and ADI of the active substances [17].

The probability of contamination of groundwater and surface water was performed using methods developed by specialists of the Institute of Hygiene and Ecology of the Bogomolets National Medical University [18].

The assessment of the migration ability of the studied herbicides in the soil-water system was carried out using the following indicators: T_{50} – the half-life period of the substance, which allows for assessing stability in the soil, K_{OC} (sorption constant by organic carbon), GUS – the index of potential leaching and $LEACH_{mod}$ – the leaching index for assessing potential contamination of groundwater and river waters.

Calculations were performed on a personal computer using MedStat v.5.2 (Copyright© 2003-2019) and Microsoft® Excel® for Microsoft 365 MSO (version 2305 build 16.0.16501.20074) program software.

RESULTS

The analysis of the physicochemical properties of the studied active substances showed that according to the migration ability, taking into account the values of $K_{oc,}$ metribuzin, thifensulfuron-methyl, tribenuron-methyl are classified as mobile (15-74 ml/g), and promethrin and terbuthylazine as moderately mobile (75-499 ml/g) (Table 1, Fig. 1).

Table 2 shows the results of the mathematical modelling of stability in the soil of the studied active substances, namely the parameters of their degradation rate (K_{oc}, T_{50}).

The calculated half-lives (T_{50}) for promethrin was 33.962.49 days, tribenuron-methyl – 12.91±5.3, terbuthylazine – 45.76±1.95, thifensulfuron-methyl – 22.01±0.36, metribuzin – 9.2±0.23, respectively, which made it possible to determine the hazard classes of these active substances.

Thus, promethrin and terbuthylazine pertain to class 2 (persistent, T_{50} 31-60 days), tribenuron-methyl and thifensulfuron-methyl to class 3 (moderately persistent, T_{50} 11-30 days), metribuzin to class 4 (slightly persistent, T_{50} less than 11 days). At the same time, in the soil and climatic conditions of other countries where similar studies were conducted, promethrin and terbuthylazine are classified as class 2 (persistent), tribenuron-methyl, thifensulfuron-methyl and metribuzin – class 4 (slightly persistent) (Table 2).

In accordance with the international classifications of IUPAC and SSLRC (Soil Survey and Land Research Centre, UK), the studied active substances are divided into 3 classes regarding stability and migration ability. The substances we studied are classified into 2-3 classes (promethrin and terbuthylazine – class 2, moderately persistent (30-100 days), tribenuron-methyl, thifensulfuron-methyl and metribuzin – class 3, poorly stable (less than 30 days)).

According to the migration ability, taking into account the K_{os} values, metribuzin, thifensulfuron-methyl, and tribenuron-methyl are classified as mobile (15-74 ml/g), promethrin and terbuthylazine as moderately mobile (75-499 ml/g).

The coefficient of determination (R^2) values ranged from 0.704 to 0.995, which allowed us to conclude a reliable relationship between the studied variables and confirm the reliability of the selected model for evaluating the results of field studies for herbicide residues (Table 2).

DISCUSSION

The behaviour of the specified active substances was also studied in the territories of such countries as the USA, Great Britain, Austria, Germany (brown forest, sod-carbonate, alluvial, acid podzolic soil types), Australia, New Zealand, Spain, Hungary (brown forest, chestnut, podzolic, chornozem), Sweden (brown forest, podzolic).

We analyzed a wide range of indices proposed by the world scientific community to assess the risks of soil and water pollution. These are the Briggs, Hamaker, Hornsby index, groundwater pollution potential index, experimental leaching index, Severo index, indices based on processes in the advection equation, YASGEP-P index and many others, and the most relevant in our case were chosen [17, 19].

With the help of mathematical modelling of the results of natural studies in Ukraine's soil and climatic conditions, we calculated the half-life periods of the studied compounds in soils (T_{50}) (Tables 2, 3). Statistical processing of the obtained results showed that T_{50} for promethrin is 50.33±5.49 days, tribenuron-methyl – 12.91±5.3, terbuthylazine – 45.76±1.95, thifensulfuron-methyl – 22.01±0.36, metribuzin – 9.2±0.23.

The obtained results made it possible to consider the peculiarities of the chemical structure and physicochemical properties of the studied substances and conclude that these indices do not affect the rate of their metabolism in the soil. Also, the obtained data show that promethrin and terbuthylazine were the most persistent in agrocenosis objects. Differences in T_{50} values of triazines in the soil, compared to the resistance of herbicides of other classes, are reliable according to Student's t-test ($p \geq 0.05$).

Considering that herbicides are the primary contaminants of the soil and can migrate from the soil to groundwater, we evaluated the possible contamination of groundwater based on the GUS index assessment in Ukraine`s soil and climate conditions. We also analysed the sources of scientific data regarding similar studies conducted in the USA and Europe (Table 2, 3).

It was established that in accordance with the SSLRC international classification, which provides for the calculation of the $K_{oc\ constant}$ by migration ability, the studied substances

Table 2. Indicators of the triazines degradation rate depending on the soils

Pesticide formulation name	Active substances	Processed culture	Application rate kg/ha	T_{50} (Ukraine)	Kinetic equation	R^2	K (day)
Gezagard 500 FW	promethrin	Soya	2.5	38.8±0.19	$y = 5.3878e^{-0.018x}$	0.9545	0.018
Triathlon Prime, WG	tribenuron-methyl	Winter wheat	0.015	6.140.104	$y = 0.0452e^{-0.113x}$	0.8337	0.112
	thifensulfuron-methyl		0.015	22.01±0.36	$y = 0.0421e^{-0.119x}$	0.8374	0.031
MNT-Star, MD	terbuthylazine	Corn	0.4	69.47±0.53	$y = 0.3735e^{-0.072x}$	0.7264	0.01
Sturnus, SC	promethrin	Sunflower	2	24.28±1.61	$y = 1.3075e^{-0.092x}$	0.7194	0.029
Parus 500, SC	terbuthylazine	Corn	0.84	42.8±6.06	$y = 0.6815e^{-0.039x}$	0.9107	0.019
Harpoon 750 WG	tribenuron -methyl	Sunflower	0.0114	11.29±1.69	$y = 0.0868e^{-0.124x}$	0.7441	0.064
Novante , SC	terbuthylazine	Corn	0.84	23.97±2.24	$y = 0.3362e^{-0.083x}$	0.7266	0.029
Bergen, SC	terbuthylazine	Corn	1.25	41.8±1.03	$y = 1.5525e^{-0.057x}$	0.7829	0.017
Barrier , SC	metribuzin	Potato	0.45	9.2±0.23	$y = 6.5945e^{-0.075x}$	0.9949	0.075
Deluge Extra 500, EC	terbuthylazine	Corn	0.84	56.13±0.55	$y = 0.5309e^{-0.049x}$	0.7742	0.012
Trigger, WG	tribenuron -methyl	Sunflower	0.0175	27.71±19.41	$y = 0.0576e^{-0.064x}$	0.7615	0.012
Floracid Aktiv, WG	tribenuron -methyl	Wheat	0.0143	6.48±0.004	$y = 0.038e^{-0.216x}$	0.7728	0.107
Propizozide, SE	promethrin	Sunflower	0.8	38.8±5.67	$y = 0.6343e^{-0.156x}$	0.7355	0.018
Starlent, SE	terbuthylazine	Sunflower	0.855	40.37±1.26	$y = 1.3204e^{-0.084x}$	0.7041	0.017

Source: compiled by the authors of this study

Table 3. Comparative data of the most important indices values for assessing the degree of active substances leaching from soils

Active substances	Pesticide formulation name	T_{50} in UA soil	T_{50} in EU soil	GUS UA	GUS EU	LEACH UA	LEACH EU	T_{50} in water	IIHW Points	SCI-GROW EU	SCI-GROW UA	P
Promethrin	Gezagard Sturnus Propizozide	33.96±2.49	50.33±5.49	2.13±0.09	0.59	2.8±0.4	4.15±0.45	56	10-11	$1.13 \cdot 10^{-02}$	$2.24 \cdot 10^{-01}$	$1.4\ 10^{-02}$
Tribenuron - methyl	Triathlon Prime Harpoon Trigger Floracid Active	12.91±5.3	5.23±2.64	2.52±0.37	1.39	915.5±359.9	371.27±187.35	86.2	11	$3.15 \cdot 10^{-03}$	$3.48 \cdot 10^{-03}$	$0.3 \cdot 10^{-05}$
Terbuthylazine	MNT- Star Sail Novante Bergen Deluge Extra Starlent	45.76±1.95	59.07±45.18	2.68±0.1	2.19	1.305±0.18	1.68±1.29	6	8-9	$8.43 \cdot 10^{-02}$	$2.57 \cdot 10^{-01}$	$1.8 \cdot 10^{-02}$
Thifensulfuron-methyl	Triathlon Prime	22.01±0.36	10.28±5.53	3.42	3.05	42.08	19.65±10.57	22	9	$8.44 \cdot 10^{-02}$	$6.24 \cdot 10^{-03}$	$2.3 \cdot 10^{-06}$
Metribuzin	Barrier	9.2±0.23	10.69±4.17	2.23	2.96	2038.1	2366.7±924.14	41	11	$1.99 \cdot 10^{-01}$	$1.89 \cdot 10^{-02}$	$5.4 \cdot 10^{-04}$

Source: compiled by the authors of this study

promethrin and terbuthylazine pertain to the III class, moderately mobile, metribuzin, tribenuron-methyl, and thifensulfuron-methyl are of II class, mobile.

An evaluation of the data received during the experimental studies was carried out, and an assessment of the obtained results was performed according to the Index of potential leaching (GUS), which characterises the probability of the migration of a substance from the soil into groundwater.

The data analysis presented in Table 3 makes it possible to conclude that such active substances as promethrin, tribenuron-methyl, terbuthylazine, and metribuzin pertain to substances with a moderate possibility of leaching into groundwater. At the same time, thifensulfuron-methyl is a herbicide potentially leaching into groundwater in the territory of Ukraine. Evaluating data on behaviour in the soils of European countries, it is possible to conclude that the GUS index for promethrin is very low (V), tribenuron-methyl – low (IV), terbuthylazine and metribuzin – moderate (III), thifensulfuron -methyl – high (II).

A comparison of the SCI-GROW index results for the studied herbicides obtained during the experiment with similar indices in the European Union countries` soil and climatic zones is given in Table 3. It was established that the maximum possible concentrations of the studied herbicides, at the maximum application rate in the soils of Ukraine, are at the level, or several times lower, than in other countries' soil and climatic conditions. Except for promethrin, which is two times higher than the similar rate in the EU.

To assess the potential contamination of groundwater and river waters in Ukraine, according to the $LEACH_{mod}$ classification, the data obtained were analyzed, and it was found that all the studied active substances, except for terbuthylazine, pertain to class I, i.e. those with a high risk of contamination of surface and groundwater, terbuthylazine – class II, of moderate risk, which coincides with the results of most EU countries.

At the final stage, using the domestic model developed by Serhiy Serheiev and co-authors, the potential hazard to the human body of surface and groundwater contamination with the studied herbicides was assessed. It was established that promethrin, tribenuron-methyl, and metribuzin are extremely hazardous for the human body (class 1A), while terbuthylazine and thifensulfuron-methyl are highly hazardous (class 1B) in different soil and climatic zones [20].

CONCLUSIONS

It was established that the majority of the studied herbicides pertained to the 1st class of hazard (highly persistent substances) according to their stability in water, except thifensulfuron-methyl (class 2) and tribenuron-methyl (class 3).

By stability in the soil, promethrin and metribuzin are pertained to the 2nd class, terbuthylazine and thifensulfuron-methyl to the 3rd class, and tribenuron-methyl to the 4th class of hazard.

According to the degree of groundwater contamination risk, estimated by calculating the GUS index, most studied substances have moderate leaching potential into groundwater, except for thifensulfuron-methyl (probably leaching). While in European countries' soils, the promethrin index is very low, tribenuron-methyl is low, terbuthylazine and metribuzin are moderate, and thifensulfuron-methyl is high.

The evaluation of similar indices we obtained during field studies according to the SCI-GROW screening model showed that most of them are significantly lower than international standards (except for promethrin). Significant dissimilarities in the maximum application rates, the frequency of treatments, and, in some cases, the different stability of substances in the soil due to the soil characteristics explain this difference in indices.

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ORIGINAL ARTICLE

Single and combined effects of lead and piroxicam on the structural and functional profiles of the liver and kidney

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ABSTRACT

Aim: Our study investigated the harmful synergistic effect of lead and piroxicam treatment on liver and kidney tissue functions and morphology.

Materials and Methods: 24 male Wister rats were allocated randomly in equal numbers n=6 into four groups: (Group1): was used as the control animal group. In the control group, 0.5 mL of distilled water was given by an orogastric tube. (Group2): for 21 days, lead acetate was administered via an orogastric tube at a dose of 4 mg/kg/day according to body weight. (Group3): is given peroxicam (0.3 mg/kg/day) by the same route and dose for 21 days. Group 4: (Group4), a synergistic mixture of piroxicam (1 mg/kg/day) and lead acetate (4 mg/kg/day) was given by an orogastric tube. Liver functions were evaluated by measuring activity of alanine aminotransferase aspartate aminotransferase and alkaline phosphatase. Kidney functions were represented by assays of blood urea nitrogen, creatinine, and urea, while antioxidant status determined by malondialdehyde catalase activity and reduced-glutathione level.

Results: Liver and renal function data were estimated in serum and organs. Group2 and Group3 caused elevated serum levels of MDA, BUN, and Cre while decreasing levels of SOD and GSH in serum and liver and kidney tissue. Group4: Administration of a synergistic mixture of lead and piroxicam caused severe elevated serum levels of MDA, BUN, and Cre, while acutely decreasing levels of SOD and GSH were found in serum and liver and kidney tissue. Generally, data supported by histological examination indicated severe damage following induced oxidative stress by a synergistic mixture of piroxicam and lead compared with other groups.

Conclusions: The synergistic treatment (group 4) resulted in the most significant effects compared to the control group, as evidenced by both biochemical and histopathological measurements. Additionally, groups 2 and 3 showed negative changes in these measurements relative to the control group, but these changes were less pronounced than those observed in group 4.

KEY WORDS: Pb, Piroxicam, liver, renal, MDA, antioxidant enzymes

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INTRODUCTION

Lead harms biological systems and has a distressing effect on all living organisms, because of its stability in polluted areas resulting in heavy metal-induced liver and kidney damage. While non-steroidal anti-inflammatory drugs (NSAIDs) as a piroxicam drug are often used for treating inflammation due to their antipyretic, analgesic, and anti-inflammatory properties, they have been shown to exert harmful adverse effects on the liver and kidneys. Currently, one can be simultaneously exposed to numerous different chemical materials and medicine drugs as natural exposure and medicine treatment in the occupational environment. Because co-exposure to two or more xenobiotics can result in unexpected harmful effects, understanding mutual interactions between hazardous compounds and recognizing their repercussions for health is a critical topic in modern toxicology [1]. Due to the excessive use of non-steroidal analgesics such as piroxicam drug by people who may be exposed to environmental and occupational heavy metals such as Cd, Pb, Zn, ...etc. [2], the idea of interest in observing the effect of interactions between lead and piroxicam, as an important epidemiological and medical factor, thus forming the topic of real interest to many researchers. According to recent research, lead may be a factor in oxidative stress by causing Reactive oxygen species (ROS) to be produced [3]. ROS levels are controlled by many defense systems, including antioxidants and detoxification enzymes [4]. Heavy metal contamination of food is an issue for human and animal health. Accordingly, an assessment is conducted on the levels of heavy metals present in food, air, and water resources [5]. Lead is a highly dangerous environmental element that poses a significant risk to multiple body organs. Although it can also be absorbed through the skin, Pb+2 is primarily absorbed by the respiratory and digestive systems. Exposure to lead (Pb) can lead to immunomodulatory, oxidative, and inflammatory reactions, which can cause respiratory, neurological, urinary, and cardiovascular issues. Additionally, Pb can disrupt the balance of the antioxidant- system and trigger inflammatory responses in various organs [6]. Because lead is so toxic, it negatively impacts the body's

neurological, biochemical, and cognitive systems. Blood level of 10 µg/dl leads poisoning is the international level of concern [7]. On the other hand, because of their antipyretic, analgesic, and anti-inflammatory properties, NSAIDs are the most commonly recommended therapeutic drugs [8]. Piroxicam (an acidic carboxamide) is a chemical subgroup of NSAIDs known as oxicam derivatives, a class of enolic acids. It is widely used to treat rheumatoid arthritis, ankylosing spondylitis, and musculoskeletal diseases [9], despite their extensive use of peroxicam, can induce undesirable consequences such as fetotoxicity, gastrointestinal toxicity, and ulcerogenic gastropathy, abnormalities in the histology of the kidney and liver, chondrocyte proteoglycan synthesis, and other processes dependent on prostaglandins [10]. When medications are consumed in excess, the liver is particularly vulnerable to their toxicity. Drugs may harm the organ and result in hepatotoxicity even if they are administered within the therapeutic levels [11]. Drugs and heavy metals can be toxic and lead to oxidative stress by releasing free radicals. These free radicals are often produced as byproducts of aerobic metabolism. When there is an excess of these free radicals at the cellular level, they can cause damage to tissue proteins, nucleic acids, and membrane lipids, and are linked to various age-related disorders [12-13]. Reactive radical species include a diverse range of oxygen, carbon, and sulfur radicals derived from the superoxide radical, hydrogen peroxide, and lipid peroxides, as well as chelates of amino acids, peptides, and protein complexes with hazardous materials. Drugs and metals' harmful effects include hepatotoxicity, neurotoxicity, and nephrotoxicity [14].

AIM

The present study aimed to evaluate the role of oxidative stress in the histophysiology changes of lead and piroxicam-induced liver and kidney injury.

MATERIALS AND METHODS

Twenty-four rats, weighing 225 ± 10.5 grams, were used in the study. Before the experiment, all animals had free access to water and were provided with a standard laboratory commercial diet during their two-week acclimation period at a temperature of approximately 25°C.

EXPERIMENTAL ANIMAL

A total of Twenty-four male Wister rats albino (*Rattus norvegicus*) of the same age were obtained from the house of the laboratory animal, Veterinary Medicine College - Basrah University. The animals were distributed into four groups of six rats each at random. Following a period of stabilization (7 days), Group1: was used as the control animal, which they given 0.5 mL of distilled water by an orogastric tube. Group 2: for 21 days, lead acetate was administered via an orogastric tube at a dose of 4 mg/kg/day according to body weight. Group 3: is given peroxicam (1 mg/kg/day) by the same route and dose for 21 days. Group 4: a synergistic mixture of piroxicam (1 mg/kg/day) and lead acetate (4 mg/kg/day) was given by orogastric tube, both substances were administered as a single dose every day for 21 consecutive days.

SAMPLING

Immediately after the experiment time ending (21 days), blood samples were taken via the caudal vena cava. All biochemical inquiry samples were maintained at ambient temperature to isolate serum without anticoagulant. The kidneys and liver were both removed and then submerged in ice-cold phosphate-buffered saline.

LIVER AND KIDNEY FUNCTION STUDIES

Liver functions were represented by an assay of the activity of the enzymes' (ALT) and (AST), which were assessed according to the IFCC standard method (without pyridoxal phosphate activation), while the IFCC recommended method (colorimetric assay) approach is used to evaluate the (ALP) enzyme in mg/100 ml. Total proteins in serum were assessed according to the colorimetric, biuret reagent method, and serum albumin according to the Colorimetric, bromcresol method. Kidney functions were represented by assays of (BUN), (Cre), and (Ur), all the values were expressed as mg/dL. The liver and kidney tissues were dissected and washed in a PBS solution with heparin (0.16 mg/mL). The tissues were then homogenized using a Sonicator homogenizer with 5 mL of buffer (50 mM potassium phosphate, pH 7.5, 1 mM EDTA) per gram of tissue. Following homogenization, aliquots of the tissue homogenate were centrifuged at 4000 rpm for 20 minutes in a chilled centrifuge and stored at -80°C until needed. The (MDA) level according to [15] and (CAT) activity according to [16], while, the (GSH) level were determined using specific diagnostic kits from the Laboratory Bio Diagnostic Company [17].

HISTOPATHOLOGICAL ASSAY

The liver and kidney were removed, preserved in formalin (10% concentration) for 24 hours, and then processed using standard paraffin-embedding methods. The paraffin blocks were sectioned into slices that were five micrometers thick for examination under a light microscope. These sections were then placed on glass microscope slides, deparaffinized, and stained with H&E stain [18].

STATISTICAL ANALYSIS

Statistical analysis was conducted using SPSS (Version 20; SPSS Inc., Chicago, USA). One-way ANOVA was used to assess group differences, followed by the Duncan test for post-hoc analysis. All values were given as mean ± SD, with a probability value of ($P \leq 0.05$) indicating a significant difference.

ETHICS APPROVAL

The protocol in this study was approved by the ethical committee of the College of Science in the University of Basrah (No. 155 in 2024).

CONSENT TO PARTICIPATE

Prior to collecting samples, all patients involved in the study were required to provide written consent for their participation.

RESULTS

BIOCHEMICAL STUDY

In table 1, the group 2, group 3, and group 4 showed renal and hepatic tissue damage, as indicated by significantly elevated levels of functional biomarkers in the serum (ALT, AST, ALP, Cre, and Ur), Furthermore, there was a significant decrease ($p \leq 0.05$) in the levels of albumin and total protein in comparison to the rats in the CON group. The group 4 exhibited more severe damage to hepatic and renal parameters and histological profiles.

OXIDATIVE STRESS INDICATORS ASSAY

The data in Table 2 show the toxic oxidative damage caused by the effects of group 2, group 3, and group 4 overdose exposure in the hepatic and renal tissues. The large increase in MDA levels was associated with a considerable decrease in GSH values and CAT activity in response to acute oxidative stress when compared to the group 1. The critical value level was increase significantly ($p \leq 0.05$) in the co-combined exposure to group 4 compared to the single exposure groups.

HISTOMORPHOMETRIC ASSAY

To confirm the results described above, the histological profile changes were showed in the liver and kidney of the groups 2, 3, and 4 . In the liver tissue sections, fig. (1-A) the group 1, displayed intact liver histological building dsgsine contain the hepatocytes exhibited uniform polygonal shapes with well-defined sinusoids and central veins. In contrast, group 2 revealed Hepatic sinus dilatation, Fusion and clumping of groups of hepatocytes, limited bleeding, acute hepatocellular degeneration, pyknosis, and Formation of bubbles on the surface of the plasma membrane, fig. (1-B). group 3 was revealed Fusion and clumping of groups of hepatocytes, pyknosis, and Limited degeneration of some hepatic cells, fig. (1-C), while the combination toxicity of the group 4 caused significant damage markers in the hepatocytes compared to their individual groups, the most prominent of these signs were loss of normal structural tissue features, cytoplasmic degeneration, and Loss of organelle content within hepatocytes, fig. (1-D), Hepatocellular necrosis accompanied by acute inflammation of the necrotic area, fig. (1-E). The sections of the kidneys, as in fig. (1-A), the group 1, showed clear renal histological architecture, Includes normal renal tubules and glomerulus. In contrast, group 2 revealed hypertrophy of the epithelial cells of some renal tubules, accompanied by narrowing and closure of some of the ducts of those tubules, and there was also severe degeneration of many renal tubular epithelial cells, atrophy and loss of normal shape of the renal glomeruli and pyknosis of the tubular epithelial cells nucleoli, fig. (2: B-C), while, group 3 revealed

Table 1. Indicators of liver and kidney function after the administration of Groups 2, 3, and 4

	Comparison groups			
Functional indicators	**Group1**	**Group2**	**Group3**	**Group4**
AST (U/L)	81.76± 6.5 a	154.6± 4.4 b	133.8± 3.6c	198.8± 5.6 d
ALT (U/L)	18.97± 0.76 a	56.7± 2.4 b	39.8± 1.9 c	79.76± 3.2 d
ALP (U/L)	197.90± 10.5 a	280.9.4±6.9b	245.6±5.9 c	313.8± 7.8 d
TP (gm/dl)	9.24± 0.45 a	6.3± 0.36 b	7.6± 0.42 b	4.1± 0.31c
Alb (gm/dl)	4.05± 0.06 a	2.9± 0.15 b	3.2± 0.17 b	1.9± 0.32 c
Cre (mg/dl)	0.81± 0.034 a	1.03± 0.05 b	0.98±0.03 a	1.2± 0.06 b
Ur (mg/dl)	20.44± 1.22a	23.42± 1.01a	21.7± 1.12a	25.51± 1.06b

Superscript letters in the same row denote a statistically significant difference ($P \leq 0.05$). All values are presented as the mean ± SD, n = 6
Source: compiled by the authors of this study

Table 2. Oxidative stress markers of Groups 2, 3, and 4

		Comparison groups			
Parameters	**Organ**	**Group 1**	**Group 2**	**Group 3**	**Group 4**
MDA (noml/g)	Liver	268.9± 12.72a	458.93±18.9b	389.7± 11.2c	571.91±23.12d
CAT(U/g)		405.8± 16.76a	279.7± 10.6b	323.6± 16.9c	258.3± 11.1b
GSH(mg/g)		78.11± 5.45a	45.8± 6.61b	61.9± 5.16c	32.7± 4.63d
MDA (noml/g)	Kidney	168.9± 8.71a	304.1±13.45b	248.4± 13.8c	380.1± 14.9d
CAT(U/g)		472.8± 20.5a	377.3± 6.93b	403.2± 16.1c	317.6± 12.4d
GSH(mg/g)		86.83± 7.72a	52.11± 2.3b	68.41± 4.4c	41.32± 3.81d

Superscript letters in the same row denote a statistically significant difference ($P \leq 0.05$). All values are presented as the mean ± SD, n = 6
Source: compiled by the authors of this study

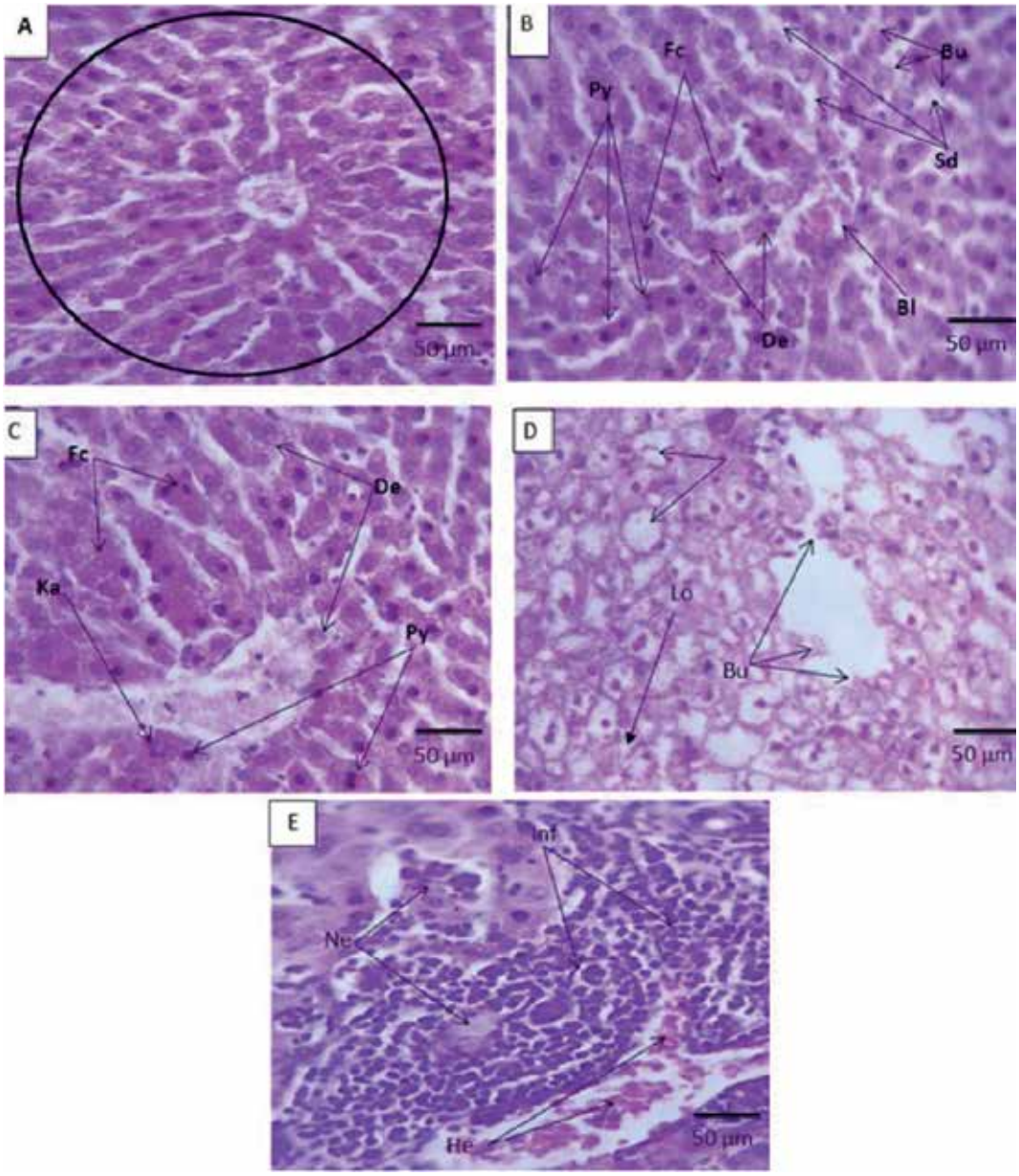

Fig 1. Micrograph sections of the liver for the toxic effects of single and synergistic doses of both Pb and piroxicam: (A) the group 1 showed intact liver histological building design (circle). (B) The group 2 shows hepatic sinus dilatation (Sd), fusion and clumping of hepatocytes (Fc), limited bleeding (Bl), degeneration (De), pyknosis (Py), and the formation of bubbles (Bu). (C) The group 3 shows fusion and clumping of hepatocytes (Fc), karyorrhexis (Ka), pyknosis (Py), and cell degeneration (De). (D and E) group 4 shows cytoplasmic degeneration (De), loss of organelle content within hepatocytes (Lo), hepatocellular necrosis (ne), haemorrhage (He), formation of bubbles (Bu), and acute inflammation (Inf) (H&E X400)

Source: compiled by the authors of this study

Slightly bleeding is noted within the renal tubules, mild degeneration of renal convoluted tubules, epithelial cell separation of some tubules, as well as hypertrophy in some of renal tubules cells, fig. (2: D-E). In the group 4, we noticed loss of normal structural tissue features and severe degradation and necrosis of the renal convoluted tubules is accompanied by severe hemorrhage, fig. (2: F).

Morphometric analysis of liver slices revealed Kupffer cells, was recorded a significant increase (P≤0.01) of its number in the Group2, Group3, and Group4 (42.51±4.2 cell/section), (35.62± 3.41cell/section), and (53.99± 5.31cell/section) respectively when compared with the Group1 (8.52± 2.31cell/section). Also, groups 2, 3, and 4 show, a markedly significant rise (P≤0.01) in the liver sinusoidal area (0.817± 0.005 mm^2/section area), (0.616± 0.007 mm^2/section area), and (0.910± 0.01mm^2/section area) respectively, compare to group1 (0.255± 0.006 mm^2/ section area), degeneration areas (2.004± 0.96 mm^2/ section area), (1.950± 0.53 mm^2/ section area), and (2.431± 0.871 mm^2/ section area) respectively compare in group1 (0.396± 0.025 mm^2/ section area), necrosis areas (0.342± 0.013 mm^2/ section are), (0.221± 0.011 mm^2/ section area), and (0.461± 0.084 mm^2/ section area) respectively compare in group 1 (0.000± 0.000 mm^2/ section area), and inflammation areas (1.072± 0.031

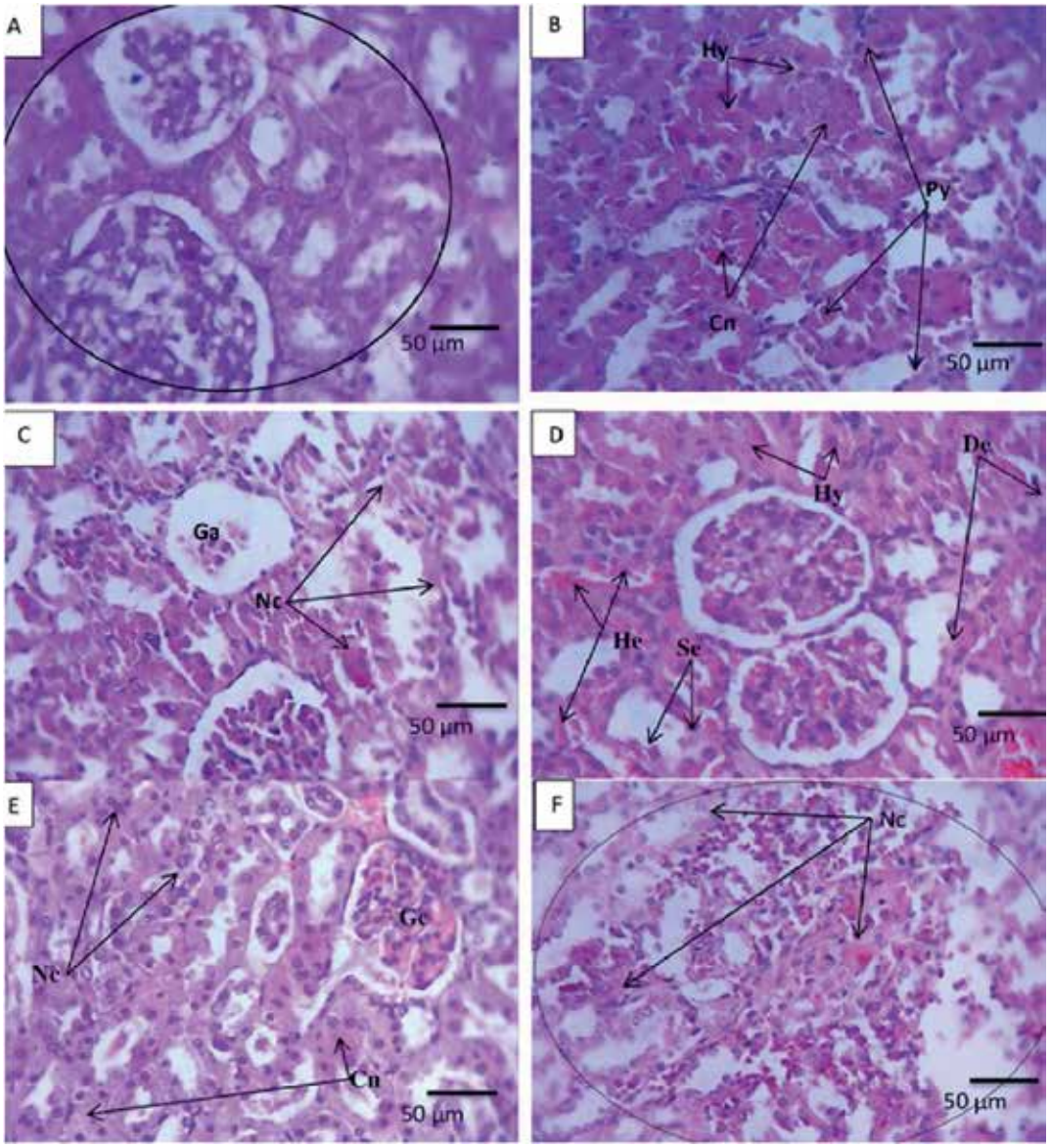

Fig. 2. Micrograph sections of the kidney for the toxic effects of single and synergistic doses of both Pb and piroxicam: (A) the group 1 showed an intact kidney histological building design (circle). (B and C) The group 2 shows tubule epithelial cell hypertrophy (Hy), narrowing and closure of renal tubules (Cn), severe epithelial degeneration (De), glomerular atroph (Ga), pyknosis (Py), and necrosis of renal tubular epithelial (Nc). (D and E) The group 3 shows Haemorrhage (He), mild renal tubules' degeneration (De), epithelial cell separation (Se), narrowing and closure of renal tubules (Cn), necrosis of renal tubular epithelial (Nc), glomerular congestion (Gc), and hypertrophy of renal tubule epithelial cells (Hy). (F and G) group 4 shows severe renal epithelial cells' necrosis (Nc), severe hemorrhage (He), tubule epithelial cell hypertrophy (Hy), metaplasia of renal epithelial cells (Mt), and loss of the normal shape of renal tubules (circle) (H&E X400)

Source: compiled by the authors of this study

mm^2/ section area), (0.872± 0.021 mm^2/ section area), and (1.36± 0.071 mm^2/ section area) of hepatic tissue, compare to group1 (0.000± 0.000 mm^2/ section area). Generally, Group 4 points to acute severe tissue morphometric alteration compared to other groups. While, the kidney sections reflect an increase of glomerular shrinkage and atrophy, hypertrophy of renal epithelial cells, degeneration, brush boundary loss, and hemorrhage, (Table 3).

DISCUSSION

Lead is a well-known hazardous environmental and occupational pollutant that reaches humans and animals through many routes of exposure, which causes severe problems in the liver and kidneys [19], while peroxicam is a non-steroid anti-inflammatory drug, PM is a widely used anti-inflammatory, analgesic, antipyretic, and antirheumatic drug, but has been shown to have adverse effects on the liver and kidneys. The current study compared the combined exposure of lead acetate (Pb) and the Piroxicam drug to their individual effects on the liver and kidneys of adult rats. According to our results, group 2 and 3 appeared a significant increase in serum AST, ALT, and ALP enzymes as well as Cre and Ur in all rats with single or co-treatment effects, TP and Alb showed decreased values compared to

Table 3. Indicator of distribution of tissues damage signs in rats after administration of the mixture of piroxicam and lead

		Comparison groups			
Pathological Signs	**Organ**	**Group1**	**Group2**	**Group3**	**Group4**
Kupffer cells	Liver	+	+++	++	++++
Sinusoids diameter		-	++	++	+++
Degeneration		-	+++	+++	++++
Necrosis		-	++	+	++++
inflammation		-	+++	++	+++
loss of glomerulus	Kidney	-	++	+	+++
Hypertrophy		+	+++	+++	++++
degeneration		-	+++	+++	++++
brush boundary loss		-	+++	+	+++
Hemorrhage		-	+++	++	+++

+ indicates the degree of damage
Source: compiled by the authors of this study

the group 1. The combined effect of group 4 represented the most severe of these changes. AST, ALT, and ALP enzymes, as well as TP and ALP, are among the hepatic function biomarkers seen in serum; an increase in them indicates liver injury [20]. Rats given piroxicam and cadmium treatment showed significant alterations in their levels of ALT, AST, ALP, total protein, albumin, creatinine, and urea, suggesting that the treatment harmed their liver [21]. The leaking of ALT, AST, and ALP enzymes, which resulted in the higher rates found in the serum, may have been facilitated by that lack of membrane integrity (Fig. 1). These results are consistent with the earlier research [22]. Hepatic cell function was correlated with total protein and albumin levels [23]. Accordingly, restoring normal protein levels is crucial for liver healing [24]. It has been established that increased ROS production leads to changes in gene expression and protein synthesis by causing damage to DNA, mitochondria, and the endoplasmic reticulum [25]. As demonstrated by a significant drop in serum albumin and total protein concentration in our study, these events result in extreme liver necrosis and disruptions of hepatic metabolism [26]. The low concentration of plasma proteins and their loss may also be been also brought on by kidney damage [27]. The level of serum creatinine and urea values used as indicators of nephrotoxicity showed a significant rise in the groups 4 as compared to the group 1. Our results confirmed the disintegrating brush boundary from the histological analysis; this contributed to tubular damage and was demonstrated by a marked increase in the levels of serum urea and creatinine. In general, Pb and PIX are known to cause oxidative damage by increasing the cell's synthesis of reactive oxygen species [28-29]. Reactive oxygen species (ROS) are the starting point of oxidative stress; they are elevated due to the depletion of endogenous antioxidant molecules, hydroxyl radical, superoxide anion, and hydrogen peroxide following this, several mechanisms, that cause tissue damage such as lipid peroxidation, DNA damage, dysfunction of mitochondria, and apoptotic cell death [30]. As a result, the present investigation demonstrated significant oxidative damage, as demonstrated by the significant declines in GSH concentrations and CAT activity and the increase of MDA concentration in the experimental group of rats [31]. Of all the ROS, OH• is the most dangerous because it directly breaks down the lipid content of the membrane, and starts producing [32]. This result was confirmed in the current investigation by the significant rise of MDA in each of the kidneys and liver, which facilitated the incidence of membrane damage. The histological investigation confirmed that hepatocyte damage may be caused by Cyclooxygenase 1 inhibition, resulting in fluid retention inside the hepatocytes [33]. Hepatocyte vacuolar degeneration may be triggered by a significant change in lipid metabolism, as Piroxicam inhibited prostaglandin synthesis leads to hepatic tissue ischemia, with consequent mitochondrial failure [34]. The current study confirmed the occurrence of programmed cell death in hepatic cells treated with lead and PIX treatment [35] explained that When a lot of hepatocytes are going through apoptotic alterations, apoptosis may convert to focal foci of coagulative necrosis, therefore, inflammation may occur as a result of the blood vessels surrounding inflammatory cells becoming infiltrated, which is an obvious reaction to any negative effects. Also, the inhibition of COXs enzyme, which is responsible for the production of prostaglandins, which are important in the process of regulating the glomerular filtration rate, which leads to a decrease in glomerular blood circulation and a higher chance of renal ischemia [36]. According to the current study, retraction of the meningeal cell processes and vasoconstriction of the glomerular capillaries, this may be due to the presence of angiotensin II in the cytoplasm, which could lead to contraction of the glomerular tuft and enlargement of Bowman's space [37]. Our histological analysis revealed that the kidney's most afflicted area is the proximal-convoluted tubule (PCT).

Generally, compared to other renal cells, PCT epithelial cells have a higher concentration of mitochondria [38], so the mitochondria are necessary for the high-energy production needed for the active transportation of amino acids with other molecules and the endocytosis-mediated reabsorption of albumin [39].

CONCLUSIONS

Lead, piroxicam and synergistic exposure caused functional and histological damage to the liver and kidney, as seen by changes in serum biochemical structure, and tissue modifications in histological profiles. This study suggested that when Pb^{+2} and PIX, when given in combination, they exert many toxic effects and severe effects on the biochemical and histological features than their administration as individuals.

ABBREVIATION:

Group1: Control Group, Group2: Lead Acetate Group, Group3: Peroxicam Group, Group4: Group of Synergistic mixture of piroxicam and lead acetate, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, ALP: Alkaline Phosphatase, BUN: Blood Urea Nitrogen, Cre: Creatinine, Ur: Urea, MAD: Malondialdehyde, CAT: Catalase, SOD: Superoxide Dimutase, TP: Total Protein, Alb: Albumin, GSH: Glutathione .

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ORIGINAL ARTICLE

Clinically significant and insignificant prostate cancer following prostate intraepithelial neoplasia

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ABSTRACT

Aim: To investigate the rate of clinical significant and insignificant Prostate Cancer (PC) in patients with initial high grade and low grade Prostatic Intraepithelial Neoplasia (PIN).

Materials and Methods: The study included 286 patients with PIN, 152 patients with HGPIN and 134 patients with LGPIN. During 3 year follow-up prostate rebiopsies with 6 months interval were performed. Each PC case was assessed in relation to its clinical significance according to such Epstein criteria as PSA density < 0,15, Gleason score < 7, number of cores positive for tumour < 3, no more than 50% involvement by tumour in every single core.

Results: During 3-year follow-up in 75 (26,2%) of 286 patients with PIN was detected PC (64 (42,1%) patients with HGPIN and in 11 (8,2%) patients with LGPIN). General estimation of clinical insignificant PC cases that were detected in patients with prior PIN amounted 12 (16%) patients. The majority of patients, namely 63 (84%) had clinical significant PC. Among 11 PC cases in patients with LGPIN 4 (36,4%) cases were insignificant. The rate of insignificant PC in HGPIN was 8 (12,5%). Statistically valid difference between HGPIN and LGPIN in rate of insignificant PC, that was 23,9% was determined.

Conclusions: PIN is a cancer precursor that progresses into PC in 26,2% of cases during 3-year follow-up. The rate of clinically insignificant PC in patients with PIN was in 23,9% more in patients witn LGPIN than in patients with HGPIN.

KEY WORDS: insignificant, cancer, prostate, intraepithelial, neoplasia

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INTRODUCTION

Prostate cancer (PC) is the second most commonly diagnosed cancer and the fifth leading cause of cancer death among men worldwide, with an estimated 1,414,000 new cancer cases and 375,304 deaths in 2020 [1]. According to Ukrainian cancer register data PC incidence in Ukraine in 2021 was 26,7 and mortality rate was 10,1 per 100000 of male population (world standard) with a tendency to increase [2].

PC incidence increased rapidly more than two decades ago because of advent of PSA testing and PC screening. This has improved the early detection of prostate cancer, leading to more men being diagnosed and treated. Despite high prevalence of disease, most prostate tumors are indolent and are unlikely to progress to clinical significance. The cause of overdiagnosis is the high prevalence of small, low-grade prostate cancers in the adult population; approximately 50% of men older than 60 years of age have such tumors. These tumors are often indolent in nature and show slow or no progression [3]. According to data of Eastham, J.A. [4] great proportion of patients with prostate cancer diagnosed at autopsy never had any clinical symptoms. Overdiagnostics and overtreatment became a significant problem because every kind of prostate cancer treatment, as well as active surveillance, may lead to comorbidity and complications [5].

For those reasons appeared the term "clinically insignificant prostate cancer" and Epstein criteria, first reported in 1994, were developed to reduce overtreatment by identifying insignificant prostate cancers that may be amenable to active surveillance therapy. Epstein criteria are based on PSA and needle biopsy examination results and include PSA density <0,15 ng/ml/cc, biopsy Gleason score ≤6, present of tumour in <3 cores and no more than 50% involvement by tumour in every single core [6, 7].

The problem of precancerous pathomorphological states, such as prostatic intraepithelial neoplasia (PIN) and its role in carcinogenesis is discussed in scientific literature. PIN consists of atypical cells with the appearance of neoplasia within prostate acini or ducts, but with minimally or non-disrupted basal membrane without evidence of invasion. PIN cells share immunohistochemical, morphologic, and genetic changes with cancer. Some researchers considere PIN to be a predecessor to overt prostate cancer-especially in case of high grade PIN (HGPIN) [8, 9]. At least 30% patients with a confirmed HGPIN will develop prostate cancer within 1 year [10].

According to data of different researchers PIN malignization rate vary from 5% to 50% depending on PIN grade, research mode and statistical peculiarties [11, 12]. Different prognostic criteria by different researchers were developed to identify potentially biologically insignificant tumours that might be

safely managed by initial surveillance or aggressive cancer that should be treated radically. Taking in the concideration role of PIN in cancerogenesis it is of great importance to determine value of PIN not only as precancerous state but also to study PIN as one of the criteria of clinical significant PC in subsequent biopsies.

AIM

To investigate the rate of clinical significant and insignificant PC in patients with initial high grade and low grade PIN.

MATERIALS AND METHODS

The study included 286 patients from 48 to 76 years old (mean age 65,6±1,4 years) with PIN, which were examined on the clinical base of State Institution of Science "Research and practical center of preventive and clinical medicine", State administrative department. All patients were divided into two groups according to PIN grade. The first group consisted of 152 patients with high grade PIN (HGPIN). To the second research group belonged 134 patients with low grade PIN (LGPIN). All patients signed informed consent before taking part in the study. Diagnosis was confirmed morphologically after transrectal multifocal prostate biopsy that was performed according to PC suspicion. The methodics of prostate biopsy and morphological diagnosis were established according to European Association of Urology (EAU) Guidelines and Transrectal Ultrasound Guided Biopsy of the Prostate [13]. During 3 year follow-up were performed prostate rebiopsies with 6 months interval. The aim of rebiopsies was to assess morphological changes in prostate tissue and to detect PC. Initial and repeated transrectal prostate biopsies were performed under transrectal ultrasonic guidance with 12 samples. Each PC new case was regarded and assessed in relation to its clinical significance according to such Epstein criteria as .

Statistical analysis of the results was performed using "Statistica" (version 6.0, Statsoft Inc, USA.) software. Comparison of two independent groups of quantitative indicators was performed using the Mann-Whitney U-test, $p < 0.05$ was considered as statistically significant.

RESULTS

During 3-year follow-up in 75 (26,2%) of 286 patients with PIN using rebiopsies was detected PC. The aim was to determine correlation between grade of PIN and rate of malignant transformation. PC was diagnosed in 64 (42,1%) patients with HGPIN and in 11 (8,2%) patients with LGPIN (fig. 1).

Therefore, it was determined statistically valid difference between HGPIN and LGPIN in malignization rate, that was 33,9% ($p<0,05$).

Analysis of all detected PC cases according to Epstein criteria (PSA density <0,15, Gleason score <7, number of cores positive for tumour <3, no more than 50% involvement by tumour in every single core) was made for each factor separately and with general assessment of each case clinical significance.

It was established that among all 75 PC cases 53 (70,7%) patients had PSA density <0,15 ng/ml/cc and 22 (29,3%) >0,15 ng/ml/cc.

By Gleason score was determined following distribution. 44 (58,7%) of patients with PC had Gleason score < 7 and 31 (41,3%) - ≥7.

Number of cores positive for tumour > 3 was observed in 32 (42,7%) of patients with PC and < 3 – in 43 (57,3%) cases.

No more than 50% involvement by tumour in every core was detected in 61 (81,3%) and in 14 (18,7%) patients had PC with spread outside of the prostate or to nearby lymph nodes or metastasis (T3-4N1M1).

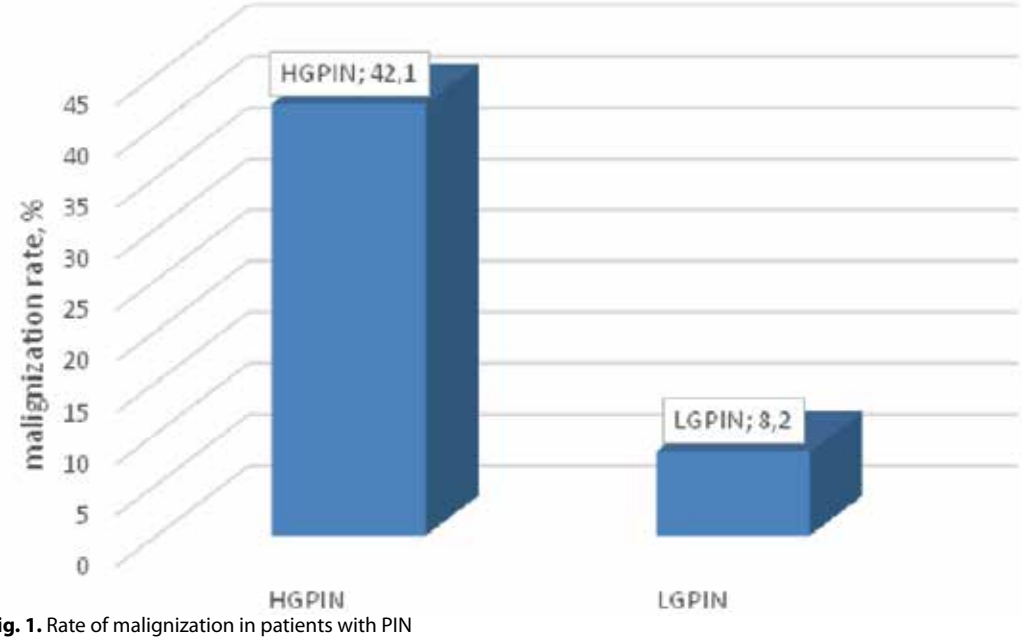

Fig. 1. Rate of malignization in patients with PIN

Source: compiled by the authors of this study

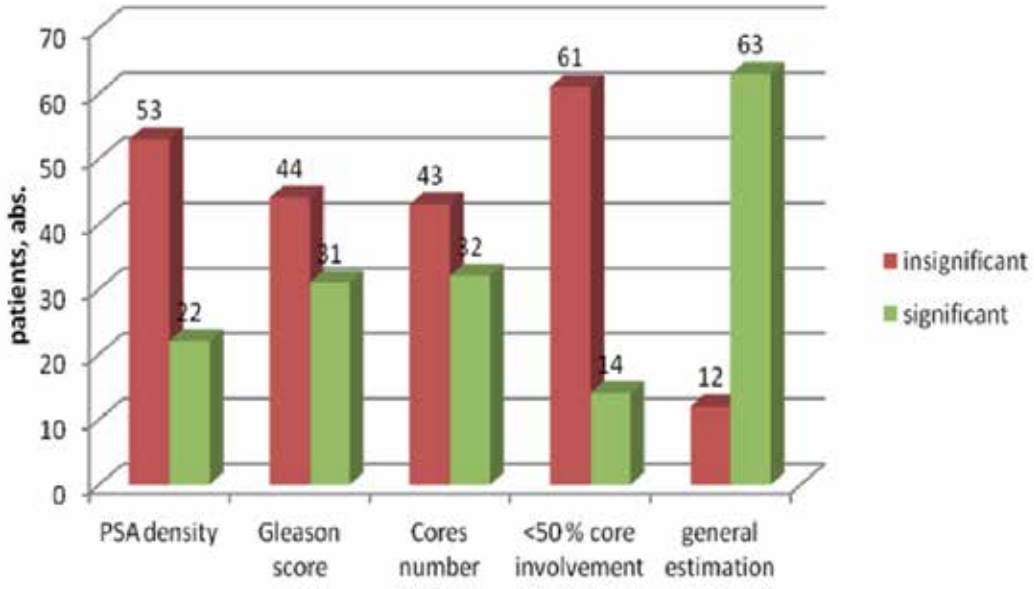

Fig. 2. Correspondence of PC cases with Epstein criteria
Source: compiled by the authors of this study

General estimation of clinical insignificant PC cases that were detected in patients with prior PIN amounted 12 (16%) patients. At the same time the majority of patients, namely 63 (84%) had clinical significant PC (Fig. 2).

After analysis of rate of insignificant cancer in patients with HGPIN and LGPIN it was established that among 11 PC cases in patients with LGPIN 4 (36,4%) cases were insignificant. Among 64 cases in patients with HGPIN 8 (12,5%) cases were insignificant. Therefore, it was determined statistically valid difference between HGPIN and LGPIN in rate of insignificant PC, that was 23,9% ($p<0,05$).

DISCUSSION

Clinical significance of precancerous disease is determined by its malignant transformation ability. In last decades of great importance is the problem of overdiagnosis and overtreatment of PC because of its indolent or slow-growing course. The advent of prostate-specific antigen (PSA) testing and its use for early detection and screening for PC increased stage migration toward more favorable stages [14]. Active surveillance, with the intent to initiate definitive treatment if there is evidence of disease progression, is currently the preferential initial management strategy in low-risk PCa to reduce overtreatment [15]. Conversely, patients with high-risk diseases appear to benefit the most from definitive therapy [16]. Such precancerous state as PIN is a stage of transformation from benign tissue to malignant. In our study it was determined that clinical significance of PIN correlates with its grade. HGPIN is a precancerous pathology with malignization rate of 42.1% while follow-up of patients with LGPIN demonstrated insignificant malignization ability of 8.2%. Obtained data correlate with those of De Marzo et al. [17]. Besides we established that in most cases HGPIN associated cancers are clinically significant and it is important to distinguish a group of high malignization risk among patients with PIN based on immunohistochemical investigation, PSA, MRI data. Controversely, to the opinion of Juan Morote et al. the risk of clinically significant PC in men with isolated HGPIN, in whom PC suspicion disappears, is extremely low. Moreover, in those men in whom PC suspicion persists, the risk of csPCa is not influenced by the previous finding of HGPIN. However, previous HGPIN increases the risk of insignificant PCa detection [18]. Mayra A. Dantés-Durán1 et al. investigated BCL2, EGFR, p53, Her2/neu, and Ki67 immunohistochemical expression patterns in acinar adenocarcinoma of the prostate and high-grade prostatic intraepithelial neoplasia. Their study focused on demonstrating the correlation between malignant and premalignant lesions immunohistochemical profile in the same patient, with expression of the markers being compared in prostatic intraepithelial neoplasia and acinar adenocarcinoma of the prostate. Higher HER2 expression was observed in high-grade prostatic intraepithelial neoplasia and acinar adenocarcinoma of the prostate. This is the evidence of close features of HGPIN and prostate adenocarcinoma [19].

As a conclusion it could be said that patients with HGPIN represent a high risk group of malignant transformation and progression namely into clinically significant PC. These need detailed precise examination and treatment to prevent malignant transformation in every individual case. More investigations with a large number of patients are needed to detect the role of theraputical influence on HGPIN in prevention of clinically significant PC.

CONCLUSIONS

During 3-year follow-up malignization rate in patients with HGPIN is 42,1% and in patients with LGPIN 8,2%.

The rate of clinically insignificant PC in patients with HGPIN is 12,5%, and in patients with LGPIN 36,4%.

PIN is a cancer precursor that progresses into PC in 26,2% of cases during 3-year follow-up. HGPIN is a precancerous state and a cause of PC in 42,1% of cases, 87,5% of which are clinically significant. Patients with HGPIN are in a high PC risk group. LGPIN progresses to adenocarcinoma in 8,2% of cases, 63,6% of which are significant. Future investigations are needed to assess the opportunities of PC prevention by HGPIN treatment.

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CONFLICT OF INTEREST

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ORIGINAL ARTICLE

The thymus structural organization in severe extracellular dehydration and during readaptation

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ABSTRACT

Aim: To investigate the changes of the thymus structure under extracellular dehydration and after rehydration in model experiment.

Materials and Methods: The study was conducted on 40 white male rats, divided into 4 groups of 10 animals each. 2 experimental groups were subjected to extracellular dehydration during 90 days. Following this period, one group was removed from the experiment and other one was switched to the vivarium's standard ration for 30 days (rehydration). The control groups of animals received the vivarium's standard ration throughout the 90 days and the 120 days respectively. The morphometric analysis, histological examination, immunohistochemical analysis were used.

Results: 90 days extracellular dehydration leads to deformation of the thymocytes nuclei of rats, vacuolation or organelle destruction in cytoplasm, apoptosis and necrosis activation. The signs of stasis and thrombosis are present in thymic vessels. Functional thymus tissues replaced with connective and adipose tissues almost completely. After 30 days of rehydration reveals partial thymus restoration with lymphoid cell density increasing, with preserved morphological signs of cellular stress and structural remodelling, vascular abnormalities and incomplete restoration of thymus mass.

Conclusions: In the context of severe extracellular dehydration, profound the depletion of functional thymus tissue, its substitution with connective and adipose tissue, and the occlusion of blood vessels. The morphological structure of the thymus doesn't complete recover during 30 days readaptation period. This suggest the need for prolonged rehydration periods or more effective interventions to fully restore thymic structure and function.

KEY WORDS: thymus, extracellular dehydration, readaptation

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INTRODUCTION

The thymus, as a primary lymphoid organ, facilitates the antigen-independent proliferation and differentiation of subpopulations of T-lymphocytes. Upon leaving thymus, T-lymphocytes enter the bloodstream and populate secondary lymphoid organs, generating responses to foreign antigen invasions [1-3].

The functional status of the thymus impacts the stability and susceptibility of the body to diseases significantly, especially during childhood and adolescence. Therefore, the investigation of the conditions that accelerate thymic involution, leading to the replacement of functional lymphoid tissue with connective and adipose tissues, is of utmost importance [3,4]. Recent studies have elucidated the cellular mechanisms underlying the replacement of functional lymphoid tissue with connective and adipose tissue in the thymus with age, indicating a complex transition within the thymic structure [5]. Furthermore, the functional decline of the thymus with aging encompasses epithelial-to-mesenchymal transitions, fibrosis, and adipogenesis, reflecting a significant deterioration in the thymic microenvironment and underscoring the contributions of various stromal cells, including adipocytes and fibroblasts, to these processes [6]. Although this transition does not lead to the complete disappearance of functional thymic tissue, it results in significant structural alterations, with notable reductions in lymphocytes and Hassall's corpuscles, highlighting the intricate dynamics of thymic involution and its implications for immunological health and disease susceptibility [7].

The apoptosis and necrosis, as mechanisms of cellular death, are critical in the context of thymic involution also, especially under the duress of environmental stressors [8-10]. Consequently, the study of structural changes in the thymus under the influence of various exogenous and endogenous factors remains a pressing concern for morphologists and other related professions [11]. One such factor is dehydration as water scarcity can have severe and potentially fatal consequences in a short timeframe [12-15].

In modern scientific literature, there is no available data regarding structural changes in the thymus parenchyma of rats under conditions of severe extracellular dehydration.

AIM

The aim of this study is to investigate the changes in the structural organization of the thymus during severe extracellular dehydration.

MATERIALS AND METHODS

The experiment involved 40 mature white male rats. The experimental group (10 animals) were subjected to extracellular dehydration by maintaining them on a

demineralised diet, namely, boiled food and bidistilled water for 90 days. Throughout the experiment, the animals were given 0.0003 g of Furosemide intraperitoneally. A severe degree of dehydration developed after 90 days.

After 90 days on a demineralised diet, the rehydration group (10 animals) was switched to the vivarium's standard food and drink ration for 30 days. The control group (C 1-10 animals) received the vivarium's standard drinking and food ration for 90 days. The control group (C 2-10 animals), received the vivarium's standard drinking and food ration for 120 days. This study follows the Animal Research: Reporting of In Vivo Experiments (ARRIVE) 2.0 guidelines [16].

All experimental animals were housed in the vivarium of Sumy State University. The study was carried out in accordance with the provisions of the European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), Council of Europe Directives 86/609/EEC (1986), Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty Handling", general ethical principles of animal experiments, adopted by the First National Congress of Ukraine on Bioethics (2001).

Prior to thymus material collection, the animals were euthanized using carbon dioxide [16]. The paraffin sections of 5-7 μm thickness were made on a Leica RM2121 rotary microtome.

Ultrathin sections were obtained using a UMTP-4 ultramicrotome. The specimens were examined and photographed utilizing a PEM-125 microscope. Semi-thin sections were prepared at a thickness of 0.5-1 μm, stained with 1% methylene blue in 1% sodium tetraborate. Examination of these sections was carried out using an Olympus BX-41 light microscope (Japan) with photographic documentation of the morphological pattern under a light microscope by a Baumer/Optronic video camera, Typ: CX05c.The morphometric study was conducted using the computer analysis system "Digimaizer."

The quantitative data were processed using the Statistica v.10 software (StatSoft Inc., USA). Descriptive statistical analysis was performed for each sample, calculating the mean (M) and standard deviation (SD). The data distribution for each sample was assessed using the Shapiro-Wilk test. ANOVA (Analysis of Variance) was used to compare the samples based on the studied parameters. Differences in the indicators were considered statistically significant if $p<0.05$.

FOOTNOTE

Animal welfare: The present study followed international, national, and/or institutional guidelines for humane animal treatment and complied with relevant legislation. All procedures performed were in accordance with the standards set forth in the Guide for the Care and Use of Laboratory Animals and Basel Declaration.

RESULTS

In terms of structure, the thymus of the control rats corresponded to the species norm. It was represented by particles of various sizes separated by thin, connective tissue transitions that left the capsule covering the organ in the parenchyma's thickness. The cortical and medullary substance of the thymus was clearly differentiated in the lobules. Individual plasma cells and thymus bodies (Hassal's bodies) were discovered in the medullary substance of thymus lobules (Fig. 1A). The semi-thin sections of the thymus confirm the typical structure of thymus cells at the light microscopy level (Fig. 1B).

At the ultrastructural level, thymocytes were found in the cortical substance of the thymus lobules, which are characterized by a rounded shape with a large, clearly defined rounded nucleus occupying almost the entire cell. The cytoplasm contains ribosomes, polysomes and a sufficient number of mitochondria. There are plasma cells. Also, epithelial reticulocytes are morphologically differentiated (forming the cortical cellular network) (Fig. 1C).

Small thymocytes have a rounded shape and a large, well-defined, rounded nucleus that occupies almost the entire cell. Cytoplasm forms a narrow rim around the nucleus, and the nuclear envelope is continuous with minor intussusceptions (Fig. 1B).

When histological sections of the thymus of rats with severe extracellular dehydrationan were examined 90 days after the start of the experiment, accidental transformation

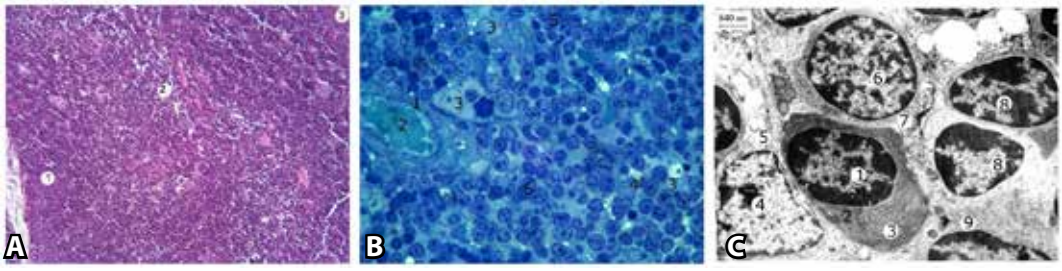

Fig. 1. Fragment of thymus lobule in a control rats: (A) location of the transition of cortical substance into medullary substance, 1 – cortical substance; 2 – medullary substance; 3 – capsule, hematoxylin and eosin, X200; (B) 1 – blood capillary wall; 2 – erythrocytes in vessel lumen; 3 – "light" epithelial reticular cell; 4 – epithelial reticular cell with vacuoles; 5 – thymocyte, methylene blue staining, X1000; (C) 1 – nucleolus in the nucleus of plasma cell; 2 – polysomes in the cytoplasm of plasma cell; 3 – tubules of the granular endoplasmic reticulum; 4 – nucleolus in the nucleus of epithelial reticular cell; 5 – cytoplasm of epithelial reticular cell; 6 – nucleus of lymphoblast; 7 – cytoplasm of lymphoblast; 8 – nucleus of thymocyte; 9 – cytoplasm of thymocyte, electronic photography, X6000

Source: compiled by the authors of this study

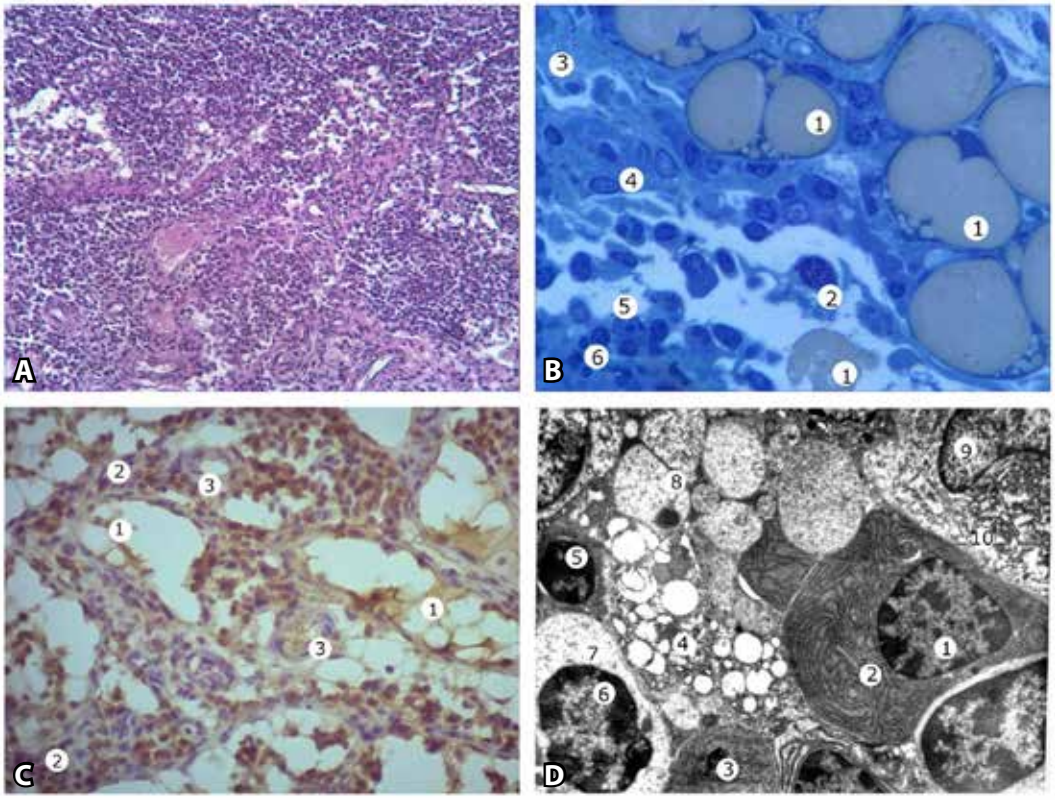

Fig. 2. Fragment of thymus lobule in rats with extracellular dehydration, 90th day: (A) absence of the boundary between the cortical and medullary substances, hematoxylin and eosin staining, X200; (B) subcapsular region of an experimental animal's thymus, 1 – fat drops, 2 – granulocytes, 3 – connective tissue fibers, 4 – vessel sections; 5 – lymphoblast, 6 – thymocytes, methylene blue staining, X1000; (C) pronounced expression of eNOS in the thymus parenchyma against the background of fatty degeneration, 1 – adipose tissue, 2 – lymphoid cells, 3 – section of a vessel, reaction from PCAt to anti-eNOS, X400

Source: compiled by the authors of this study

was noted in the majority of the observations. The boundary between the cortical and medullary substances of the thymus lobules was not contoured in separate fields of vision (Fig. 2A).

Amongst the experimental group of rats, the third showed lobule collapse, sclerosis, and atrophy of the parenchyma of thymus. Connective tissue and fatty replacement were seen in areas of atrophic parenchyma (Fig. 2B). The capsule of the organ had thickened, the connective tissue fibres within it were distended, and the vessels were full of blood with signs of stasis and thrombosis. The density of lymphoid cells in the gland's parenchyma decreased. The proportion of connective tissue and adipose tissue also increased. Thymus bodies were deformed and single. Cytokeratin PAN+ thymus bodies were homogeneous, with sharply flattened epithelial cells on the periphery, some cystically expanded, and some calcified.

All vessels underwent changes. Veins and venules were deformed, dilated, and contained formed blood elements with stasis signs. Sludge syndrome was characterised by thickened walls in the lumen of arteries and arterioles. Some blood capillaries were "empty" with a narrowed lumen, while others had deformed erythrocytes.

The severe degree of extracellular dehydration of rats at the time of withdrawal from the experiment was characterized by a decrease in their absolute body weight by 28.49% ($p<0.001$) in comparison with the constant body weight of animals in the control group. According to the data of the organometric study, a significant ($p<0.001$) decrease in the absolute mass of the thymus by 39.92% in comparison with the animals of the corresponding control group ($p<0.001$) (Table 1).

The relative area of the cortical substance of the thymus lobules decreases by 13.36% ($p<0.001$) and, accordingly, the relative area of the medullary substance of the thymus lobules increases by 20.14% ($p<0.001$).

A decrease in cell density on an area of 0.009 mm^2 of the cortical substance of thymic lobules by 59.28% ($p<0.001$) was observed compared to the control group of animals. Additionally, there was a decrease in cell density on an area of 0.009 mm^2 of the medullary substance of thymic lobules by 43.48% ($p<0.001$) compared to the control group of animals (Table 1).

The preserved thymus lobules were primarily composed of single CD20+ B-lymphocytes and numerous CD68+ macrophages. When compared to the control group of animals, the number of dendritic cells (S100+) decreased significantly. Ki-67, a mitotic activity indicator, was also significantly reduced. Endothelial nitric oxide synthase (eNOS) with severe extracellular dehydration was found in

Table 1. Parameters of the thymus of the studied animals (M±SD)

Observation group, indicator	Control 1	Experimental	Control 2	Rehydration
Body weight of the rat, g	226,50±6,45	161,97±5,35 p<0,001	232,42±9,58	200,18±13,21 p<0,001
Thymus mass, mg	257,51±17,46	154,72±12,11 p<0.001	262,65±12,08	193,40±9,45 p<0,001
The relative area of the cortical substance	60,12±1,76	52,09±1,12 p<0,001	61,10±2,06	56,33±1,24 p<0,001
The relative area of the medullary substance	39,88±1,37	47,91±1,23 p<0,001	38,90±1,64	43,67±1,21 p<0,001
Lymphocytes density in the cortical substance, Lymphocytes/0.009 mm2	218,39±13,68	88,93±11,74 p <0,001	220,77±9,17	149,30±6,88 p<0,001
Lymphocytes density in the medullary substance, Lymphocytes/0.009 mm2	117,70±7,63	66,53±17,68 p <0,001	115,04 ±8,41	81,61±8,69 p <0,001

p – comparison with animal indicators from the control group

Source: compiled by the authors of this study

endotheliocytes of thymus vessels, marked macrophages, and plasma cells, and was found in areas of fatty degeneration (Fig. 2C). Inducible nitric oxide synthase (INOS) was found in the thymus capsule, intergranular septa, and lipocytes, as well as endothelium, macrophages, and numerous plasma cells.

During ultrastructural examination, it was discovered that areas, where functional thymus tissue have been replaced with connective and adipose tissue, alternate with areas where lymphoid tissue is preserved. The nuclei of the cells were deformed and shrunken, and the cytoplasm was frequently vacuolated or contained damaged organelles. Many cells were undergoing apoptosis or necrosis. Epithelioreticulocytes had a deformed, reduced nucleus, a thin nuclear envelope, a non-pronounced perinuclear space, clear cytoplasm, and almost no organelles. The structure of the cells in the preserved gland fragments was nearly identical to the previous period of study, but their number was much lower (Fig. 2D).

The number of macrophages and plasma cells increased significantly (Fig. 2D). The cytoplasm of macrophages was completely filled with fat inclusions and fragments of other cells, many of which were in a state of apoptosis. Plasma cells had a reduced, deformed nucleus, and the cytoplasm was completely filled with expanded tubules of the granular endoplasmic reticulum.

Thymocytes in the cortex and medulla had a pyknotic nucleus, a thin rim with areas of cytolysis, are electron-dense, and contain destructed organelles. Most of the time, these are deformed vacuolated mitochondria arranged in a chain. The perinuclear space was not expressed. Many cells had vacuolated cytoplasm.

Epithelial reticular cells of both the medullary and the cortical substance of the thymus lobules were observed with both a light and a "dark" nucleus, and contain diffusely located heterochromatin. Cytoplasm underwent compaction, was vacuolated, filled with fine granular material. As in the previous term of the study, areas of cytolysis with the formation of electron-transparent vacuoles and vacuoles with fine-grained content of low electron density were visualised in the cytoplasm (Fig. 2D).

Severe extracellular dehydration, accompanied by stasis and thrombosis in the hemomicrocirculatory vessels, is characterized by the release of blood elements into the organ parenchyma, complete occlusion of capillary lumens by deformed erythrocytes forming "coin columns," deformation and swelling of endotheliocyte nuclei.

Histological analysis of thymus parenchyma in the experimental group after one month of readaptation revealed that, as in cases of severe extracellular dehydration, the boundary between the cortex and medulla in some thymic lobules remained indistinct. (Fig. 3A).

Some thymic lobules displayed atrophy and sclerosis, with lymphoid tissue replaced by connective and adipose tissue, reducing its proportion. The thickened, fibrous capsule showed congested vessels with stasis and thrombosis. Lymphoid cell density increased, with isolated thymic bodies observed in the medulla and cortex. Hemomicrocirculatory changes included dilated veins and venules, thickened arterial walls, stasis, sludge syndrome, and narrowed hemocapillary lumens.

After a month of readaptation following severe extracellular dehydration, the average absolute body mass of the rats increased by 35.4% (p=0.001) compared to the group that underwent severe dehydration, which is 13.78% less (p=0.001) than the control group. The absolute thymus mass of the rats increased by 25.0% (p=0.001), which is 26.37% less (p=0.001) than the control group (Table 1).

Morphometric analysis revealed a 7.53% increase (p<0.001) in the relative area of the thymus cortex compared to the group of animals subjected to severe general dehydration. However, this value is 7.81% lower (p<0.001) than that of

the control group. Additionally, there is an 8.85% decrease ($p<0.001$) in the relative area of the medulla, which is 10.92% higher ($p<0.001$) than in the control group. (Table 1).

An increase in cell density within the 0.009 mm^2 area of the thymus cortex was observed by 32.38% ($p<0.001$), though this parameter remains lower than in the control group. Additionally, there is an increase in cell density within the 0.009 mm^2 area of the thymus medulla by 22.67 % ($p<0,001$) which is на 29.06% ($p< 0,001$) lower than the control group (Table 1).

No increase in the number of dendritic cells (S100+) was observed, and the Ki-67 mitotic activity index remained low. Endothelial nitric oxide synthase (eNOS) accumulated in some endothelial cells, macrophages, plasma cells, and adipocytes. Inducible nitric oxide synthase (iNOS) accumulated in the thickened organ capsule, septa, endothelium, macrophages, plasma cells, and adipocytes.

Ultrastructural analysis of the thymus parenchyma revealed alternating zones of cell destruction, fibrous and adipose accumulation, preserved lymphoid tissue with disturbances, and widespread apoptosis and necrosis in both cortical and medullary regions, accompanied by vacuolated cytoplasm, deformed nuclei, and intercellular spaces filled with vacuole-like structures and cellular debris (Fig. 3A).

The structure of cells in preserved fragments of the gland in the readaptation group is nearly indistinguishable from that in the group of animals with severe extracellular dehydration (Fig. 3B).

The thymic parenchyma shows a high number of macrophages, many undergoing apoptosis with vacuolated cytoplasm, condensed heterochromatin, and indistinct nucleoli. Plasma cells exhibit cytoplasm densely filled with dilated cisternae of the granular endoplasmic reticulum and irregularly rounded nuclei (Fig. 3B, C).

The hemomicrocirculatory vessels predominantly exhibit stasis and thrombosis, with less frequent extravasation of blood elements into the organ parenchyma.

DISCUSSION

Our experiment revealed that severe extracellular dehydration in rats showed structural changes in thymus parenchyma and blood vessels, leading to the replacement of functional thymus tissue with connective and adipose tissue.

The studies investigating extracellular dehydration in rats revealed structural changes in the vascular bed of the thyroid gland, including the congestion of veins, blood capillaries, vessel stasis, sludge syndrome, thrombosis, and microhemorrhages in the organ parenchyma [20] and the replacement of functional tissue by connective tissue, defibrillation, fibre delamination, edema, and clear separation from follicular parenchyma [21]. Similarly, our findings indicate the presence of blockages in the lumen of blood capillaries in rats experiencing severe extracellular hydration and replacement of functional thymus tissues with connective and adipose tissues. Despite the distinct functional roles of the thymus, responsible for protecting the body against foreign antigens, compared to the thyroid gland, these findings support the claim that the parenchyma and blood vessels structural changes in response to extracellular dehydration are not organ-specific.

Furthermore, the observed thymus parenchymal atrophy and decreased number of S100+ dendritic cells reflect the compounded effects of reduced blood flow, nutrient delivery, and oxygen supply to the thymus, exacerbating the degradation of cellular structures and diminishing the organ's immunological functions.

The thymus loses functional capacity as we age, resulting in a progressive decrease in the output of naive T cells. The deterioration of the thymus microenvironment, including limited epithelial-mesenchymal transitions, fibrosis, and adipogenesis, indicates organ atrophy. The investigation of cellular changes in the thymus at various stages of development, including mouse models with single-cell RNA sequencing, reveals an increase in the number of different cell types that influence thymus function [21-23]. Severe cellular dehydration seems to have an analogous effect to ageing on the thymus. Our experiment observed lobule collapse, sclerosis, and atrophy of the parenchyma of the thymus in as many as a third of the dehydrated group of rats. This is accompanied by reduced and deformed nuclei in plasma cells, and cytoplasm completely filled with fat inclusions and fragments of other cells in apoptosis

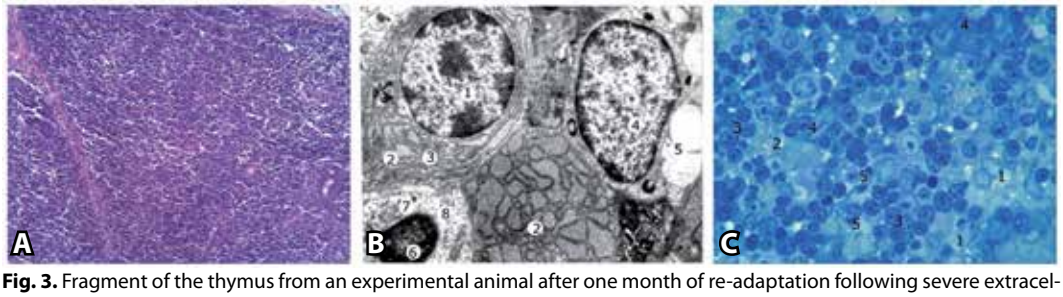

Fig. 3. Fragment of the thymus from an experimental animal after one month of re-adaptation following severe extracellular dehydration: (A) absence of the boundary between the cortical and medullary substances, hematoxylin and eosin staining, X200; (B) 1 – nucleus of a plasma cell, 2 – dilated cisternae of the granular endoplasmic reticulum in the cytoplasm of a plasma cell, 3 – destructured mitochondrion, 4 – nucleus of a macrophage, 5 – vacuolated cytoplasm of a macrophage, 6 – cell in a state of karyopyknosis, 7 – swollen mitochondrion, 8 – cleared cytoplasm, electron micrograph, X8000; (C) 1 – lymphoblast with vacuolated cytoplasm, 2 – vacuolated intercellular space, 3 – thymocyte accumulation, 4 – epithelial reticular cell, 5 – cell in apoptosis, methylene blue staining, X1000

Source: compiled by the authors of this study

in macrophages. In order to understand the correlation between severe extracellular dehydration, ageing, and thymus function, similar experiment designs focused on the analysis of the functional capacity of the thymus need to be conducted. Dehydration causes similar changes in various glands and organs [21, 22].

The relationship between immunosuppression and thymic lymphocyte apoptosis and proliferation has been investigated through the infection of animals with reticuloendotheliosis virus (REV), an avian retrovirus. Findings reveal that REV led to an increase in apoptotic and necrotic cells in the thymus parenchyma, inhibiting T-lymphocyte proliferation, changing T-cell subsets, and increasing immunosuppression [24]. Severe extracellular dehydration yielded a similar outcome in terms of immune cells, including decreased density of lymphoid cells in the parenchyma of the thymus gland, and significant decrease of the number of S100+ dendritic cells and Ki-67. When exposed to dehydration, the thymus gland undergoes ultrastructural changes, that indicate the onset of cellular senescence, which is strongly linked to thymus involution [25].

The results of the study on the structural changes in the thymus after one month of rehydration following severe extracellular dehydration show findings from morphometric analyses, which are supported by histological data. In the thymic lobules, there is no clear boundary between the cortical and medullary substances. For instance, researchers have demonstrated that dehydration induces significant changes in the proteome of the hypothalamo-neurohypophysial system in rats, likely reflecting similar stress responses in the thymus, where cellular stress and structural remodeling persist even after rehydration [26]. Similar results were obtained by other authors studying the recovery of cerebral circulation after dehydration. Their research indicates the need for prolonged periods of rehydration for complete recovery [14].

The observed partial restoration of the thymus after rehydration, including increased lymphoid cell density and improved corticomedullary differentiation, aligns with findings from studies on the rehydration of various organs after dehydration. For example, a study on the pancreas showed that rehydration could restore some pancreatic structures, but often this restoration was incomplete [21].

Vascular abnormalities, including stasis and thrombosis, also resonate with studies on immune and endocrine organs, emphasizing the long-term impact of dehydration on microcirculation [26].

Despite the increase in lymphoid cell density, the presence of cell stress markers, such as vacuolization and necrosis, indicates incomplete morphological recovery of the thymus. In their study of rehydration strategies, it is noted that while the restoration of water balance may reduce structural damage, it does not always lead to complete recovery of organ functions, especially after severe dehydration [27].

CONCLUSIONS

The results of our study demonstrate structural changes in the parenchyma and blood vessels of the thymus in white male rats following 90 days of severe extracellular dehydration. Key findings included thymus parenchymal atrophy, a decreased number of S100+ dendritic cells, deformed and shrunken nuclei in cells, vacuolated cytoplasm with damaged organelles, and a continuous "coin column" arrangement of deformed erythrocytes causing complete blockages in the blood capillary lumen. The areas of complete replacement of functional tissue with connective and adipose tissue alternated with small areas of preserved lymphoid tissues. During re-adaptation, the morphological structure of the thymus significantly recovered, but full restoration was not achieved. Therefore, further research is needed to explore longer periods of rehydration or alternative strategies to accelerate the recovery process.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Respiratory dysfunction in patients with irritable bowel syndrome

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ABSTRACT

Aim: To study the respiratory symptoms and respiratory functions markers in patients with irritable bowel syndrome (IBS).

Materials and Methods: The study included 40 patients with IBS, and 30 apparently healthy subjects. Pulmonary functions are performed using spirometry, including forced expiratory volume, forced vital capacity, Forced Expiratory Volume/Forced Vital Capacity, peak expiratory flow rate. Classification of Irritable Bowel Syndrome subtypes performed according to the Rome III criteria.

Results: Respiratory symptom among patients with IBS was sputum in the most common 67.5%, cough 50%, and wheeze 30% and dyspnea 37.5%. The results of the current study also revealed a significant decrease in most markers of respiratory functions markers, these include: Forced Vital Capacity which was (2.59±0.12 L) in Irritable Bowel Syndrome patients as compared to healthy control group (2.82±0.06 L). A significant decrease in Forced Expiratory Volume was also recorded in patients (2.18±0.12 L) comparing to control subjects (2.42±0.73 L). Accordingly, the Forced Expiratory Volume/Forced Vital Capacity was also significantly decreased in patients 83.98%±1.21% in comparison to healthy individuals 85.85%±1.35%.

Conclusions: It was concluded that patients with irritable bowel syndrome exhibited a marked decreased in respiratory functions. It also seems that constipation is one of the underlining mechanisms for deterioration in the pulmonary functions and appearance of respiratory symptoms.

KEY WORDS: Forced Vital Capacity, Peak Expiratory Flow Rate, Irritable Bowel Syndrome, respiratory symptoms

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INTRODUCTION

One of the most common gastrointestinal disorders is irritable bowel syndrome (IBS), which is characterized by a symptom complex of abdominal pain, bloating, and altered bowel habits. The varied pathophysiological mechanisms underlying IBS symptomatology explain why both diagnosis and treatment are far from straightforward processes [1]. An extra prevalence of bronchial hyper-responsiveness has been shown among patients with IBS. A review of the literature reveals that these two conditions merely coexist and have no relationship. For asthma, studies have been conducted among asthma patients for IBS and among IBS patients for asthma to explain this association but came up with conflicting results [2]. Recent research has begun to uncover the bidirectional relationship between the gut and lung health often referred to the gut-lung axis. Enaud et al., (2020) explain the potential influence of respiratory health by dysbiosis in gut microbiota, leading to inflammation and exacerbation of respiratory conditions. This interorgan communication suggests that gastrointestinal health may play a role in respiratory disorders. Irritable Bowel Syndrome (IBS) is a common gastrointestinal disorder manifested with a variety of symptoms including periodic abdominal pain as well as bloating and variable bowel habits. Though the majority of focuses for IBS-oriented research works are centered within gastrointestinal symptoms, extra-intestinal symptoms have been increasingly recognized; specifically respiratory symptoms like dyspnea cough and wheeze [4]. The heterogeneity of IBS is reflected in its different subtypes; diarrhea-predominant IBS (IBS-D), constipation-predominant IBS (IBS-C), and IBS-M. Recognition of these subtypes is essential for any appropriate management and therapeutic strategy. Likewise, respiratory syndromes may likewise take different forms and some may share common pathophysiological mechanisms with the disorders of the gastrointestinal tract [5]. Indeed, very little is known about the possible relationship between the respiratory disorder and IBS, though few works have been done in this field. While the exact pathways that drive disease remain poorly understood, research has suggested that it is likely to involve shared inflammatory pathways. Patients suffering from respiratory disorders most probably will have some kind of gastrointestinal symptoms. For instance, insight to the underlying inflammatory mechanisms in COPD has been given by Agustí and Hogg (2019), and they could run in parallel to those of IBS [6]. According to Hert et al. (2011), the relation between physical sickness and mental disorders has been studied concerning the fact that chronic organic

diseases could have concurring psychosomatic aspects related to functional gastrointestinal disorders, as in IBS. This does not solve the idea that holistic nursing must keep an eye on the whole respiratory and gastrointestinal function in these patients. Comprehending the interaction between gastrointestinal health and respiratory function is of immense help in coming up with all-inclusive management strategies for a patient who presents with both conditions.

AIM

Therefore, the aim of this research is to study the respiratory symptoms and respiratory functions markers in patients with irritable bowel syndrome (IBS).

MATERIALS AND METHODS

PATIENTS AND DATA COLLECTION

A cross-sectional descriptive study was conducted at Al-Sadr Medical City in Al-Najaf City, Iraq in the period from August 2023 to July 2024. Forty patients with a confirmed diagnosis with irritable bowel syndrome, aged 16-45 years were included in the current study. The general information (age, respiratory symptoms) was collected from the records of the patients in the hospital. Evaluation of pulmonary functions are performed using spirometry, these markers include: forced expiratory volume (FEV1), forced vital capacity (FVC), FVC%, FEV1/FVC, peak expiratory flow rate (PEF). Regarding classification of IBS subtypes, patients were primarily diagnosed according to the Rome III criteria of IBS by the gastroenterologists in the hospital, and classified into three types: diarrhea-predominant IBS (IBS-D), constipation-predominant IBS (IBS-C), and mixed IBS (IBS-M) [5].

STATISTICAL ANALYSIS

The SPSS software version 25.0 (SPSS, Chicago) was used for all the appropriate statistical analyses, the normality test which was used to test who want to continuous data for Shapiro Wilk test. All normally distributed data are acted as mean ± standard deviation. Independent t test was used to find out the statistical difference in the respiratory function markers between patients' and control. ANOVA test was used to find out the statistical difference in the respiratory function markers among patients' subgroups classified according to IBs subtypes. A p-value less than 0.05 were considered to indicate a statistically significant difference.

ETHICS APPROVAL

The protocol in this study was approved by the ethical committee of the Medical College in the University of Kufa (No. 122 in 2024).

RESULTS

The results indicated that sputum in the most common respiratory symptom among patients with IBS constituting (67.5%), the other recorded symptoms are: cough (50%), wheeze (30%), dyspnea (37.5%), while about (15%) of them exhibited no symptoms. These symptoms tend to show a significant difference (chi square = 36.47; p value <0000) in patients comparing to those recorded in control groups (table 1).

Patients with IBS have been classified into three subgroups according to IBS subtypes: 18(45%) with IBS-D, 16(40%) with IBS-C and 6(15%) with IBS-M, as illustrated in fig.1.

The results of the current study also revealed a significant decrease in most markers of respiratory functions markers, these include: FCV which was (2.59 ± 0.12 L) in IBS patients as compared to healthy control group (2.82 ± 0.06 L). A significant decrease in FEV1 was also recorded in patients (2.18 ± 0.12 L) comparing to control subjects (2.42 ± 0.73 L), accordingly, the FEV1/FVC was also significantly decreased in patients 83.98% ± 1.21% in comparison to healthy individuals 85.85 ± 1.35, table 2.

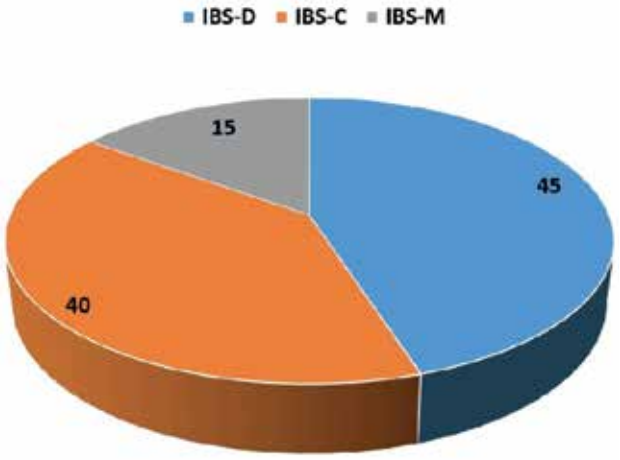

Fig. 1. Distribution of patients according to subtypes of irritable bowel syndrome
Source: compiled by the authors of this study

Table 1. Descriptive statistics for respiratory symptoms among irritable bowel syndrome

Symptoms*	Patients N=40	Control N=30	Chi Square (P value)
Wheeze	12(30%)	3(10%)	36.47 0.000
Cough	20(50%)	9(50%)	
Dyspnea	15(37.5%)	0(0%)	
Sputum	27(67.5%)	6(20%)	
No Symptoms	6(15%)	21(70%)	

** Choices applied more than once*
Source: compiled by the authors of this study

Table 2. Differences in respiratory functions markers between patients and control groups

Parameter	Control (n=30)	Patients (n=40)	Independent T Test	P value
	Mean±SD	Mean±SD		
FVC (L)	2.82 ± 0.06	2.59 ± 0.12	8.8	0.000 HS
FEV1 (L)	2.42 ± 0.73	2.18 ± 0.12	9.37	0.000 HS
FEV1/FVC (%)	85.85 ± 1.35	83.98 ± 1.21	5.96	0.000 HS
PEF (L)	4.39 ± 0.23	4.33 ± 0.06	1.53	0.13 NS

NS: Not significant difference at $p > 0.05$; HS: High significant difference at $p \leq 0.01$
Source: compiled by the authors of this study

Table 3. Differences in respiratory functions markers among patients' subgroups classified according to IBS subtypes

Parameter	IBS-D (n= 18)	IBS-C (n=16)	IBS-M (n=6)	F Test	P value
	Mean±SD	Mean±SD	Mean±SD		
FEV1 (%)	2.61 ± 0.55	2.58 ± 0.35	2.59 ± 0.23	0.019	0.98 NS
FVC (mlU/mL)	2.14 ± 0.19	2.17 ± 0.18	2.18 ± 0.32	0.12	0.88 NS
FEV1/FVC	82.79 ± 4.8	83.66 ± 8.5	84.98 ± 9.11	0.22	0.80 NS
PEF (µg/L)	4.31 ± 0.18	4.34 ± 0.35	4.35 ± 0.46	0.059	0.94 NS

NS: Not significant difference at $p > 0.05$; HS: High significant difference at $p \leq 0.01$
Source: compiled by the authors of this study

The comparison in respiratory functions markers has also been achieved among patients' subgroups classified according to IBs subtypes, the results exhibited no significant differences in these markers , as shown in table 3.

DISCUSSION

It has been brought out that the patients with IBS may have respiratory symptoms which could render their general health status and quality of life complicated. A greater volume of literature is suggesting that the gastrointestinal symptoms of IBS may be tightly associated with other systemic manifestations, including respiratory issues. It was highlighted that self-reported food-related gastrointestinal symptoms in IBS patients relate to more severe symptoms and, eventually, a reduced quality of life. If indeed the deterioration of gastrointestinal symptoms were to cause respiratory discomfort, then a plausible explanation would be shared pathophysiological mechanisms, like inflammation or dysbiosis [8]. According to Halmos et al., (2014), a low FODMAP diet effectively reduces gastrointestinal symptoms in patients with IBS, which also has a reflection on decreasing related respiratory symptoms, such as dyspnea and cough. It has been proposed that through the lessening of fermentable carbohydrates, the low FODMAP diet could reduce gastrointestinal distress, hence resulting in lower respiratory discomfort. Though scanty, if any, is the direct evidence that links this dietary intervention to symptom relief in respiration. In 2015, Böhn and colleagues carried out a randomized controlled trial that proved how low fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) diet not only eases IBS symptoms but also may go extend to respiratory symptoms. The mechanisms underlying IBS that are considered to play a major role in the pathogenesis of respiratory symptoms include visceral hypersensitivity and the gut-brain axis, point towards inextricable connections between the systems of gastrointestinal and respiratory health [10]. This in turn indicates that through dietary means, it may be possible to influence simultaneously both the system of the gastrointestinal tract and the system of the respiratory tract in patients with IBS. The interconnections between gastrointestinal and pulmonary symptoms are rather complicated. It is possible, as Ong and his colleagues did in 2010, to manipulate dietary short-chain carbohydrates to alter gas production, which might influence IBS symptom genesis. This suggests a role for dietary intervention not only in managing gastrointestinal symptoms but also in reducing respiratory discomfort associated with gastrointestinal distress [11]. Second, the role of the transient receptor potential vanilloid 1 has come into the center of attention. According to Staudacher et al., transient receptor potential vanilloid-1 (TRPV1) is sensitized by histamine and has a contribution to visceral hypersensitivity, which, in turn, can explain accompanying respiratory symptoms such as cough or wheeze [12]. That is important because a key to understanding how TRPV1 may contribute to the symptomatology of IBS is provided through the linkage between visceral pain and hypersensitivity with respiratory discomfort. Potential mechanisms that connect IBS to respiratory symptoms may be via histamine release and visceral hypersensitivity. According to Wouters et al., (2016), sensitization mediated by the histamine receptor H1 might induce histamine to cause visceral hypersensitivity, which could present itself as both gastrointestinal and respiratory symptoms [13]. Major et al., (2017) supported this by stating that hypersensitivity of the colon to their studies rather than excessive gas production actually accounts for the symptoms

related to carbohydrates in irritable bowel syndrome, supporting that comparable hypersensitivity mechanisms could play a role in respiratory symptoms [14]. The discussion of gut microbiota has been increasingly realized in various diseases, including IBS. The composition of the gut microbiota is altered in patients with IBS, with potential systemic effects acting via the respiratory system. As the authors put it, dysbiosis is defined as a departure from a balanced composition and is associated with different respiratory conditions, such as asthma and allergies, providing evidence of communication between two highly varied systems, namely the gastrointestinal and respiratory tracts [15]. In this line of thought, Vila and his colleagues found very dissimilar gut microbiota compositions when comparing patients with inflammatory bowel diseases to those with IBS. Even though still focusing on gastrointestinal diseases, more research may dissect a role that these microbiota alterations play in respiratory functions [16]. The knowledge of these microbial differences could easily explain how IBS might cause respiratory-associated symptoms, leading to treatments that focus on the two kinds of complaints, both gastrointestinal and respiratory. Physiological responses of IBS patients to carbohydrate intake suggest that gastrointestinal hypersensitivity may also extend to respiratory responses. It hints that dietary triggers might link both sets of symptoms to a common locus: the gastrointestinal tract and decline in respiratory function. Understanding in what way dietary components play the role in the exacerbation of both gastrointestinal and respiratory symptoms will allow fighting against two problems simultaneously. The problem with killing two birds with one stone is that the birds might be in different places [18]. It seems likely to be a result of facial eczema and nutritional management for cattle and sheep in East Coast North Island (ECNI) of New Zealand suggested by a string of words you might be likely ranching if you say them: "weaner, yards, cattle stop. The gut-brain axis is of prime consideration as changes in gut microbiota are capable of manifesting both gastrointestinal and respiratory symptoms. Indeed, as research indicates, the composition of gut microbiota might be involved in systemic inflammation; this may have a direct effect on lung function and FEV1 among patients with IBS [19]. Besides, chronic in Flammarion in the gut might be considered the source of other systemic effects, like respiratory problems. Studies on these pathways could clarify the way in which IBS affects respiratory health [20]. FVC is the most widely used key efficacy variable in clinical trials for respiratory diseases, ipf in particular [21]. Fundamental IBS pathophysiology specifically sets the stage for notable symptom presentation in the various subtypes of the disorder. In their study, Johannesson et al. (2011) put into question whether the effectiveness of histamine receptor antagonists in reducing visceral sensitive-related symptoms would in one way or another elicit an explanation in terms of a possible cause effect mechanism that could ease ways for more effective treatment interventions targeted at specific pathways that underlie symptom generation of different subtypes of IBS [22]. Such distinctions are crucial in finding tailored therapeutic options that act on particular IBS symptoms. A relationship with disease progression and patient outcomes gives some background information as to why FVC should be carefully assessed over time. Some of these key issues can guide targeted therapy development for IBS based on homing in on unique symptom profiles. Especially to relate it to the conditions of the gastrointestinal tract, such as constipation and their possible effects on FVC, is important since conditions related to the functioning of the respiratory system may act in response to a wide range of factors that include pressure over the abdomen and movement of the diaphragm. There has also been other research which proposed constipation to be related to elevated pressures inside the abdominal cavity, which is likely to compromise lung volumes as well as FVC [21]. These mechanical effects limit the diaphragm's movement due to abdominal distension and reduce ultimately effectiveness concerning respiratory mechanics. While literature shows effects of diseases of respiration on functions of the alimentary canal, no reference concerns the converse: how gastro-intestinal dysfunction, constipation inclusive, may condition respiratory performance [24].

CONCLUSIONS

It was concluded that patients with irritable bowel syndrome exhibited a marked decreased in respiratory functions (especially FEV and FVC). It also seems that constipation is one of the underlining mechanisms for deterioration in the pulmonary functions and appearance of respiratory symptoms. Further investigations are recommended, with including the possible impacts of body mass index, duration of the diseases, and medication used, on the respiratory functions of the patients with IBS.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Sex features of chewing pressure in youth of the Ukrainian ethnic group

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ABSTRACT

Aim: Determin the magnitude of chewing pressure between different teeth on the right and left parts of the dental arches between young people of different sex of general groups and individual craniotypes.

Materials and Methods: The study used a quantitative approach, in particular, Fuji technology, Prescale MS. The system consists of a film, a scanner and special software, which allows you to obtain data on the distribution of pressure and its exact values. The participants were divided by gender – 118 girls and 104 boys, in whom one of the physiological bite types was detected. The determination of craniotypes was carried out using a cranial index. The following distribution of craniotypes among adolescents was established: dolichocephalyc, mesocephalyc, brachycephalyc.

Results: We found a significant difference between youth in chewing efficiency, there were sex differences in the values of occlusal load between the first, second, third, fourth and fifth teeth of the left and right parts of the dental arches. We analyzed the value of chewing pressure in boys and girls belonging to different craniotypes, but significant sex differences were not found in all craniometric groups.

Conclusions: Gender differences were established in the magnitude of chewing pressure of incisors, canines, and premolars of the left and right parts of the dental arches when comparing groups of girls aged 16-20 and boys aged 17-21. The most significant sex differences were found in individuals with mesocephaly, the smallest in dolichocephaly.

KEY WORDS: masticatory efficiency, craniotype, sexual dimorphism, Ukrainian youth, gnathodynamometry

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INTRODUCTION

In modern dentistry, one of the problems that requires urgent solution is the determination and assessment of chewing efficiency, in particular, a reliable assessment of chewing pressure at the moment of occlusion. The practical significance of determining chewing pressure is due to the fact that a significant influence of ethno-territorial features on the value of this indicator has been determined, in particular W. Ma et al. [1] identified a difference in the magnitude of chewing pressure between Chinese Han and Chinese Mongols, explaining the presence of ethnic differences in oral physiological properties by different eating habits of the subjects. Therefore, the study of the characteristics of the pressure distribution on individual teeth in representatives of a certain ethno-territorial region is relevant and has undeniable practical significance.

There are various methods for determining chewing pressure [2], because in dental practice, bite force is significant for assessing the therapeutic effect during dental restoration, periodontal treatment and in the professional activities of orthodontists [3]. In modern dentistry, many devices are used to determine chewing pressure. These devices mostly use force sensors, in particular transducers: strain gauge, piezoresistive, piezoelectric and pressure-sensitive films. The relevance of the use of film Dental Prescale II and T-Scan equipment is emphasized in their work by YF Huang et al. [4], which does not contradict our chosen method of assessing chewing pressure in the Fuji system. Prescale MS with Fuji film coating. One of the leading factors influencing chewing efficiency is belonging to a certain age range of ontogenesis of male and female individuals. In particular, sex differences in chewing pressure are determined, in the study of the authors WH Shi et al. [5], where it is noted that the chewing pressure and occlusal surface in boys was significantly greater than in girls and the main point of the greatest pressure was noted in the molar region. In the work of JN Su et al. [6], determined a negative correlation between the number of missing teeth and the sum of missing teeth and filled teeth and as a conclusion proved that the number of cariogenic bacteria in the oral cavity increases the risk of tooth loss and, accordingly, leads to a decrease in chewing pressure. Detection of deviations in the normative indicators of chewing pressure, especially in middle-aged and elderly people requires correction, according to the work of the authors K. Kashiwazaki et al. [7], it is possible to improve the maximum chewing pressure through training exercises using chewing gum of different hardness. Having processed the results of modern

scientific research, attention was drawn to the fact of the significance of chewing pressure and chewing efficiency in diagnostics, treatment [8] and rehabilitation [9] in dental practice for the diagnosis and assessment of risk factors [10]. Studies are constantly being conducted on the influence of chewing or occlusal pressure on the course of various diseases of the maxillofacial region, such as periodontitis and osteoporosis Rui Zhu et al. [11], some authors separately distinguish osteoporosis of the alveolar bone associated with "underloading". Y. Chen [12]. According to AM Atlas et al. [13], this method can help in future planning of treatment with removable and non-removable prosthetics, in particular implant-based prosthetics. Unfortunately, data on the peculiarities of chewing pressure in representatives of the Ukrainian ethnic group were not found during the analysis of literary sources.

AIM

The aim of the work was to determine the magnitude of chewing pressure between different teeth on the right and left halves of the dental arches between young people of different sex of general groups and individual craniotypes.

MATERIALS AND METHODS

On the basis of the Department of Orthopedic Dentistry of Vinnytsia National Medical University named after M.I. Pirogov, an examination of the oral cavity of 264 young people of the Ukrainian ethnic group was conducted: boys aged 17 to 21 years inclusive and girls (from 16 to 20 years). As a result of the examination, it was found that 54 people had anomalies and deformations of the maxillofacial region, namely: acute and chronic diseases of the temporomandibular joint; pathological types of occlusions; trigeminal neuritis, acute and chronic inflammatory processes of the maxillofacial region (apical periodontitis, previously identified cyst granuloma, as well as exacerbation of chronic periodontitis), bruxism. Thus, 222 people participated in the further examination, including 118 girls and 104 boys, in whom one of the physiological bite types was detected. In particular, among girls, it was found that 106 people had an orthognathic bite, 10 girls had a direct bite and 2 had a biprognathic bite. Among young men, 72 people had orthognathic bite, 27 people had direct bite, and 5 people had biprognathic bite.

In order to determine chewing efficiency, chewing pressure indicators were determined using the Fuji program. Prescale MS (license number 4761-0000-0404-C67B-CAB2-57A2). Fuji Prescale is a masticatory pressure indication system based on Fuji's advanced film coating technology, which allows for the assessment of pressure distribution across the entire measurement surface. In the case of MS film, the pressure range is from 10 to 50 MPa (Fig. 1).

The principle of action is to destroy microcapsules of the color-forming layer as a result of the action of pressure, at the same time the dye is absorbed (Fig. 2). Microcapsules with color-forming material are adapted to different sizes and applied in a uniform layer, as a result of which, when the capsules are destroyed, staining occurs, the intensity of

Fig. 1. Fuji film Prescale cut in the shape of a dental arch and film holder
Source: compiled by the authors of this study

Fig. 2. Fuji film Prescale after pressure measurement
Source: compiled by the authors of this study

which can be judged by the increase in pressure. After taking the impression with film, the data is scanned, digitized and visualized. According to the visualized data, pressure points can be determined along the entire chewing surface of each tooth (Fig. 3). In the study, the value of the maximum pressure on the occlusal surface of the tooth was chosen.

Craniotypes were assessed using the cranial index (ratio of maximum width to maximum length of the head), which has three main gradations: brachycephaly – cranial index more than 80.0%; mesocephaly – cranial index within 75.0 – 79.9%; dolichocephaly – cranial index is 74.9% and below. The following distribution of craniotypes was established: dolichocephaly – 33 boys and 33 girls, mesocephaly – 37 and 39, respectively, brachycephaly – 34 and 46, respectively.

The significance of the difference between the chewing pressure indicators of individual teeth in boys and girls was determined using the Mann-Whitney U-test using the licensed software package "Statistica 5.5". All examinations

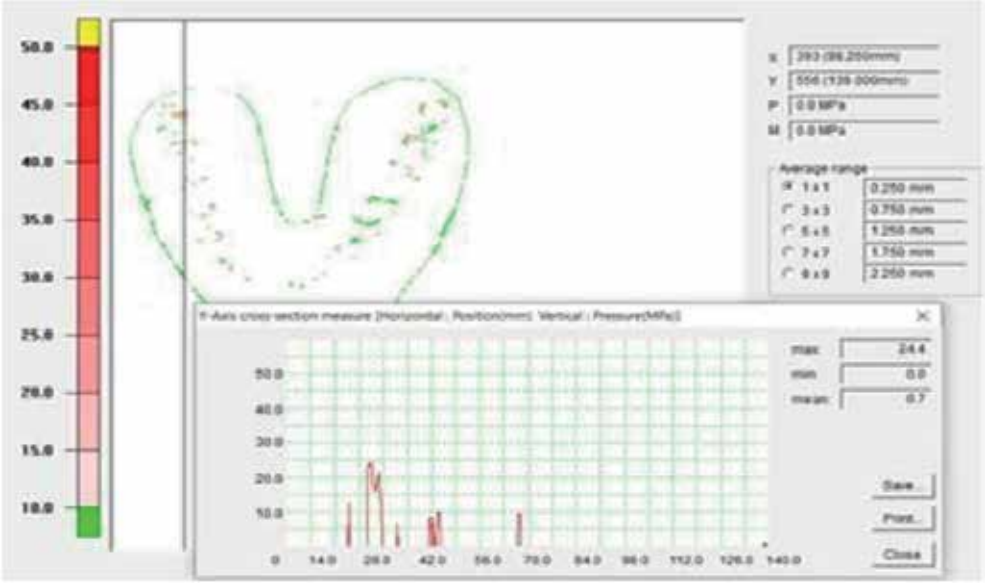

Fig. 3. Methodology for determining pressure on scanned and processed prints in the vertical plane
Source: compiled by the authors of this study

were conducted on the basis of the principle of voluntary informed consent. The work is a fragment of the planned scientific research work "Somatoviscerometric features of the human body in different periods of ontogenesis" (state registration number 0121U113772). The Bioethics Committee of Vinnytsia National Medical University named after M. I. Pirogov established that the conducted gnathodynamometric studies do not contradict basic bioethical norms (Protocol № 3, from 12.03.2024).

RESULTS

Significant gender differences in chewing efficiency in terms of chewing pressure between adolescent girls and boys were found. Thus, in girls, the chewing pressure created by the right canines of the upper and lower jaw was 14.94±4.22 MPa, which is less than in boys 18.16±5.24 MPa, the difference between the comparison groups was significant ($p<0.001$) (Fig. 4). The chewing pressure on the right second incisor in boys (14.61±5.44 MPa) was higher ($p<0.05$) compared to the group of girls (12.88±4.57 MPa). A significant difference ($p<0.001$) was found when studying the pressure between the right first incisors, in girls – 12.95±5.34 MPa, in boys – 16.39±5.85 MPa (Fig. 4).

It was found that the chewing pressure on the left first incisor was 12.66±4.71 MPa in girls and 15.65±6.08 MPa in boys, the difference between the comparison groups was statistically significant ($p<0.001$) (Fig. 5). The gender differences in chewing pressure between the second left incisors of the upper and lower jaws were even more significant, the difference was significant ($p<0.001$), the value of this indicator in females was 12.55±5.03 MPa, in males – 16.49±4.75 MPa. In girls, the chewing pressure on the left canine was 15.19±4.23 MPa, which is less than in boys 17.54±4.95 MPa, the difference between the comparison groups was significant ($p<0.001$).

Analyzing the sexual characteristics of masticatory efficiency indicators, it was noted that there were no

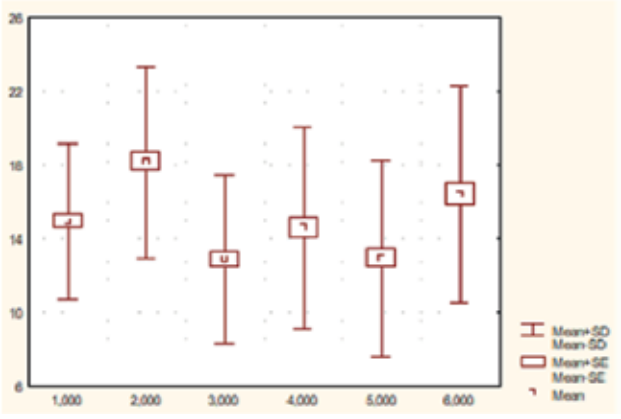

Fig. 4. Sex-specific characteristics of chewing pressure (MPa) of right incisors and canines
Note: here and hereafter: Mean Mean – sample mean; Mean±SE – ± error of the mean; Mean±SD – ± standard deviation. 1 – right canine (girls), 2 – right canine (boys), 3 – right second incisor (girls), 4 – right second incisor (boys), 5 – right first incisor (girls), 6 – right first incisor (boys)
Source: compiled by the authors of this study

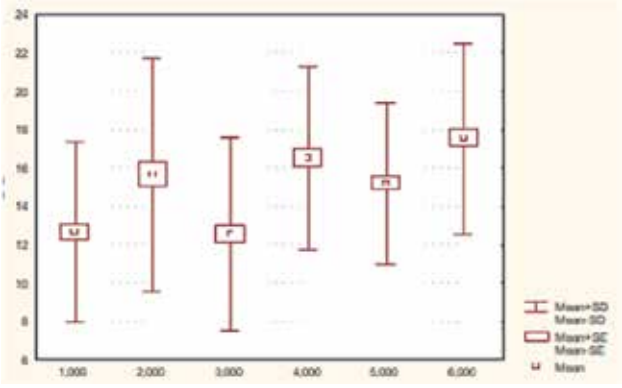

Fig. 5. Sex-specific characteristics of chewing pressure (MPa) of left incisors and canines
Note: 1 – left first incisor (girls), 2 – left first incisor (boys), 3 – left second incisor (girls), 4 – left second incisor (boys), 5 – left canine (girls), 6 – left canine (boys)
Source: compiled by the authors of this study

significant differences between the groups of boys and girls of adolescent age in the magnitude of masticatory pressure on the right first and second molars, although there was a tendency to higher values of these indicators in male representatives. Thus, the right second molars between themselves in the group of boys created an average pressure within 24.29±4.11 MPa, and in girls – 23.21±3.94 MPa. The masticatory pressure between the right first molars of the lower and upper jaw in boys was 22.21±4.39 MPa, in girls – 21.04±5.06 MPa.

It was found that the magnitude of chewing efficiency of premolars, in particular, chewing pressure on the right second premolar was 19.23±4.88 MPa in girls, 20.96±3.81 MPa in boys, the difference between the comparison groups was statistically significant ($p<0.01$). The magnitude of chewing pressure between the right first premolars in the group of girls (17.03±4.82 MPa) was significantly lower ($p<0.001$) compared to boys (19.59±3.89 MPa) (Fig. 6).

It was determined that boys (19.65±4.26 MPa) had a higher ($p<0.001$) chewing pressure created by the left first premolars of the lower and upper jaw, compared to girls (17.20±4.97 MPa). In girls, the chewing pressure on the left second premolar was 18.42±5.66 MPa, which is less than in boys (20.42±4.86 MPa), the difference between the comparison groups was significant ($p<0.05$). The chewing pressure on the left first molar in girls was 21.63±4.56 MPa, in boys – 22.14±4.93 MPa, the difference between the comparison groups was insignificant. The pressure between the left second molars of the lower and upper jaw in girls was 23.30±4.10 MPa, in boys – 24.18±4.27 MPa, there were also no significant differences ($p>0.05$) (Fig. 7).

Having analyzed the magnitude of chewing pressure in boys and girls belonging to different craniotypes, we found statistically significant gender differences not in all craniometric groups (Table 1). Thus, chewing pressure on the right canine was significantly higher only in boys with brachycephalic and mesocephalic skull types (in both cases $p<0.001$). There were no significant gender differences between representatives with pronounced dolichocephaly, and the average values of chewing pressure on the right canine in boys were slightly lower than in girls. Chewing pressure on the right second incisor in boys with mesocephalic skulls was significantly ($p<0.01$) higher than in girls with mesocephalic skulls, and there were no significant gender differences between adolescents with brachycephalic and dolichocephalic skull types. A significant gender difference was found when studying the pressure between the right first incisors in representatives with pronounced brachycephaly ($p<0.05$) and dolichocephaly ($p<0.001$), despite the fact that in mesocephalic boys the average values of chewing pressure on the right first incisor were higher than in girls with this craniotype, the difference between them was not significant (see Table 1).

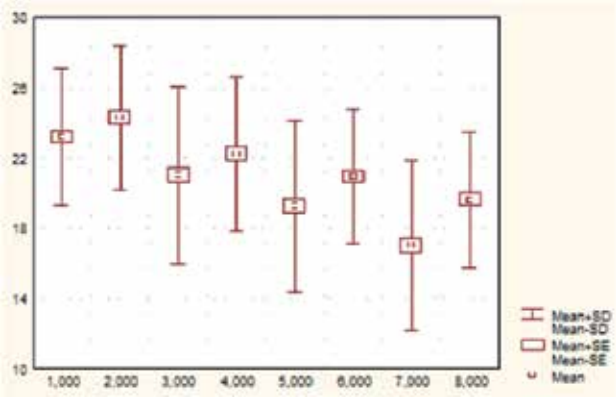

Fig. 6. Gender-specific characteristics of chewing pressure (MPa) of right canines

Note: 1 – right second molar (girls), 2 – right second molar (boys), 3 – right first molar (girls), 4 – right first molar (boys), 5 – right second premolar (girls), 6 – right second premolar (boys), 7 – right first premolar (girls), 8 – right first premolar (boys)

Source: compiled by the authors of this study

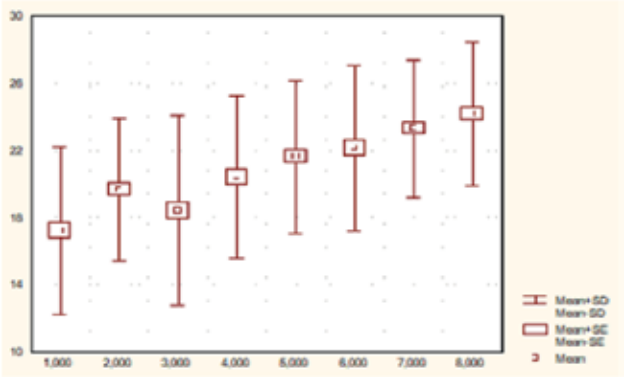

Fig. 7. Gender-specific characteristics of chewing pressure (MPa) of left canines

Note: 1 – left second molar (girls), 2 – left second molar (boys), 3 – left first molar (girls), 4 – left first molar (boys), 5 – left second premolar (girls), 6 – left second premolar (boys), 7 – left first premolar (girls), 8 – left first premolar (boys)

Source: compiled by the authors of this study

It was found that the chewing pressure on the left first incisor was greater in males of all craniotypes, but significant sex differences were determined only between girls and boys belonging to brachycephals and dolichocephals (in both cases $p<0.05$). We found sex differences in the magnitude of chewing pressure between the second left incisors of the upper and lower jaw in representatives of all craniotypes; in brachycephalic boys the pressure on this tooth was greater ($p<0.05$) than in brachycephalic girls, in dolichocephalic boys it was greater ($p<0.001$), in mesocephalic boys it was greater ($p<0.01$). The magnitude of chewing pressure on the left canine was practically no different between girls and boys who had a dolichocephalic craniotype, while brachycephalic ($p<0.001$) and mesocephalic ($p<0.05$) boys had greater pressure on this tooth than girls of the corresponding craniotypes (see Table 1).

Analyzing the sex-specific features of chewing pressure on canine teeth in representatives of different craniotypes, differences were not uniform (Table 2). In particular, the pressure between the right second molars of the upper and lower jaws in brachycephalic boys were even slightly lower than in girls of the same craniotype, but the difference between the comparison groups was not

Table 1. Sex-specific characteristics of chewing pressure (MPa) of incisors and canines in representatives of different craniotypes (M± σ)

Indicator	Group	Girls	Young men	p
Right fang	Brachiocephalians	12.53±3.75	16.76±5.91	p<0.001
	Dolichocephalic	17.00±4.02	16.96±5.54	p>0.05
	Mesocephalians	16.20±3.51	20.46±3.33	p<0.001
Right second incisor	Brachiocephalians	11.76±3.98	11.31±3.95	p>0.05
	Dolichocephalic	11.79±3.42	13.41±5.26	p>0.05
	Mesocephalians	14.87±5.26	18.78±4.06	p<0.01
Right first incisor	Brachiocephalians	11.13±5.12	14.37±6.71	p<0.05
	Dolichocephalic	10.46±3.49	16.12±5.86	p<0.001
	Mesocephalians	16.92±4.44	18.51±4.21	p>0.05
Left first incisor	Brachiocephalians	10.16±3.27	14.27±6.91	p<0.05
	Dolichocephalic	11.18±3.47	14.67±5.65	p<0.05
	Mesocephalians	16.60±4.34	17.87±5.05	p>0.05
Left second incisor	Brachiocephalians	12.13±4.63	14.97±4.08	p<0.05
	Dolichocephalic	10.90±3.55	15.99±5.34	p<0.001
	Mesocephalians	14.35±5.95	18.43±4.24	p<0.01
Left fang	Brachiocephalians	12.14±3.14	16.23±5.27	p<0.001
	Dolichocephalic	16.89±3.85	16.93±4.99	p>0.05
	Mesocephalians	16.99±3.75	19.28±4.17	p<0.05

Note: p – is an indicator of the statistical significance of the difference in chewing pressure between representatives of different sexes of a particular craniotype
Source: compiled by the authors of this study

significant (p>0.05). A higher indicator of chewing pressure on this tooth was determined in dolichocephalic boys, but the difference when compared with girls is also not significant (p>0.05), only males with a mesocephalic skull type had a significantly higher (p<0.05) chewing efficiency of the second right molar than females with a mesocephalic craniotype. The population average values of the pressure between the right first molars of the lower and upper jaw in brachycephalic and dolichocephalic boys were slightly (p>0.05) lower than in girls of the same craniotype, only in mesocephalic boys this indicator was significantly higher (p<0.01).

It was determined that brachycephalic boys had greater (p<0.05) chewing pressure generated by the left first premolars of the lower and upper jaws compared to girls. brachycephalic, among dolichocephalic sex differences were even more significant (p<0.01), and among representatives of mesocephaly no significant sex differences were found. Young men with different craniotypes had greater chewing pressure on the left second premolar, but significant differences (p <0.05) were found only when comparing individuals of different sexes of the mesocephalic craniotype. Chewing pressure on the left first molar in young men of brachycephalic was even slightly lower (p>0.05) than in girls of brachycephalic; in young men of dolichocephalic, this indicator was higher, but the difference when compared with girls was also insignificant (p>0.05); Only males with mesocephalic skull type had significantly greater (p<0.05) masticatory efficiency of the left first molar than females of the same craniotype. The magnitude of masticatory pressure on the left second molar did not differ significantly between males of all craniotypes (see Table 2).

DISCUSSION

Gnathodynamometry, allows to determine the chewing pressure and reflects the chewing function, is determined by two important factors, such as the development of the masticatory muscles and the parameters of craniofacial development. Knowledge of the functional state of the masticatory muscles, which must be considered in interaction with the peculiarity of innervation and the state of the dento-maxillary system, in combination with data on facial morphology, has practical significance for understanding the complex, balanced functioning of the entire stomatognathic system [12, 14]. In addition, it has been proven that oral health correlates with the general health and general well-being of a person [6]. The functioning of the masticatory complex, which is mainly characterized by bite force, as one of the key indicators of chewing, is influenced by a significant number of factors, among which ontogenetic changes (actually, growth and development processes) [7] and sexual dimorphism [5] occupy a prominent place. Age-related features of masticatory function are associated with

Table 2. Sex-specific characteristics of chewing pressure (MPa) of canines in representatives of different craniotypes (M± σ)

Indicator	Group	Girls	Young men	p
Right second molar	Brachiocephalians	23.02±3.95	21.99±4.32	p>0.05
	Dolichocephalic	23.36±3.77	25.12±3.44	p>0.05
	Mesocephalians	23.31±4.15	25.78±3.56	p<0.05
Right first molar	Brachiocephalians	21.43±5.34	21.17±4.52	p>0.05
	Dolichocephalic	21.96±3.94	21.54±4.82	p>0.05
	Mesocephalians	19.81±5.42	23.71±3.49	p<0.01
Right second premolar	Brachiocephalians	18.24±5.41	19.69±4.07	p>0.05
	Dolichocephalic	20.46±4.57	20.44±3.94	p>0.05
	Mesocephalians	19.40±4.25	22.63±2.81	p<0.001
Right first premolar	Brachiocephalians	16.90±5.31	19.27±2.99	p<0.05
	Dolichocephalic	17.07±4.30	17.35±4.45	p>0.05
	Mesocephalians	17.15±4.74	21.76±2.87	p<0.001
Left first premolar	Brachiocephalians	16.70±5.07	19.57±4.03	p<0.05
	Dolichocephalic	16.28±4.56	19.70±5.05	p<0.01
	Mesocephalians	18.51±5.04	19.68±3.83	p>0.05
Left second premolar	Brachiocephalians	18.20±6.01	19.83±4.88	p>0.05
	Dolichocephalic	18.20±5.85	19.34±5.22	p>0.05
	Mesocephalians	18.85±5.19	21.94±4.21	p<0.05
Left first molar	Brachiocephalians	22.16±4.30	20.83±4.90	p>0.05
	Dolichocephalic	21.23±5.20	21.95±5.13	p>0.05
	Mesocephalians	21.24±4.35	23.62±4.50	p<0.05
Left second molar	Brachiocephalians	23.37±3.90	23.73±4.51	p>0.05
	Dolichocephalic	23.18±3.34	24.12±3.68	p>0.05
	Mesocephalians	23.33±4.93	24.70±4.61	p>0.05

Note: p – is an indicator of the statistical significance of the difference in chewing pressure between representatives of different sexes of a particular craniotype
Source: compiled by the authors of this study

a change in the dentition (temporary to permanent) and age-related involution. There are studies that confirm the increase in the force of chewing pressure from childhood to adulthood.

The work of M. Palinkas and co-authors [15] regarding the study of the influence of age and gender on the chewing force of molar bite made it possible to find that the bite force indicators on both sides in the studied Brazilians aged 7–12 years were significantly lower compared to other age groups (13–20 years; 21–40 years; 41–60 years), except for the group of those examined aged 61–80 years, which makes it possible to speak of an increase in chewing force of bite in childhood and its decrease in old age. M.S. Nawaz et al. [16] evaluated the chewing force of the molar bite in individuals aged 13 to 40 years, who were divided into three age groups: I (13-20 years), II (21-30 years), III (31-40 years). It was found that the average value of this indicator depending on age is 476.11 N ± 181.27 N for group I, 550.93 N ± 191.83 N for II and 573.21 N ± 171.18 N for III, that is, it can be stated that the bite force is positively correlated with age, however, only up to a certain point. According to the study by Y. Hama and co-authors [17], it was found that a significant increase in oral functions with age is characteristic of men under the age of 40.

As a result of our research, it was found that boys have mostly higher chewing pressure indicators compared to girls. In particular, boys had higher chewing pressure by 17.73% on the right canine; by 11.84% on the right second incisor; by 20.99% on the right first incisor; by 19.11% on the left first incisor; by 23.89% on the left second incisor; by 13.40% on the left canine; by 8.23% on the right second premolar; by 13.07% on the right first premolar; by 12.47% on the first left premolar; by 9.79% on the second left premolar. The most significant difference was found when examining the pressure between the left second incisors, right first incisors, and left first incisors. The results of our study do not contradict the data obtained by other scientists, in particular W.H. Shi et al. [5] noted that chewing pressure in boys was significantly greater than in girls, explaining

this by a greater occlusal surface is especially in males. A.H. Medhat and A.H. Al Haidar [18] found a significant positive correlation between bite force and maximum mouth opening for boys, as they had higher mean values of these indicators than girls. A study of this indicator in children of different ages found that the magnitude of bite force is directly proportional to age, height, and body weight [19].

S.I. Roldan et. al. [20] drew attention to the fact that there are pronounced sex differences in the ontogenetic development of masticatory muscle strength, and therefore masticatory pressure, because it was found that the strength of the incisors reached a peak at the age of 14.3 for females and 15.3 for males, and the canines at the age of 16 in both sexes. The maximum bite force of the molars increased by 2.6 kg for each year [20]. As a result of our study, it was found that the values of chewing pressure on the left first and second molars and on the right first and second molars did not have significant gender differences. In the work of Y. Shoji et. al. [21] determined the difference in chewing pressure between healthy individuals and individuals with toothache; individuals with pain had lower chewing pressure, and despite the fact that the average chewing pressure was greater in men than in women, the difference between the comparison groups was not significant.

The significant differences we found in the magnitude of chewing pressure between most teeth of the upper and lower jaws prompted us to think about the causal factors of influence. On the one hand, sexual dimorphism in the magnitude of chewing pressure can be explained by the larger total body size (length, mass, body surface area) in males, as noted in their studies by individual authors [19, 20] and the larger amount of muscle mass in men of different periods of ontogenesis [22]. At the same time, there is information that a certain shape of the face [23-25] and the skull in general [26, 27] have a significant influence on a wide range of dental indicators [28]. Therefore, we analyzed the magnitude of chewing pressure in boys and girls belonging to different craniotypes. And significant sexual differences were not found in all craniometric groups. The chewing pressure on the right canine was significantly higher only in boys with brachycephalic (by 33.76%) and mesocephalic (by 26.30%) skull types compared to girls of the same craniotypes. The chewing pressure on the right second incisor was significantly higher by 26.29% only in mesocephalic boys compared to mesocephalic girls, and on the right first incisor this indicator was significantly higher in boys with brachycephalic (by 29.11%) and mesocephalic (by 24.48%) skull types compared to girls of the same craniotypes. Chewing pressure on the left first incisor was higher only in representatives of two male craniotypes: brachycephalic (by 40.45%) and dolichocephalic (by 31.22%) compared to girls who had the same type of skull shapes. The magnitude of chewing pressure on the second left side incisors and was greater in brachycephalic boys by 23.41%, in dolichocephalic boys by 46.70%, in mesocephalic boys by 28.43% compared to girls of the same craniotypes. The chewing pressure on the left canine in brachycephalic boys was significantly greater by 33.69%, and in mesocephalic boys by 13.48% than in girls of the corresponding craniotypes.

It is shown that only boys with a mesocephalic skull type compared to girls of the same craniotype had significantly higher chewing efficiency of the second right molar (by 10.60%) and the first right molar (by 19.69%), among representatives of other craniotypes there was no significant gender difference between the comparison groups. However, it is noteworthy that the chewing pressure on the right molars in brachycephalic boys is slightly lower. Chewing pressure on the left first premolar was significantly higher only in boys with brachycephalic (by 17.19%) and dolichocephalic (by 21.01%) skull types compared to girls of the same craniotypes. Boys with different craniotypes had higher chewing pressure on the left second premolar, but only boys with a mesocephalic craniotype had this indicator significantly higher (by 16.39%) than girls. Chewing pressure on the left first molar in mesocephalic boys was significantly higher by 11.21% than in mesocephalic girls, brachycephalic boys had insignificantly lower values of this indicator compared to girls. Masticatory pressure on the left second molar did not differ at all between individuals of different sexes of all craniotypes.

Thus, the use of the craniometric approach made it possible to confirm the sexual dimorphism in the amount of chewing efficiency of different teeth of the left and right part of the dental left and right parts of the dental arches, previously identified in general groups of young men and women of the Ukrainian ethnic group. Definition and assessment of chewing efficiency taking into account the factor of sexual dimorphism has direct practical importance for orthopedic and orthodontic treatment, as well as theoretical significance in such fields as human anatomy and physiology, dietetics, and pharmacology.

CONCLUSIONS

1. Significant differences in chewing efficiency are evident when comparing individuals of different sexes in the juvenile period of ontogenesis. Significant gender differences were found in the magnitude of chewing pressure between the left and right first and second incisors, canines, and first and second premolars. The most significant gender difference in masticatory pressure indicators was recorded between the left second incisors, right first incisors, and left first incisors (in all cases $p<0.001$). It was found that the magnitude of chewing pressure on the right and left molars did not significantly differ between the general groups of boys and girls.
2. Sex differences in the magnitude of chewing pressure were found in brachycephals on the right and left canines, right and left first incisors, left second incisor, right and left first premolars. In dolichocephals – on the right and left first incisors, left second incisor, left first premolar. In mesocephals – on the right and left canines, right and left first incisors, left second incisor, right first and second molars and premolars, left second premolar, left first molar.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Assessment of the complex-forming capacity of pectins obtained from various vegetable and fruit raw materials as a basis for creating special food products

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ABSTRACT

Aim: To study the complexing ability of pectins obtained from various vegetable and fruit raw materials with metal ions.

Materials and Methods: The content of metals was determined by the atomic emission method (SHIMADZU ICPE–9820 spectrometer); heavy metal measurement methods used were MI.C3.7.2.01-017 and MI.C3.7.2.01-018. Statistical analysis of the obtained data was performed using the IBM SPSS Statistics version 29.0.0.0 program; correlation analysis was conducted using the Kendall Tau-b method.

Results: It was found that the highest complexing ability towards the mixture of Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+} ions was demonstrated by low-esterified sugar beet pectin (r=0,600; p<0.01). Citrus (r=0,312; p<0.01), quince (r=0,292; p<0.01), and apple (r=0,271; p<0.01) pectins also exhibited high complexing rates, while pumpkin (r=0,214; p<0.01) and carrot (r=0,156; p<0.01) pectins had slightly lower rates. In addition, pectin is found in smaller quantities in carrots and pumpkins than in sugar beet, citrus fruits, quince, and apples. To improve nutritional characteristics, reduce allergenicity, and maintain high complexing ability of the final product, the authors proposed a combination of raw materials from sugar beet and pumpkin, enriched with apple pectin and/or pectin-containing products and juices of pumpkin and apples.

Conclusions: It was established that the highest complexing ability in relation to a mixture of lead, cadmium, mercury, zinc, and copper ions is possessed by low-esterified sugar beet pectin. The addition of this pectin to apple pectin and/or pectin-containing apple products and juices increases the potential non-specific detoxification properties of the final food product.

KEY WORDS: pectins, complexation, heavy metals, food products

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INTRODUCTION

Pectin is an important polysaccharide used in the food, pharmaceutical, and several other industries. In the food industry, pectin is added to jams, jellies, frozen foods, and, more recently, to fat and sugar substitute formulations. In the pharmaceutical industry, it is used to lower blood cholesterol levels and treat gastrointestinal disorders. Other uses of pectin include its application in edible films, paper substitutes, plasticizers, and more. Although pectin is present in the cell walls of all plants, apple pomace and orange peel are the main sources of commercial pectin [1].

The functionality of the complex macromolecule pectin in products and ingredients derived from fruits and vegetables is largely determined by the presence of the polymer homogalacturonan, which undergoes various enzymatic and non-enzymatic transformation reactions during food processing, altering its structural and functional properties [2].

Natural polysaccharides are recommended for long-term chronic diseases, such as diabetes and heart disease [3, 4]. Studies on cell and animal models, as well as clinical trials on humans, have provided supporting data that modified citrus pectin improves the course of oncological diseases [5]. The important role of pectin as a probiotic has also been proven [6].

The relevance of preserving and improving the health of the population of Ukraine today is due to the negative consequences of the Chornobyl disaster, the COVID-19 pandemic, and the catastrophic impact on the health of the population from the large-scale war unleashed by the Russian Federation against Ukraine. Natural pectin-containing products are of particular scientific interest in terms of restoring the functions of the human body. The value of pectin-containing products lies, along with the above-mentioned qualities, in their detoxification and radioprotective properties, which are attributed to the

ability of pectin molecules to complex with both metal cations and cations of organic origin [7].

AIM

The purpose of this work is to study the complexing ability of pectins obtained from various vegetable and fruit raw materials to metal ions (Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+}) for further development of food formulations for special medical purposes.

MATERIALS AND METHODS

An analysis of domestic and foreign sources of scientific information on the topic of the study was carried out; methods of comparative analysis and a systematic approach were applied; experimental studies were conducted to assess the compatibility of components and select the optimal content of the ingredients in the recipe; statistical methods of information collection and processing were used.

The main recipe components of the final products were pectin-containing pumpkin puree, apple pectin extract, pumpkin and apple juices, low-esterified sugar beet pectin and its puree; the manufacturer of raw ingredients (semi-products and products) LLC "MANZANA-FOOD" LLC "MANZANA-FOOD" according to TU U 15.3-35422486-001:2009 "Juices, nectars, fruit, vegetable, vegetable-fruit juice drinks".

Technical conditions. The safety of the raw materials was investigated according to microbiological and sanitary-chemical safety indicators using instrumental methods of chemical analysis and for compliance with the State Sanitary Rules and Norms "Maximum permissible levels of individual pollutants in food products" [8], the requirements of GN 6.6.1.1-130-2006 "Permissible levels of radionuclides ^{137}cesium (^{137}Cs), ^{0}strontium (^{90}Sr) in food products and drinking water" [9] and the manufacturer's specifications. Also, in the process of work, when selecting raw pectin-containing components, the physicochemical quality indicators of the recipe components and the complexing ability of pectins from various vegetable and fruit raw materials to a mixture of Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+} ions were investigated.

The choice of pectin-containing raw materials was due to the fact that the initial products, in terms of their chemical composition, should have the least probable allergenicity, which is a very important factor when consuming final food products for special medical purposes. The safety of the raw materials was investigated according to sanitary-chemical and microbiological indicators. To determine the content of pesticide residues, the method of ultra-high-performance liquid chromatography with electron spray ionization mass spectrometry (UPLC-MC/MC, Waters, USA) and the method of gas chromatography according to DSTU EN 12393-1:2003, DSTU EN 12393-2:2003, DSTU EN 12393-3:2003 and GOST 30349-96 were used. The content of lead, cadmium, mercury, zinc was determined by the atomic emission method (device – atomic emission spectrometer "SHIMADSU ICPE-9820"); methods for measuring heavy metals – MI.C3.7.2.01-017 and MI.C3.7.2.01-018. To determine radionuclides, the spectrometer "CEG-001", "AKP-C" and spectrometer "CEB-01-70" were used (Methodology for measuring the specific activity of ^{137}Cs – MI 12-04-99. Methodology for measuring the specific activity of ^{90}Sr – MI 12-05-99).

Statistical analysis of the obtained data was carried out using the IBM SPSS Statistics version 29.0.0.0 program; correlation analysis was applied using the Kendall Tay-b method.

RESULTS

According to the results of the studies, it was found that the raw material components in terms of the content of heavy metals, radionuclides and pesticide residues meet the established medical safety criteria. The complexing ability of pectins from various vegetable and fruit raw materials to a mixture of Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} ions was (mg meth/g pectin / mmol meth/g pectin):

- from sugar beet pulp (Cu^{2+} – 148/2,32; Zn^{2+} – 85,0/1,30; Cd^{2+} – 154/1,17; Pb^{2+} – 147/0,71);
- citrus (Cu^{2+} – 127/2,00; Zn^{2+} – 82,4/1,26; Cd^{2+} – 115/1,02; Pb^{2+} – 126/0,61);
- quince (Cu^{2+} – 82,7/1,30; Zn^{2+} – 85,7/1,31; Cd^{2+} –130/1,16; Pb^{2+} – 143/0,69);
- apple (Cu^{2+} – 99,2/1,56; Zn^{2+} – 97,5/1,49; Cd^{2+} – 88,8/0,79; Pb^{2+} – 82,9/0,40);
- carrot (Cu^{2+} – 50,9/0,80; Zn^{2+} – 46,4/0,71; Cd^{2+} – 79,8/0,71; Pb^{2+} – 78,7/0,38);
- pumpkin (Cu^{2+} – 76,3/1,20; Zn^{2+} – 53,6/0,82; Cd^{2+} – 67,4/0,60; Pb^{2+} – 66,3/0,32).

The above results are shown in Fig. 1, Fig. 2, Fig. 3, Fig. 4.

According to the Kendall Tay-b rank correlation method (two-way correlation is significant at $p<0,01$), it was found that the lowest esterified sugar beet pectin has the highest complexing ability towards the mixture of Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} ions ($r=0,600$; $p<0,01$). Apple pectin has a lower complexing ability for Pb^{2+} than sugar beet, citrus and quince pectins ($r=0,560$; $p<0,01$).

Pumpkin ($r=0,314$; $p<0,01$) and carrot ($r=0,156$; $p<0.01$) pectins have a lower complexing ability towards heavy metals than the other pectins mentioned above.

DISCUSSION

It is known that pectin is contained in carrots and pumpkins in smaller quantities than in sugar beets, citrus fruits, quinces and apples. However, as raw materials for food formulations for special medical purposes, pectin-containing pumpkin products enriched with apple pectin and/or pectin-containing pumpkin and apple juices were chosen, since they have the least probability of causing an allergic reaction. The addition of apple pectin and/or pectin-containing apple products and juices was intended to increase the detoxification capacity of the pectin-containing food product in relation to heavy metal ions, which will further ensure the possibility of its use for special medical purposes.

The action of pectin begins from the moment it enters the stomach; the full complexing effect occurs in the colon and ends in the rectum. The most favorable conditions for

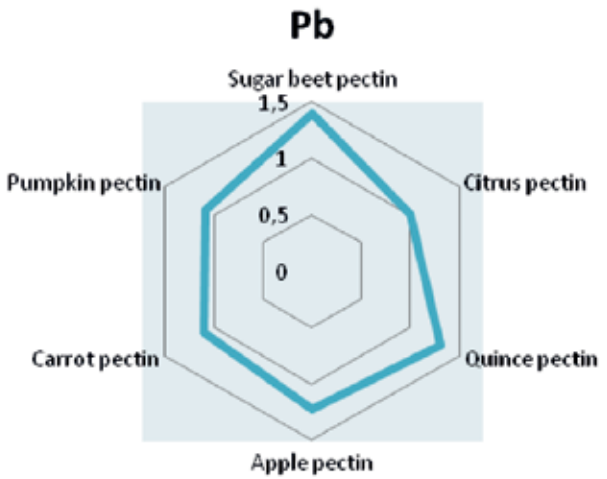

Fig. 1. Comparative characteristics of the complexing ability of pectins (mmol met/g pectin) from various vegetable and fruit raw materials to metal ions (Pb^{2+})
Source: compiled by the authors of this study

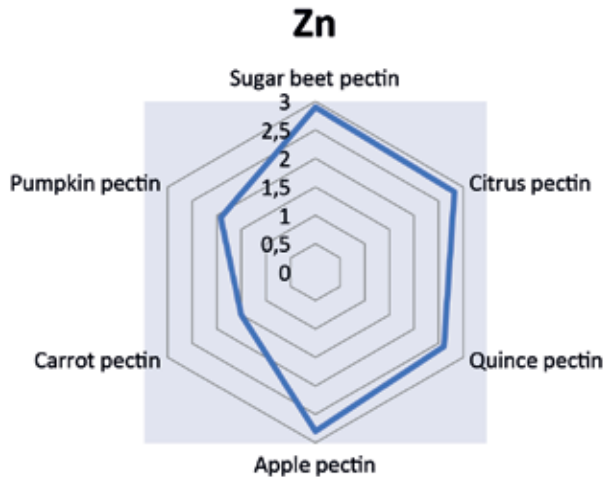

Fig. 3. Comparative characteristics of the complexing ability of pectins (mmol meth/g pectin) from various vegetable and fruit raw materials to zinc ions (Zn^{2+})
Source: compiled by the authors of this study

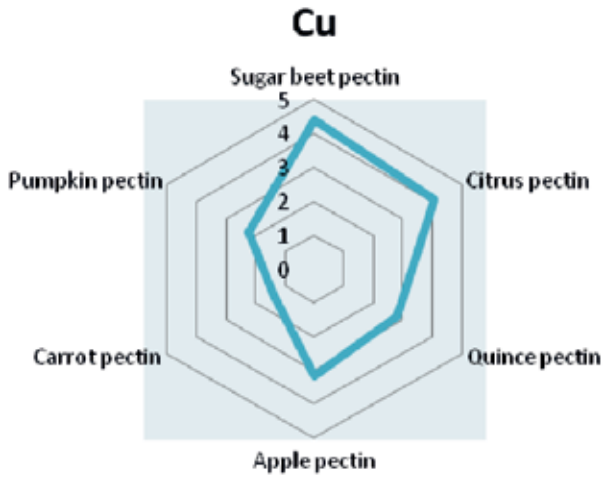

Fig. 2. Comparative characteristics of the complexing ability of pectins (mmol meth/g pectin) from various vegetable and fruit raw materials to copper ions (Cu^{2+})
Source: compiled by the authors of this study

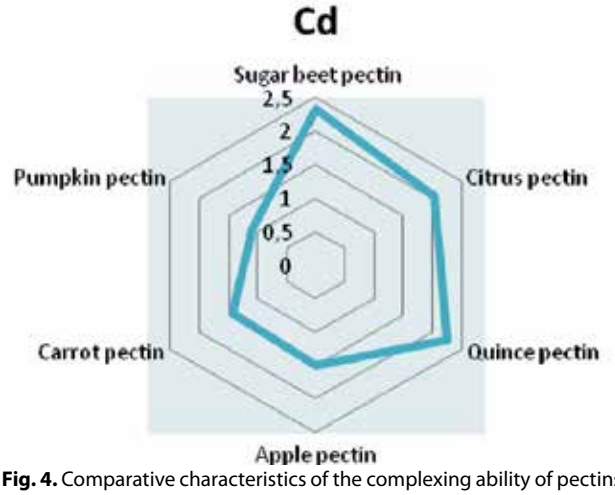

Fig. 4. Comparative characteristics of the complexing ability of pectins (mmol meth/g pectin) from various vegetable and fruit raw materials to metal ions (Cd^{2+})
Source: compiled by the authors of this study

the complexation of pectins with metals in the intestine are an alkaline environment. The acidity rate in the stomach is 1,5–2,0 (pH units). The acidity of the small intestinal juice is 7,2–7,5 (pH units); with increased secretion it increases to 8.6 (pH units). The acidity of the large intestinal juice is 8,5–9,0 (pH units). The effectiveness of apple pectin for complexation decreases under the influence of hydrochloric acid and gastric juice enzymes during food digestion, so we have developed a number of product formulations for special medical purposes with an additional content of low-esterified sugar beet pectin (and/or its puree), which is known to form complexes with metals in a wide pH range.

Also, when developing food formulations for special medical purposes that contained pectin-containing carrot raw materials, the need to maintain shelf life and increase the detoxification capacity of the final product was taken into account by adding low-esterified sugar beet and apple pectins (or their pectin-containing purees) under the condition of maintaining the pH of the product within 4,8–5,0 (pH units).

Based on the results of studies of the complexing ability of pectins from various vegetable and fruit raw materials to each of the Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} ions separately, which are shown in Figs. 1-4, it is possible to assess the feasibility of using a particular pectin-containing raw material (or the corresponding pectin) for the purpose of removing a certain metal from the body.

The term "food products for special medical purposes" is defined in paragraph 93 of part one of article 1 as amended by the Law of Ukraine "On Amendments to Certain Legislative Acts of Ukraine on Bringing the Legislation of Ukraine in the Field of Providing Baby Food in Accordance with the Requirements of the Legislation of the European Union" No. 1822-IX dated 10/21/2021). The creation of food products

for special medical purposes containing pectin-containing extracts, purees and pectins is due to the need to restore the functions of the human body after acute infectious and non-infectious diseases and to support the body weakened by long-term and chronic diseases.

The complex-forming properties of low-esterified pectins allow them to be considered as a valuable component of special nutrition aimed at non-specific detoxification of the body in a wide range of acute and chronic diseases accompanied by metabolic disorders and multi-organ failure in a wide range of infectious, non-infectious diseases and poisonings.

The production of food products for special medical purposes in Ukraine is most economically profitable due to the use of semi-finished products and products (from pumpkin, apples, sugar beet) as raw materials, in the creation of which there is almost no production waste [2, 10, 11]. Food products manufactured according to the proposed approaches, along with the content of pectin substances, also have a valuable composition of biologically active substances (organic acids, glucose, fructose, proteins), macro- and microelements. Such food products can be intended for partial feeding of patients with various needs that cannot be satisfied by modifying the usual diet [12]. The proposed pectin-containing products should be consumed as prescribed by a doctor in a healthcare facility and/or outside it.

In many countries of the world, the search for new natural components for the creation of special nutrition formulations continues. The complexing properties of low-esterified pectins allow us to consider them as a valuable component of special nutrition aimed at non-specific detoxification of the body in a wide range of diseases.

Today, natural macromolecules are used for the adsorption of pollutants, including heavy metal ions and organic dyes, due to their low cost, availability, biodegradability and environmental friendliness. Pectin, an important natural polymer, has a large number of carboxyl and hydroxyl functional groups that can interact with inorganic and organic cations [13].

The economic efficiency of the production of pectin-containing food products is due to the fact that a cheap source of pectin is beet pulp. During the processing of sugar beet in the sugar industry, a huge amount of beet pulp is formed as waste, which can be considered a valuable by-product and a source of cellulose, hemicellulose and pectin. The transformation of sugar beet pulp into value-added products is carried out using acid hydrolysis, hydrothermal methods and enzymatic hydrolysis. Modern methods of processing raw materials allow to obtain value-added products [14].

Scientific and technological progress has allowed to create hybrid materials of hydrogels and metal organic pectin frameworks with high adsorption capacity. For example, pectin hydrogel was obtained from by-products of tangerine peel, and calcium chloride was used as a cross-linker to combine with metal organic Fe-TAC frameworks to form a framework-pectin composite by in situ synthesis [15]. Another direction for increasing the antioxidant properties of sugar beet pectin is the integration of enzymatic modification and ultrafiltration for the production of pectin fractions with high antioxidant capacity as a green valorization of sugar beet pulp [16]. The positive effect of ultrasound treatment on the structure and antioxidant properties of sugar beet pectin has been proven [17].

Studies aimed at studying the structural characteristics and mechanisms of antioxidant activity of sugar beet pectin, in particular its subfraction rich in ferulic acid, have been conducted [18]. A separate important argument for the value of pectin, in addition to its complex-forming properties, is its high probiotic capacity [19, 20].

PERSPECTIVES FOR FURTHER RESEARCH

The large-scale war unleashed by the Russian Federation against Ukraine has created an extremely high level of chemical, biological, and radiological and nuclear threats due to the use of explosives, the destruction of critical infrastructure, and the terrorist seizure of the Zaporizhzhia NPP by Russian forces. All this confirms the need to develop effective means of protecting the health of military personnel and civilians from the effects of chemicals and ionizing radiation. A special role in this aspect belongs to food pectin-containing products with high complexing properties.

CONCLUSIONS

1. It was established that the lowest esterified sugar beet pectin has the highest complexing ability towards the mixture of Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} ions.
2. Adding apple pectin and/or pectin-containing apple products and juices to sugar beet pectin helps to reduce its allergenic properties and increases the nonspecific detoxification properties of the final product.
3. The complexing properties of low esterified pectins allow us to consider them as a valuable component of special nutrition aimed at nonspecific detoxification of the body in a wide range of infectious, non-infectious diseases and poisonings accompanied by metabolic disorders and multiorgan failure.

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In this study, the authors adhered to current Ukrainian regulatory documents. The study protocol was approved by the Commission on Ethics of Medical and Biological Research of the L.I. Medved's Research Center of Preventive Toxicology, Food and Chemical Safety of the Ministry of Health of Ukraine (State Enterprise). The studies were conducted within the framework of the research work "Scientific Substantiation of Measures for the Toxicological Safety of the Human Environment in the Health Care System" (№ 0123U102087). The study did not require additional funding.

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

The impact of colectomy on the quality of life in patients with chronic slow-transit constipation according to the pac-qol scale, depending on the histological subtype of the colonic wall

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ABSTRACT

Aim: To assess the quality of life using the PAC-QOL scale in patients with chronic slow-transit constipation depending on the histological subtypes of the colonic wall after colectomy.

Materials and Methods: The dynamics of quality-of-life scores were studied in 107 patients with slow-transit constipation resistant to conservative treatment after colectomy using PAC-QOL questionnaire.

Results: Significant improvement in quality of life was observed for all PAC-QOL subscales one year after surgery: physical discomfort improved by 58.7% to 67.7%, psychological discomfort – by 50.5%-67.2%, worries and concerns – by 53.3%- 67.6%, satisfaction with treatment – by 80.7%-82.6%, the PAC-QOL score – by 54-67.9%. The best outcomes for the PAC-QOL scale were demonstrated in patients with cajal subtype – 67,9% compared to the neuropathic (54,0%), myopathic inflammatory (56,1%), histologically intact (61,2%). Cajal histological subtype showed a greater improvement in the physical discomfort score – 67,7%, compared to the histologically intact subtype (58,7%), and the neuropathic subtype (58,9%); for Psychosocial discomfort – 67,2% compared to the dystrophic myopathic (55,5%), the neuropathic (50,7%), and the myopathic inflammatory subtype (50,5%); for the worries and concerns – 67,6% compared to the neuropathic subtype (53,3%).

Conclusions: In patients with STC resistant to conservative treatment, colectomy leads to significant improvement in quality of life according to the Pac-Qol questionnaire, one year after surgery with the best improvement for the Cajal subtype.

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

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INTRODUCTION

Chronic constipation is one of the most common diseases in clinical practice. The prevalence of constipation increases with age. [1, 2] The prevalence of constipation in a population aged 60 years or older was 32.6% in Chinese study; it increased with age, being 44.8% in those aged 80 years and older [3]. Constipation manifests as a variety of symptoms, such as infrequent bowel movements, hard stools, feeling of incomplete evacuation, straining, a sense of anorectal blockage during defecation, and use of digital maneuvers to assist defecation [4]. These symptoms persist chronically, lowering their quality of life (QoL), resulting in social and economic burdens [5]. Chronic constipation negatively affects various aspects of patients' quality of life, including both mental and physical health [4]. The impact of different treatment methods on quality of life in patients with chronic constipation, including surgery, is insufficiently observed in modern literature. The clinical and social impacts of functional constipation neccessitate an accurate diagnosis and effective treatment of CSTC [4]. Standardized patient evaluation allows more accurate assessment of patients and selection of the most appropriate treatment. Several tools exist for the specific evaluation of constipation, one of which is the "Patient Assessment of Constipation Quality of Life" (PAC-QOL) questionnaire for patients with CSTC, but the last was rarely observed in literature [6]. Typically, the SF-36 scale [7, 8] was used to assess QoL in patients with CSTC, although it is not disease-specific. However, there are currently no studies assessing the impact of surgical treatment on the QoL in CSTC patients using the PAC-QOL scale.

AIM

The aim was to assess the quality of life using the PAC-QOL scale in patients with chronic slow-transit constipation depending on the histological subtypes of the colonic wall after colectomy.

MATERIALS AND METHODS

107 patients with chronic slow-transit constipation (CSTC) were studied and operated at the O.O. Bogomolets National Medical University clinics between 2011 and 2023.

CSTC in patients was diagnosed using the Rome IV criteria [9].

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. Women were predominant: 102 (95.3%) The average age was 43.1±13.6 years; the body mass index was 22.9±4.5 kg/m^2.

QUALITY OF LIFE ASSESSMENT

The quality of life was evaluated using the disease-specific PAC-QOL questionnaire, developed and validated by Marquis et al [10] 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 [10]. QoL was assessed before surgery and one year after the surgery.

The patients were divided into four groups based on the histological type of the colonic wall. The frequency of different histological variants is presented in Table 1.

The most common histological variant was the myopathic type, identified in 59 patients (55.1%), with the dystrophic myopathic subtype being the most prevalent, found in 47 patients (43.9%). Inflamative myopathic subtype was found in 12 (11,2%) patients. The neuropathic type of constipation was registered in 20 (18,7%) patients and constipation associated with Cajal cell pathology was observed in 21 patients (19.6%).

In 7 patients (6.5%), no histological or immunohistochemical changes were detected and this group was classified as the "histologically intact type."

The age of patients with the Cajal type was significantly lower than that of those with the myopathic ($p = 0.006$) and neuropathic ($p < 0.001$) types. Patients with the neuropathic type were significantly older compared to those with the myopathic type ($p = 0.003$) [11].

QUALITY OF LIFE WAS ASSESSED BEFORE AND ONE YEAR AFTER SURGERY

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.

The duration of anamnesis before surgery ranged 1-50 years, with an average of 21.0±14.0 years.

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

Quality of life was assessed using the PAC-QOL questionnaire in 107 patients after surgical treatment.

One year after surgery, a significant improvement in all PAC-QOL scale indicators was observed compared to preoperative values (Table 2).

The highest percentage of improvement was observed in satisfaction scale (81,18 ± 7,94 %), the lowest – in psychological (56,65±16,03 %), table 3.

Table 1. Frequency of different histological variants in patients with chronic slow-transit constipation

The main histological variant in patients with chronic slow-transit constipation		Number of patients	%	Mean age	Age mediana
Myopathic	Inflammative	12	11,2	36,9±8,5	34,5
	Dystrophic	47	43,9	43,7±12,8	44
	Total	59	55,1	43,1±12,7	43
Cajal cells pathology		21	19,6	33,9±10,3	35
Neuropathic		20	18,7	52,5±13,5	54
Histologically intact		7	6,5	49,214,8	43,5

Source: compiled by the authors of this study

Table 2. Average scores of PAC-QOL scales before and after surgical treatment in patients with CSTC. Minimum and maximum scores for PAC-QOL scales before and after surgical treatment in patients with CSTC

PAC-QOL scale		M	SD	Min	Max	m	P
Physical	Before	2,78	0,52	1,50	3,75	0,05	p<0.005
	After	1,01	0,32	0,50	2,25	0,03	
Psychological	Before	1,90	0,48	0,88	2,88	0,05	p<0.005
	After	0,83	0,41	0,25	2,25	0,04	
Worries and concernes	Before	1,99	0,31	0,73	2,36	0,03	p<0.005
	After	0,72	0,34	0,27	2,27	0,03	
Satisfaction	Before	2,35	0,60	0,60	3,40	0,06	p<0.005
	After	0,84	0,47	0,14	3,22	0,05	
PAC-QOL	Before	2,14	0,23	1,57	2,71	0,02	p<0.005
	After	0,82	0,35	0,46	2,54	0,03	

Source: compiled by the authors of this study

Table 3. Percentage of improvement in individual PAC-QOL scales after surgical treatment in patients with CSTC

PAC-QOL scale	M	SD	Min	Max	m
Physical	63,14	11,23	30,00	81,82	1,09
Psychological	56,65	16,03	0,00	78,57	1,55
Worries and concerns	64,00	15,75	0,00	82,35	1,52
Satisfaction	81,18	7,94	33,33	88,24	0,77
PAC-QOL	61,51	14,96	0,00	77,19	1,45

Source: compiled by the authors of this study

In our study there was an improvement of the average quality of life scores in all PAC-QOL scales one year after surgery.

For the Physical discomfort scale, the average score for the histologically intact subtype before surgery was 2.69±0.53, ranging from 1.75 to 3.5, and after surgery, it was 1.08±0.4, ranging from 0.5 to 1.75 (p<0.005). For the dystrophic myopathic subtype, the average score before surgery was 2.67±0.46, ranging from 1.5 to 3.75, and after surgery, it was 0.95±0.27, ranging from 0.75 to 2 (p<0.005). For the inflammatory myopathic subtype, the average score before surgery was 2.58±0.49, ranging from 1.75 to 3.25, and after surgery, it was 0.96±0.28, ranging from 0.75 to 1.75 (p<0.005). For the Cajal subtype, the average score before surgery was 3.24±0.44, ranging from 2 to 3.75, and after surgery, it was 1.05±0.26, ranging from 0.5 to 1.5 (p<0.005). For the neuropathic subtype, the average score before surgery was 2.74±0.53, ranging from 2 to 3.75, and after surgery, it was 1.1±0.45, ranging from 0.5 to 2.25 (p<0.005), (Fig. 1).

For the Psychosocial discomfort scale, the average score for the histologically intact subtype before surgery was 1.88±0.65, ranging from 0.88 to 2.75, and after surgery, it was 0.72±0.44, ranging from 0.38 to 1.75 (p<0.005). For the dystrophic myopathic subtype, the average score before surgery was 1.91±0.47, ranging from 1.13 to 2.88, and after surgery, it was 0.85±0.35, ranging from 0.5 to 2.25 (p<0.005). For the inflammatory myopathic subtype, the average score before surgery was 1.91±0.5, ranging from 1.13 to 2.75, and after surgery, it was 0.96±0.5, ranging from 0.38 to 2.13 (p<0.005). For the Cajal subtype, the average score before surgery was 1.84±0.58, ranging from 1.13 to 2.88, and after surgery, it was 0.61±0.22, ranging from 0.25 to 1.0 (p<0.005). For the neuropathic subtype, the average score before surgery was 1.94±0.31, ranging from 1.25 to 2.38, and after surgery, it was 0.98±0.51, ranging from 0.5 to 2.0 (p<0.005), (Fig. 2).

For the Worries and concerns scale, the average score for the histologically intact subtype before surgery was 1.92±0.25, ranging from 1.55 to 2.27, and after surgery, it was 0.72±0.23, ranging from 0.45 to 1.18 (p<0.005). For the dystrophic myopathic subtype, the average score before surgery was 1.98±0.33, ranging from 0.73 to 2.36, and after surgery, it was 0.05±0.65, ranging from 0.29 to 0.27 (p<0.005). For the inflammatory myopathic subtype, the average score before surgery was 1.94±0.39, ranging from 1.09 to 2.27, and after surgery, it was 0.11±0.65, ranging from 0.3 to 0.45 (p<0.005). For the Cajal subtype, the average score before surgery was 2.05±0.24, ranging from 1.45 to 2.27, and after surgery, it was 0.67±0.14, ranging from 0.36 to 0.91 (p<0.005). For the neuropathic subtype, the average score before surgery was 2.04±0.27, ranging from 1.27 to 2.27, and after surgery, it was 0.96±0.5, ranging from 0.45 to 1.91 (p<0.005), (Fig. 3).

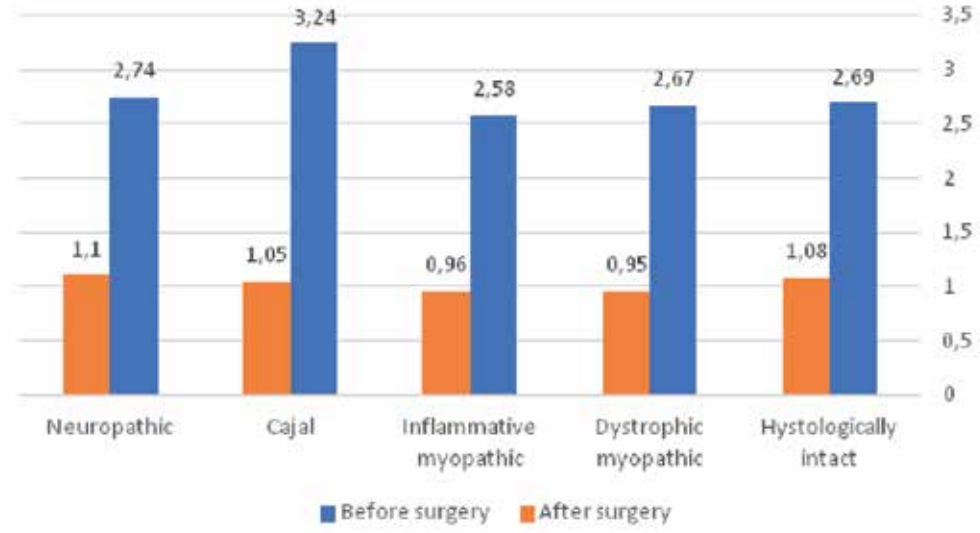

Fig. 1. Distribution of the average score on the Physical discomfort subscale in patients with different histological subtypes before and after surgery
Source: compiled by the authors of this study

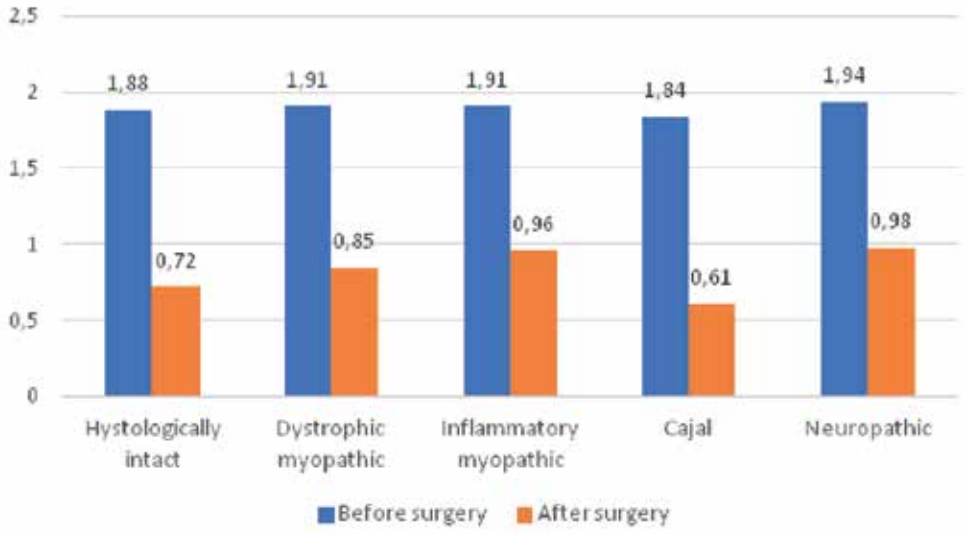

Fig. 2. Distribution of the average score for the Psychological discomfort subscale in patients with different histological subtypes before and after surgery
Source: compiled by the authors of this study

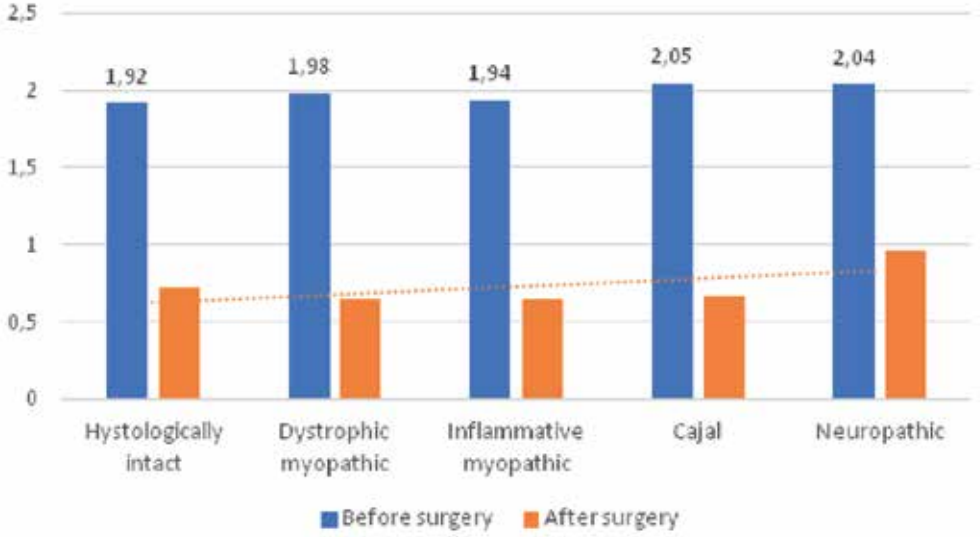

Fig. 3. Distribution of the average score for the Worries and concerns subscale in patients with different histological subtypes before and after surgery
Source: compiled by the authors of this study

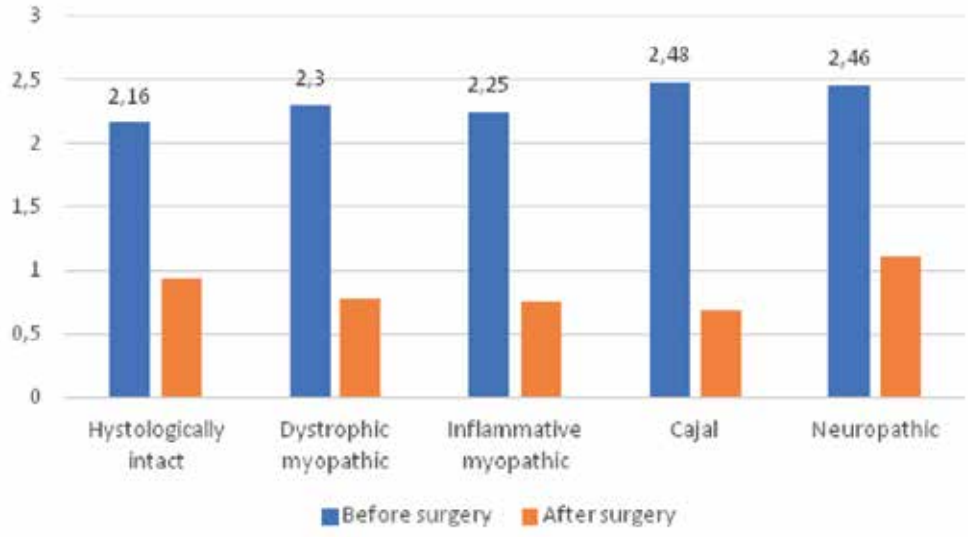

Fig. 4. Distribution of the average score for the Satisfaction with treatment subscale in patients with different histological subtypes before and after surgery
Source: compiled by the authors of this study

For the Satisfaction and treatment scale, the average score for the histologically intact subtype before surgery was 2.16±0.42, ranging from 1.6 to 2.8, and after surgery, it was 0.94±0.4, ranging from 0.5 to 1.8 ($p<0.005$). For the dystrophic myopathic subtype, the average score before surgery was 2.3±0.6, ranging from 0.8 to 3.2, and after surgery, it was 0.78±0.37, ranging from 0.28 to 2.8 ($p<0.005$).

For the inflammatory myopathic subtype, the average score before surgery was 2.25±0.55, ranging from 1 to 3, and after surgery, it was 0.75±0.56, ranging from 0.36 to 2.4 ($p<0.005$). For the Cajal subtype, the average score before surgery was 2.48±0.59, ranging from 1 to 3.4, and after surgery, it was 0.69±0.26, ranging from 0.2 to 1.35 ($p<0.005$). For the neuropathic subtype, the average score before surgery was 2.46±0.71, ranging from 0.6 to 3.4, and after surgery, it was 1.11±0.67, ranging from 0.14 to 3.22 ($p<0.005$), (Fig. 4).

For the PAC-QOL scale, the average score for the histologically intact subtype before surgery was 2.06±0.23, ranging from 1.68 to 2.5, and after surgery, it was 0.79±0.22, ranging from 0.47 to 1.17 ($p<0.005$). For the dystrophic myopathic subtype, the average score before surgery was 2.12±0.22, ranging from 1.57 to 2.5, and after surgery, it was 0.77±0.25, ranging from 0.52 to 2.2 ($p<0.005$). For the inflammatory myopathic subtype, the average score before surgery was 2.08±0.19, ranging from 1.71 to 2.54, and after surgery, it was 0.92±0.61, ranging from 0.47 to 2.54 ($p<0.005$). For the Cajal subtype, the average score before surgery was 2.24±0.25, ranging from 1.86 to 2.71, and after surgery, it was 0.71±0.12, ranging from 0.46 to 0.98 ($p<0.005$). For the neuropathic subtype, the average score before surgery was 2.18±0.23, ranging from 1.64 to 2.64, and after surgery, it was 1.01±0.47, ranging from 0.55 to 2.04 ($p<0.005$), (Fig. 5).

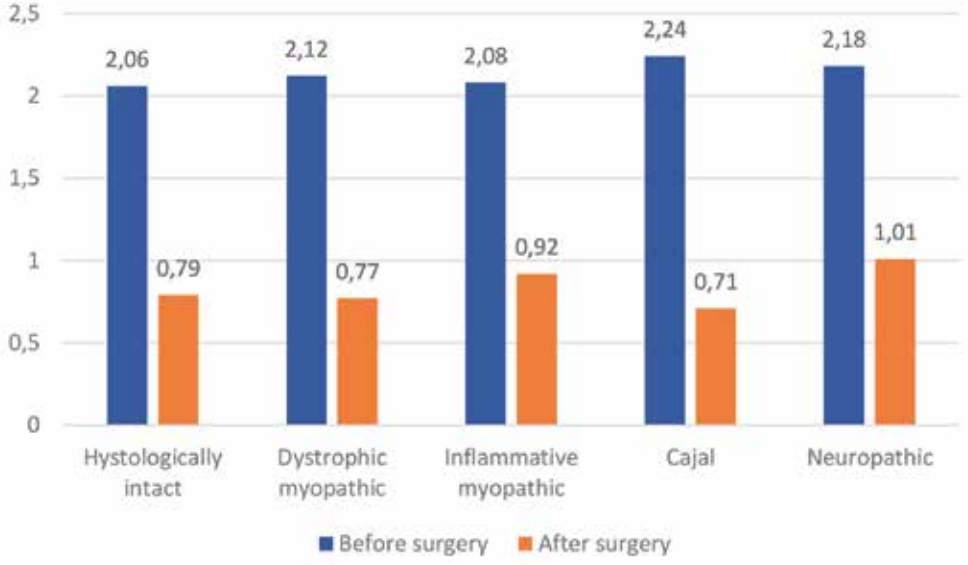

Fig. 5. Distribution of the average score for PAC-QOL scale in patients with different histological subtypes before and after surgery
Source: compiled by the authors of this study

According to the Physical discomfort scale, an improvement was observed across all histological subtypes of CSTC, ranging from 58.7% to 67.7% (Fig. 6). At the same time, the Cajal histological subtype showed a greater percentage improvement in the physical component score compared to the histologically intact subtype (p=0.04) and the neuropathic subtype (p=0.026). Other pair comparisons of quality of life improvement percentages in the physical component between the remaining histological subtypes did not reveal any statistically significant differences (p>0.05), Table 5.

For the Psychosocial discomfort component of the PAC-QOL scale, an improvement was observed across all histological subtypes of CSTC, ranging from 50.5% to 67.2% (Fig. 7). At the same time, the cajal histological subtype demonstrated a significantly higher percentage improvement in the Psychosocial discomfort component compared to the dystrophic myopathic subtype (p = 0.001), the neuropathic subtype (p = 0.001), and the myopathic inflammatory subtype (p = 0.003). Other pair comparisons of the percentage improvement in quality of life for this component among different histological subtypes did not reveal significant differences (p > 0.05), see Table 6.

For the Worries and concerns scale, improvement was observed in all histological subtypes of CST from 53.3% to 67.6%, as shown in Fig. 8. Meanwhile, the cajal histological subtype demonstrated a greater percentage improvement

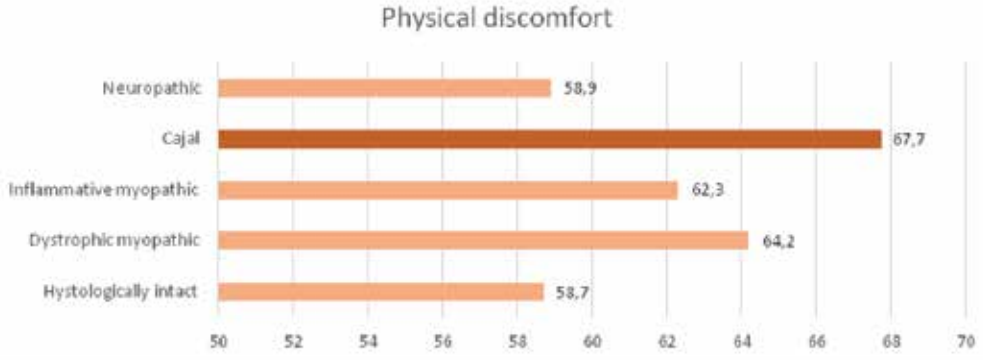

Fig. 6. Percentage of improvement in the Physical discomfort subscale among patients with different histological subtypes after surgery
Source: compiled by the authors of this study

Table 5. Statistical significance (P-values) of differences in the percentage of improvement in quality of life for the Physical discomfort subscale, depending on histological subtypes

	Hystologically intact	Dystrophic myopathic	Inflammative myopathic	Cajal	Neuropathic
Hystologically intact	9	0,158	0,537	0,04	0,975
Dystrophic myopathic	0,158	47	0,532	0,074	0,087
Inflammative myopathic	0,537	0,532	12	0,067	0,503
Cajal	0,04	0,074	0,067	19	0,026
Neuropathic	0,975	0,087	0,503	0,026	20

Source: compiled by the authors of this study

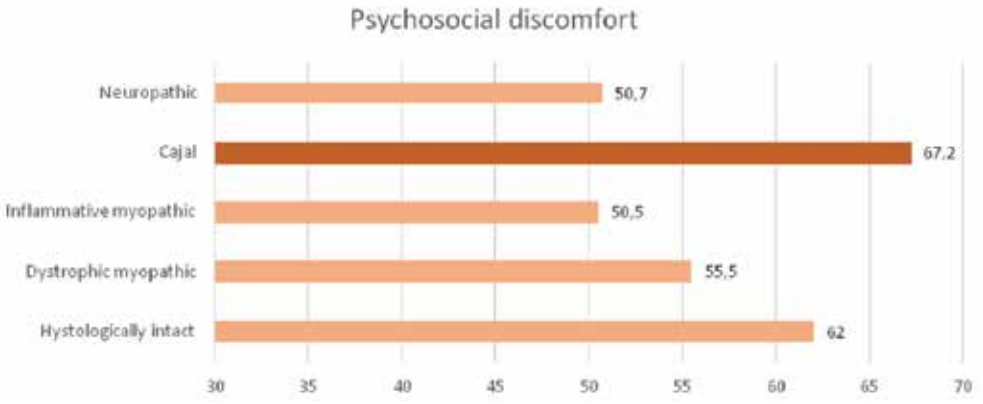

Fig. 7. Percentage of improvement for the *Psychosocial discomfort* subscale in patients with different histological subtypes after surgery
Source: compiled by the authors of this study

Table 6. Significance of differences (*P*) in the percentage of quality of life improvement for the *Psychosocial discomfort* subscale depending on histological subtypes

	Hystologically intct	Dystrophic myopathic	Inflammative myopathic	Cajal	Neuropathic
Hystologically intact	9	0,191	0,183	0,148	0,143
Dystrophic myopathic	0,191	47	0,323	0,001	0,263
Inflammative myopathic	0,183	0,323	12	0,003	0,978
Cajal	0,148	0,001	0,003	19	0,001
Neuropathic	0,143	0,263	0,978	0,001	20

Source: compiled by the authors of this study

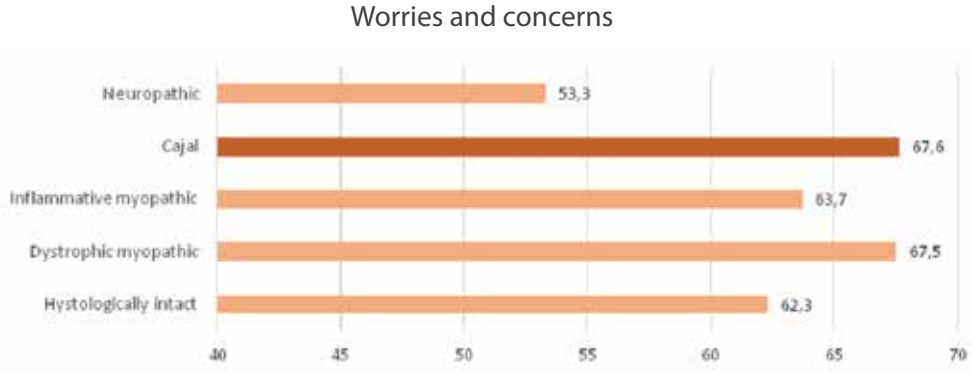

Fig. 8. Percentage of improvement for the *Worries and concerns* subscale in patients with different histological subtypes after surgery
Source: compiled by the authors of this study

Table 7. Significance of differences (*P*) in the percentage of quality of life improvement for the *Worries and concerns* subscale depending on histological subtypes

	Hystologically intact	Dystrophic myopathic	Inflammative myopathic	Cajal	Neuropathic
Hystologically intact	9	0,243	0,875	0,123	0,249
Dystrophic myopathic	0,243	47	0,437	0,966	0,001
Inflammative myopathic	0,875	0,437	12	0,491	0,21
Cajal	0,123	0,966	0,491	19	0,008
Neuropathic	0,249	0,001	0,21	0,008	20

Source: compiled by the authors of this study

in the score compared to the neuropathic subtype (p=0.008), and the myopathic dystrophic subtype also showed a greater percentage improvement compared to the neuropathic subtype (p=0.001). Other pair comparisons in the percentage of improvement for this component between the other histological subtypes did not show significant differences (p > 0.05), as presented in Table 7.

For the Satisfaction with treatment scale, improvement was observed in all histological subtypes of chronic slow transit constipation (CST) from 80.7% to 82.6%, as shown in Fig. 9. Pair comparisons of the percentage improvement in quality of life for this scale between the histological subtypes did not reveal significant differences (p > 0.05), as presented in Table 8.

For the PAC-QOL scale, an improvement in scores was observed in all histological subtypes of CSTC ranging from 54% to 67.9%, as shown in Fig. 10. At the same time, the cajal histological subtype showed a higher percentage of score improvement compared to the neuropathic (p=0.04), myopathic inflammatory (p=0.037), histologically intact (p=0.046) subtypes. The myopathic dystrophic subtype also showed a higher percentage of score improvement compared to the neuropathic (p=0.009). Other pair comparisons of improvement percentages in quality of life for this component between the other histological subtypes showed no statistically significant differences (p > 0.05), as shown in Table 9.

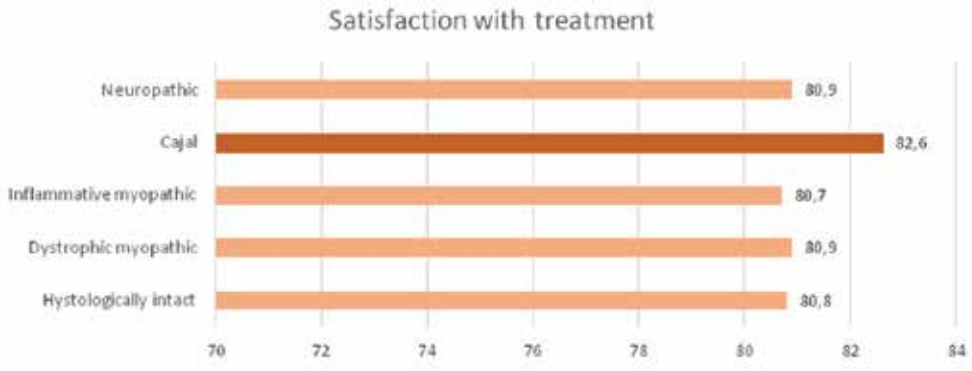

Fig. 9. Percentage of improvement in the Satisfaction with treatment subscale for patients with different histological subtypes after surgery
Source: compiled by the authors of this study

Table 8. Statistical significance (P) of differences in the percentage of improvement in quality of life for the Satisfaction with treatment subscale of PAC-QOL depending on histological subtypes

	Hystologically intact	Dystrophic myopathic	Inflammative myopathic	Cajal	Neuropathic
Hystologically intact	9	0,97	0,979	0,438	0,988
Dystrophic myopathic	0,97	47	0,945	0,377	0,99
Inflammative myopathic	0,979	0,945	12	0,452	0,973
Cajal	0,438	0,377	0,452	19	0,585
Neuropathic	0,988	0,99	0,973	0,585	20

Source: compiled by the authors of this study

Table 9. Statistical significance (P) of differences for the percentage improvement in quality of life based on the PAC-QOL scale indicator depending on histological subtypes

	Hystologically intact	Dystrophic myopathic	Inflammative myopathic	Cajal	Neuropathic
Hystologically intact	9	0,53	0,61	0,046	0,303
Dystrophic myopathic	0,53	47	0,132	0,091	0,009
Inflammative myopathic	0,61	0,132	12	0,037	0,798
Cajal	0,046	0,091	0,037	19	0,004
Neuropathic	0,303	0,009	0,798	0,004	20

Source: compiled by the authors of this study

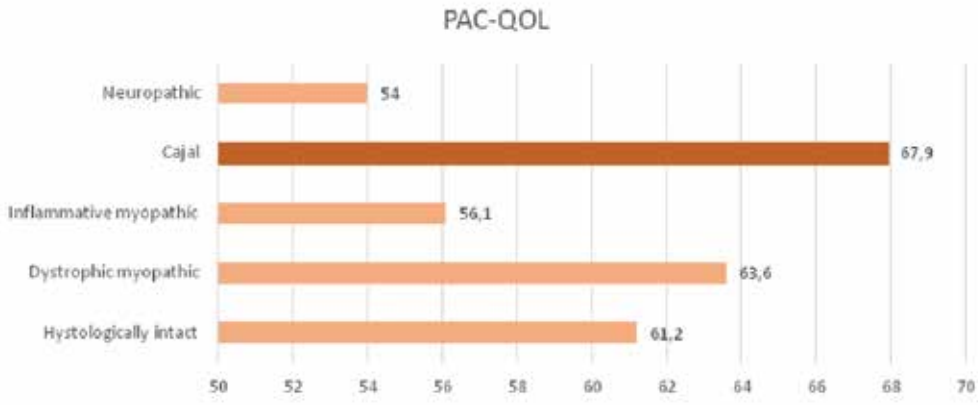

Fig. 10. Percentage improvement in the PAC-QOL scale for patients with different histological subtypes after surgery
Source: compiled by the authors of this study

DISCUSSION

Chronic constipation affects 10–15% of the population [11,12], with a higher prevalence in women than in men [1]. Slow-transit constipation accounts for 15–30% of chronic constipation [13] and is associated with reduced quality of life, anxiety, and depression [14, 15]. The diagnosis of CSTC requires functional studies of intestinal motility, histological analysis, and clinical evaluation [12, 16-18].

Conservative therapy is not always effective, and the proportion of patients with constipation refractory to conservative treatment who require surgical intrusion remains high [19]. The choice of colectomy technique is critically important, as surgical outcomes do not always meet expectations [7, 19-26]. The choice of surgery should be guided by histological changes in the intestinal wall, as it has been suggested by some researchers [27]. However, this topic remains underexplored. Moreover, modern preoperative diagnostic methods for slow-transit constipation do not provide information about histological changes in the colonic wall, complicating the development of a personalized treatment strategy.

This study analyzed the impact of colonic histological patterns on the quality of life of patients with slow-transit constipation who had failed conservative treatment, leading to the decision to perform colectomy.

Histological examination of the colonic wall allowed differentiation into five previously described [11] histological patterns of colonic wall changes: Cajal type – 19.6%, neuropathic – 18.7%, myopathic dystrophic – 43.9%, myopathic inflammatory – 11.2%, and histologically intact – 6.5%.

One year after surgery, the mean percentage improvement in quality of life (QoL) scores, assessed using the Pac-Qol questionnaire, significantly increased compared to preoperative values. The positive impact of colectomy on QoL in patients with chronic slow-transit constipation (STC) has also been reported in other studies [7, 8, 28].

There was notable variability in QoL subscale scores depending on the histological subtype of STC. Patients with the Cajal subtype demonstrated the best outcomes for the psychological subscale compared to those with myopathic dystrophic, myopathic inflammatory, and neuropathic subtypes. This finding is unexpected, as the Cajal subtype, associated with a deficiency of interstitial cells of Cajal (pacemakers regulating intestinal peristalsis), is typically characterized by the most severe form of STC. These patients often experience prolonged intervals between bowel movements (ranging from two to three weeks) and poor response to conservative treatment [29]. Taken these factors into considerations, it was expected that colectomy might not fully resolve the problem in this group of patients.

One year after surgery, constipation was resolved in this group of patients, as well as in others. Patients with the Cajal subtype demonstrated better results on the psychological subscale. The last can be explained by the more pronounced contrast in their level of social adaptation after surgery compared to other constipation subtypes. Before surgery, these patients experienced significant limitations in daily life due to severe symptoms, which greatly affected their quality of life and social interactions. As a result, they may have experienced greater psychological relief and enhanced social functioning after surgery. Similarly, patients with the Cajal histological subtype showed greater mean improvements in quality of life (QoL) scores on the Physical Discomfort subscale compared to those with histologically intact and neuropathic subtypes, for the Worries and Concerns subscale compared to the neuropathic subtype, and in the overall PAC-QOL score compared to the neuropathic, myopathic inflammatory, and histologically intact subtypes.

Relatively less positive changes for the PAC-QOL scale scores were observed in patients with the neuropathic and myopathic inflammatory histological subtypes, with improvements of 54.0% and 56.1%, respectively. This was primarily due to a less improvement for worries and concerns, psychological discomfort, and physical discomfort subscales compared to other histological subtypes.

In our study, unlike the findings of Gonçalves [30], the neuropathic subtype of constipation was registered in the oldest patients with a longer anamnesis. This may have led to a certain degree of adaptation to constipation symptoms, which resulted in a less noticeable surgical effect by patient.

Regarding the myopathic inflammatory subtype of constipation, the presence of concomitant inflammatory processes in the intestine should be considered. Even after surgical intervention, these patients may experience residual symptoms such as periodic bloating and colic. These manifestations could be linked to food intolerance or poor nutrient absorption, necessitating dietary adjustments and supportive pharmacological therapy.

This study has certain limitations, including a small patient sample and a single-center design. Further prospective studies are required to confirm the effectiveness of preoperative histological assessment. The histological characteristics of the colonic wall may influence better surgical outcomes and increase patients' quality of life.

CONCLUSIONS

Chronic slow-transit constipation (STC) significantly reduces patients' quality of life. In patients with STC refractory to conservative treatment, colectomy leads to a statistically significant improvement in quality of life indicators, as assessed by the Pac-Qol questionnaire, one year after surgery.

The histological subtype of STC influences surgical outcomes and quality of life improvements**.** The best results in psychological well-being and physical comfort were observed in patients with the Cajal subtype of constipation, due to a significant contrast in social adaptation after surgery. In contrast, patients with neuropathic and myopathic inflammatory subtypes had less benefits from surgical treatment, potentially due to symptom habituation or the presence of concomitant inflammatory processes in the intestine, which required additional therapy.

Histological analysis of colonic biopsies may serve as a essential criterion for selecting patients for colectomy. The identification of significant alterations in Cajal cells may indicate the appropriateness of total colectomy.

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CONFLICT OF INTEREST
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ORIGINAL ARTICLE

Genotypes of *Candida albicans* and its cooperative interaction with *Streptococci* isolated from throat infections

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ABSTRACT

Aim: This study was aimed to detect and determine genotypes of *Candida albicans* and its cooperative interaction with streptococci isolated from throat infections.
Materials and Methods: This survey was carried out during November 2023 and March 2024 to collect a total of 80 throat swab samples from patients in in Al-Sadr Medical City in Najaf during. *Candida* was isolated from culturing throat swab samples on Sabouraud agar, and Blood agar. Each isolate *(Candida & Streptococci)* enhanced in monoculture using enrichment media; Potato Dextrose broth. Molecular assay included detecting three of biofilm forming genes; *Als1, Als2, Als3*.
Results: Twelve out of fifteen *Candida* isolates showed increase in number after mixing with *Streptococci* and incubation. In contrast, three isolates showed no change or decrease after mixing in co-culture media. Five *Candida* isolates (out of 15 isolates) were positive in gel electrophoresis to three biofilm genes classified as first genotype (CALSG1). Four *Candida* isolates were negative in gel electrophoresis to three biofilm genes, classified as second genotype (CALSG2). Other *Candida* isolates were positive to one or two of three biofilm genes, classified with genotypes (CALSG3, CALSG4, CALSG5 and CALSG6).
Conclusions: *Candida albicans* has biofilm formation genes *(Als)*, which attract other organisms, like *Streptococci* resulting in synergistic interaction. Despite the presence of some *Als* genes, it's not necessary to found strong biofilm results as *AIs* genes may not be translated to form biofilm.

KEY WORDS: Streptococcus species, *Candida albicans, Streptococci*

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INTRODUCTION

Streptococcus species, particularly Group A *Streptococcus* (GAS), are significant pathogens in throat infections, leading to conditions such as pharyngitis and potentially serious sequelae like rheumatic fever and rheumatic heart disease. The prevalence of GAS positive pharyngitis has been documented at about 24.1% in clinical settings, stressing the need for diagnosis and management to be accurate and effective [1-2]. Pharyngitis is a common condition experienced by children and adolescents, often manifested as a sore throat with or without fever and lymphadenopathy. Among the recent ones, clinical pretense of GAS over other pathogens such as *Fusobacterium* may complicate the diagnosis in real-time clinical settings [3]. Moreover, GAS infections are of extensive epidemiological concern in different populations that identify significant implications regarding the dynamics of transmission and risk factors for infection [4]. *Candida albicans* is the species most frequently found in cases of oral candidiasis, with the possibility of it progressing to throat infection, especially among people with weakened immune systems [5]. Symptoms of *Candida* infection in the throat usually are the appearance of white patches in the mouth, tongue, and throats accompanied by itching or burning sensation in other area site like genitalia [6]. Pathogenicity for *Candida albicans* is coordinated by numerous virulence factors, including biofilm formation and the ability to switch between the yeast and filamentous forms. In vivo and in vitro, the compound phloretin, previously investigated for its antifungal properties, has been shown to inhibit these virulence factors. Thus, the potential therapeutic treatment options managing available for managing infections caused by *Candida* species throat infection can interfere with these virulence factors [7]. The balance between *Candida* and the host immune response is really important for an understanding of the dynamics of infection. It underlines that aberrant type 1 immunity is propagative of susceptibility to fungal infections at mucosal sites, which in its turn indicates the question of immune modulation in their prevention and treatment [8]. One of the features that make *Candida* species pathogenic is their ability to form biofilms, making them resistant to external factors of protection against these pathogens, such as antifungal drugs and host immune system defenses [9]. In *Candida* species' genome, there are eight genes that encode large cell-surface glycoproteins and belong to the Als family. This family consists of eight members (Als1 to Als7 and Als9), which show a certain degree of similarity in function and structure [10]. There are several infectious diseases

caused by *Streptococci* strains, such as throat infection [11]. In other words, by forming biofilms with *Candida*, it creates a more supportive environment for bacterial infections to develop.

AIM

Therefore, this study was aimed to detect and determine genotypes of *Candida albicans* and its cooperative interaction with *Streptococci* isolated from throat infections.

MATERIALS AND METHODS

SAMPLING AND IDENTIFICATION

Throat swab samples were collected from patients in Al-Sadr Medical City in Najaf during November 2023 and March 2024. All throat swab samples (120 samples) were cultured on Blood agar, MacConkey, and Sabouraud agar. The isolates of *Candida* and *Streptococci* were purified and stored for in vitro investigation. *Candida* isolates were identified based on colony staining on chromogenic agar as well as germ tube test for *Candida albicans* [12]. Biochemical tests and API system were used to identify bacterial isolates.

CO-CULTIVATION METHOD

Candida and *Streptococci* were subcultured as mono-fermentation and co-fermentation in Potato Dextrose Broth (PDB) medium and incubated in 37°C with shaking for 24 hrs. Serial dilthroat infections of the culture should be made on MacConkey agar (for *Streptococci*) and Sabouraud agar (for *Candida*). *Candida* and *Streptococci* were separated from mix growth by adding Ampicillin and amphotericin. Ampicillin was added in Sabouraud agar petridishes to inhibit *Streptococci* and allow the growth of *Candida*, and amphotericin was added in MacConkey agar to quantitate *Streptococci*. Finally, colony forming unit (CFU) was calculated by colony counter.

ESTIMATION OF *CANDIDA* BIOFILM BY CONGO RED AGAR

Brain Heart Infusion Agar (BHIA) with 0.08% (w/v) Congo red supplemented with 5% (w/v) sucrose was prepared [13]. The medium boiled on stirring hot plate. Then the medium transported to the autoclave at 121°C for 15 minutes. The six color tones of the scale were as follows: very black (vb), black (b), almost black (ab), which were considered as positive results, and Bordeaux (brd), red (r), and very red (vr), interpreted as negative [14-15].

DETECTION OF ALS GENES

DNA molecules were extracted from fifteen *Candida* isolates by AccuPower® PCRPreMix (Geneaid, Korea) .The final volume was (25 µl) which was used in PCR. Amplifications of three *Als* regions were performed in thermocycler (Invetrogen-USA). The first cycle of denaturation was for 5 min at 94 °C followed by 35 cycles of denaturation at 94 °C for 30 sec and annealing at 36 °C for30 sec. Final step was extension at 72 °C for 7 min. Amplified DNA products were separated by electrophoresis in a 1.5% agarose gel (Biolife EGY-Italy) with 10X TBE (Tris. Boric acid. EDTA), and stained with Ethidium bromide (Biotium, USA).

RESULTS

PATIENTS' CHARACTERISTICS

During the study period, 80 throat samples from throat infection patients were collected and cultured for bacterial and fungal isolation. Forty-six throat infection patients were positive for bacterial and *Candida* growth; 21(45.65%) patients were female and 25(54.35%) were male. The patients' ages ranged from 11 to 62 years, with a mean of 26.5 ± 12 years. Fifteen (32.16%) patients were aged between 11 and 23 years, 14(39.43%) between 24 and 36 years, 11(23.91%) between 37 and 49 years, and 6 (13.04%) were between 52 to years, as shown in table 1.

BACTERIAL AND *CANDIDA* IDENTIFICATION

Bacterial growth was more likely to be observed in THROAT INFECTION patients when compared to *Candida* growth (62.96% *versus* 37.04%, respectively). *The Group A beta-hemolytic Streptococci,* constitute 36% of the cases for acute throat infections. Other bacterial species include streptococcal species 22%*, staphylococcus aureus* 13.2%, *Haemophilus influenzae* 6.4%*, Fusobacterium necrophorum* 1.4%, *Arcanobacterium haemolyticum* 3.7%*, Mycoplasma pneumonia* 0.5%, *and Chlamydia pneumonia* (0.7%), as shown in fig. 1.

Candida albicans was the most frequent isolated from throat infection patients, accounting for 84.72% of all *Candida* infections, when compared to *C. glabrata* (8.5%), *C. parapsilosis* (3.78%), C. tropicalis (2.9%).

Table 1. Descriptive statistics (frequency and percentage) for patients' characteristics

Patients' Characteristics	Rating	Frequency N=46	Percentage
Age / Years	11-23	15	32.61
	24-36	14	30.43
	37-49	11	23.91
	50-62	6	13.04
	Mean ± SD (Min-Max) : 26.5 ± 12 (11-62)		
Gender	Male	25	54.35
	Female	21	45.65

Source: compiled by the authors of this study

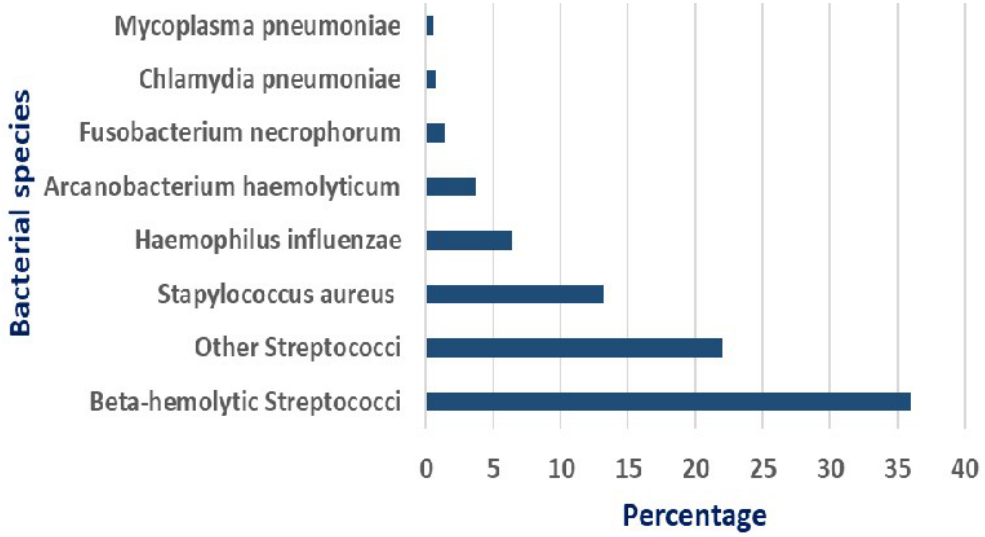

Fig.1. Bacterial growth from urine samples on different culture media

Source: compiled by the authors of this study

CO-CULTIVATION OF *STREPTOCOCCI* AND *CANDIDA*

This method was used to investigate the cooperative growth between *Candida* and *Streptococci in vitro*. Fifteen isolates were selected to perform this test. The growth of many isolates from both *Candida* and *Streptococci* has increased when they subcultured together in a single flask liquid medium in compared with control. In contrast, the growth of some isolates have decreased fig. 2. The CFU percentages of *Streptococci* isolates, for example, no. 1, 3, 4, and 5 were very low in control (*Streptococci* was alone), while the percentage has increased when the same isolates subcultured with *Candida* indicating for occurring the cooperation or synergism among the isolates from both *Candida* and *Streptococci* fig. 3. In another way, we found that growth is synchronous between bacteria and yeast due to the cooperative between them in mix growth depending on the compatible among the isolates from both microorganisms.

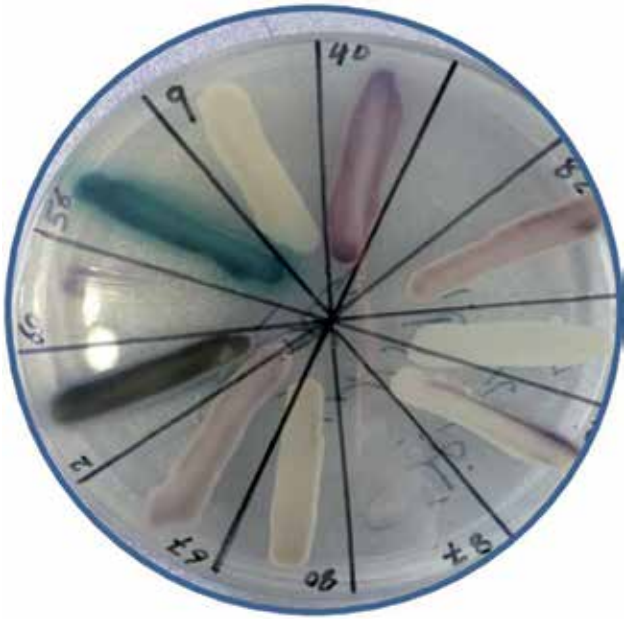

Fig.2. Growth of *Candida* species on CHROM Agar, (C. albicans = Green, C. parapsilosis = Creamy, C. tropicalis = blue, C. glabrata = purple)

Source: compiled by the authors of this study

CANDIDA BIOFILM ESTIMATION

Candida albicans on Congo red agar showed different tones of two colors, black and red. Black represent the positive to biofilm formation and it was in three degrees, as a result graduated as (+, ++, +++). The negative result (red) was in tow tones in our survey, written as weak and (-). Four isolates were the darkest with three plus. Less darkness with two plus was reported in another four isolates. Least in darkness as a positive for biofilm formation with one plus (+) was observed in three isolates. The negative graduated in three tone degree. The first negative group was arranged as weak darkness. The second group was negative (pure red color). Finally, fourteen *Candida* isolates were positive to biofilm formation in different levels, Table 1.

GENOTYPING OF *CANDIDA* AND THEIR COOPERATION WITH *STREPTOCOCCI*

The genotypes of *Candida* isolates were determined according to three genes of *Als* family (*Als1, Als2* and *Als3*). Five *Candida* isolates (out of 15 isolates) were positive in gel electrophoresis to three biofilm genes classified as first genotype (CALSG1). Four *Candida* isolates were negative in gel electrophoresis to three biofilm genes, classified as second genotype (CALSG2). Other *Candida* isolates were positive to one or two of three biofilm genes, classified with genotypes (CALSG3, CALSG4, CALSG5 and CALSG6). In general, six genotypes were determined in the present study based on presence or absence of three biofilm genes above, which do not effect on the ability of *Candida* isolates in biofilm formation. However, some *Candida* isolates were negative to presence of all three biofilm genes but these isolates were positive to biofilm formation using Congo red

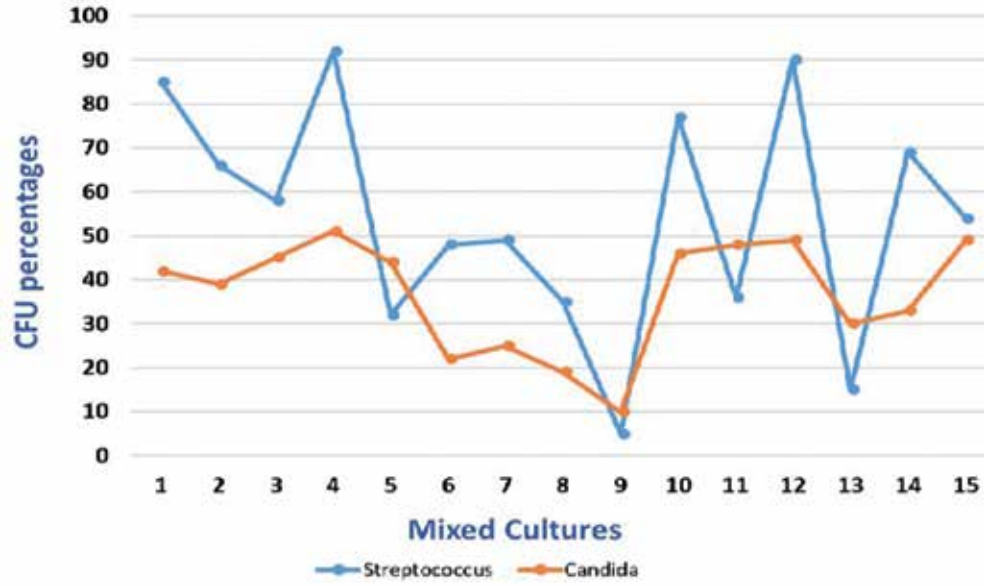

Fig. 3. The CFU for each sample mixed with its partner
Source: compiled by the authors of this study

Table 2. Determination of the genotypes of *Candida* isolates according to three genes of Als family using gel electrophoresis

Samples	Als1	Als2	Als3	Degree of darkness in culture with Congo red stain	Genotype
1	1	1	1	+	CALSG1
2	0	0	0	+	CALSG2
3	1	1	1	++	CALSG1
4	0	0	0	++	CALSG2
5	1	1	1	-	CALSG1
6	0	0	1	+++	CALSG3
7	1	0	0	+++	CALSG4
8	1	1	1	+	CALSG1
9	1	1	1	+	CALSG1
10	0	0	0	-	CALSG2
11	0	1	1	-	CALSG5
12	1	0	0	++++	CALSG4
13	0	0	0	+++	CALSG2
14	1	1	0	-	CALSG6
15	1	1	0	+	CALSG6

(1) Presence of gene, (0) Absence of gene, (+) Presence and degree of darkness, (-) absence of black stain in culture
Source: compiled by the authors of this study

method. In contrast, the presence of three biofilm genes in *Candida* isolates, which were negative to biofilm formation by Congo red method. *Candida* isolates that did not have the ability to biofilm formation by Congo red method did not show growth cooperative with *Streptococci*. While *Candida* isolates that were positive to biofilm formation by Congo red method have shown positive effect on the growth of *Streptococci*. Therefore, we believe that the ability of *Candida* on biofilm formation increase the *Streptococci* growth throughout create an environment conducive to bacterial growth by comparison with the growth of bacteria alone (Table 2).

DISCUSSION

The interaction between *Candida* species, particularly *Candida albicans*, and *Streptococci* has garnered increasing attention in microbiological research, particularly in understanding the implications for oral and systemic health.

Candida albicans is a commensal organism that can become pathogenic under certain conditions, contributing to various infections, especially in immunocompromised individuals. *Streptococci*, also prevalent in oral microbiota, play essential roles in maintaining oral health but can contribute to disease under favorable conditions [16]. Recently, *Candida albicans* has been found to have synergy with oral *Streptococci* in relation to biofilm formation. According to Diaz et al. (2011), *C. albicans* enhances the capacity of *Streptococci* for biofilm formation on both abiotic surfaces and analogues of oral mucosa. Thus, this happens through a mechanism of cooperation capable of allowing colonization and infection. Such relationship means that *C. albicans* is likely to be a key participant in the increase in streptococcal adherence and biofilm development, cited as crucial in the pathogenesis of a number of oral diseases [17]. In addition, Xu et al. (2013) had shown that streptococcal co-infection enhances *C. albicans* pathogenicity by increasing the host inflammatory response at the mucosal surfaces. From this interaction, one can hypothesize that the presence of *Streptococci* may increase not only the virulence of *C. albicans* alone but also elevate the inflammatory reactions of the host, leading to more severe clinical symptoms. The combination of these works really flaunts an undescribed pathogenic cooperative relationship between *C. albicans* and oral *Streptococci*; it signals towards an intricate interplay for therapeutic insight [18]. The existing literature primarily discusses the pathogenic mechanisms of *C. albicans* individually and not how the streptococcal species might influence these mechanisms. For example, although *Enterococcus* faecalis can indeed counteract the virulence of *C. albicans* through bacteriocin production, which inhibits its biofilm, formation and morphogenesis, mechanisms whereby oral *Streptococci* may modulate virulence of *C. albicans* are poorly defined [19]. In the present study, we found that *Candida* species have the ability to attribute *Streptococci* cells by biofilm formation to maintain their microfiche. The role of *Als* genes in co-adhesion of mixed microbial communities are potentially important [20]. The co-adhesion between *Candida* and *Streptococci* may be limited to some genotypes of *Candida*. This fact because some isolates has biofilm formation genes, but when we detect biofilm, it where absent. The explanation for previous is *Candida* may has biofilm formation gene but it is pronounced (not expressed). In the other hand, there is *Candida* does not have any of the three biofilm genes, and it has biofilm in detection. It could be acceptable result as *Candida* biofilm formation genes; *Als* (Agglutinins Like Sequence) family comprise 8 genes [21]. Research indicates that specific Als proteins, including Als1 and Als3, play a fundamental role in the adhesion of *Candida albicans* to epithelial surfaces. Waechtler et al. (2011) identified that defined genes of *Candida albicans*, including Als1 and Als3, mediate adhesion and invasion during interactions with oral epithelial cells [22]. This finding underscores the importance of these proteins in the initial stages of colonization. The process of biofilm formation better explains the role of Als genes. In their study, Zago et al. (2015) tested the interactions between *Candida albicans* and *Staphylococcus aureus* to state that biofilm formation is due to other underlying pathogens. The research paper pointed out that co-adhesion of *Candida* with methicillin-resistant and susceptible strains of S. aureus is important in the development of mixed-species biofilms [23]. In addition to their adhesion properties, Als proteins also contribute to the yeast-to-hypha transition, a process critical for biofilm maturation. These two researchers also showed that compounds like magnolol and honokiol influence adhesion and yeast-hyphal transition of *C. albicans*, indicating that regulation of Als proteins could serve as a possible therapeutic target [24].

CONCLUSIONS

Candida albicans has biofilm formation genes (Als) that are adhesions for other organisms, including *Streptococci*. In addition to its own ability to form biofilm examined by Congo red *C. albicans* also has the ability to increase the number of *Streptococci* in co-cultivation. Even with some Als genes present, it is not obligatory to search for strong biofilm because it might be that not all Als genes are translated into proteins constituting the biofilm. On the other hand, *C. albicans* may have one Als gene and display a firm biofilm.

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ORIGINAL ARTICLE

Hepcidin as a marker of iron status in patients with chronic kidney disease

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ABSTRACT

Aim: To investigate hepcidin as a marker of iron status in chronic kidney disease (CKD) patients (stage 5 vs. stage 3), and to assess its association with iron injection status within the maintenance hemodialysis group.

Materials and Methods: This cross-sectional study compared 69 hemodialysis (stage 5 CKD [G1]) and 19 non-dialysis (stage 3 CKD [G2]) patients, assessing hepcidin, ferritin and hemoglobin. As a part of their standard anemia management, patients requiring iron administration received scheduled injections of ferric carboxymaltose.

Results: Hemodialysis patients (G1) had significantly lower hemoglobin and higher anemia prevalence than non-dialysis patients (G2), while baseline hepcidin and ferritin levels were comparable. Importantly, hepcidin levels were above the normal range in 85,5% and 84,2% of G1 and G2 patients, respectively. Hepcidin correlated positively with ferritin in both groups (G1: ρ=0,66, p<0,001; G2: ρ=0,87, p<0,001). Within G1, recent iron injections, administered in 24 patients, were significantly associated with higher hepcidin and ferritin, but not hemoglobin, as compared to patients without additional ferric therapy (n=45) (effect size: r=0,09 [by hemoglobin], r=0,80 [by hepcidin] and r=0,58 [by ferritin]).

Conclusions: Significant iron metabolism impairment, marked by high hepcidin and ferritin prevalence, exists in both CKD stages studied. Although hemodialysis patients had lower hemoglobin, baseline hepcidin/ferritin levels were similar between groups. Within the hemodialysis group, recent iron injections were associated with increased hepcidin/ferritin but not hemoglobin. Findings suggest hepcidin may be a crucial indicator of functional iron availability in CKD, potentially offering more insight than ferritin, particularly reflecting acute changes following iron administration in hemodialysis patients.

KEY WORDS: hepcidin, ferritin, chronic kidney disease, hemodialysis

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INTRODUCTION

Iron is an essential element involved in oxygen transport and numerous metabolic processes in the body. Unlike many other minerals, the human body lacks an active mechanism for excreting excess iron, making regulated absorption and utilization critically important for maintaining balance. Maintaining this balance between iron need and supply is vital to prevent both iron deficiency and iron overload [1].

The assessment of iron indices is complicated by the presence of inflammatory processes, which can alter the levels of biomarkers such as ferritin and hepcidin. Inflammation-induced elevation of hepcidin leads to iron sequestration within macrophages and hepatocytes, causing functional iron deficiency. This creates an anemia-inflammation association, necessitating high doses of iron administration to maintain normal hemoglobin levels [2].

Hepcidin binds to ferroportin and forces it to internalize into the cell, where it is tagged for destruction and degraded. This halts the export of iron from intestinal cells, liver cells, and macrophages into the blood. Hepatocytes respond to iron stores and begin to release hepcidin. In iron deficiency, hepcidin is low, and therefore iron is released by ferroportin into the bloodstream from enterocytes, hepatocytes, and macrophages, promoting iron availability for erythropoiesis [3]. In iron overload, hepcidin release from hepatocytes increases. Elevated hepcidin binds to ferroportin and inhibits ferroportin-mediated iron export into the bloodstream to reduce transferrin saturation, leading to further inhibition of iron absorption in the duodenum. When iron deficiency occurs, the liver begins to synthesize less of the specific protein BMP-6. This protein helps control hepcidin production, which, in turn, regulates how much iron is released into the blood. Therefore, when BMP-6 levels are low, hepcidin levels also decrease [4].

Hepcidin, like ferritin, is simultaneously not only a marker of iron stores but also a marker of inflammation. The use of hepcidin as a marker of iron status in the context of hemodialysis has been insufficiently studied. Chronic inflammation caused by chronic kidney disease (CKD) significantly complicates the interpretation of these biomarkers.

AIM

The aim of the study was to investigate hepcidin as a marker of iron status in CKD patients (stage 5 vs. stage 3), and to assess its association with iron injection status within the stage 5 CKD (maintenance hemodialysis) group.

MATERIALS AND METHODS

A cross-sectional observational study was conducted involving 69 patients with CKD stage 5 receiving regular maintenance hemodialysis as renal replacement therapy (Group 1). A comparison group of 19 hospitalized patients with stage 3 CKD (Group 2) was also selected.

Among the examined patients, there were 36 (40,9%; 95% Confidence Interval [CI] 30,64-51,18) women and 52 (59,09%; 95% CI 48,82-69,36) men, with a mean age of (mean [M] ± standard deviation [SD]) 55,8±13,10 years. Inclusion criteria were: receiving ongoing maintenance hemodialysis as renal replacement therapy for at least 6 months, age between 18 and 85 years, patient consent to participate in the study, and ability to cooperate adequately during the study process. Exclusion criteria were: patient refusal to participate, age <18 years, evidence of acute infectious processes of any etiology, oncological diseases, and mental disorders.

In all patients, the diagnosis of CKD was established according to the recommendations of the Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group 2024 [5].

All patients underwent standard examination, including general clinical, biochemical, and instrumental investigations. Biochemical parameters were determined in the laboratory of St. Panteleimon Hospital of the 1st Territorial Medical Union (TMU) of Lviv. Glomerular Filtration Rate (GFR) was calculated using the CKD-EPI (2021) formula with a calculator.

Hepcidin and ferritin levels were determined using an enzyme-linked immunosorbent assay (ELISA). According to KDIGO recommendations, the target ferritin ranges for patients were adopted as follows: 200-500 µg/L for Group 1, and 100-500 µg/L for Group 2. A reagent kit manufactured by FineTest (China) was used for hepcidin determination. Reference values were 5,5-47 ng/mL for healthy individuals. As a part of their standard anemia management protocol, patients requiring iron administration received scheduled injections of ferric carboxymaltose at a dose of 500 mg. For the purposes of this study, these administrations were timed such that blood sampling for hepcidin and ferritin occurred at least 2 weeks after the most recent infusion.

The study was conducted in accordance with international standards regarding the coordinated participation of respondents, the ethical component of research, and biomaterial collection (Declaration of Helsinki of the World Medical Association – "Ethical Principles for Medical Research Involving Human Subjects" and the "Universal Declaration on Bioethics and Human Rights" [UNESCO]). The research protocol was reviewed and approved by the Local Ethics Committee of Danylo Halytsky Lviv National Medical University (meeting on March 18, 2024, protocol number 3). All patients signed written informed consent to participate in the study.

The R 4.4.2 and RStudio 2025.05.0+496 programs were used for calculating statistical indicators. General statistical indicators were calculated in "Microsoft Excel" using built-in formulas. The frequency of qualitative indicators was presented as absolute (n) and relative (%) values, as well as the 95% CI in the format "n (%; 95% CI)". The normality of quantitative data distribution was checked using the Shapiro-Wilk test. Normally distributed data were presented as mean ± standard deviation (M±SD), while data with a non-normal distribution were presented as median and interquartile range (Me [Q25; Q75]). To compare two independent groups based on quantitative indicators, depending on the data distribution type, the parametric Student's t-test or the non-parametric Mann-Whitney U-test was used. Fisher's exact test was used to compare frequencies between the two groups for qualitative indicators. Correlation analysis between quantitative indicators was performed using Spearman's rank correlation coefficient (ρ). To assess the effect size for group comparisons using the Mann-Whitney U-test, the rank biserial correlation coefficient (r) was calculated. The critical significance level (p) for testing statistical hypotheses was set at 0,05.

RESULTS

The following parameters were calculated for all patients (Table 1). It should also be noted that C-reactive protein (CRP) levels in all patients in each group were <10 mg/mL, so we can state the absence of acute inflammatory processes.

In group 1 (n=69), hepcidin levels were above the normal range in 59 patients (85,5%; 95% CI 76,3-92,8), compared to 16 patients (84,2%; 95% CI 65,0-96,7) in group 2 (n=19); (Fisher's exact test: NS). Elevated ferritin levels (above the target range) were observed in 23 patients in group 1

Table 1. General parameters and iron metabolism indicators in both groups

Parameter	Group 1 n=69	Group 2 n=19	p
Hepcidin, ng/mL	138,0 [57,2; 327,8]	130,9 [87,6; 159,5]	NS
Ferritin, µg/L	129,0 [65,8; 382,0]	156,0 [99,5; 193,5]	NS
Creatinine, µmol/L	783 [540; 971]	158 [150; 169]	<0,05
Urea, mmol/L	23,1±6,45	11,1±1,42	<0,05
Hemoglobin, g/L	85,8±18,37	112,3±15,84	<0,05
Age, years	57,1±12,93	51,2±13,17	NS

Note: NS – non-significant
Source: compiled by the authors of this study

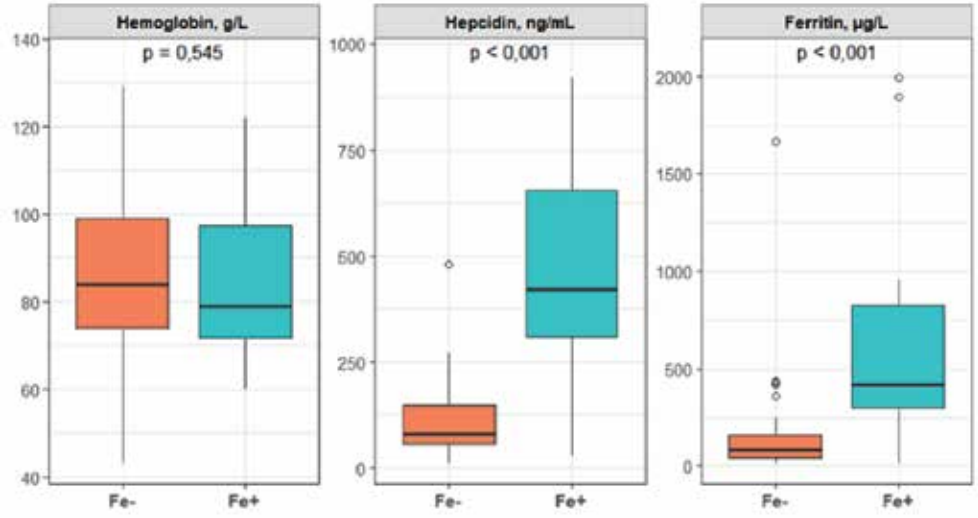

Fig. 1. Comparison of hemoglobin, hepcidin and ferritin levels between patient groups receiving (Fe+) (n=24) and not receiving (Fe–) (n=45) iron injections in CKD stage 5 group

Source: compiled by the authors of this study

(33,3%; 95% CI 22,8-44,8) and 5 patients in group 2 (26,3%; 95% CI 9,5-47,8) (Fisher's exact test: NS). However, anemia was significantly more prevalent in group 1, observed in 61 patients (88,4%; 95% CI 79,9-94,8), compared to only 6 patients in group 2 (31,6%; 95% CI 13,2-53,6) (Fisher's exact test: p<0,001).

We revealed direct correlations of hepcidin and ferritin in the group 1 (ρ=0,66, p<0,001) and group 2 (ρ=0,87, p<0,001). Besides, we did not observe any significant correlations between hemoglobin levels and iron metabolism markers (hepcidin or ferritin) in both study groups.

To assess the association between iron injections and the levels of hemoglobin, hepcidin, and ferritin within stage 5 CKD group, we compared the groups of patients receiving iron injections (Fe+) (n=24) with those not receiving them (Fe–) (n=45). The results showed: no statistically significant difference for hemoglobin (Fe+: 79,0 [71,8; 97,5] g/L *vs.* Fe–: 84,0 [74,0; 99,0] g/L; p=0,545), but statistically significant differences for hepcidin (Fe+: 421,8 [308,1; 654,3] ng/mL *vs.* Fe–: 81,4 [55,2; 147,0] ng/mL; p<0,001) and ferritin (Fe+: 416,0 [302,5; 828,3] µg/L *vs.* Fe-: 85,7 [41,7; 162,0] µg/L; p<0,001) (Fig. 1).

Additionally, the magnitude of the difference (the effect size) within stage 5 CKD group between patients receiving and not receiving iron injections was as following: r=0,09 (by hemoglobin), r=0,80 (by hepcidin) and r=0,58 (by ferritin).

DISCUSSION

The high prevalence of elevated hepcidin in both hemodialysis (85,5%) and stage 3 CKD patients (84,2%) suggests early dysregulation of iron homeostasis. Interestingly, despite differing CKD stages and dialysis status, median hepcidin concentrations were comparable between groups between these two groups. This finding appears somewhat contrary to reports suggesting hepcidin levels tend to increase with advancing CKD stage [6-8]; the lack of a significant difference in our cohort might be attributable to specific patient characteristics, sample size limitations (particularly in Group 2), or other unmeasured factors.

Similarly, elevated ferritin levels were prevalent in substantial proportions of patients in both groups (33,3% in Group 1; 26,3% in Group 2) without a statistically significant difference between them. Given that ferritin acts as both an iron storage marker and an acute-phase reactant [9], these findings likely reflect underlying inflammation and disrupted iron storage processes occurring relatively early in CKD and persisting through to the dialysis stage. Our observation aligns with some previous research suggesting hyperferritinemia may not strongly correlate with CKD stage [10, 11].

As expected, hemoglobin levels were significantly lower and the prevalence of anemia was markedly higher in the hemodialysis group compared to the stage 3 CKD group. This is consistent with the well-established association between declining kidney function and the increasing burden of anemia in CKD [12].

Consistent with the known interplay between iron stores and regulation, a significant positive correlation between hepcidin and ferritin was observed within both patient groups [13], potentially reflecting inflammation and hepcidin's action on ferroportin. This relationship appeared numerically stronger in the non-dialysis CKD group, possibly suggesting a more direct link between storage and hepcidin synthesis in earlier stages of CKD, though this warrants further study. Importantly, and perhaps highlighting the complexity of anemia in CKD, no statistically significant associations were found between hemoglobin levels and either hepcidin or ferritin in either group.

Confirming the expected physiological response within the hemodialysis group, recent intravenous iron administration was associated with significantly higher levels of both hepcidin and ferritin, as compared to patients without the additional ferric therapy. This aligns with extensive evidence showing that parenteral iron rapidly influences these markers [14, 15]. Assessment of the magnitude of this association revealed

a large effect size for both hepcidin (r= –0,80) and ferritin (r= –0,58) in additional ferric therapy group.

In contrast, this cross-sectional comparison within the hemodialysis group did not reveal a statistically significant difference in haemoglobin levels between those who had recently received iron and those without the additional ferric therapy, with a negligible effect size (r = 0.09). This short-term lack of haemoglobin association may reflect the challenges of functional iron deficiency in this population, where high hepcidin levels, potentially driven by both iron load and inflammation, can restrict the utilisation of administered iron for erythropoiesis [2].

These findings lend support to the concept of hepcidin as a potentially sensitive indicator of functional iron status in CKD. While ferritin reflects iron stores, its level is also strongly influenced by inflammation, common in CKD, which can complicate its interpretation for iron availability [9]. High hepcidin directly restricts iron release via ferroportin blockade [16], potentially causing functional iron deficiency even when ferritin levels seem adequate [7]. The observation within the hemodialysis group that the association between recent iron administration and hepcidin showed the previously noted larger effect size for hepcidin's response compared to ferritin further suggests hepcidin might be a more dynamic marker reflecting acute changes in iron availability in these patients.

Study limitations. Despite the obtained results, our study has certain limitations that should be considered when interpreting the data: cross-sectional design, small sample size, which may limit the statistical power of the analysis, the analysis of the effect of iron injections was performed only 2 weeks after drug administration, and it did not account for the potential influence of other factors that could affect iron metabolism and haemoglobin levels.

PERSPECTIVES FOR FURTHER RESEARCH

Further, large-scale, and longitudinal studies are needed for a deeper understanding of hepcidin's role in regulating iron metabolism and anemia development in patients at different CKD stages, considering concomitant factors. Additional thorough investigation of chronic inflammatory status and its relationship with hepcidin levels is warranted to optimize therapy in the context of inflammation. Assessing the impact of erythropoiesis-stimulating agents on hepcidin levels will provide important data for improving existing patient treatment strategies.

CONCLUSIONS

This study confirms that significant iron metabolism impairment occurs in patients with both stage 3 CKD and stage 5 CKD on hemodialysis, marked by a high prevalence of elevated hepcidin and ferritin levels. Despite significantly lower hemoglobin and higher anemia prevalence in hemodialysis patients, baseline hepcidin and ferritin concentrations did not significantly differ between the studied CKD stages. A strong positive correlation between hepcidin and ferritin was observed in both patient groups.

Short-term intravenous iron injections were associated with significantly increased hepcidin and ferritin levels within the hemodialysis group. However, no significant association between recent iron administration and hemoglobin levels was detected in this group.

These findings, particularly the observed sensitivity of hepcidin to recent iron administration within the hemodialysis group, support the concept that hepcidin may serve as a crucial indicator of functional iron availability, potentially offering more insight than ferritin alone in the complex setting of CKD.

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ORIGINAL ARTICLE

Emotional and pain experiences reported by Ukrainian healthcare workers during wartime

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ABSTRACT

Aim: To assess the emotional and pain experiences of Ukrainian healthcare workers during the war to identify key targets for multidisciplinary prevention approaches.

Materials and Methods: The pilot study enrolled 99 healthcare professionals (69 women, 30 men), aged 23-65 years. Participants included doctors (n=69), nurses, and physical therapists (n=30), with experience ranging from under 10 to over 25 years. A 49-item questionnaire was distributed via Google Forms. The survey assessed demographics, workload, emotional stress, pain, and attitudes toward working under wartime conditions. The variables were ranked using categorical and numerical discrete scales, and the data were analyzed using Spearman's rank correlation.

Results: Emotional exhaustion was reported by nearly half of the healthcare professionals at least 2-3 times per week, and pain syndrome was common, particularly lower back pain. Emotional exhaustion showed positive correlations with job dissatisfaction, workload, anxiety, and apathy. Higher pain intensity was linked to decreased productivity and emotional strain, including depression. The impact of war correlated with emotional and physical disruption, yet professionals remained empathetic toward patients. War-induced insomnia was associated with a higher desire to leave the profession, highlighting the long-term risks to healthcare workforce sustainability.

Conclusions: The pilot study highlights the urgent need for preventive multidisciplinary interventions to address emotional strain and pain among healthcare workers. Tailored support strategies may enhance well-being and professional sustainability in high-stress environments.

KEY WORDS: healthcare workers, wartime, pain, emotional distress

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INTRODUCTION

Healthcare professionals face immense psychological and physiological stress, leading to burnout, reduced career engagement, and compromised patient care quality. A systematic review and meta-analysis by A. Hodkinson et al. [1] emphasize the strong associations between physician burnout and decreased professional satisfaction, increased medical errors, and suboptimal patient care. Furthermore, the ongoing challenges of sleep deprivation, poor dietary habits, and lack of physical activity exacerbate occupational stress, as highlighted by J. Park and K. McElveen [2]. The COVID-19 pandemic has further intensified mental health burdens among medical professionals, with studies reporting heightened levels of depression, anxiety, and sleep disorders [3, 4]. Psychological distress and resilience among healthcare workers were notably studied by T. Jeamjitvibool et al. [5], reinforcing the need for structured psychohygienic interventions. Implementing comprehensive mental health and well-being programs is essential to sustaining the healthcare workforce and ensuring the provision of high-quality patient care [6].

The ongoing war in Ukraine has placed additional, unprecedented demands on medical professionals, further exacerbating workplace stress and health-related challenges [7]. Burnout and pain are among the most pressing concerns affecting healthcare workers, as they navigate long hours, emotionally taxing patient interactions, and the physical strain of their duties [8]. Research suggests that prolonged exposure to high-stress environments increases the risk of burnout, leading to emotional exhaustion, reduced job satisfaction, and impaired professional performance [7].

Pain, both acute and chronic, is another critical factor influencing medical professionals' well-being. Repetitive physical strain, inadequate rest, and psychological distress contribute to high pain prevalence, further diminishing work efficiency and overall quality of life. Despite these challenges, healthcare workers adopt various coping strategies, including professional support programs, psycho-physical hygiene measures, and resilience-building techniques [7, 8].

This study examines the emotional and pain experiences of Ukrainian medical professionals during wartime.

AIM

The aim of the study was to assess the emotional and pain experiences of Ukrainian healthcare workers during the war in order to identify key targets for multidisciplinary prevention approaches.

MATERIALS AND METHODS

A pilot study enrolled a total of 99 healthcare professionals aged 23–65 years (69 women, 30 men), of whom 69 were doctors, and 30 were nurses and physical therapists. Experience levels ranged from under 10 years (n=38) to 11–25 years (n=25) to over 25 years (n=36).

After reviewing the relevant literature, a questionnaire consisting of 49 items was developed and distributed via Google Forms. Healthcare professionals were invited to participate by completing the questionnaire, which assessed the following domains: demographics (age, gender, and years of professional experience); workload (average working hours, shift duration, and perceived emotional stress at work); physical health and pain (location, intensity, and frequency of pain, as well as its association with stress); psychological distress (anxiety, depression, job satisfaction); and healthcare professionals' reactions to living and working in a country subjected to prolonged aggression from a neighboring state over the past ten years.

Data for this study were collected using an online survey administered through Google Forms. The survey was sent to respondents who were healthcare professionals in outpatient clinics. The responses were then exported to Google Sheets for further analysis. The data were organized and prepared for analysis in a structured format.

The data were analyzed using Statistica v. 14.0 (TIBCO Software Inc., USA). The Spearman's rank correlation coefficient (ρ) was calculated using standardized categorical rankings, aiming at analyzing the relationships between responses received from healthcare workers. The following indicators — 'Emotional exhaustion', 'Job dissatisfaction', 'Anxiety', 'Apathy', 'Depression', 'War-induced insomnia', 'Indifference toward patients', 'Decreased productivity due to pain', 'Desire to change profession' — were ranked on a descending frequency scale: 'every day', '2–3 times per week', 'rarely', and 'never'. Such parameters as 'Workload', 'Pain intensity', 'Impact of war on well-being', and 'Recovery quality' were assessed using a numeric discrete scale ranging from 1 to 10 points. The indicator 'Sufficient time for recovery' and 'Sufficient time for rest' was ranked as follows: 'sufficient', 'sometimes sufficient', 'insufficient'. A p-value <0.05 was considered statistically significant.

The authors take full responsibility for ensuring compliance with ethical and legal standards in biomedical research. The study adheres to Articles 43 and 45 of the Law of Ukraine "Fundamentals of Health Legislation of Ukraine," the principles of the Council of Europe Convention on Human Rights and Biomedicine, as well as the ethical principles outlined in the World Medical Association Declaration of Helsinki regarding biomedical research involving human participants. Additionally, it complies with Directive 2001/20/EC of the European Parliament and the Council of the EU (April 4, 2001, as amended). The research strictly follows bioethical principles and aligns with both national and international regulatory requirements. The study protocol was approved by the local ethics committee. All responses were provided voluntarily and anonymously by healthcare workers.

RESULTS

The ongoing war in Ukraine has profoundly impacted the healthcare system, placing extraordinary emotional and physical demands on medical personnel. Healthcare workers continue to operate under conditions of chronic stress, high workload, and limited resources, often leading to emotional exhaustion and work-related pain. Despite increasing recognition of these challenges, empirical data on the subjective experiences of professionals in wartime settings remain limited.

This pilot study aimed to explore emotional and pain-related experiences among Ukrainian healthcare workers and identify key correlations that may inform future preventive strategies. The findings offer an initial evidence base for the development of tailored multidisciplinary support approaches. Of the various questions about the emotional experiences of healthcare workers, the results regarding emotional exhaustion deserve special attention (Fig. 1).

According to Fig. 1, the following data were obtained from a survey of 99 healthcare professionals: 22% report experiencing emotional exhaustion every day, 24% 2-3 times a week, 50% of healthcare professionals experience emotional exhaustion rarely, while only 4% of healthcare professionals report no symptoms of emotional exhaustion.

Fig. 2 illustrates the distribution of pain syndromes among healthcare workers. Sixty percent of respondents reported experiencing pain syndrome 2-3 times a week, 5% reported "every day," 30% experienced them rarely, and only 5% reported no pain at all (Fig. 2).

These results indicate that pain syndrome is a common issue among medical personnel, with the vast majority experiencing it at least occasionally. This highlights the need for targeted preventive measures and ergonomic interventions in clinical settings. Among the 94 healthcare workers who reported pain, the most common type was low back pain (dorsalgia), affecting 54 (57.5%) of respondents. Neck pain (cervical pain) was reported by 44 (46.8%), followed

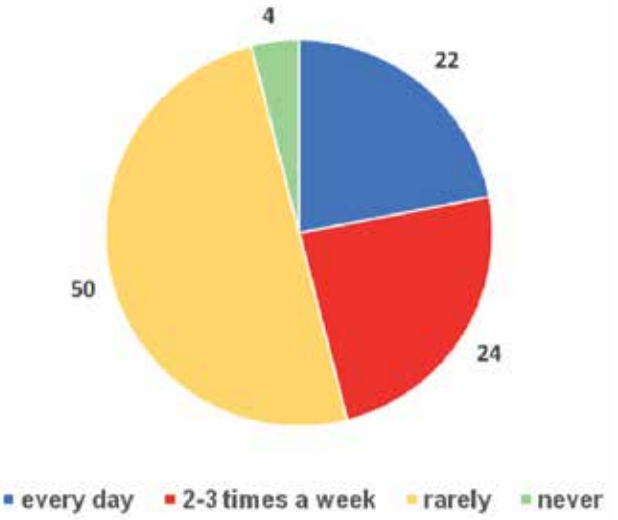

Fig. 1. Frequency (%) of emotional exhaustion among healthcare workers
Source: compiled by the authors of this study

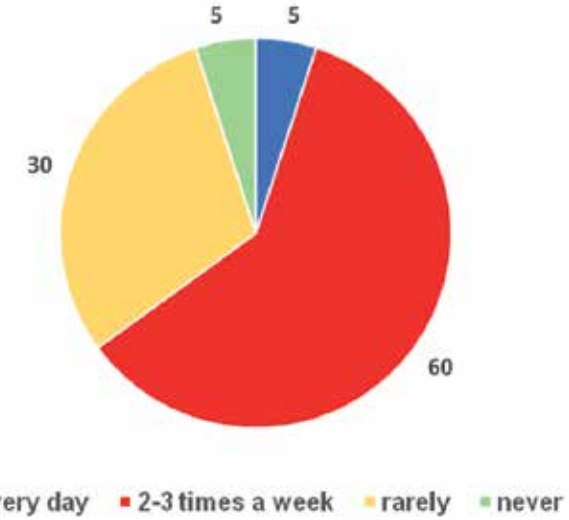

Fig. 2. Frequency (%) of pain syndrome in healthcare workers
Source: compiled by the authors of this study

by shoulder pain in 36 respondents (38.3%). Headaches were also frequent, affecting 33 (35.1%) of respondents who reported pain. Pain in multiple anatomical locations was commonly observed. These findings suggest that musculoskeletal pain is a prevalent issue, likely associated with the physical demands of healthcare work, prolonged standing, and stress-related factors.

Therefore, emotional exhaustion and pain syndromes are highly prevalent among healthcare workers. Emotional exhaustion is the most common burnout symptom, while lower back pain is the most frequently reported physical issue. These findings highlight the urgent need for workplace interventions, such as stress management programs and ergonomic improvements, to support healthcare professionals' well-being.

The following information presents an analysis of some of the correlation results obtained from a survey of 99 medical professionals. The data presented in Tables 1–3 demonstrate various interrelations between emotional state, physical symptoms, and external stressors, particularly those associated with wartime conditions.

The data from Table 1 illustrate that emotional exhaustion among healthcare professionals positively correlated with job dissatisfaction and workload. The link with years of medical experience suggests that emotional fatigue may accumulate over time. Notably, emotional exhaustion is accompanied by psychological symptoms such as anxiety and apathy, indicating its multidimensional and persistent nature.

The correlation between apathy and job dissatisfaction reinforces the impact of negative workplace attitudes, while the connection between anxiety and limited recovery time highlights the crucial need for adequate rest and recovery. Overall, the findings indicate a high prevalence of emotional disturbances among medical workers and underscore their urgent need for systemic support and psychological restoration (Table 1).

The results from Table 2 demonstrate that higher pain intensity among healthcare professionals is associated with decreased productivity, indicating a direct functional impact. Positive correlations with war-induced insomnia and emotional exhaustion suggest that pain is closely tied

Table 1. The correlations (ρ) beetween emotional and associated factors

Variables		ρ	p
Emotional exhaustion	Years of medical experience	0,35	< 0,01
	Job dissatisfaction	0,39	< 0,01
	Workload (scored)	0,35	< 0,01
	Anxiety	0,28	< 0,05
	Apathy	0,27	< 0,05
Apathy	Job dissatisfaction	0,41	< 0,01
Anxiety	Time for recovery	0,27	< 0,05

Source: compiled by the authors of this study

Table 2. The correlations (ρ) between pain syndrome intensity and functional state

Variables		ρ	p
Pain intensity	Decreased productivity due to pain	0,50	< 0,01
	War-induced insomnia	0,37	< 0,01
	Emotional exhaustion	0,33	< 0,01
	Depression	0,26	< 0,05

Source: compiled by the authors of this study

Table 3. The correlations (ρ) of war-related and other associated factors

Variables		ρ	p
Impact of war on well-being	Recovery quality	–0,42	< 0,01
	Indifference toward patients	–0,37	< 0,01
	Emotional exhaustion	0,30	< 0,05
	Apathy	0,28	< 0,05
	Sufficient time for daily routine	–0,25	< 0,05
War-induced insomnia	Desire to change profession	0,35	< 0,01

Source: compiled by the authors of this study

to both physical and psychological fatigue. The association with depressive symptoms further highlights the emotional exhaustion linked to chronic pain. These findings underscore that pain syndromes not only impair physical functioning but are also intertwined with emotional strain, reinforcing the need for integrated approaches to both physical and psychological recovery in medical staff.

The data, presented in Table 3, reflect the considerable emotional and functional toll that prolonged war-related stress has on healthcare professionals. In particular, a negative correlation was revealed between the impact of war on well-being and the recovery quality, stability of daily routine, and rest, highlighting the presence of significant lifestyle disruptions. Interestingly, the negative correlation of the impact of war on well-being with indifference toward patients suggests that, despite being under significant stress, many professionals maintain or even reinforce their sense of empathy and professional responsibility.

Furthermore, emotional exhaustion and apathy both correlate with the perceived impact of war, emphasizing its role in exacerbating psychological strain. Importantly, war-induced insomnia was linked to an increased desire to leave the profession, pointing to longer-term consequences for workforce sustainability (Table 3). These findings underline the urgent need for targeted psychological support and institutional interventions in wartime healthcare settings.

DISCUSSION

Emotional exhaustion and pain among medical professionals remain critical issues, particularly during wartime, when healthcare workers face heightened psychological and physical stress. This study confirms that a significant proportion of medical professionals experience emotional exhaustion and pain, negatively impacting job performance, emotional well-being, and overall health. Addressing these challenges requires a multidisciplinary approach, integrating psychohygienic strategies, physical rehabilitation, and organizational support measures [8].

The results show that 46% of healthcare worker respondents experience emotional stress daily (22%) or 2–3 times a week (24%) under conditions of high workload and stress. Chronic stress, emotional exhaustion, and depersonalization are well-documented consequences of this condition, leading to decreased job satisfaction, reduced patient care quality, and increased medical errors [9, 10].

War-related stressors exacerbate these issues, leading to heightened anxiety, depression, and post-traumatic stress disorder symptoms. Despite the importance of psychological resilience, our study highlights a significant gap in access to psychological support programs, with many healthcare workers either unaware of available resources or lacking time to participate. Studies emphasize that structured psychohygienic interventions, including mindfulness training, stress management workshops, and peer support programs, can enhance emotional stability and reduce exhaustion [7, 8, 10].

Psychohygienic interventions play a pivotal role as a means of mitigating emotional exhaustion and enhancing resilience. Research highlights the effectiveness of workplace interventions such as mindfulness training, structured exercise programs, and organizational strategies fostering a supportive work environment [9, 10]. Structured well-being programs improve mental health and lifestyle behaviors among healthcare workers, reducing stress and promoting resilience [11].

Addressing sleep disturbances is another crucial aspect, given their high prevalence among medical personnel [15, 16]. Future research should focus on scalable, evidence-based interventions tailored to the unique challenges of high-stress environments.

Pain is an important but often forgotten factor contributing to emotional exhaustion. Sixty percent of the surveyed healthcare professionals experience pain 2–3 times a week, and 5% report constant pain. The correlation between pain intensity and emotional distress underscores the necessity of integrating physical rehabilitation into workplace well-being programs.

Work-related musculoskeletal pain is prevalent among medical professionals, particularly in physically demanding specialties [11]. Studies suggest that ergonomic workplace modifications, structured physical exercise programs, and physiotherapy interventions can reduce occupational pain and improve work efficiency [12].

A comparative analysis explored the links between physical rehabilitation and psychological support needs, revealing gaps in access to recovery resources. Encouraging participation in support programs and improving coping mechanisms may enhance resilience among medical professionals.

Given the strong correlation between pain, emotional exhaustion, and the stress, a multidisciplinary rehabilitation approach is essential.

Physical therapy, preformed physical factors (e.g., electrotherapy, magnetotherapy, ultrasound therapy), massage therapy, and structured exercise programs have demonstrated efficacy in improving pain management, stress resilience, and professional longevity [7, 8, 17].

CONCLUSIONS

This pilot study found a high prevalence of emotional exhaustion and work-related pain among Ukrainian healthcare workers working in war-torn areas. The correlations found between workload, stress, emotional instability, and pain highlight the complex interplay of physical and psychological challenges faced by healthcare workers. These findings highlight the critical need for a unified system that incorporates multidisciplinary preventive and restorative approaches that integrate physical conditioning, psychological support, psycho-physical rehabilitation and workload management. Implementation of tailored, evidence-based interventions can improve the well-being, resilience, and professional longevity of healthcare workers in high-stress environments.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Conflict-induced increase in viral hepatitis and human immunodeficiency virus infection among internally displaced persons: a retrospective study in Volodymyr community, Ukraine

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ABSTRACT

Aim: To study the trends in the prevalence of hepatitis B (HBV), hepatitis C (HCV), and human immunodeficiency virus (HIV) infection during 2020-2024 in the rear region of Ukraine, in the city of Volodymyr, and assess the impact of internal displacement on the spread of infectious diseases.

Materials and Methods: A retrospective analysis in Volodymyr Hospital (Volodymyr, Ukraine) was conducted using data from the official clinical registry and ELISA (enzyme-linked immunosorbent assay) for 2020-2024. Comparative literature and statistical data from infection prevention programmes were also analyzed.

Results: The decline in officially reported new HIV infections (from 29 in 2020 to 7 in 2024) contrasted with a marked increase in the number of ELISA-tested cases of hepatitis B and C (7,974 and 6,705 in 2024, respectively). Internally displaced persons (IDPs) accounted for a significant proportion of new diagnoses after 2022, including 22 HBV and 17 HCV cases in 2022. The discrepancy between laboratory results and official reporting suggests underdiagnosis. Additionally, Poisson regression analysis demonstrated that IDP status was a significant factor for increased risk of blood-borne infections.

Conclusions: Military conflict and population displacement significantly exacerbate the problem of blood-borne infections. Countries with successful infection control systems, such as Canada and the Netherlands, have implemented universal HBV vaccination, national HCV elimination strategies, and mobile care for vulnerable populations. These examples highlight the need to replicate such models in conflict-affected regions. National plans should be based on international recommendations, such as the WHO Global Health Strategy 2022 and the US Centers for Disease Control and Prevention's viral hepatitis elimination roadmap.

KEY WORDS: quality, medical care, displaced persons, hostilities

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INTRODUCTION

Armed conflicts drastically impact global populations, leading to widespread displacement and worsening public health issues. By mid-2024, around 122,6 million people (1,5% of the world's population) have been forcibly displaced due to persecution, conflict, violence, human rights abuses, or public order disturbances. This is nearly double the number from a decade before [1].

In Ukraine, the ongoing military conflict has led to one of the largest displacement crises in the world. By the end of 2023, more than 9 million people had been forcibly displaced internally and externally. This massive displacement has put a huge strain on the healthcare infrastructure, leading to increased vulnerability to infectious diseases [2].

The link between armed conflict and infectious disease outbreaks is well documented. A systematic review of the literature highlights that conflicts disrupt health systems, reduce vaccination coverage and deteriorate sanitation, all of which contribute to the resurgence and spread of infectious diseases [3]. For example, the Democratic Republic of the Congo (DRC) experienced a significant Ebola outbreak from 2018 to 2020 amid ongoing conflict, which complicated containment efforts and resulted in more than 3000 cases [4]. Similarly, Yemen faced a cholera outbreak between 2016 and 2022 during its civil war, with more than 2,5 million suspected cases and over 4000 deaths [5].

In the context of Ukraine, the conflict has disrupted routine immunization programmes and hindered access to health services, thereby increasing the risk of outbreaks of vaccine-preventable diseases such as measles and polio [6]. The World Health Organization highlights that the combination of displacement, overcrowded housing and limited access to clean water and sanitation increases the risk of infectious disease transmission in conflict zones [7].

Understanding the relationship between armed conflict, population displacement, and infectious disease dynamics is crucial to developing effective public health responses. Addressing these challenges requires a multifaceted approach that includes restoring health services, ensuring access to vaccination, improving sanitation infrastructure, and implementing disease surveillance systems to mitigate the impact of conflict on public health.

AIM

The aim of the study was to evaluate the trends in the prevalence of hepatitis B (HBV), hepatitis C (HCV) and human immunodeficiency virus (HIV) infection during 2020-2024 in rear region of Ukraine in the city of Volodymyr and assess the impact of internal displacement on the spread of infectious diseases.

MATERIALS AND METHODS

We conducted a retrospective study using data from regional public health surveillance and ELISA-based laboratory diagnostics for 2020-2024. Data on internally displaced persons (IDPs) were analyzed separately. Comparative literature and statistical data from infection prevention programmes in Germany, Sweden, Canada and the Netherlands were analyzed.

Statistical processing was carried out in StatPlus v. 7.8.4 (AnalystSoft Inc., USA) and Microsoft Excel v. 16.96 (25041326), which allowed combining tabular analysis with advanced statistical methods. Poisson regression was used to assess the impact of IDP status on HIV, HBV and HCV infection prevalence, followed by calculation of coefficients, p-values and incidence rate ratios (IRRs) with 95 % confidence intervals (CIs). Additionally, IRRs module was used to compare the IRR in IDP and local population groups. To predict the incidence of HCV infection, the ARIMA (1,1,1) model was used, which, based on ELISA data for 2020-2024, provided a forecast for 2025-2026 with 95 % CIs and graphical visualization. Linear regression was also used to plot the dynamics of infection in the general population and among IDPs. A p-value <0,05 was considered as statistically significant.

The ethical approval was not required as anonymized, aggregated surveillance data were used.

RESULTS

Surveillance data in Ukraine illustrate the impact of the conflict on infectious disease trends. Table 1 presents data on reported cases of HIV, HBV, and HCV infection from 2020 to 2024 in one of Ukraine's regional health jurisdictions, including a subset of cases identified among IDPs. Several patterns emerge: overall, the number of new HIV infections declined markedly during the peak of the conflict, while IDPs accounted for an increasing proportion of newly diagnosed cases.

As shown in Table 1, in 2020, before the escalation of the war, there were 29 new HIV infections in the region, while in 2023, a year after the outbreak of full-scale conflict, there were only 5 new diagnoses. This sharp decline in new cases does not necessarily reflect a true decline in transmission; rather, it reflects the deterioration in access to testing and diagnostic services during the conflict, as healthcare priorities shifted to emergency care and many at-risk people were displaced or unable to get tested. In parallel, the destruction of the healthcare system led to a decrease in the number of routine hepatitis screenings: the number of new HCV cases also initially stagnated (8 cases in 2020 vs. 12 in 2023 in this region). However, in 2024, when some medical services were restored, the number

Table 1. The number of HIV, HBV and HCV infection cases during the period 2020-2024

Year	HIV Cases (Total)	New HIV Cases (Total)	New HIV Cases (IDPs)	Hepatitis B Cases (Total)	New Hepatitis B Cases (Total)	Hepatitis B (IDPs)	Hepatitis C Cases (Total)	New Hepatitis C Cases (Total)	Hepatitis C (IDPs)	Hepatitis B test	Hepatitis C test
2020	183	29	0	313	12	0	258	8	0	1760	N/A
2021	173	13	0	327	8	0	265	7	0	2600	N/A
2022	170	7	12	332	10	22	270	8	17	1761	N/A
2023	172	5	8	328	15	16	272	12	15	3028	2218
2024	149	7	9	330	22	12	275	28	14	7974	6705

Note: N/A – not available

Source: compiled by the authors of this study

of new HCV cases increased to 28, possibly indicating that undiagnosed infections are gradually being detected.

Notably, the data show that IDPs have contributed significantly to the local burden of disease since 2022. In this region of Ukraine, at least 12 HIV-positive IDPs were identified in 2022, which is more than the 7 new local HIV cases diagnosed that year. Many of these displaced people were likely already living with HIV and hepatitis prior to displacement (diagnosed elsewhere or only discovered after moving), highlighting how conflict-induced migration can redistribute health problems across the country. In terms of hepatitis B, 22 IDP-related cases were reported in 2022, which indicates a significant influx of infection into the host region's patient cohort. Some of these are newly diagnosed infections among IDPs, while others may be known chronic cases that have come under local care after people fled the fighting in other parts of Ukraine. In 2023-2024, the number of IDPs under HBV surveillance in this region decreased slightly (from 22 to 12), possibly due to some IDPs returning home or moving to another location, or due to deaths among the cohort. A similar trend was observed for HCV among IDPs (17 cases in 2022 decreased to 14 in 2024).

These patterns highlight two important points. First, conflict can hide new cases in official records (giving the false impression that the situation has improved), when in fact it is worsening surveillance and allowing unchecked transmission to continue. Secondly, when IDPs move, they take their health status with them – regions that receive large numbers of IDPs may suddenly see a sudden increase in the number of patients in need of HIV, HBV or HCV treatment. In Ukraine, relatively peaceful regions in the west of the country have experienced an influx of patients from the war-torn east and south, requiring local health systems to adapt quickly (for example, by expanding the capacity of HIV clinics and ensuring the supply of hepatitis drugs). This redistribution of the patient population within the country demonstrates how internal displacement can geographically spread the burden of infectious diseases.

Despite these challenges, Ukraine – with the support of international organizations – has made significant health efforts to assist IDPs and conflict-affected communities. Mobile clinics and telemedicine have been used to continue HIV treatment for those on the move, and vaccination campaigns have been launched to compensate for missed doses (including hepatitis B immunization for unvaccinated adults in IDP centers). By the end of 2023, these measures helped prevent major outbreaks. However, the Ukrainian case highlights the need to build conflict-sensitive health systems: ones that support surveillance and treatment of chronic infections even in times of instability, and that are specifically geared to provide health services to displaced populations.

The official registry data indicate a downward trend in the number of new HIV diagnoses in the region (from 29 in 2020 to 7 in 2024), likely due to service disruptions and lower screening rates. Hepatitis B and C show the opposite trend with an increase in the number of laboratory-confirmed cases.

To estimate the dynamics of HIV and viral hepatitis among internally displaced persons (IDPs) and the host population in 2020–2024, three complementary modelling approaches were applied. First, adjusted incidence rate ratios (IRRs) were used to assess the association between displacement status and infection risk. Second, Poisson regression analysis was used to assess the impact of IDP status on the risk of blood-borne infections (vs. non-IDP status) (Table 2, Fig. 1).

The analysis demonstrated that IDP status was a significant factor for increased risk of blood-borne infections. Adjusted IRRs from the Poisson regression model revealed that IDPs were: 2,72 times more likely to be diagnosed with HIV infection (95% CI: 1,64-4,49; p<0,001), 8,82 times more likely to be diagnosed with hepatitis B (95% CI: 5,09-15,3; p<0,001), and 8.12 times more likely to be diagnosed with hepatitis C (95% CI: 4,65-14,16; p<0,001) (Table 2, Fig. 1). The presented data underscore both higher transmission and increased diagnostic detection of blood-borne infections following mass displacement.

At the third final step, ARIMA time-series forecasting was used to project future trends based on historical surveillance data (Fig. 2). The model included annual data on ELISA-confirmed cases of HIV and viral hepatitis B and C during the period 2020-2024. The best fit was the ARIMA(1,1,1) model with the optimal (lowest) Akaike information criterion (AIC = 37,2), which showed a reliable approximation of the trend. According to the forecast, in the absence of interventions, the projections for 2026 suggest that more than 10 new HIV, more than 30 new hepatitis B and more than 50 new hepatitis C cases are expected. The forecast includes 95 % CIs, enabling public health authorities to plan for both favorable and unfavorable scenarios in terms of diagnostic needs, treatment resources and preventive strategies.

DISCUSSION

The gaps in surveillance in conflict zones often disrupt disease monitoring. This was evident in Ukraine, where the

Table 2. The adjusted IRRs for blood-borne infections among IDPs (vs. non-IDPs) (2020-2024)

Condition	Total cases	IDP cases	Non-IDP cases	Adjusted IRR	95 % CI	p
HIV	61	29	32	2,72	[1,64 – 4,49]	< 0,001
Hepatitis B	67	50	17	8,82	[5,09 – 15,3]	< 0,001
Hepatitis C	63	46	17	8,12	[4,65 – 14,16]	< 0,001

Source: compiled by the authors of this study

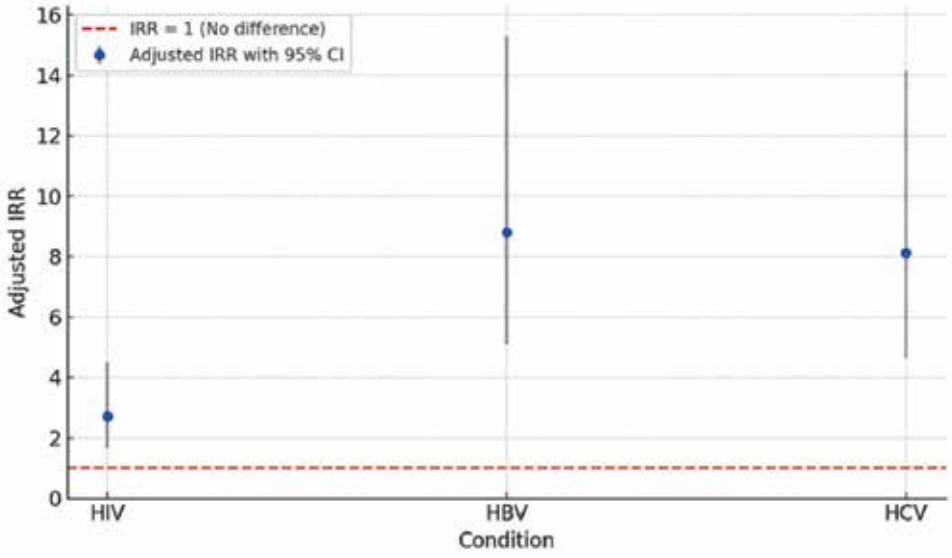

Fig. 1. Adjusted IRRs for IDPs (vs. non-IDPs) with 95 % CI for HIV, HBV, and HCV among IDPs (vs. non-IDPs): Poisson regression analysis (2020-2024)

Source: compiled by the authors of this study

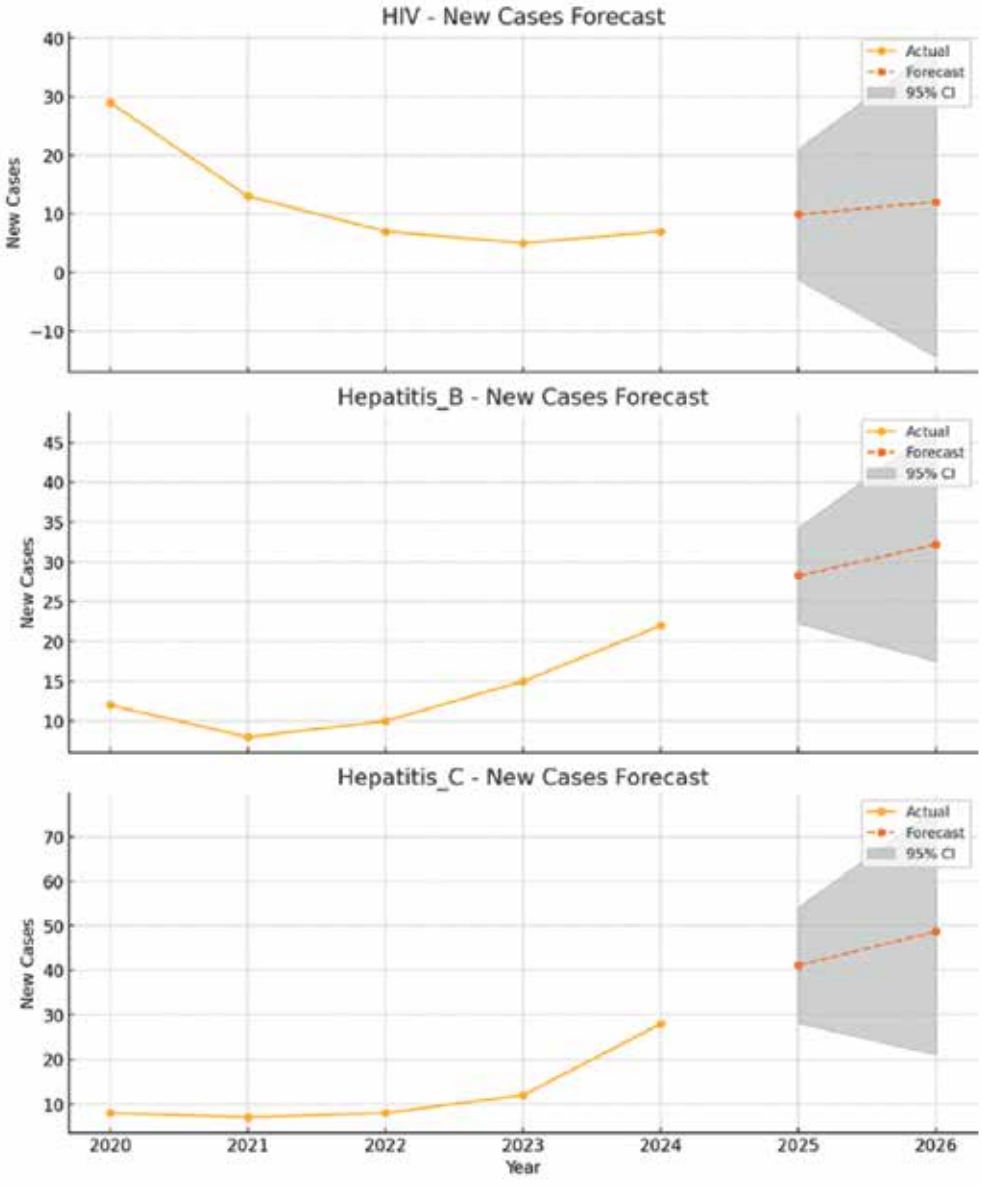

Fig. 2. The ARIMA (1,1,1) model for the forecasting of HIV, HBV and HCV cases (2020-2026)

Source: compiled by the authors of this study

official number of new cases fell not because transmission stopped, but because reporting became difficult. Syria and Yemen are also likely to underreport HIV/HBV/HCV cases due to the collapse of their surveillance systems [8]. Re-establishing reliable disease monitoring in conflict zones (even through minimalist, innovative means such as mobile reporting or community health workers) is crucial [9]. Early warning systems for disease outbreaks should also cover blood-borne infections, not just water- or airborne diseases.

CONTINUITY OF CARE

Perhaps the most important need is to ensure continuity of care for those who are already infected. Conflict should not mean a death sentence for people with HIV or hepatitis. Mechanisms need to be developed to support the provision of treatment, whether through pre-positioning of medicines in conflict-prone areas, buffer stocks that patients can take with them if they flee, or cross-border health programmes that allow IDPs/refugees to continue treatment in neighboring countries. Ukraine's experience with the emergency distribution of antiretroviral therapy is a positive example, but many other places (e.g., Yemen, Sudan) have experienced dangerous supply disruptions. Innovative approaches, such as telemedicine consultations, drone drug delivery, or encrypted digital health records that move with patients, could be scaled up in war settings to support continuous treatment [10].

TARGETED WORK WITH IDPS

IDPs are often overlooked by health systems. They cannot be reached by standard public services [11]. They may not be reached by standard health messages or services (especially if they settle outside of official camps) [12]. National programmes and international organizations need to actively work with IDP communities to provide HIV and hepatitis testing, counselling and treatment. For example, establishing rapid clinics in IDP settlements that offer rapid testing for HIV, HBV and HCV can help to detect infections at an early stage among IDPs [13]. Combining these measures with immediate care (possibly through referrals to the nearest functioning hospital or field clinic) can reduce the increase in untreated cases.

VACCINATION AND PREVENTION SERVICES

Many conflict-affected countries need catch-up vaccination campaigns against hepatitis B. Adults who missed hepatitis B vaccination as children (or children in current conflict zones who are currently missing it) should be offered the vaccine at the earliest opportunity, whether in an IDP camp, host community or refugee reception center [14]. This is a long-term investment to prevent future infections. Similarly, HIV prevention programmes should be re-established in war zones: condom distribution (even in conservative societies, discrete methods can be used), blood safety and the provision of clean needles for those who inject drugs are vital [15]. These measures can be sensitive in conflict settings, but humanitarian health organizations increasingly recognize that neglecting them can lead to a surge in HIV/HCV that will burden societies for decades. New interventions such as portable rapid test kits and mobile blood screening laboratories can help maintain standards of care in makeshift health facilities [16].

DATA AND RESEARCH

There is a need for more research and data collection on the relationship between conflict and chronic infectious diseases. While data on outbreaks of acute diseases (e.g. cholera, etc.) in humanitarian emergencies are routinely collected, chronic infections often go unmeasured [17]. Encouraging and funding research (even small-scale) among IDPs to estimate the prevalence of HIV and hepatitis can help to develop targeted responses. For example, knowing the prevalence of hepatitis C in an IDP camp can help justify the deployment of a hepatitis C treatment programme that might otherwise be overlooked [18]. In addition, studying the long-term treatment outcomes of displaced patients (do they continue treatment? are the outcomes different from those who were not displaced?) will help to improve future interventions [19].

COLLABORATION AND INTEGRATION

The burden of managing health in conflict cannot be borne by national authorities alone; they are often overburdened or are parties to the conflict. International organisations (WHO, UNHCR, UNICEF) and non-governmental organizations play a crucial role in bridging the gaps [20]. Better integration of services is needed – for example, combining HIV services with general primary health care in IDP camps or integrating hepatitis screening into nutrition programmes [21]. When agencies work together, displaced people can receive more comprehensive care rather than a fragmented approach. The example of European countries integrating refugee health care into their national systems is instructive. Similarly, governments in conflict countries should integrate IDPs into their health planning as part of the national population, with the support of international partners [22].

Ukraine is a prime example of how conflict contributes to infectious disease problems among IDPs and the civilian population at large. The country has been facing a conflict since 2014, which was initially confined to the eastern regions and escalated into a full-scale war in 2022 [23]. This upheaval has caused one of the largest displacement crises in recent history. As of 2023, an estimated 5-6 million people were internally displaced within Ukraine due to the conflict, in addition to millions who fled as refugees. These IDPs, along with their host communities, have had a significant impact on health services and disease patterns [24].

Even before the invasion in 2022, Ukraine had a significant HIV and viral hepatitis burden. It was among the countries with the highest HIV prevalence in Eastern Europe (approximately 0,9 % of the adult population in 2020, according to UNAIDS) and a high prevalence of viral hepatitis C due to unsafe medical practices and past injecting drug use. The war has jeopardized the progress made in combating these epidemics. In the conflict-affected areas, many HIV clinics and opioid substitution treatment centers were forced to close or relocate, and thousands of patients were at risk of

interrupting their treatment. For example, during intense fighting in 2022, a significant number of people living with HIV in eastern Ukraine were left without antiretroviral drugs for weeks or months, highlighting the fragility of wartime continuity of care. Health authorities and international organizations have attempted to deliver antiretroviral drugs across the front line and evacuate patients whenever possible to prevent a spike in HIV incidence and further transmission.

Conflict-related displacement has increased vulnerability to blood-borne infections due to interruptions in health care, limited access to diagnostics and poor living conditions. In Ukraine, while the official number of HIV cases has declined, the number of IDP-related cases has increased since 2022. The large discrepancy between hepatitis cases detected by ELISA and official data indicates significant underdiagnosis. Unreported or undiagnosed cases likely reflect failures in the screening infrastructure and overloaded primary healthcare in host regions [25].

Germany and Sweden demonstrated how surveillance and inclusive care prevented the spread of infectious diseases among displaced populations. Their programmes offered screening for HIV and hepatitis upon arrival, routine hepatitis B vaccination for adults and adolescents, mobile testing, and access to direct-acting antivirals for hepatitis C treatment [26]. Canada's strategy includes mobile screening in underserved areas and mandatory reporting systems, while the Netherlands uses geospatial health registries to track clusters of asylum seekers [27].

The case of Ukraine highlights both the epidemiological risk and the logistical burden on host communities hosting IDPs [28]. Health authorities should expand mobile diagnostic units, access to telemedicine, and apply surveillance and prevention approaches aligned with those of WHO and CDC [29].

CONCLUSIONS

The armed conflicts of the last decade have undoubtedly contributed to the growing challenges of fighting hepatitis B, hepatitis C and HIV among internally displaced and war-affected civilians. The case of Ukraine (2020-2024) has demonstrated how quickly progress in disease control can be reversed when war disrupts health systems and displaces millions of people, leading to lower testing rates, interrupted treatment and a shift in the concentration of cases. Comparisons with Syria, Iraq, Sudan, Yemen and Afghanistan reveal a recurring pattern: conflict creates conditions conducive to the spread of blood-borne infections through the destruction of health infrastructure and the dispersal of populations in vulnerable situations. Under these conditions, IDPs often bear a double burden – they are at greater risk of infection and have less access to healthcare.

However, amidst these challenges, there are lessons of resilience and effective response. The experience of Germany and Sweden shows that with foresight and resources, the risks of infectious diseases associated with displaced populations can be managed. The key to success is to view displaced people not as outsiders who pose a threat, but as part of the population whose health needs have to be addressed. When health systems provide inclusive health care – offering screening, prevention and treatment to all, regardless of status – the outcome is better for both displaced people and host communities.

The analysis in this section highlights the urgent need for improved control measures in conflict settings, from supporting vaccination programmes and medicine supply lines to protecting the human rights of vulnerable populations (e.g. providing HIV and hepatitis prevention treatment to survivors of sexual violence). Innovative healthcare interventions will be crucial in the most dangerous regions, using technology and new methods to help people who have been cut off from traditional medical care. In addition, the international community should continue to advocate for and provide better living conditions for IDPs: adequate housing, clean water and sanitation not only prevent epidemic diseases such as cholera, but also create an environment in which overall health improves and people are more likely to seek health services (including for HIV/HBV/HCV).

To sum up, conflicts pose a serious threat to the control of chronic infectious diseases, but this threat can be reduced by working together. The combination of strengthening health systems to make them more resilient to conflict, prioritizing the health needs of displaced populations and learning from successful interventions can reduce the incidence of HIV, hepatitis B and hepatitis C in war-torn regions. Unfortunately, as conflicts continue in different parts of the world, these lessons are crucial to protecting public health and maintaining the well-being and dignity of people displaced by war.

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REVIEW ARTICLE

Odontogenic infections in pregnant women

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ABSTRACT

Odontogenic infections in pregnant women pose a threat to the health of both the mother and her unborn child. Adjusting diagnostics and treatment for this group of patients presents numerous challenges for dental practitioners. The National Health Fund in Poland provides additional dental services for pregnant and also postpartum women. Access to these services is critical for the well-being of pregnant women and their future children, on condition of providing safe treatment. Despite the reduction in the risk of dental complications, there is concern that some patients may delay treatment until pregnancy. Therefore, a key aspect is educating the public on the risks of untreated oral pathologies, promoting oral hygiene, and encouraging regular dental check-ups. This article analyzes the key aspects related to the dangers of untreated dental infections, presents the mechanisms of the most common diseases, and outlines basic forms of treatment, diagnostic and therapeutic limitations due to pregnancy, as well as the influence of the legal system in Poland on the potential decisions of pregnant women to undergo treatment.

KEY WORDS: dentistry, odontogenic infections, pregnancy

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INTRODUCTION

Odontogenic infections constitute a significant portion of dental issues. These are infections originating primarily not from teeth, but they often involve the surrounding supporting tissues as well [1]. Although most odontogenic infections cause mild symptoms, they can also lead to serious complications [1]. A particularly vulnerable group of patients are pregnant women, for whom treatment options are limited to methods that have minimal or no impact on the health of both the mother and the child. Examples include amalgam fillings, substances in anesthetics such as epinephrine or antibiotics such as tetracycline, doxycycline, streptomycin, sulfonamides and others [2]. This issue presents a considerable challenge for dentistry. It is important to promote preventive strategies, educate the public, and develop increasingly effective treatment methods for such conditions.

AIM

The aim of this paper is to analyze the problems associated with dental treatment in women during different periods of pregnancy and to review the most common dental infections occurring in this group of patients. This publication is based on studies published in PubMed and Google Scholar.

A thorough search of the literature was conducted, covering publications from 2002 to 2022, to provide a comprehensive and up-to-date overview of the relevant findings. The search strategy included the use of specific keywords such as "odontogenic infections in pregnancy," "dental treatment during pregnancy," and "effect of pregnancy on periodontal and dental health." These terms were chosen to capture both the general challenges and specific considerations for managing dental health during pregnancy.

The initial search yielded approximately 50 articles. After a detailed review, 19 scientific papers were selected based on their relevance to the subject matter and their adherence to methodological and content quality criteria. The selection process prioritized studies that provided direct insights into the effects of pregnancy on oral health and the safety and efficacy of dental treatments during this period.

Of the 50 articles initially identified, 11 were excluded due to irrelevance or methodological limitations, such as a lack of focus on pregnancy-specific dental issues or insufficient detail regarding clinical outcomes. Among the selected papers, full texts were accessed and analyzed to ensure a comprehensive understanding of the findings, while abstracts were only used as supplementary references for some excluded works.

The resulting selection of 19 key materials forms the foundation of this review, ensuring that the conclusions drawn are both well-supported and aligned with current scientific knowledge.

REVIEW AND DISCUSSION

THE MOST COMMON ODONTOGENIC INFECTIONS AND BASIC TREATMENT

The oral cavity is inhabited by over 700 species of bacteria that constitute the natural bacterial flora [1]. Among the main groups of bacteria are *Streptococcus, Actinomyces, Porphyromonas*, and *Fusobacterium* [3]. The proper balance of the bacterial flora is important for maintaining oral health. This microbiome is responsible for various functions, including mobilizing the host's immune system, participating in the metabolism of nutrients, and playing a role in the processes of enamel remineralization and demineralization [4]. Disruptions in the composition and function of the bacterial flora can lead to the development of various oral diseases, including caries, gingivitis, and periodontitis [1]. If left untreated, the condition leads eventually to the pathogens' penetration into deeper tissues via the bloodstream or lymphatic system, resulting in the development of odontogenic infections.

ODONTOGENIC INFECTIONS IN THE HEAD AND NECK REGION

Neglecting oral hygiene and untreated pathologies can exacerbate infections and increase the risk of odontogenic infections. According to available reports, periodontal diseases affect between 20% and 50% of the global population [5]. The condition is caused by the accumulation of dental plaque along the gumline, leading to infection and gingival inflammation, which results in marginal periodontitis and ultimately tooth loss [1, 6]. Treatment involves the removal of dental calculus, and in cases of severe inflammation, antibiotic administration if necessary [7].

Through the tooth's root canal bacteria are able to penetrate deeper tissues, most commonly via dental caries but also through fractures or leaking restorations [1]. As the infection progresses, it can lead to pulp necrosis, periodontal ligament inflammation, and eventually apical periodontitis. This condition, if left untreated, may result in serious complications, such as odontogenic abscesses, and possibly can spread to various structures of the head and neck, affecting bones, soft tissues, and sinuses [8]. Infections in the head region can lead to purulent meningitis, brain swelling, or inflammation of the cranial nerves, posing a serious threat to the patient's life. In the case of odontogenic infections, it is estimated that they account for 5–40% of all cases of chronic maxillary sinus infections [9]. The primary treatment is root canal therapy, removal of abscesses and implementation of antibiotic use [1, 8]. In some cases, extraction of the infected tooth is required.

Necrotizing fasciitis occurs much less frequently than the aforementioned conditions, but if left untreated, it can lead to sepsis within 48 hours, posing a life-threatening situation for the patient [10, 11]. Data presented in the work of Pang-Yun Chou et al. indicate that group A β-hemolytic streptococci were the most common pathogen causing necrotizing fasciitis, accounting for 51.1% of all reported cases. 31% of those infected developed toxic shock syndrome [11]. This condition arises when thrombosis of blood vessels occurs around the site of infection, leading to swelling and ultimately tissue necrosis. The ongoing inflammation causes pain, neck swelling, anatomical distortion, and often dysphonia, dysphagia, and airway obstruction. The latter is a significant threat to the patient's life [12]. Particularly notable forms of this condition include Ludwig's angina, which affects the floor of the mouth, and Lemierre's syndrome, characterized by thrombophlebitis of the internal jugular vein [1]. Prompt surgical intervention to remove necrotic tissues is crucial [13]. The patient also undergoes antibiotic therapy and dental treatment to address the infection source.

The neglect of odontogenic infections can have serious consequences. Prevention, public education, early diagnosis, and appropriate treatment are essential for preventing complications.

ODONTOGENIC INFECTIONS OF DISTANT TISSUES, ORGANS AND BODY PARTS

When detected at an early stage oral diseases are relatively easy to manage. However, if appropriate treatment is not applied, the infection may spread to neighboring tissues, extending even beyond the head and neck region.

An infection descending through the tissues of the neck can potentially reach the thoracic cavity [12]. One of the most serious health conditions is necrotizing mediastinitis poses a life-threatening risk. Odontogenic necrotizing fasciitis can also spread to body parts, such as the limbs, trunk, and perineal areas. This condition requires immediate diagnostic intervention, surgical treatment, and administration of broad-spectrum antibiotics.

In individuals with predisposing factors such as immunosuppression, children or elderly, or with general weakness, the infection may progress to sepsis, characterized by the presence of bacteria in the bloodstream. This life-threatening condition arises from the body's dysregulated response to infection, leading to organ dysfunction [12, 14]. Pathogens triggering the immune system with toxins cause an excessive release of pro-inflammatory cytokines. In result, blood vessels are damaged, tissue perfusion is impaired, and a systemic inflammatory response ensues. Sepsis manifests with symptoms such as fever, tachycardia, altered mental status, hypotension, rapid breathing, and multiorgan failure. Early recognition and swift treatment of sepsis are crucial to improving patient outcomes, regardless of the infection's origin [14].

Prompt diagnosis, appropriate surgical intervention, and antibiotic therapy are essential for effectively managing odontogenic infections and preventing their spread to other parts of the body. It is therefore important that patients suspected of having an odontogenic infection are quickly diagnosed and treated, especially those with risk factors that predispose them to infectious complications.

LEGAL SITUATION IN POLAND REGARDING DENTAL CARE SERVICES DURING PREGNANCY

According to the Act of August 27, 2004, on healthcare services (Journal of Laws of 2004, No. 210, item 2135, as amended), pregnant women are guaranteed the right to receive healthcare services and pharmaceutical services in pharmacies out of turn [15]. This entitlement is designed to provide faster access to essential healthcare for pregnant women, acknowledging the unique medical needs during this period.

In Poland, the National Health Fund (NFZ) extends additional dental services to pregnant women and women in the postpartum period (up to 42 days after delivery) [16]. This includes a range of treatments aimed at maintaining oral health and preventing complications that could impact both the mother and the unborn child. To access these benefits, patients need only present a pregnancy card confirming their status. The services available to pregnant women under the NFZ include temporary and permanent fillings of tooth canals, removal of live and dead tooth pulp, root canal treatments (including treatment of infected canals), removal of dental deposits once every six months, and dental check-ups every three months. These entitlements supplement the basic dental care package, offering significant support to ensure adequate oral health during pregnancy.

Maintaining oral health during pregnancy is critical, as untreated dental issues can pose serious risks to both the mother and the fetus. The NFZ's expanded dental care program not only provides access to essential treatments but also underscores the importance of regular preventive care, such as frequent check-ups and timely removal of dental deposits. It should be noted that all diagnostics and treatments under this program must prioritize the safety of both the mother and the unborn child.

By ensuring accessible and safe dental care, these regulations aim to support pregnant women in maintaining their health and that of their unborn children. Public awareness of these entitlements is crucial to encourage pregnant women to take full advantage of the services available to them.

CHANGES DURING PREGNANCY AFFECTING ORAL HEALTH

Pregnancy is a state that requires special care for the health of both the mother and the developing fetus. A crucial aspect of healthcare during this period is oral health, as untreated odontogenic infections can pose a serious threat. Scientific studies emphasize the connection between severe odontogenic infections and the risk of complications for both the mother and the fetus.

Hormonal changes during pregnancy can increase susceptibility to oral health problems, including odontogenic infections [17]. Estrogen and progesterone, through vasodilation and increased capillary permeability, cause increased fluid flow, along with white blood cells, into tissues. This can lead to various inflammatory conditions and periodontal diseases.

Studies related to this issue highlight the influence of diet and eating habits changes during pregnancy, contributing to oral infections [18]. Particularly the increased consumption of sweets and fruit juices, which raises the risk of dental caries.

Pregnancy remodels the immune system, which can also affect the body's response to infections [19, 20]. Some studies suggest that this process may make the body more susceptible to certain infections, increasing the risk of complications related to inflammation.

Many women experience issues such as vomiting and heartburn during pregnancy, which directly impact oral health [21]. Frequent vomiting exposes teeth to acidic stomach contents, increasing the risk of enamel erosion and the development of caries [22]. Moreover, constant exposure to stomach acids due to recurring heartburn episodes can irritate the oral mucosa and increase the likelihood of gingival inflammation. Recurrent vomiting episodes may also disrupt daily oral hygiene practices, further raising the risk of gum disease and caries. Preventive measures, such as regularly rinsing the mouth with water after vomiting, using acid-neutralizing preparations, or avoiding acidic foods right before bedtime, can help reduce the risk of oral health problems in pregnant women affected by these conditions [23].

Odontogenic infections in pregnant women pose a significant threat that requires special attention and appropriate treatment. A lack of proper prevention and treatment can lead to serious health complications. Therefore, regular dental check-ups are essential to detect and address any potential issues early. Pregnant women should be encouraged to schedule dental visits as part of their routine prenatal care, ensuring both prevention and timely treatment of oral health issues.

In addition to professional dental care, maintaining proper oral hygiene is crucial. Pregnant women should be educated on the importance of brushing teeth at least twice a day with fluoride toothpaste, flossing daily, and using mouthwash if necessary. Proper oral hygiene can significantly reduce the risk of infections and maintain overall oral health during pregnancy.

Another important factor to consider is the fear of dental procedures that many women experience during pregnancy. Anxiety related to dental visits may be intensified by hormonal changes, fear of potential pain, or concerns about the safety of treatments for both the mother and the baby. Addressing this fear through clear communication, reassurance, and ensuring that dental procedures are safe during pregnancy is essential. It is important that healthcare providers emphasize the safety of dental care during pregnancy and offer emotional support to help women feel comfortable and confident in seeking treatment.

In conclusion, prevention, including regular dental check-ups and proper oral hygiene practices, is vital in maintaining good oral health during pregnancy. Overcoming the fear of dental visits and ensuring that women feel supported and informed about the safety of dental treatments during pregnancy can greatly contribute to better health outcomes for both the mother and the developing fetus.

RISKS FOR MOTHER AND CHILD ASSOCIATED WITH DENTAL TREATMENT

Some women delay seeking treatment until pregnancy to access free and expedited care, which they are legally entitled to. However, it is important for women's health and well-being in the reproductive period to be monitored and adequately treated before pregnancy begins, to avoid more severe complications. It seems reasonable, therefore, to create the possibility of proper prevention and dental treatment already at the stage of pregnancy planning, which the gynecologist should draw the patient's attention to. One of the issues emphasized in the literature is the importance of close collaboration between the pregnancy care specialist and the dentist in the patient's care. Regular dental visits early in pregnancy allow for early detection and successful treatment of potential dental issues, which minimizes the risk of complications for both the mother and the child.

Pregnancy is a unique condition requiring special attention during diagnosis and treatment. Thus, healthcare providers are obligated to tailor care to the patient's safety. If pharmacological treatment is required, the potential risks to the developing fetus and the welfare of the mother should be taken into account when making therapeutic decisions with due caution. Pregnant women face several challenges in dental care, including delayed treatment access, potential risks of radiographic imaging, concerns about dental materials, and the safety of local anesthesia.

Dental radiography (X-ray) can be required for the treatment of oral pathology in pregnant patients [24, 25]. This diagnostic method enables a precise assessment of dental health, allowing the dentist to identify various conditions such as cavities, periodontal disease, or root infections. X-rays are also helpful to identify hidden problems, such as retained teeth or cysts that may not be visible during a routine dental examination. Radiographs aid in planning dental treatment and monitoring its effects, allowing the dentist to tailor procedures to the patient's individual needs and ensure effective therapy. It is generally common knowledge to avoid exposure to X-rays for pregnant patients. There is also uncertainty regarding the efficacy of lead shielding during dental radiography in pregnancy. A study examining estimated radiation doses to the fetus and the breasts of pregnant patients across different dental exams, including intraoral, panoramic, cephalometric, and CBCT scans, both with and without lead shielding, found that the upper estimates of radiation doses to the fetus and maternal breast were relatively low [26]. Even without lead shielding, fetal radiation exposure did not exceed 1% of the annual dose limit. Although lead shields offer some protective effect, the risk from radiation exposure shows minimal impact on the health of the patient and the unborn child. However, due to the potential non-stochastic risks of radiation exposure, it remains essential to minimize radiation doses as much as possible to further reduce any potential harm to both the mother and the fetus. Another aspect of dental care that raises concerns among pregnant patients is the effect of certain substances and therapeutic methods on both maternal and fetal health.

During dental treatment materials potentially harmful to the fetus should be avoided. There is growing interest in the possible effects of substances released from dental fillings on fetal development. Traditional amalgam fillings were popular due to their durability and resistance to abrasion [27]. A Norwegian cohort study of mothers and children conducted between 1999 and 2008 suggested a possible correlation between the placement of amalgam fillings during pregnancy and the risk of perinatal mortality. Analysis indicated an increased risk of perinatal mortality with a higher number of teeth filled with amalgam. Adjusted logistic regression models showed that women with 13 or more amalgam-filled teeth had nearly twice the risk of perinatal mortality compared to women without such fillings. On July 1, 2018, the Polish Medical Council's Dental Commission banned the use of amalgam fillings in primary teeth, children under 15, and pregnant or breastfeeding women, with exceptions allowed only if deemed medically necessary by the dentist due to specific patient needs [28]. The decision stems from EU regulations and the International Convention on the Reduction of Mercury Use, to which Poland is a signatory. Some studies also suggest that some components in polymer-based filling materials may release substances that could interfere with the endocrine system, which are raising concerns about fetal safety. A 2018 study based on data from a Norwegian cohort study of mothers and children was conducted to assess the association between placement of polymer-based dental fillings during pregnancy and adverse birth outcomes [29]. These studies did not show statistically significant connections between the placement of white dental fillings and stillbirth, congenital abnormalities, preterm births, or low or high birth weights. Although no association was detected between these fillings and adverse birth outcomes in the study, there is a strong need for further research in this area. It is important that dentists and medical personnel make decisions regarding dental treatment in pregnant women in a prudent and cautious manner. It is important to consider the potential risks to the fetus and the available scientific evidence regarding the safety of the dental materials used.

Local anesthesia is a common practice in dentistry to ensure patient comfort and minimize pain during dental procedures. The choice of anesthetic during dental procedures for pregnant women prioritize the safety of both mother and fetus. Commonly, locally administered anesthetics such as lidocaine or mepivacaine are preferred, as they are relatively safe and have a short duration of action [30, 31]. The use of anesthetics containing epinephrine is usually limited in pregnant women, especially during the first trimester. Although studies do not conclusively indicate harmful effects of epinephrine on fetal development, it is recommended to avoid its use, especially in large doses. In cases where dental anesthesia is necessary for a pregnant woman, it is advised to minimize the dosage and frequency of anesthetic use to limit drug exposure.

In summary, dental anesthesia for pregnant women requires special consideration for safety. An individualized

approach, risk minimization, and consultation with the pregnancy care provider are essential to ensure effective dental treatment during pregnancy. All decisions regarding dental treatment for pregnant women should be made on an individual basis such as pregnancy trimester or comorbidities, taking into account the patient's health and the recommendations of the pregnancy care provider. In cases of doubt or concern, consulting with a physician before proceeding with any dental actions is always advised.

CONCLUSIONS

In consideration of the issues surrounding dental infections in pregnant women, it is essential to highlight the importance of a comprehensive approach to dental care during this critical period. While healthcare providers play a pivotal role, the primary responsibility for maintaining health lies with the women themselves. Pregnant women must take an active role in safeguarding their own well-being and that of their unborn child by prioritizing preventive measures, attending regular dental check-ups, and addressing any existing health issues promptly. Healthcare professionals, in turn, should focus on empowering and supporting women in these efforts by providing accurate information, practical advice, and accessible care.

Gynecologists, for instance, should encourage women planning pregnancy to address any existing dental issues beforehand and emphasize the importance of maintaining good oral hygiene and health throughout the reproductive period. Without the willingness of women to engage in proactive health management, even the most advanced healthcare systems cannot ensure optimal outcomes.

Pharmacological and diagnostic restrictions during pregnancy present additional challenges for dental practitioners, but these can be managed effectively through adherence to evidence-based guidelines. Collaboration among healthcare providers, including dentists, gynecologists, and midwives, plays a crucial role in supporting women's efforts to maintain their health by facilitating early detection, appropriate intervention, and ongoing education.

Public education campaigns are also vital in encouraging women to take responsibility for their oral health and to understand the risks of untreated dental infections during pregnancy. Accessible information about the availability of reimbursed health services can further motivate women to seek timely and appropriate care. At the same time, continuous professional development for dentists ensures that they are well-prepared to address the unique needs of this patient group.

Ultimately, improving maternal and fetal health outcomes depends on a shared commitment. Women must lead the way in prioritizing their health, while healthcare providers must focus on enabling and supporting these efforts through education, collaboration, and high-quality care. By fostering such a partnership, we can reduce the risk of complications and ensure better health for both mothers and their babies.

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CONFLICT OF INTEREST
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REVIEW ARTICLE

Study of abortion material in idiopathic habitual miscarriage of pregnancy: Literature review

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ABSTRACT

Aim: The study aims to review current scientific publications on the genetic study of foetal material in habitual miscarriage with an emphasis on a comparative analysis of the methods used traditional karyotyping and chromosomal microarray analysis. The material in this paper is based on an analysis of scientific publications covering the issues under study over the past five years.

Materials and Methods: This study reviewed cytogenetic diagnostic methods for detecting genetic abnormalities in foetal material from recurrent miscarriages. A comparative analysis of the widely used methods, karyotyping and chromosomal microarray analysis (CMA), was conducted to assess their strengths and limitations.

Conclusions: Given its superior diagnostic capabilities, CMA should be adopted as the first-line method for investigating genetic abnormalities in cases of habitual miscarriage. Its ability to detect a wider range of chromosomal abnormalities allows for more accurate diagnosis and better-informed clinical decisions. The use of CMA in routine practice can significantly improve the management of future pregnancies, offering couples a greater chance of successful outcomes and providing reproductive health specialists with a more reliable tool for evaluating repeated pregnancy loss.

KEY WORDS: chromosomal microarray analysis; karyotyping; reproductive health; laboratory diagnostics; genetic abnormalities

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INTRODUCTION

Habitual miscarriage, especially its idiopathic form, is one of the most difficult challenges in modern reproductive medicine, affecting many couples in their reproductive years. According to various studies, up to 4% of couples planning to have children suffer from habitual miscarriage [1]. At the same time, the definition of "habitual miscarriage" varies depending on the authoritative sources. E. Dimitriadis et al. [2] and European Society of Human Reproductive and Embryology (ESHRE) Guideline Group on Recurrent Pregnancy Loss et al. [3] note that according to the recommendations of influential medical organisations such as the American Society of Reproductive Medicine (ASRM) and ESHRE, habitual miscarriage is two or more cases of spontaneous pregnancy loss before 22 weeks. However, the World Health Organization sets a stricter criterion: a diagnosis of habitual miscarriage is made when there are three or more confirmed cases of miscarriage.

The 2012 ASRM opinion contains recommendations for a comprehensive examination of couples with habitual miscarriage, including diagnosis of uterine anatomical abnormalities, antiphospholipid syndrome, endocrine disorders, luteal phase disorders, infections, and karyotyping of the couple. At the same time, ESHRE in its 2017 guidelines excludes the need for parental karyotyping in a standard examination, except for "standard risk assessment" [4]. This difference in the evolution of approaches emphasises the importance of individualised assessment of each couple with habitual miscarriage and the need to flexibly adapt recommendations to specific clinical cases, covering the latest scientific evidence and practical experience in reproductive medicine. Thus, despite a common understanding of the urgency of the problem of habitual miscarriage, approaches to the diagnosis and management of such patients may vary between different reputable medical organisations and expert groups. This underscores the need for further research into this issue and the development of uniform standards of examination and treatment to ensure the most optimal pregnancy outcomes for couples suffering from habitual miscarriage.

G. Svyatova et al. [5] in a study on the etiopathogenesis of habitual non-pregnancy emphasised that more than half of the episodes of non-pregnancy are due to an unspecified cause. This idiopathic form of habitual miscarriage, after secondary diagnostic tests, is often justified by genetic, chromosomal, disorders. This fact determines the need to include genetic analysis in the primary diagnostic algorithm for managing couples facing the problem of habitual pregnancy loss. Identification of genetic, including chromosomal, abnormalities in abortion material allows us to determine the potential cause of miscarriage and to plan further patient management. Timely cytogenetic and molecular genetic testing can provide valuable information for assessing the risk of repeated reproductive failure and

help in choosing the best reproductive interventions. In this regard, K.T. Boboyev et al. [6] noted that a significant proportion of genetically determined cases of miscarriage, especially when using assisted reproductive technologies, are associated with haemostatic disorders. The diagnosis of these disorders is possible by conducting a complex of preimplantation genetic diagnostics using karyotyping or microarray analysis. The prospect of using cytogenetic methods of examination for couples facing the problem of habitual non-pregnancy was considered in the studies of M.M. Bolatovna et al. [7] and A.N. Rybina et al. [8]. Both researcher groups concluded that comprehensive pregravid preparation for such couples is important, especially emphasising the importance of cytogenetic analysis to determine the genetic causes of miscarriage.

Despite the abundance of research and significant progress in the field of cytogenetics, scientists often overlook one important area – direct comparative study and evaluation of various diagnostic methods and approaches used in this field. Conducting such comparative studies is vital, as it identifies the most accurate, effective and informative diagnostic methods for different clinical cases and types of genetic disorders. Comparison of the diagnostic capabilities, advantages and limitations of different approaches could significantly optimise the management of idiopathic forms of habitual miscarriage.

AIM

The study aims to analyse the current literature on the genetic analysis of foetal material in cases of habitual miscarriage and to compare the methods used, especially karyotyping and microarray analysis.

MATERIALS AND METHODS

The present study is based on the results of a large-scale bibliographic search of scientific literature aimed at forming a holistic view of modern views on the problem of habitual pregnancy loss, as well as methods of identifying foetal genetic abnormalities and their comparative assessment. The search focused on two key diagnostic approaches: classical cytogenetic karyotyping and the new chromosomal microarray analysis (CMA).

An extensive search for relevant sources was carried out in leading abstract and scientometric databases, such as ScienceDirect, PubMed, EMBASE, Ovid, Google Scholar and Web of Science. A wide range of keywords was used to extract the necessary information, including "chromosomal microarray analysis", array comparative genomic hybridisation (aCGH), "genetic abnormalities", "karyotyping", "recurrent miscarriage", "abortion material", "prenatal diagnosis", "non-invasive testing". This covered the widest range of scientific publications on the issues under study. In addition, the official guidelines and websites of the leading professional societies and associations in the field of reproductive medicine, genetics and cytogenetics around the world were analysed. This provided up-to-date information on current clinical guidelines and standards in the field of diagnosis and treatment of habitual pregnancy loss.

To ensure the relevance, reliability and practical significance of the selected scientific publications, strict criteria for inclusion and exclusion were formed. Priority was given to original empirical work and large-scale meta-analyses published in reputable peer-reviewed journals over the past five years, from 2019 to 2024. This prioritised the most recent and relevant data, backed by strict scientific standards. In addition, the study included works conducted by research teams from different countries and regions of the world. This ensured geographical and socioeconomic heterogeneity of the sample, which made it possible to evaluate the effectiveness of the studied cytogenetic diagnostic methods in a variety of medical and economic conditions typical of different national healthcare systems.

One of the key criteria for including publications in the study was the presence of practical data from real clinical practice regarding the use of classical karyotyping and CMA in the examination of women or couples experiencing idiopathic habitual miscarriage. Thus, empirically verifiable results of direct clinical value were addressed. At the same time, the study intentionally excluded theoretical papers and reviews that were not supported by practical data, as well as articles that were not of direct practical value for clinical practice and research. This included studies with an excessively theoretical focus or limited relevance to the issues under study.

An additional criterion for exclusion was publications published before 2019. This restriction was introduced to ensure that the data analysed is up-to-date and reflects the most recent trends in the field of study. Finally, the focus was on English-language sources available for analysis by the international scientific community. This provided an opportunity for a comprehensive study of global trends and achievements in the diagnosis of habitual pregnancy loss. Some national works in other languages of particular interest were also included. The final review included more than 50 eligible scientific articles by authors from around the world on modern clinical practices of genetic testing of abortion material in case of miscarriage.

REVIEW AND DISCUSSION

MAIN KNOWN DETERMINANTS OF HABITUAL MISCARRIAGE AND THE PROPORTION OF GENETIC PATHOLOGY AMONG THE CAUSES OF THIS PHENOMENON

The significance of chromosomal changes in the context of recurrent miscarriage is a complex issue that requires analysis of both the genetic background of the parents and the molecular genetic composition of the foetal material. When studying the genetic profile of the parents, it is noted that couples experiencing repeated problems with pregnancy are more likely than the general population to have significant chromosomal abnormalities, including structural abnormalities such as balanced translocations or inversions. On average, up to 5% of such pairs have noticeable changes in the chromosomal apparatus [9]. However, despite the increased risk of miscarriage in the presence of such genetic abnormalities, they do not

Table 1. The main known causes of habitual miscarriage are

Group of etiological factors	Share of the group in the structure of causes of habitual miscarriage	Brief description
Chromosomal abnormalities	40-70%	It includes qualitative and quantitative changes in the chromosomal apparatus of both parents and foetus. It is the most common cause of not only recurrent but also sporadic pregnancy loss.
Immunological factors	10-20%	Certain pathologies of the mother's immune system, such as antiphospholipid syndrome, systemic lupus erythematosus, etc., can initiate foetal rejection in the first trimester of pregnancy.
Anatomical features of the mother's reproductive apparatus	10-20%	Structural disorders of the female reproductive system, such as uterine malformations, polyps and fibroids, can interfere with the development of pregnancy both during implantation and during its development.
Disorders of the mother's endocrine system	10-20%	Disorders of endocrine regulation, both at the level of sex hormones and thyroid hormones, prolactin, etc., can induce pregnancy disorders.
Infections	0.5-5%	Sexually transmitted infections in either partner can affect the development of pregnancy.
Epigenetic factors	Not determined	Factors such as impaired sperm deoxyribonucleic acid integrity and impaired X-chromosome inactivation can contribute to repeated episodes of miscarriage.
Factors related to parental lifestyle and environment	Not determined	A wide range of environmental factors, the role of each of which is difficult to assess, can affect the risk of pregnancy loss.

Source: compiled by the authors of this study

explain all such cases. The main known causes of habitual miscarriage, which can explain up to half of the cases, are shown in Table 1.

As pointed out by C. Gug et al. [10] and D. Lei et al. [11] in a study on molecular cytogenetic analysis of foetal material, chromosomal defects are the main cause of both sporadic and habitual miscarriages, including those, the primary diagnosis of the causes of which did not accurately determine the aetiology of miscarriage. Studies of foetal material show a high level of chromosomal abnormalities [12]. Despite advances in genetic analysis methods, there are still certain challenges to studying the genetic details of abortion tissue. These include difficulties in obtaining suitable samples, contamination by maternal cells, and complex phenomena such as mosaicism and polyploidy, which can render the results interpretation difficult [13].

EVOLUTION OF CYTOGENETIC ANALYSIS METHODS USED IN THE STUDY OF FOETAL TISSUES

Over the past decade, the field of molecular genetic diagnostics has undergone significant changes. This has become possible due to the introduction of a wide range of advanced technologies that ensure accurate detection and analysis of genetic changes [14]. These innovations expanded the possibilities for diagnosing genetically determined diseases, understanding their mechanisms of development, implementing individualised treatment approaches and making informed medical decisions.

As G. Murugappan et al. [15] noted, karyotyping, a method of imaging and subsequent qualitative and quantitative analysis of chromosomes, has traditionally been the standard method for diagnosing genetic changes in foetal material for many years. This procedure involves the microscopic examination of chromosomes isolated from human cell culture, usually peripheral blood lymphocytes. The chromosomes are subjected to a special stain to create a unique pattern of bands that allows the identification of individual chromosomes and their structural features. After staining, the chromosomes are arranged in a certain order to form a karyotype. This method can be used to detect major chromosomal abnormalities. However, despite its usefulness, karyotyping has limited resolution [16]. It can detect changes larger than 3-5 megabases (Mb), but cannot detect smaller genetic disorders such as point mutations or small deletions/duplications. Nevertheless, as M.I. Hanif et al. [17] noted, karyotyping remains an important diagnostic tool in such areas as prenatal diagnosis (detection of chromosomal abnormalities in the foetus), oncogenetics (study of genetic disorders in cancer) and the study of developmental disorders associated with chromosomal defects. Despite its limitations, this method remains central in clinical practice. It provides quick and efficient detection of major chromosomal abnormalities that cause many hereditary diseases and syndromes, especially when, for one reason or another, access to more labour-intensive diagnostic interventions is not possible.

Later, more advanced methods were introduced for the genetic study of foetal material in the case of habitual miscarriage. Fluorescence in situ hybridisation (FISH) is widely used, a highly efficient molecular cytogenetic method that allows visualising specific chromosomal regions using fluorescently labelled probes [18]. This method is based on the hybridisation of these probes with complementary deoxyribonucleic acid (DNA) sequences in preparations of

metaphase chromosomes or interphase nuclei. Fluorescence-labelled probes are fragments of DNA or ribonucleic acid conjugated to fluorochromes that emit a characteristic glow when irradiated with a specific wavelength. These probes specifically recognise and bind to homologous sequences in the genome, visualising them as discrete fluorescent signals. If it is necessary to detect aneuploidy, especially gonosomes and 21, 18 and 13 pairs of somatic chromosomes, the method of quantitative fluorescent polymerase chain reaction is used [19]. This method is implemented by amplifying short, paired-end sequences (STRs) on target chromosomes, which determines its main advantages and disadvantages: high specificity within exclusively targeted chromosomes. To decipher the protein-coding segments of the genome, whole exome sequencing (WES) is used, which allows to identification of most of the significant mutations that are localised in only a few hundredths of the genetic material [20]. In turn, for a broader analysis of the genetic profile of foetal material, whole genome sequencing (WGS) is used, which involves a larger amount of genetic material [21]. The multiplex ligand-dependent probe amplification (MLPA) method is a universal method that allows for the simultaneous quantitative analysis of several dozen (up to 60) nucleotide sequences [22]. This method is used to detect copy number variations (CNVs) associated with rare hereditary diseases. At the same time, the method is not effective in detecting point mutations and balanced chromosomal rearrangements. A summary of the above methods with their advantages and disadvantages is shown in Table 2.

Each of the methods of molecular cytogenetic analysis described here has certain advantages and limitations due to the specifics of its principle of operation and technical features. On the one hand, combining several methods would compensate for their shortcomings and expand analytical capabilities through complementary approaches. However, such a strategy involves a significant increase in the cost of reagents, equipment and staff labour, which makes it economically unfeasible in most cases.

FEATURES OF CMA AS AN INDEPENDENT METHOD AND ITS PLACE IN THE ALGORITHM OF PREIMPLANTATION GENETIC TESTING

According to the observations of L. Lovrečić et al. [23], a new and promising method, CMA, gained particular emphasis. This method combines several advantages of previous methods, such as FISH and karyotyping, but lacks some of their significant disadvantages [24, 25]. CMA is based on the use of high-density DNA microarrays containing many unique oligonucleotide probes covering the genome with high resolution. According to the results of N. Raouf et al. [26], the patient's genomic DNA is hybridised with these probes after special sample preparation, and the intensity of hybridisation signals is analysed using specialised software. The key advantages of CMA are high resolution and complete coverage of the genome up to

Table 2. Summary characteristics of the most common modern methods of molecular cytogenetic diagnostics used in connection with idiopathic habitual miscarriage

Name	Resolution	Pros	Cons	Target pathologies
WES	Exon only (1-2% of the genome)	Fast results, ability to detect contamination of foetal material with maternal cells, possibility to use both canned and fresh material	Ignoring non-coding genes, significant error rate in detecting deep CNV variants, failure to detect balanced rearrangements	Monogenic diseases, rare genetic diseases.
WGS	The entire genome, including both coding and non-coding regions	Full genomic coverage of both canned and fresh material, ability to detect contamination of foetal material by maternal cells, high-resolution	High cost, failure to identify balanced re-arrangements	Mono- and polygenic hereditary diseases.
FISH	Chromosome-specific loci	Fast results, high specificity, no need to culture the material	A significant proportion of false positives and material coverage is limited by sensor specificity	Chromosomal abnormalities including all chromosomes (poly and aneuploidies)
QF-PCR	Exclusively targeted chromosomes	High specificity within the target chromosomes, fast results, locus specificity, ability to detect contamination of foetal material by maternal cells	Restriction to target chromosomes	Chromosomal abnormalities (mainly aneuploidies) of the target chromosomes
MLPA	CNVs specific to a gene or locus	Low-cost, one-step analysis of a wide range of nucleotides	Inability to detect contamination of foetal material by maternal cells, mosaicism and balanced rearrangements	Detection of rare hereditary diseases, including those associated with mental retardation

Source: compiled by the authors of this study

the detection of submicroscopic deletions/duplications of only a few kilobases, simultaneous analysis of the entire genome, without the need for preliminary mapping of the target locus, quantitative assessment of the number of DNA copies for each probe sequence, high degree of automation and standardisation of the procedure, the ability to analyse not only deletions/duplications but also complex chromosomal rearrangements, both balanced and not [27]. At the same time, CMA does not have some of the disadvantages of other methods. Unlike FISH, it provides whole-genome analysis without the need for multiple locus-specific probes. Compared to karyotyping, CMA has a much higher resolution (up to 100kb), does not require culturing, and allows for reliable detection of submicroscopic changes [28].

As R.S. Papas and W.H. Kutteh [29], S. D'Ippolito et al. [30], in the field of reproductive medicine and genetics, diagnosis and therapy of habitual miscarriage are carried out using various methods of cytogenetic analysis. Among them, traditional karyotyping and microarray analysis are of paramount importance, the former being the gold standard in detecting chromosomal abnormalities in fetal material. These methods are recommended in the presence of two or more consecutive idiopathic cases of miscarriage and are also actively used in preimplantation genetic testing (PGT), which requires embryo biopsy and in vitro fertilisation [31, 32]. Even though numerous studies have demonstrated the impressive effectiveness of CMA in detecting chromosomal abnormalities in miscarriage, especially in comparison with traditional karyotyping, some technical aspects of this method remain controversial. Although the effectiveness of CMA in detecting chromosomal abnormalities is not in doubt, questions arise regarding several ancillary factors that may affect the results of the test. One of these factors is the choice of microarray platforms (matrices) and their resolution. Different commercial matrices may have different sensitivities to certain types of genomic variations, which should be addressed when interpreting the results. In addition, there is a risk of contamination of the test material with foreign DNA, which can lead to false-positive or false-negative results. Finally, the need to use assistive technologies is being debated. These methods can be costly and time-consuming but are often necessary to accurately interpret the results and develop an optimal patient management strategy.

While the results of the karyotyping method, due to its well-established standardised methodology and simpler implementation, do not raise significant questions, the analysis of the effectiveness of CMA in different situations, especially when comparing the experience of researchers from different countries, faces some problems. As J.O. Olayiwola et al. [33] demonstrated, the use of microarray platforms from different manufacturers can lead to different results. The use of NimbleGen 135K matrices was less effective compared to Agilent 180k (detection of pathogenic variations in 8.7% and 11.25% of cases, respectively). This fact illustrates the main limitation of this study: most of the scientific studies conducted by different authors around the world use different technical components, such as microarray platforms, data analysis software, and criteria for interpreting the results. This diversity of methodological approaches makes it very difficult to form a holistic view of the real value of CMA in diagnosing the genetic causes of miscarriage. Although the advantage of this method over classical karyotyping is undeniable, it is difficult to determine the exact extent of this advantage. Thus, to obtain more objective and comparable data on the effectiveness of CMA, further standardisation of research protocols, the use of microarray platforms, criteria for interpreting results, as well as international cooperation and exchange of experience between different research centres is required. Only with the unification of methodological approaches will it be possible to conduct reliable meta-analyses and develop generally accepted clinical guidelines for the use of CMA in the diagnosis of the causes of habitual miscarriage.

COMPARISON OF THE DIAGNOSTIC POTENTIAL OF KARYOTYPING AND CMA IN THE FRAMEWORK OF CYTOGENETIC EXAMINATION OF ABORTED MATERIAL

Addressing the priority of CMA and karyotyping in the cytogenetic study of foetal material, R.J. Martinez-Portilla et al. [34] pointed out that the use of technologies based on genomic hybridisation can increase the efficiency of detecting chromosomal abnormalities by about 15%, which is reflected in the optimisation of anomaly detection from 75% to 90% compared to classical karyotyping. In addition, CMA can detect additional structural abnormalities in cases where a normal karyotype was previously detected using traditional methods, which is typical for up to 8% of all cases [35]. Studies have confirmed the feasibility of CMA in the case of habitual miscarriage, even with a preliminary detection of a normal foetal karyotype [36]. As for the detection of variants of unknown clinical significance, the method, compared to karyotyping, increases the detection rate by about 10% among structurally normal abortions.

The meta-analysis demonstrated that in cases where developmental disorders are manifested without karyotype changes visible to classical methods, their detection is possible with CMA. When detecting a single anomaly, CMA can detect karyotype abnormalities in 3-8% of cases, and when detecting two or more anomalies, it can detect them in 10% of cases [37]. The method is also more effective in terms of genomic imbalance, which is a disturbance in the quantitative content of genetic material in the cells of the body, which can lead to various genetic and chromosomal abnormalities. It can be manifested in CNVs, which include deletions or duplications of DNA. The size of the genomic imbalance plays a significant role in determining the pathogenicity of such abnormalities. Studies have shown that approximately 15.24% of miscarriages can be associated with pathogenic copy number variations, and this association does not always correlate with clinical manifestations. In particular, the study determined that CNVs exceeding 3 Mb in size have a high probability of pathogenicity in miscarriage. In addition, about 40% of CNVs between 1 and 2 Mb in size can

also be pathogenic. One of the most common pathogenic copy number variations found in conception products is a deletion at 8p23.3, which is especially common in women of Chinese descent [38]. Analysis of the size of genomic imbalances provides valuable information for assessing the risk of developing genetically determined conditions and determining appropriate strategies for their diagnosis and treatment in cases of miscarriage. CMA proves to be more effective for these purposes due to its higher resolution compared to traditional karyotyping [39].

CMA demonstrates not only high efficiency in identifying genetic abnormalities but also has significant economic benefits for both the healthcare system and patients. F. Popescu et al. [40] in a cost study based on the use of 24-chromosome microarrays for the investigation of products of conception established that the use of this method contributes to a 50% reduction in financial costs compared to traditional methods of diagnosing genetic abnormalities in products of conception. These savings are achieved by reducing the need for repeated medical interventions, reducing the time for diagnosis and treatment, and optimising the process of identifying potential genetic problems, which allows for more efficient allocation of resources to targeted therapeutic and preventive measures. In addition to direct financial benefits, the use of this method contributes to improving the quality of patient care by providing faster test results and reducing psychological stress from waiting for a diagnosis.

Thus, the introduction of CMA into clinical practice represents an important step forward in the field of genetic diagnosis of miscarriage, providing not only increased efficiency in detecting genetic abnormalities, but also significant cost savings for the entire healthcare system and, most importantly, for patients. However, further discussion is required on the application of this method, considering the peculiarities of tissue culture, some specific genetic anomalies and the potential need for additional interpretation of the results.

PROSPECTS FOR THE DEVELOPMENT OF MULTIMETHOD DIAGNOSTIC ALGORITHMS, INCLUDING CMA IN CONJUNCTION WITH OTHER RESEARCH METHODS

It is important to note that the use of CMA, unlike most other cytogenetic tests, suffers to a much lesser extent from contamination of foetal material by foreign (maternal) cells. Many of the studies analysed in this literature review highlight this as one of the main advantages of CMA but do not demonstrate the exact measure of this advantage. However, M. Vinciguerra et al. [41] illustrate the high resolution of CMA in detecting foetal chromosomal abnormalities even with a minimal amount of foetal material. The researchers were able to perform a full cytogenetic analysis of the foetus based on the collection of whole blood containing only a few foetal cells. These results are consistent with the findings of this literature review and demonstrate the outstanding ability of CMA to detect foetal genomic variations in samples with low foetal cell counts. This aspect is often overlooked by other authors when they focus too much on comparing CMA with other cytogenetic techniques in terms of the overall efficiency of chromosomal abnormalities detection. However, the ability of CMA to perform highly accurate analysis even in conditions of significant maternal cell contamination is an undeniable advantage, especially valuable in the study of miscarriage products, where limited amounts of foetal material are often available.

The use of cytogenetic methods is often considered exclusively in the context of their comparison with each other, without addressing additional instrumental techniques that can be used in combination. However, in practice, cytogenetic analysis is often performed after a preliminary ultrasound examination (ultrasound) to detect possible anatomical abnormalities of the foetus. K. Gajjar et al. [42] demonstrated that the isolated use of cytogenetic methods, such as karyotyping and FISH, in combination with ultrasound data is not sufficiently effective for detecting chromosomal abnormalities. At the same time, chromosomal CMA was able to detect pathogenic variations in 38% of cases missed by karyotyping and FISH. This study, which is comparable to the results of the present literature review, clearly demonstrates that CMA has a significantly higher diagnostic value than traditional cytogenetic methods, even with additional information obtained by ultrasound. These findings highlight the importance of a comprehensive approach to the diagnosis of chromosomal abnormalities in miscarriage, including not only cytogenetic studies but also other auxiliary methods such as ultrasound. However, they also point to the need to consider CMA as a primary genetic screening method for habitual pregnancy loss, as it can detect abnormalities missed by other approaches.

The development of multimethodological diagnostic approaches integrating CMA and cytogenetic methods that complement its results for couples with habitual miscarriage seems to be a promising area. The integrated use of various diagnostic technologies will allow us to identify a wider range of genetic abnormalities, including difficult-to-diagnose variants, and thus contribute to a deeper understanding of the aetiology of this disorder. At the same time, optimised monitoring of a new pregnancy involving regular ultrasound support with an increased frequency of scheduled imaging is also relevant. This will provide timely identification of potential complications at early stages and promptly take the necessary therapeutic measures, reducing the risk of pregnancy termination.

The introduction of such multi-level diagnostic protocols, combined with close monitoring of a new pregnancy, could be a fundamentally new stage in the practical management of habitual miscarriage. The comprehensive nature of this approach, which combines modern molecular genetic methods and optimised instrumental diagnostics, can provide a higher level of identification of causative factors and allow for the development of individualised pregnancy management regimens based on the specifics of each clinical case. Such a strategy has the potential to significantly increase the success rate of gestation in couples with a history of reproductive pathology.

PROBLEMS IN THE USE OF MICRO-MATRIX ANALYSIS TECHNOLOGY, DETERMINED BY THE SPECIFICITY OF METHODOLOGICAL APPROACHES AND THE PRESENCE OF DIFFICULT-TO-INTERPRET GENETIC DISORDERS

For many decades, despite significant progress in the field of cytogenetics, as demonstrated in the present study, there has been an active debate about the diagnosis of mosaic forms of chromosomal pathologies, including in aborted material. As P. Tulay et al. [43] noted, the diagnosis of mosaic forms of chromosomal abnormalities depends on the number of tissues examined, their culture conditions and the degree of mosaicism. Considering the data presented in this literature review, it is worth noting that these diagnostic conditions are extremely unfavourable for classical karyotyping, but do not represent significant limitations for CMA. Due to its high sensitivity and ability to detect genomic variations even at low levels of mosaicism, CMA is well suited for the diagnosis of mosaic forms of hereditary diseases and is therefore a more optimal means of primary diagnosis compared to karyotyping. Traditionally, the diagnosis of mosaic forms of chromosomal abnormalities has been associated with a range of difficulties due to the limitations of classical cytogenetic methods. However, the introduction of CMA has significantly improved the accuracy and reliability of mosaic variation detection, which is especially important in cases of habitual miscarriage, when one often has to deal with a limited amount of foetal material and the possible presence of mosaicism.

Addressing the technical issues discussed, such as the choice of microarray platforms, the risk of contamination of the test material, the prospect of combining with instrumental methods such as ultrasound, and the difficulties of diagnosing mosaic forms of chromosomal abnormalities, CMA has proven to be a highly effective genetic diagnostic tool for common miscarriage, significantly outperforming classical karyotyping. The lack of standardisation of CMA protocols makes it difficult to compare data from different sources and complicates meta-analyses and the development of accepted clinical recommendations. To properly implement the potential of CMA in diagnosing the causes of habitual miscarriage, it is essential to achieve global standardisation of methodological approaches. This includes the unification of microarray platforms, software, criteria for interpreting results, and the development of common research and quality control protocols.

CONCLUSIONS

The literature review demonstrates that CMA is an effective diagnostic tool that not only provides information on copy number variations (deletions and duplications) but also allows for the identification of heterozygosity status, which facilitates the detection of new genetic changes associated with recurrent miscarriage. This additional information is essential for improving the management of subsequent pregnancies and developing personalised therapeutic approaches.

When CMA is used to analyse foetal material, a significantly higher detection rate of chromosomal abnormalities is observed compared to traditional karyotyping. This advantage is primarily due to the success of CMA in cases where cell culture is difficult or impossible. Due to the above, as well as the ability to analyse contaminated material, CMA can be considered a first-line method for determining the cause of miscarriage with higher accuracy and sensitivity than karyotyping. However, it is important to note that, despite its undoubted advantages, the CMA is not without certain limitations. This method does not allow the detection of polyploidies and balanced translocations, as they do not lead to changes in the number of gene copies. Thus, for the comprehensive diagnosis of chromosomal abnormalities in habitual miscarriage, it is advisable to use CMA in combination with other advanced cytogenetic methods.

A significant limitation of this study was the lack of standardisation of methodological approaches to CMA in different laboratories, which made it difficult to compare and interpret the results. Further research in this area should focus on the development of uniform protocols for CMA, including the unification of microarray platforms, data analysis software and criteria for interpreting the identified genomic variations. Further work is also needed to evaluate the clinical significance of various genomic variations detected by CMA to better define their association with habitual miscarriage and develop effective therapeutic strategies.

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CONFLICT OF INTEREST
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REVIEW ARTICLE

The right to privacy in medical legal relations in the practice of the European court of human rights: Experience for Ukraine

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ABSTRACT

Aim: To analyse the problems of implementing an individual's right to privacy in medical legal relations and to characterise the practice of the European Court of Human Rights (ECtHR).

Materials and Methods: The methodological basis of the article is a set of methods and techniques of scientific knowledge. Methods of theoretical analysis, system-analytical and comparative-legal methods made it possible to characterise the problems of protecting the right of an individual to privacy in medical legal relations, taking into account the practice of the ECtHR.

Results: The right to privacy in medical legal relations is a fundamental principle of the legal systems of the states parties to the European Convention. The right to privacy in medical legal relations is complex and forms part of the right to health. The ECtHR, in many of its decisions in the field of healthcare, has emphasised the existence of both negative and positive obligations of the state to ensure the right to respect for the private and family life of patients. States parties to the European Convention must not only refrain from unlawful interference, but also create mechanisms for the effective protection of patients' privacy, including the protection of confidential data.

Conclusions: The practice of the ECtHR in implementing an individual's right to privacy in medical legal relations is a dynamic source of law and contributes to the formation of uniform judicial practice in the context of protecting the rights, freedoms and interests of individuals in the field of healthcare.

KEY WORDS: European Court of Human Rights, privacy, healthcare

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INTRODUCTION

Ukraine's ratification of the Convention for the Protection of Human Rights and Fundamental Freedoms allows all persons under its jurisdiction to apply to the European Court of Human Rights if they consider their rights to have been violated. Part 4 of Article 55 of the Constitution of Ukraine enshrines the right of every citizen, after exhausting all national methods of legal protection, to apply for the restoration of their violated rights and freedoms to the relevant international judicial institutions or bodies of international organisations of which Ukraine is a member or participant. One of the most important personal rights, which most clearly reflects human freedom and the right to health, is the human right to privacy in medical legal relations.

Both domestic and foreign scientists have drawn attention to certain legal problems in the field of healthcare in the context of the implementation of the right to innovation [1], and have investigated the legal issues of judicial protection of patients' rights [2]. In particular, there is increased interest in the legal problems of implementing individual rights in the field of healthcare, taking into account the practice of the European Court of Human Rights, from both domestic [3] and foreign scholars. However, these studies did not conduct a comprehensive analysis of the practice of the European Court of Human Rights on the implementation of the right to privacy in medical legal relations in modern conditions, in particular regarding the protection of the right to information and the confidentiality of patient data.

AIM

To analyse the problems of implementing an individual's right to privacy in medical legal relations and to characterise the practice of the European Court of Human Rights (ECtHR).

MATERIALS AND METHODS

The methodological basis of the article is a set of methods and techniques of scientific knowledge. Methods of theoretical analysis, system-analytical, and comparative legal methods made it possible to characterize the problems of protecting the individual's right to privacy in medical legal relations, taking into account the practice of the European Court of Human Rights. The comparative legal method was used to identify trends and compare the approaches of various European Court of Human Rights cases in the context of protecting the individual's right to privacy in medical legal relations. The norms of the European Convention on Human Rights regarding the implementation of the

individual's right to privacy in medical legal relations were analyzed using a formal-logical method, the content of the main categories was determined, and problematic issues were systematized in order to obtain conclusions within the framework of the research task. The method of systems analysis made it possible to identify problematic issues of protecting the right of an individual to privacy in medical legal relations.

REVIEW

The general norms that define a person's right to privacy in medical legal relations should be understood as the right to the inviolability of personal and family life, respect for one's home, and the secrecy of correspondence. This right of an individual in the field of healthcare is defined in the provisions of international documents such as the Universal Declaration of Human Rights (Article 12) and the International Covenant on Civil and Political Rights (Article 17). The provisions of the Convention for the Protection of Individuals with regard to Automatic Processing of Personal Data, as well as the Lisbon Declaration on the Rights of the Patient [4], which establish a patient's right that is subordinate to the doctor's obligation to ensure confidentiality, are of great importance. This document expands the understanding of information that is not subject to disclosure, indicating the confidentiality of medical and personal information about a patient.

The Declaration on the Promotion of Patients' Rights in Europe states that every person has the right to respect for their privacy. The Declaration defines a list of information that is confidential: "All information about the patient's health status, diagnosis, prognosis and treatment of his or her disease, as well as any other information of a personal nature, must be kept secret, even after the patient's death" (Clause 4.1). It also contains a provision on the mandatory consent of the patient to the disclosure of information; establishes the possibility of adopting exceptions to the disclosure of information without consent at the national legislative level (Clause 4.2); and protects the patient's personal data (Clause 4.3) [5].

Article 40 of the Law of Ukraine "Fundamentals of the Legislation of Ukraine on Health Care" indicates that "medical workers and other persons who, in connection with the performance of their professional or official duties, become aware of the illness, medical examination, examination and their results, the intimate and family aspects of a citizen's life, do not have the right to disclose this information, except in cases provided for by legislative acts." [6]. Article 286 of the Civil Code of Ukraine provides that an individual has the right to confidentiality regarding their health status, the fact of seeking medical care, diagnosis, and information obtained during his or her medical examination [7].

The issue of protecting the right to privacy in medical legal relations has been repeatedly addressed by the European Court of Human Rights. According to Article 8 of the Convention, everyone has the right to respect for their private and family life, their home and their correspondence [8]. Based on the content of Article 8 of the Convention, the following areas of private life and family life are distinguished. The following areas of private life are distinguished (physical, psychological, and moral integrity): (1) victims of violence; (2) reproductive rights; (3) compulsory treatment and mandatory medical procedures; (4) mental illness; (5) health care and treatment; (6) end-of-life issues; (7) disability issues; (8) burial issues; (9) environmental issues; (10) sexual orientation and sexuality; (11) professional and business activities [6]. And also includes the following components (Fig. 1) [9].

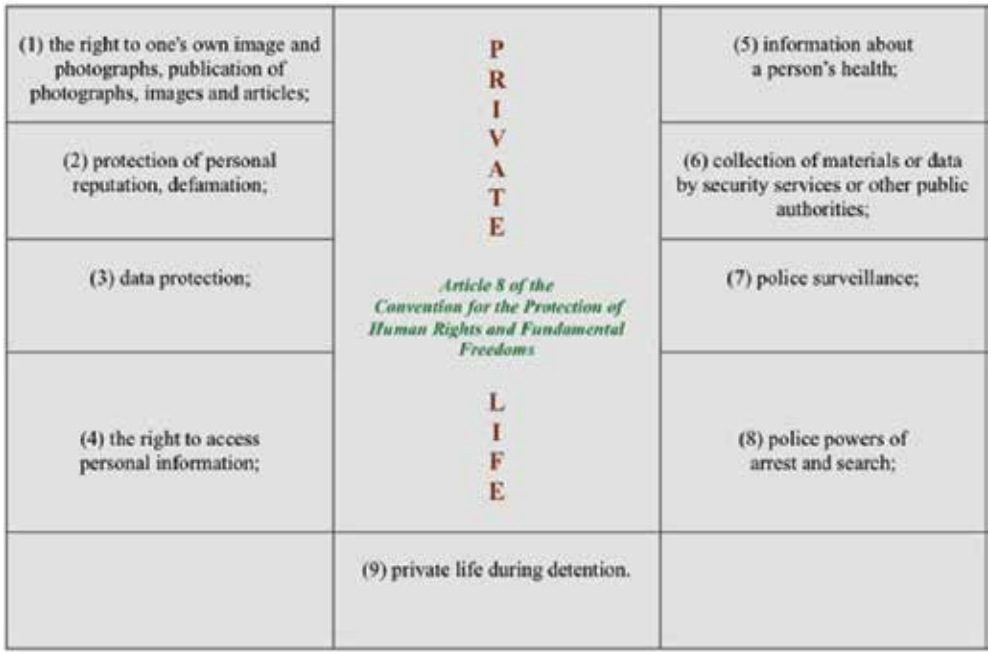

Fig. 1. The main components of the right to privacy in medical-legal relations
Source: compiled by the authors of this study

In particular, the "right to privacy" has been analysed in many decisions of the European Court of Human Rights and encompasses many components, namely: the physical and psychological integrity of a person ("Pretty v. The United Kingdom"), numerous aspects of a person's physical and social identity ("Mikulić v. Croatia"), gender identification, name, sexual orientation, and sex life, all of which fall within the scope protected by Article 8 of the Convention ("Bensaid v. the United Kingdom"). A person's name, private life, and other means of personal identification and contact with family also fall within the scope of protection ("Burghartz v. Switzerland"). Information about a person's health is included ("Z v. Finland"), as are certain structural components of the right to one's image ("Sciacca v. Italy"), etc. According to Article 8 of the Convention, the state must not only refrain from unlawful interference but also create a mechanism for the effective protection of private life [8].

Let us consider a number of decisions on the protection of patient data in the European Court of Human Rights' rulings regarding the confidentiality of patient health information. First, we note that the European Charter of Patients' Rights proclaims that everyone has the right to the confidentiality of personal information, including information about their health status and possible diagnostic or therapeutic procedures, as well as to the protection of their privacy during diagnostic examinations (Article 6) [10]. We also point out that confidential information is information to which access is restricted by an individual or legal entity, other than those in authority, and which may be disseminated in a manner determined by them at their request and under the conditions they stipulate [11].

Personal information concerning a patient falls within his "private life" (I. v. Finland, § 35 [12], and L.L. v. France, § 32 [13]). The protection of personal data, not only medical data, is fundamental to the exercise by an individual of his right to respect for private and family life, guaranteed by Article 8 of the Convention [14]. In the case of Mayboroda v. Ukraine, the applicant complained that the State had failed to protect her right to informed consent in connection with a nephrectomy (surgical removal of a kidney) performed during emergency care while she was unconscious, and against doctors' concealment of information about the removal of the kidney in the postoperative period. The Court considers that the respondent State has failed to fulfil its positive obligation to establish an adequate legal framework to protect the applicant's right to informed consent [15]. There has therefore been a violation of Article 8 of the Convention [8].

For example, in the case of "Radu v. the Republic of Moldova", the applicant alleged interference in her private life by a medical institution, in particular when the applicant was on sick leave due to a threatened miscarriage. The employer contacted the clinic to clarify the reason for his employee's absence. In response, the clinic provided the employer with all the information, including how the woman became pregnant. According to the applicant, the employer told all the employees about this, and due to the stress she suffered, she had a miscarriage. The woman then accused both the clinic and the employer of interfering in her private life and disclosing private information. The court found this claim admissible [16].

It is important not only to respect the patient's medical confidentiality, but also to maintain his trust in the medical profession and the medical services in general (P. and S. v. Poland, § 128[17]; and L.H. v. Latvia, § 56 [18]). Without protection, individuals in need of medical care may avoid disclosing such personal and intimate information as may be necessary to obtain appropriate treatment, and may even avoid seeking such care, thereby endangering their health (Z v. Finland, § 95) [19]. In this case, during criminal proceedings against the applicant's ex-husband, who knowingly infected others with HIV, the courts authorised the taking of statements from the applicant's doctor and psychiatrist regarding her medical condition, despite her objection.

Furthermore, the courts authorised the seizure of the applicant's medical records without her consent and attached them to the criminal case file. The local court ruled that the full text of the decision, which mentioned the applicant's name, as well as the case documents, would remain confidential for 10 years. Instead, the Court of Appeal issued a full judgment, a copy of which was made available to the media, which mentioned the applicant's name and her HIV status. The applicant complained about these facts to the Court under Article 8 of the Convention (Z v. Finland, § 95) [18]. Therefore, national legislation must provide appropriate safeguards to prevent the transfer or disclosure of personal health data that may conflict with the guarantees of Article 8 of the Convention [8].

Violation of a person's rights to confidentiality in medical-legal relations is also possible when communicating with representatives of state authorities and law enforcement agencies. All information must be provided by medical professionals under an official request. In particular, in the European Court of Human Rights decision in the case of Avilkina and Others v. Russia, the Court noted that "the violation of the right to respect for private life, as guaranteed by Article 8 of the Convention for the Protection of Human Rights and Fundamental Freedoms, consisted in the fact that information about the applicants' refusal of blood transfusion was provided by the Health Committee at the request of the prosecutor's office. The request for the information was submitted by the prosecutor's office as part of an investigation conducted against a religious organization of which the applicants were members. The European Court of Human Rights found such interference in the applicants' private lives to be unjustified" [19].

International law also contains provisions on ensuring the confidentiality of medical information about a patient during scientific research. At the same time, the European Court of Human Rights has also issued contradictory decisions on its application in practice. Thus, in the decision in the case of Gilberg v. Sweden, it was this aspect, namely the right not to disclose information that was collected in confidence, as opposed to the right of researchers to have access to medical research materials, that became the cause of the dispute that the European Court of Human Rights resolved in its decision [20]. Therefore, the European Court

of Human Rights, in many of its decisions, emphasized the existence of both a negative and positive obligation of the state to ensure the right to respect for private and family life in the field of healthcare. In turn, the negative obligation is embodied in the protection of private and family life, home, and correspondence from arbitrary interference by the state. The European Court of Human Rights considers that there is a certain line between the negative and positive obligations of the state in protecting the individual's right to privacy in medical legal relations. By its established practice, when determining obligations, it is necessary to take into account a fair balance in this area, which should be established between the competing interests of society and individuals when exercising the right of an individual to privacy in medical legal relations.

DISCUSSION

Some researchers have paid attention to the study of the protection of patients' rights in the healthcare sector [1, 2] and have also studied the practice of the European Court of Human Rights [3, 21]. Other scholars point out that one of the important areas of the right to privacy is a patient's medical data, which is subject to legal protection [22]. The protection of confidential medical information is reflected in national legislation. Importantly, medical data is particularly sensitive as it concerns the most intimate sphere of a person, revealing information about their physical and mental health [21]. The protection of personal medical data in national legislation has many problems, including legal uncertainty about the scope of information subject to medical confidentiality and the lack of a clear indication of the range of subjects who, when performing professional or official duties, must adhere to the principle of confidentiality [23]. Determining exactly what amount of information about a patient's health status may be harmful to health or the treatment process is an evaluative process. Each treating physician, based on their professional experience, knowledge of the disease, the risks associated with it, and other factors, may determine differently what information about the patient's health may be unfavorable to them and their relatives. At the same time, the doctor is also guided by the subjective perception they have of the patient. The doctor provides the patient with incomplete information about their health status, limiting the patient's real opportunities to exercise their other rights (for example, the right to be examined by another specialist or the choice of treatment).

The problem remains the lack of a comprehensive approach to defining the definitions of "medical confidentiality" and "medical information." Such uncertainty creates problems in law enforcement when exercising a person's right to privacy in medical legal relations. Among such problems of law enforcement, it is worth noting the existence of several definitions that express the identical content of one legal phenomenon. In addition to the above categories, medical legislation uses the terms "health secret", "confidential information about the mental health of a person", and "medical information". These terms are used as synonyms in national legislation. Yes, it is a deficiency of legal regulation that Article 39-1 of the Law of Ukraine "Fundamentals of the Legislation of Ukraine on Healthcare" indicates that "a patient has the right to confidentiality about his health status, the fact of seeking medical care, diagnosis, as well as information obtained during his medical examination." And Article 40 of this Law of Ukraine contains the same data, only with an indication of the prohibition of disclosure, which is already considered data constituting a medical secret [6]. Some scientists determine that the most successful definition today is the one used in the provisions of the Civil Code of Ukraine - "the right to confidentiality of health." [7]. It should be noted that the right to confidentiality covers: the fact of a person's application to a medical institution for medical care; the person's health status; the diagnosis; the circumstances that preceded or provoked the disease; functional features of the body; bad habits, psychological characteristics, property status, other information obtained during a medical examination, in particular information about the person's family and intimate life, as well as the health status of relatives and close friends of the patient

The acceptability of the name "medical confidentiality" is actively debated in scientific works. Many scientists consider it unsuccessful and insist on introducing the concept of "medical confidentiality". We are also inclined to such an interpretation [24], since the term "medical" has a significant drawback - the subject of confidentiality of confidential information is only a doctor, with a broad interpretation - a medical worker. In addition, when it comes to relatives or legal representatives who are informed about the health status of a patient or a loved one, this does not always coincide with the patient's wishes. There are known cases when a patient does not want to inform his relatives about his illness, and wants to keep it a secret. Therefore, the right to information about a person's health and to ensure its confidentiality is legally enshrined and guaranteed by national legislation, but it needs to be improved, taking into account the practice of the European Court of Human Rights.

CONCLUSIONS

The practice of the European Court of Human Rights in the application of an individual's right to privacy in medical legal relations is a dynamic source of law and contributes to the development of a unified judicial practice in the context of protecting the rights, freedoms, and interests of an individual in the field of healthcare. An individual's right to privacy in medical legal relations is complex and is part of the right to health. A key component of an individual's right to privacy in medical legal relations is the right to information and the protection of confidential data. Respect for the confidentiality of health information is a fundamental principle of the legal systems of all States Parties to the Convention for the Protection of Human Rights and Fundamental Freedoms. The legal regime for the confidentiality of patient information involves its non-disclosure to any third party. The disclosure of confidential patient information is permitted only in the interests of national security, economic well-being, and human rights.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

The impact of caffeine consumption on reproductive health. A literature review

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ABSTRACT

Aim: Caffeine is popular stimulant added to a lot of drinks, such as coffee, tea, sodas, and energy drinks. However, there is a dispute over how caffeine affects reproduction and fertility for both men and women. This review aims to provide an insight into this matter including the impact of caffeine on male and female fertility, primary infertility, fertility treatment outcomes, time to pregnancy, spontaneous abortion, sperm ploidy, assisted reproductive technologies, sperm DNA and semen quality.

Materials and Methods: The review is based on the thorough analysis of the materials selected from "PubMed" and "Google Scholar" scientific databases using the following key words: caffeine; coffee; fertility; infertility; tea. These key words were chosen based on their relevance to the matter of an article.

Conclusions: According to the researched studies, moderate caffeine consumption does not pose a hazard for fertility neither regarding men, nor women. However, high caffeine intake, may pose reproductive risks, including an increased miscarriage risk in women and compromised semen quality in men. These findings support the current health guidelines recommending caffeine intake moderation for individuals of reproductive age. Nonetheless, further studies are required to clarify the nuanced effects of caffeine on fertility and assisted reproductive technologies outcomes to provide individuals with the essential knowledge to make proper dietary choices during family planning.

KEY WORDS: caffeine, coffee, fertility, infertility, tea

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INTRODUCTION

Globally, between 15% and 26% of couples face fertility issues at some point during their reproductive years [1–3]. One of the issues worth discussing while considering this matter is a decline of semen quality over the recent decade [1–3]. This important observation has prompted the investigation regarding the impact of lifestyle factors on men's reproductive health, such as for example caffeine consumption [4–6]. It is worth to notice that the impact of caffeine, not only on male but also on female fertility can have major public health implications [7] and as global fertility rates decline, it is vital to draw more attention to all the potential factors that may influence one's reproductive health [1, 3, 8].

AIM

The aim of this review is to focus mainly on one of them and to provide an insight into the matter of the influence of caffeine consumption on fertility in terms of primary infertility, fertility treatment outcomes, time to pregnancy (TTP), spontaneous abortion (SAB), sperm ploidy, assisted reproductive technologies (ART), sperm DNA and semen quality by investigating findings from multiple research studies

MATERIALS AND METHODS

The review is based on the thorough analysis of the materials selected from "PubMed" and "Google Scholar" scientific databases using the following key words: caffeine; coffee; fertility; infertility; tea. These key words were chosen based on their relevance to the matter of an article.

REVIEW AND DISCUSSION

CAFFEINE- DEFINITION AND INFLUENCE ON GENERAL HEALTH

Caffeine (1,3,7-trimethylxanthine) is an organic chemical compound contained in coffee, tea, sodas, energy drinks, and other food products [9]. Known for its stimulating effects on the central nervous system, caffeine has become an integral part of many people's daily routines [10]. It ought to be highlighted that caffeine influences human organism in a wide range of ways, for instance, it increases secretion of catecholamine, relaxes muscle tissue and stimulates the heart [11]. Furthermore, moderate caffeine consumption

may lead to decreased risk of Parkinson's disease [12], liver disease, cardiovascular diseases such as heart failure, coronary heart disease and arrhythmia, as well as diabetes [10]. A study conducted by Agardh et al. [13] indicate that people who consume large amounts of coffee have a reduced risk of type 2 diabetes and impaired glucose tolerance, which may be related to an improved insulin sensitivity as well as an increased insulin response. Nevertheless it is important to emphasize that coffee consumption may provide not only beneficial, but also negative implications as extended amounts of caffeine may contribute to the damage of human health including the matter of fertility which is going to be the main focus of this review.

Coffee consumption can have both negative and positive effects, also on fertility where evidences are mixed [8, 11, 14]. There is no specific mechanism for the effects of caffeine, coffee, tea on fertility in women or men [7, 9].

CAFFEINE'S IMPACT ON FEMALE FERTILITY

Primary Infertility

Lív í Soylu et al. (2017) investigated the potential correlation between caffeine intake from coffee and tea and the risk of primary infertility [7]. This Danish cohort study followed 7,574 nulliparous women aged 20–29 over two decades, linking self-reported caffeine consumption to primary infertility diagnoses. The findings revealed no significant association between coffee or tea intake and increased infertility risk. Neither additional servings of these beverages nor overall caffeine intake were linked to a higher infertility risk, suggesting that moderate caffeine intake, particularly from coffee and tea, does not adversely affect female fertility. However there are numerous discrepancies in the available articles. A number of studies have indicated that the consumption of coffee, tea or caffeine may have a detrimental effect on female fertility [14,15]. However, other studies have not found any correlation between caffeine intake and fertility, or have even suggested that caffeine may have a beneficial effect [4].

Fertility treatment outcomes

Lyngsø et al. (2019) examined the effects of coffee consumption on women undergoing assisted reproductive technology (ART), including intrauterine insemination (IUI), semen - *in vitro* fertilization (IVF), and frozen embryo transfer [8]. In women undergoing IUI, there was an association between low to moderate coffee consumption and increased pregnancy and live birth rates after the first cycle of treatment. This association decreased in subsequent IUI cycles [8]. A cohort of 1,708 women revealed that those consuming 1–5 cups of coffee daily (moderate consumers) experienced a 1.5-fold increase in clinical pregnancy and live birth rates during IUI treatment, compared to non-drinkers [8]. However, this association was not observed in more invasive treatments like IVF and embryo transfer, suggesting that moderate coffee intake may support less intensive fertility procedures like IUI but may not affect outcomes for other ART techniques [8]. Additionally, Lyngsø et al. (2017) stated that ART outcomes were not significantly impacted by caffeine intake [16].

What is interesting, Huang et al. [17] observed that coffee or tea consumption was associated with better pregnancy outcomes and live births, but the study had limitations due to a lack of detailed analysis of caffeine sources and amounts.

In addition, studies such as Al-Saleh et al. [18] and Choi et al. [19] found no association between caffeine consumption and pregnancy outcomes during IVF or IVF. Al-Saleh linked higher serum caffeine levels to a decrease in the number of oocytes [18].

In addition, an earlier study by Schliep et al. [20] indicated an influence of caffeine on luteal progesterone levels and a reduced risk of sporadic ovulation. Other studies have shown an association between coffee consumption and improved insulin sensitivity [13] and reduced risk of type 2 diabetes in both caffeinated and decaffeinated coffee [21]. All of the above may be potential mechanisms to explain the apparent benefits during IUI [8].

Time to pregnancy (TTP) and spontaneous abortion (SAB)

The meta-analysis by Bu et al. (2020) investigated the effects of caffeine on waiting time to pregnancy (TTP) and spontaneous abortion (SAB) [22]. Findings demonstrated a dose-dependent increase in SAB risk, as suggested by previous reviews and meta-analyses [6, 23, 24], with relative risks of 1.37 for 300 mg/day and 2.32 for 600 mg/day. However, the authors caution that SAB results should be interpreted with caution due to significant heterogeneity and risk of bias, including publication bias [16]. Moreover, Lyngsø et al. (2017) described mechanisms that may influence the increased risk of SAB [16]. Caffeine easily crosses the placental barrier in pregnant women and thus enters all fetal tissues [25]. Ultimately the fetus is exposed to the same levels of caffeine as the mother [25].

It is worth highlighting that no significant impact was noted on TTP, indicating that caffeine does not seem to affect the likelihood of conception per cycle in natural fertility [16]. These findings emphasize a potential miscarriage risk at high caffeine levels. However they also suggest minimal influence on other fertility outcomes.

It ought to be emphasized that pregnant women should apply guidelines of The World Health Organization (WHO) and European Food Safety Authority (EFSA) that recommend caffeine intake to 200-300 mg per day [5, 26]. The caffeine content of coffee is approximately 100 mg per cup [16], which equates to two to three glasses of coffee per day. However, some authors suggest that this limit may be too high [16].

CAFFEINE'S IMPACT ON MALE FERTILITY

Sperm ploidy

Caffeine consumption may provoke changes in sperm ploidy, mainly regarding chromosomal integrity. DNA integrity may suggest male infertility [9,11]. Aneuploidy is the most common genetic abnormality and the main type of chromosomal aberration [11,27]. It involves an abnormal number of chromosomes and it has the potential to play a role in pregnancy loss [28]. Aneuploidy occurs during

meiosis, where it is disrupted by various environmental and lifestyle factors, resulting in an incorrect separation of sister chromatids/chromosomes [11]. Most of the aneuploidies that occur in embryos and newborns are derived from the father [29]. Ricci et al. (2017) confirmed an association between caffeine consumption and sperm aneuploidy and DNA damage [11]. Jurewicz et al. (2014) [30] demonstrates the impact of caffeine intake on chromosomal aneuploidy. Caffeine consumption by daily coffee drinking, may lead to an increase in incidents of missing X or Y chromosomes [30]. According to a study by Jurewicz et al. [30], people who drank coffee 1–6 times per week had more often additional chromosome 18 in sperm cells. In addition, Robbins et al. (1997) [31] connected caffeine consumption with an increased frequency of sperm aneuploidy XX18 and XY18 as well as diploidy XY18-18 and the duplication phenotype YY18-18.

Time to Pregnancy (TTP)

Results of the examined studies concerning the influence of caffeine consumption on time to pregnancy (TTP) are various and inconclusive. Wesselink et al. (2016) [14] links higher caffeine intake, mainly from sodas and energy drinks, with a reduced man's fecundability rate (FR). Basing on this statement, the authors concludes prolonged time to pregnancy [14]. In contrast, this study indicated that caffeine intake from coffee and black tea had no consistent effect on TTP [14]. These results suggest that various caffeine sources may have potentially different impact on male fertility. However Curtis et al. (1997) [32] found no direct correlation between caffeine intake and time to pregnancy. Another interesting study was conducted by Florack et al. [33] who classified men by their caffeine consumption. Those with low or moderate caffeine intake had no significant differences in fertility [33], whereas high caffeine consumption was more often associated with reduced fertility.

Assisted reproductive technologies (ART)

Karmon et al. (2017) [34] have reported that men with caffeine intake more than 272 mg/day have had significantly lower live birth rates in comparison with those who have consumed less caffeine. This inverse relationship emphasizes the possible impact of caffeine on sperm quality and fertility success in clinical reproductive settings, though caffeine did not directly affect semen parameters in these studies [34]. Another study taken into consideration was a prospective cohort study by Klonoff-Cohen et al. [35] who investigated the rate of successful ART among coffee drinkers. The authors concluded that male caffeine consumption had no effect on fertilization, pregnancy or live birth delivery [35]. However, when caffeine was analysed as a continuous variable, men's caffeine intake increased by an additional 100 mg per day which was a factor that increased the possibility of multiple pregnancy by 2.2 times (95% CI 1.1-4.4) for usual lifetime consumption and by 3.0 times (95% CI 1.2-7.4) for consumption during the week of the initial clinic visit [35].

Sperm DNA

The association between caffeine and DNA integrity varies according to different studies that show diverse results depending on the dose and source of caffeine intake. Schmid et al. (2007) [36] suggested a correlation between high caffeine intake in 80 healthy non-smokers with increased sperm DNA fragmentation, specifically double-strand DNA breaks. Nevertheless, other studies showed no significant association between moderate caffeine intake and DNA fragmentation [37]. Coffee drinking was not associated with parameters of sperm DNA damage and DNA stainability, including the percentages of DNA fragmentation index (DFI), the medium DNA fragmentation index (MDFI), the high DNA fragmentation index (HDFI) and the high DNA stainability index (HDS—percentage of immature sperms) [37].

Semen quality

Caffeine's effects on semen quality (sperm concentration and motility) are inconsistent [11]. Two studies observed a positive correlation between the highest intake of coffee/caffeine and an increasing percentage of motile sperm in men [38, 39]. For example Sobreiro et al. (2005) found that sperm motility increased with greater coffee consumption. The study indicated that in the group of medical individuals who did not drink coffee, progressive motility averaged 57.1 per cent, while in the group of those who consumed over six cups of coffee per day (and thereby provided large amount of caffeine to the organism) it was 62.4 per cent on average ($p < 0.05$) [38].

It is important to highlight the influence of different sources of caffeine on the semen volume as some research indicates that cola and soft drink consumption might lower semen volume, sperm count and concentration, whereas coffee intake does not consistently show adverse effects on these variables [11, 22].

A study by Jensen et al (2010) does not indicate any correlation between low (101-200 mg) or moderate (201-800 mg) daily caffeine intake and reduced semen quality [40]. However, it highlights that daily intake of above 800 mg of caffeine may cause a non-significant reduction in semen quality [40]. Moreover it investigates the impact of cola consumption on semen parameters indicating that male cola drinkers show a decrease in semen volume, sperm concentration, total sperm count and percentage of spermatozoa with normal morphology in comparison to those who do not consume cola [40].

CONCLUSIONS

The influence of caffeine consumption on fertility is a complex matter which depends on a wide range of factors such as the amount of daily consumption, different caffeine sources and gender. Moderate caffeine intake, which is recommended by the World Health Organisation (WHO) and the European Food Safety Authority (EFSA), appears to be safe regarding human health. Moreover, it should be emphasized that pregnant women ought to follow specific guidelines that recommend caffeine intake of 200-300 mg per day. In addition, investigated studies lead

to conclusion that individuals who would like to prioritize their reproductive health should limit the consumption of energy drinks and sodas, as they pose a greater risks regarding the matter of fertility than coffee and tea.

The implications of caffeine intake vary among women and men therefore they ought to be separately discussed.

Taking into consideration the influence of caffeine on the matter of female fertility, it has an inconclusive effect on risk of primary infertility. Other investigated parameters such as time to pregnancy (TTP) and assisted reproductive technologies (ART) outcomes have not been significantly impacted by caffeine intake, however in women undergoing IUI, there has been an association noticed between low to moderate coffee consumption and increased pregnancy as well as live birth rates after the first cycle of treatment. The situation was different for SAB, where a dose-dependent increase in SAB risk was suggested.

In contrast, considering male it ought to emphasized that caffeine consumption may change sperm ploidy, mainly chromosomal integrity. However the impact of men's caffeine consumption on time to pregnancy (TTP), assisted reproductive technologies (ART), sperm DNA, semen quality (sperm concentration and motility) is still inconsistent, therefore further studies and research need to be conducted to provide more information and increase knowledge in this field.

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CONFLICT OF INTEREST

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CASE STUDY

Functioning of the patient after four-finger amputation in his dominant hand due to necrosis caused by a streptococcal infection: a case report

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ABSTRACT

A case of a 40-year-old patient who presented to the clinic due to carpal tunnel syndrome of the left hand was presented. Four years earlier, the same patient underwent four-finger amputation of his right dominant hand due to fulminant necrosis caused by Streptococcus pyogenes infection. Descriptive history and the results of examination indicated that the patient adapted very good to significant disability of the hand. He had no constant pain, but only when the weather changed, which he rated at 3-4 points on the Numeric Rank Scale. The hand function examined by the DASH questionnaire showed a significant impairment – 42 points, but the patient declared that he functioned well in everyday life and even found a permanent job in a security company.

KEY WORDS: multiply finger amputation, disability, adaptive (coping) strategies

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INTRODUCTION

Amputation of a hand, several fingers or other significant mutilation is the cause of major disability that can make it difficult or impossible to perform work, many activities of daily life, deteriorate the overall quality of life and cause a depression. The hand is an extremely complex and perfect organ, which is a mechanical manipulator controlled by bioelectrical stimuli conducted by nerves. Treating hand injuries is difficult, and in complex injuries, it is often impossible to reconstruct its anatomical structure and regain normal function. The progress of microsurgery and hand surgery allows for the replantation of amputated hands and fingers, repair of nerves and tendons so that the patient regains the greatest possible function of the crippled limb [1, 2]. For over 25 years, even a hand transplant from a cadaver has been performed, which allows the patient to regain partial mobility even after many years after the amputation. Significant progress has also been made in the field of hand prostheses, mechanical and bioelectric [3].

On the other hand, life sometimes provides evidence of the perfect adaptation of patients to even significant disabilities and the normal functioning in society of people with one amputated arm or other significant disability. An example is one of the deceased professors of Pomeranian Medical University, who lost his arm during the war from a bomb explosion, when he was a young, newly certified doctor and, despite such a significant disability, remained in the profession, obtained a specialization in paediatrics, became an outstanding scientist, obtained the title of professor and the position of the head of the clinic.

AIM

The aim of this case report study was presentation of a patient who lost 4 fingers of his right (dominant) hand as a result of fulminant necrosis caused by streptococcus purulent infection (only the thumb survived). This patient 4 years after the amputation presented to the author's clinic due to symptoms of carpal tunnel syndrome of the other (left) hand. In addition to performing the surgery, an examination of the function of the mutilated hand was performed to check how the patient was coping in everyday life. The results of this study form the basis of this article.

CASE REPORTS

FIRST PRESENTATION

A 36-year-old man was admitted to the authors' institution in 2020 year, with a diagnosis of phlegmon in his right (dominant) hand and necrosis of the fingers II-V. The patient was a homeless chronic alcoholic. On admission the whole upper extremity was red and swollen, but some areas of the skin were pale, in a mosaic manner. In the area of redness, a multiple small blisters were seen (Fig. 1). The fingers II-V were dark, cold and showed no signs of vitality, while the thumb looked normal. The clinical picture suggested Streptococcal aetiology of the infection. Considering necrosis of the fingers II-V, an informed consent on amputation was obtained. The fingers were amputated first, followed by wide, longitudinal incision on the dorsal and palmar side of the metacarpus, wrist and the forearm (Fig. 2). At the incision a muddy fluid was found and evacuated from the subcutaneous tissue and taken for bacterial culture. which

showed Streptococcus pyogenic infection, sensitive to all standard antibiotics. Post-operative course was uneventful and the patient was released home 2 weeks after admission with further care to be continued in the outpatient clinic. The wounds eventually healed, the thumb remind alive and had full range of motion (Figs 3 a, b).

SECOND PRESENTATION

In April 2024, four years after the amputation of his fingers, the same patient (40 years-old at this presentation) presented to the clinic with symptoms of carpal tunnel syndrome in his left, healthy hand. Typical symptoms: numbness of the I-IV fingers occurring mainly at night, nocturnal pain in the hand and reduction of grip strength began 2 years before the presentation. The patient rated his symptoms as troublesome, out of 6 points on the NRS scale. Because he presented classical symptoms and signs of the syndrome, no other examination was performed and he was offered surgical treatment.

During the visit, the function of the patient's mutilated right hand was examined. The questions concerned pain and how the patient how the patient copes with everyday life using the DASH questionnaire. When asked about the pain in the metacarpal stump, he reported no constant pain, only when the weather changed, then he rated it at 3-4 points on the NRS. The pain was not troublesome enough to take medication. He assessed function of his mutilated hand as good, but the result of the DASH questionnaire – 42 points, indicated a significant impairment (range 0 – normal function to 100 – total disability). Analysing the individual items of the questionnaire, it was found that the patient had the greatest problems with opening a jar, turning the key in the lock, preparing a meal, changing a light bulb, using a knife to cut meat and driving a car – in these items the patient assessed that these activities were difficult or impossible to perform (4 or 5 points). Figures 4 a-c illustrate appearance of the mutilated hand at second presentation. Asked about work status he reported

Fig. 1. A 36 years-old patient's hand and forearm at a first presentation

Source: compiled by the authors of this study

Fig. 2. A 36 years-old patient's hand after amputation of the fingers and incisions at the forearm

Source: compiled by the authors of this study

Fig. 3a. The hand and forearm in the course of healing

Source: compiled by the authors of this study

Fig. 3b. The hand and forearm in the course of healing

Source: compiled by the authors of this study

that had full-time employment in a security company and that performing duties at work does not cause him much difficulty.

DISCUSSION

The presented case is an example of a patient's significant adaption to physical disability and the potential to regain good function of the mutilated hand with the use of limited resources. Nowadays, a patient after extensive trauma to the hand, also after amputation, has the possibility of immediate reconstruction and/or replantation, which significantly increases his chances of regaining at least partial hand function [1, 2]. Even in the event of failure, technically and logistically advanced operations such as toe-to-hand transfer, or hand transplantation, are available [3]. However, indications for such operations should be made carefully, especially for transplantation, which is associated with the need for lifelong immunosuppressive treatment. The described case is an example that a significant injury to the hand, even in a patient burdened with chronic alcoholism, can be effectively compensated, allowing him to function relatively normally in everyday life and even to perform work.

There are no many cases in the literature similar to presented here. In our earlier work (2016), we reported a case of a man who suffered the amputation of the thumb and fingers II-IV of his left (non-dominant) hand in similar circumstances and who spent his entire adult life being fit enough to perform his professional work and pursue his life passions [4]. In the another article (2017) we presented a case of 26 year-old patient who sustained a four-finger amputation in his dominant, right hand due to fulminant necrosis caused by streptococcal infection, similar to presented here. This patient was extremely motivated to return to his original job as a lorry-driver and, in spite of substantial mutilation of his hand, he returned to work within 3 months after finger amputation [5]

Dec et al., (2013) we compared the results of replantation or revascularization with simple terminalisation (fitting

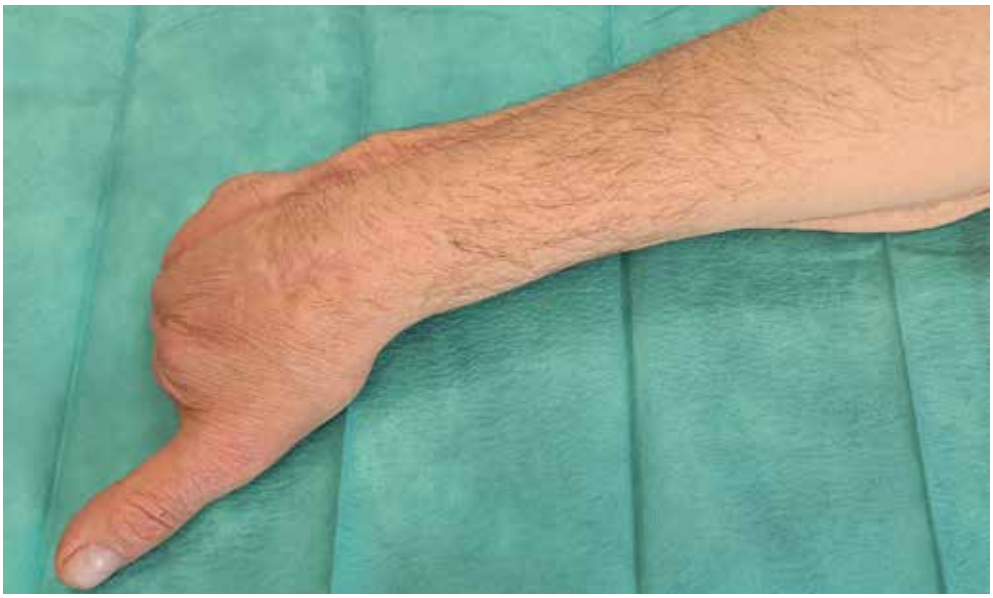

Fig. 4a. Appearance of the hand of a 40 years-old patient with amputated four fingers at second presentation

Source: compiled by the authors of this study

Fig. 4b. A 40 year-old patient during the period of adaptation – holding a bottle

Source: compiled by the authors of this study

Fig. 4c. A 40 year-old patient during the period of adaptation – writing

Source: compiled by the authors of this study

the stump) of the amputated finger(s). Patients after terminalisation had a stronger global grip – 72% of the strength of the healthy hand, compared to the replantation group – 48%, and slightly better hand dexterity in activities of daily living as assessed with the DASH questionnaire – 29 *vs* 33 points. These patients also returned to work earlier – at a mean of 6 months, versus 12 months after replantation or revascularization of the fingers. These results indicate that – considering hand function – replantation of the amputated finger, or advanced reconstruction were less beneficial for the patient than simple terminalisation [6].

Burger et al., (2007) reported results of the study investigated return to work of patients who sustained fingers, or partial hand amputations. They noted that half of the patients who did not receive a replantation, returned to their previous work. Only 30% of them used a simple cosmetic prostheses regularly, primarily in contact with other people, but not at work or at home [7].

Najman et al., (2013) reported treatment outcomes of 40 patients at a mean age of 50 years, who sustained single finger amputation, who received only terminalisation of their fingers under local anaesthesia. At the 6 months follow-up examination most patients adapted very well to the use of the hand with the loss of finger(s) and returned to work [8]

This work does not present any novelty or description of a new treatment technique. It only describes how great are the adaptability of a person to a severe hand injury, which – it seems – should result in permanent inability to work and cause very significant problems in everyday life. Both the described case and others cited in the discussion contribute to a different approach to the life situation of young people who suffered severe hand injuries, impossible to avoid due to the type of disease that caused them. Fulminant necrosis of fingers in the course of streptococcal infection does not give any chance to save involved fingers. The situation is different in the cases of traumatic amputations, where – in some cases – there is a chance of finger replantation. The presented cases indicate that the will to regain an acceptable functioning in everyday life and to take up professional work is a very important factor in regaining a place in social life for such severely crippled young patients, without regard for sophisticated surgical reconstructions and social support of the community.

In conclusion, the presented case show very good adaptation of the patient to physical disability – loss of four fingers of the dominant hand, and the potential to regain good function of the mutilated hand with the use of limited resources.

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CONFLICT OF INTEREST

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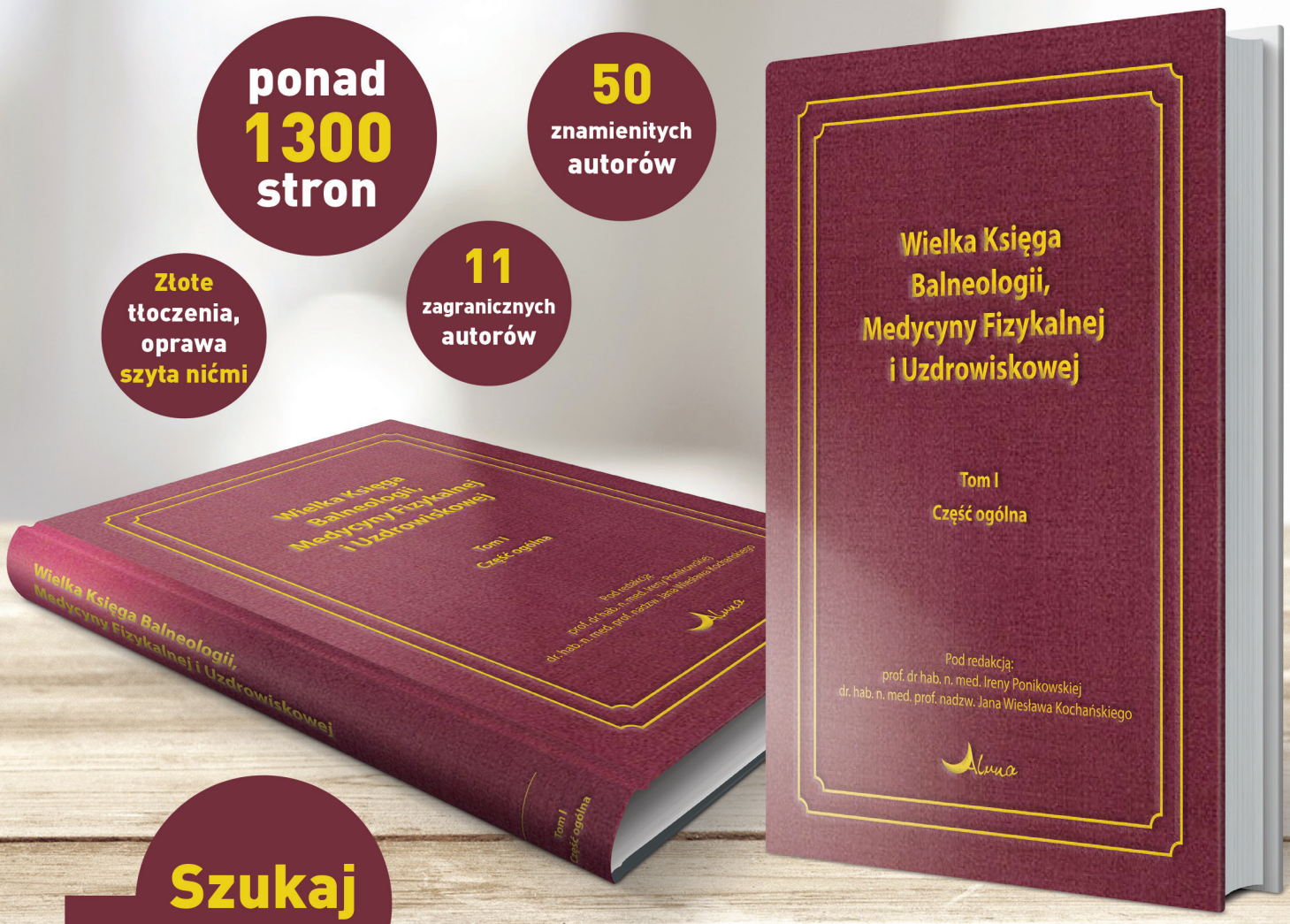

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