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ORIGINAL ARTICLE

Comparative effects of intravenous furosemide and torsemide on survival and length of hospital stay in acute heart failure

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ABSTRACT

Aim: To investigate the impact of the initial choice between intravenous furosemide and torsemide on mortality outcomes and duration of hospital stay.

Materials and Methods: The prospective, open-label, comparative study included 68 adult patients hospitalized with acute heart failure (AHF). Furosemide was administered to 35 patients (51.5%) and torsemide to 33 patients (48.5%) during the first 48 hours after admission. Diuretic dosing was titrated according to urinary sodium concentration. After the initial 48 hours, further diuretic therapy was administered at the discretion of the attending physician. The primary endpoint was all-cause mortality, and the secondary endpoints were length of hospital stay and in-hospital mortality.

Results: All-cause mortality was 51.4% in patients treated with furosemide and 42.4% in those treated with torsemide (p=0.478). The median survival time was 633 days (95% CI 178-1,259) in the furosemide group and 1,093 days (95% CI 541-1,239) in the torsemide group, with no statistically significant difference in survival between the two treatment groups (p=0.44). No in-hospital deaths occurred in the torsemide group, whereas four in-hospital deaths (11.4%) were observed in the furosemide group (p=0.115). The mean length of hospital stay was 12±7 days in both groups (p=0.551).

Conclusions: Both furosemide and torsemide showed no statistically significant differences in all-cause mortality, in-hospital mortality, and length of hospital stay.

KEYWORDS: all-cause mortality, length of hospital stay, acute heart failure, Intravenous furosemide and torsemide

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INTRODUCTION

Current clinical practice guidelines for the treatment of both AHF and chronic HF (CHF) endorse the use of loop diuretics, most notably furosemide and torsemide, as first-line agents to restore and maintain euvolemia. These drugs act by inhibiting sodium and water reabsorption in the renal tubules. Nevertheless, torsemide differs pharmacologically from furosemide, exhibiting greater plasma protein binding and a prolonged half-life, features that contribute to more sustained natriuresis and diuresis. Beyond its diuretic efficacy, torsemide has been shown to exert additional pleiotropic effects, including attenuation of renin–angiotensin–aldosterone system and reduction of sympathetic nervous system activity. Its anti-aldosterone properties are associated with a decreased incidence of hypokalemia. Moreover, torsemide therapy has been correlated with a lower risk of myocardial fibrosis development. Importantly, torsemide appears metabolically neutral, without clinically meaningful effects on bicarbonate balance or lipid profiles [1-7]. The pharmacokinetic and pharmacodynamic superiority of torsemide over furosemide has led to the hypothesis that these properties may result in better clinical outcomes. However, there is limited data demonstrating the superiority of one loop diuretic over the other. While a few studies have compared mortality rates between torsemide and furosemide in CHF, comparable evidence in AHF is lacking.

AIM

The study aimed to evaluate the impact of the selected loop diuretic administered during the first 48 hours of hospitalization for AHF on all-cause mortality as the primary endpoint. Secondary endpoints included length of hospital stay and in-hospital mortality.

MATERIALS AND METHODS

This prospective, open-label, two-arm study enrolled 68 patients aged 30 to 94 years who were admitted with AHF to the 2nd Department of Cardiology at Poznan University of Medical Sciences, Poland, between June 2022 and September 2024. Clinical follow-up was maintained through September 2025. Participants were consecutively recruited at the time of urgent hospitalization by presentation of AHF, including acute decompensated heart failure, acute pulmonary oedema, or isolated right ventricular failure. The diagnosis of AHF was established in accordance with the clinical criteria defined in the 2021 European Society of Cardiology Guidelines for the diagnosis and treatment of acute and chronic heart failure [1, 2]. At hospital admission, laboratory assessments included measurement of N-terminal pro-B-type natriuretic peptide (NT-proBNP) and estimation of glomerular filtration rate (eGFR), calculated using the Modification of Diet in Renal Disease (MDRD) equation. All patients underwent a standard 12-lead electrocardiogram and transthoracic echocardiographic evaluation. Participants

were consecutively assigned to either the furosemide or torsemide treatment group. Patients who were not previously receiving oral loop diuretics were administered either 40 mg of intravenous furosemide or 20 mg of intravenous torsemide. In contrast, patients already treated with oral loop diuretics received an initial dose equivalent to twice their usual oral regimen. Diuretic therapy was subsequently titrated based on urinary sodium concentration. Spot urinary sodium levels were assessed 2 hours after drug administration and again at 24 and 48 hours. If urinary sodium concentrations were below 70 mEq/L, the dose was doubled and could be repeated at intervals of 2 to 12 hours until a maximum daily dose of 500 mg of furosemide or 100 mg of torsemide was reached. When urinary sodium concentrations exceeded 70 mEq/L, the same dose was repeated every 12 hours. After 48 hours of admission, further diuretic therapy was administered at the discretion of the attending physician (Fig.1).

ETHICAL APPROVAL

The local bioethics committee has approved this study, and written informed consent has been obtained from each patient.

STATISTICAL ANALYSIS

Data were processed using IBM SPSS Statistics version 29, which was employed to generate basic descriptive summaries and to evaluate data distribution patterns using the Shapiro-Wilk test. To verify treatment effectiveness, Kaplan-Meier survival analysis was performed. Additionally, differences in the length of hospitalization were evaluated using the Mann–Whitney U test. In the last step, Cox regression analysis was conducted to identify additional factors affecting patient survival. The level of statistical significance was set at the conventional threshold of α=0.05.

RESULTS

BASELINE CHARACTERISTICS OF THE SAMPLE

The study cohort included data from 68 adult patients. Furosemide was administered to 51.5% (n=35) of the patients, while 48.5% (n=33) received torsemide. In the furosemide group, 48.6% were female and 51.4% were male, whereas in the torsemide group, 51.5% were female and 48.5% were male. The age distribution in the furosemide group ranged from 42 to 92 years, with a median age of 73 years. In the torsemide group, the age range was 30 to 94 years, with

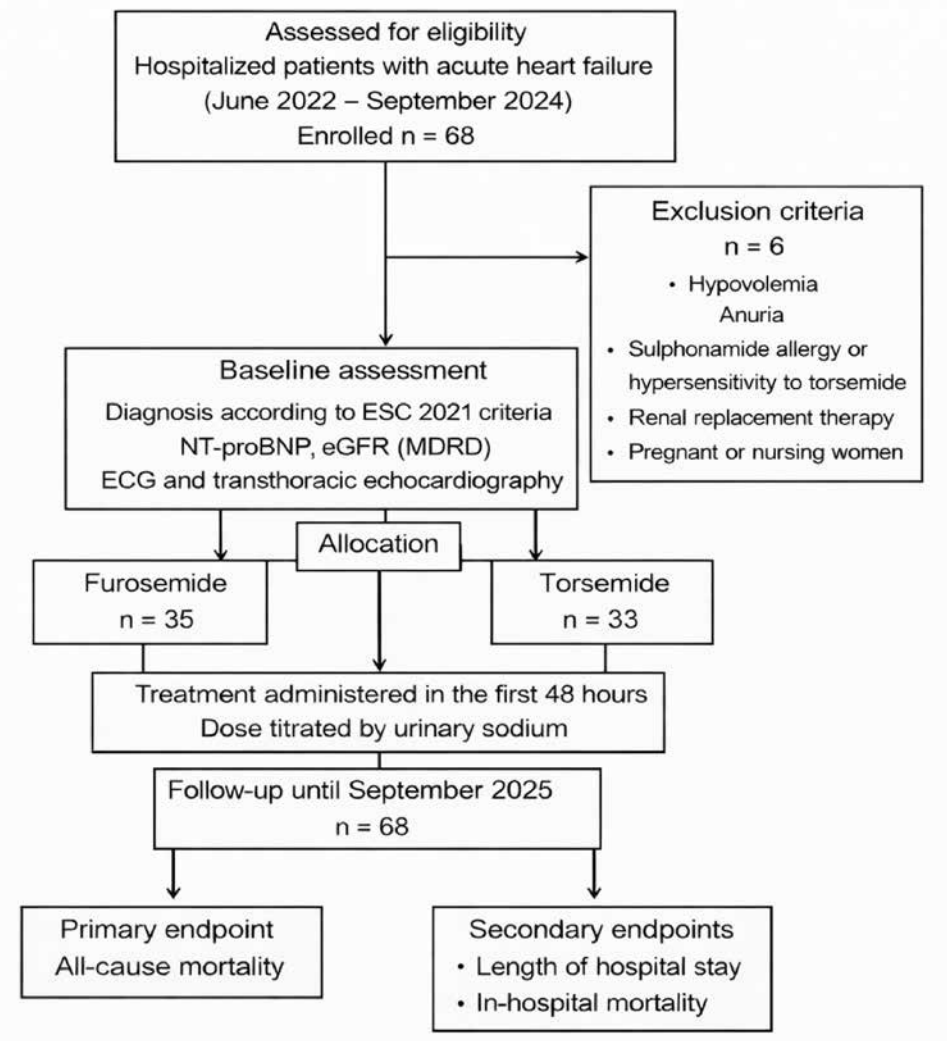

Fig. 1. Study flow diagram
Legend: n, number of participants; ESC, the 2021 European Society of Cardiology Guidelines for the diagnosis and treatment of acute and chronic heart failure; NT-proBNP, N-terminal pro-B-type natriuretic peptide; eGFR, estimated glomerular filtration rate; MDRD, the Modification of Diet in Renal Disease equation; ECG, electrocardiogram

Source: Own materials

a median age of 70 years. The median body mass index (BMI) was 26.85 kg/m^2 in the furosemide group and 29.71 kg/m^2 in the torsemide group. The etiology of AHF in the furosemide group was ischemic heart disease in 40% of patients, hypertension in 25.7%, and other causes in 34.3%. In the torsemide group, 48.5% of patients had ischemic heart disease, 18.2% had hypertension, and 33.3% had other etiologies. Regarding left ventricular ejection fraction (LVEF) categories, both the furosemide and torsemide groups demonstrated a similar predominance of HF with reduced EF (HFrEF), accounting for 57.1% and 51.5% of patients, respectively. In the furosemide group, HF with preserved EF (HFpEF) was present in 40% of patients, while 8.6% were classified as having HF with mildly reduced EF (HFmrEF). In the torsemide group, 21.2% had HFpEF and 27.3% had HFmrEF. The clinical profile characteristics of the furosemide and torsemide groups demonstrated similar prevalence rate regarding diabetes mellitus 45.7 vs 51.5%, atrial fibrillation 48.6 vs 54.5% , hypertension 45.7 vs 54.5% and chronic obstructive pulmonary disease 48.6% vs 36.4%. Median LVEF in the furosemide group was 35%, and in the torsemide group 38%. Kidney function, assessed by eGFR, blood pressure measurements, NT pro-BNP levels showed similar values (Table 1).

Table1. Baseline characteristics of the sample

Characteristic	Fursemide n = 35; 51.5%	Torsemide n = 33; 48.5%	p-value
Sex:			1.0
Female	17 (48.6%)	17 (51.5%)	
Male	18 (51.4%)	16 (48.5%)	
Age/years			0.399
M (SD)	73.11 (10.33)	70.27 (16.39)	
Me (IQR)	74.00 (16.00)	71.00 (16.00)	
Min. – Maks.	42-92	30-94	
Etiology			0.065
Hypertension	9 (25.7%)	6 (18.2%)	
Ischemic heart disease	14 (40.0%)	16 (48.5%)	
Other	12 (34.3%)	11 (33.3%)	
BMI; kg/m2			0.060
M (SD)	26.85 (4.89)	29.71 (7.14)	
Me (IQR)	25.70 (6.40)	27.80 (9.50)	
Min. – Maks.	19.5-38.6	21.0-48.4	
Lenght of hospitalization			0.679
M (SD)	12.63 (6.45)	11.94 (7.19)	
Me (IQR)	10.00 (9.00)	11.00 (8.00)	
Min. – Maks.	4-28	3-38	
Classification of heart failure			0.068
HFpEF	14 (40%)	7 (21.2%)	
HFmrEF	3 (8.6%)	9 (27.3%)	
HFrEF	20 (57.1%)	17 (51.5%)	
Comorbidities, cardiac functions, and clinical conditions			
Atrial fibrillation	17 (48.6%)	18 (54.5%)	0.803
Hypertension	16 (45.7%)	18 (54.5%)	0.627
COPD	17 (48.6%)	12 (36.4%)	0.440
Diabetes mellitus	16 (45.7%)	17 (51.5%)	0.814
NT-pro BNP; pg/mL			0.608
M (SD)	10502.74 (8959.25)	9418.79 (8394.67)	
Me (IQR)	8504.00 (9599)	5770.00 (11585)	
Min. – Maks.	281-35000	252-35000	
eGFR; mL/min/1.73m2			0.372

Table1. Cont.

M (SD)	4.,68 (13.70)	46.61 (14.45)	
Me (IQR)	59.50 (21.50)	49.00 (26.60)	
Min. – Maks.	12.10-60.00	19.60-60.00	
SBP; mmHg			0.335
M (SD)	130.31 (28.44)	136.97 (28.10)	
Me (IQR)	125.00 (42)	135.00 (45)	
Min. – Maks.	95-200	94-192	
DBP; mmHg			0.490
M (SD)	76.51 (13.65)	79.03 (16.11)	
Me (IQR)	74.00 (19)	80.00 (21)	
Min. – Maks.	54-103	50-130	
LVEF			0.777
M (SD)	35.94 (16.35)	37.00 (14.50)	
Me (IQR)	35.00 (30)	38.00 (23)	
Min. – Maks.	10-62	15-65	

n – number of observations; M – mean; SD – standard deviation; Me – median; IQR – interquartile range; Min. and Max. – the lowest and highest values in the distribution.

Legend: Other, severe aortic stenosis, severe tricuspid regurgitation, severe mitral regurgitation, dilated cardiomyopathy, right ventricular heart failure, alcohol-induced cardiomyopathy, left bundle branch block – induced cardiomyopathy, anthracycline-induced cardiomyopathy, peripartum cardiomyopathy; BMI, body mass index; COPD, chronic obstructive pulmonary disease; HFpEF, heart failure with preserved ejection fraction; HFmrEF, heart failure with mildly reduced ejection fraction; HFrEF, heart failure with reduced ejection fraction; NT-proBNP, N- terminal pro-B-type natriuretic peptide; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; DBP diastolic blood pressure; LVEF, left ventricular ejection fraction

Source: Own materials

SURVIVAL ANALYSIS

Death occurred in 18 of 35 patients (51.4%) treated with furosemide and 14 of 33 patients (42.4%) treated with torsemide (p=0.478). The median survival time was 633 days (95% CI 178-1,239) in the furosemide group and 1,093 days (95% CI 541-1,259) in the torsemide group. Survival outcomes were not significantly different between the two treatment groups (p=0.44) (Fig. 2). The conducted Cox regression analysis did not demonstrate a significant effect of the type of loop diuretic used (furosemide vs. torsemide) on patient survival. Likewise, the phenotype of HF (HFpEF, HFmrEF, HFrEF) was not a significant predictor of mortality risk (Fig. 3). No significant interactions were found between the type of drug and the HF phenotype (Fig. 4). No in-hospital deaths occurred in the torsemide group, whereas four in-hospital deaths (11.4%) were observed in the furosemide group. Although a numerical trend toward lower in-hospital mortality was observed with torsemide, this difference did not reach statistical significance on Fisher's exact test (p=0.115).

DISCUSSION

The European Society of Cardiology guidelines highlight the importance of the initial management phase in AHF, emphasizing that the first 24-48 hours after admission are critical for patient stabilization and treatment optimization; however, they do not provide specific recommendations on whether the choice of loop diuretic influences outcomes [1].

The principal finding of our study was the absence of a statistically significant difference in long-term survival between patients treated with furosemide and those treated with torsemide, despite a numerically lower mortality rate in the torsemide group (51.4% vs. 42.4%). Similarly, median survival was longer in the torsemide group (1,093 days vs. 633 days), but this difference was also not statistically significant. These findings were further supported by Cox regression analysis, which demonstrated no independent effect of loop diuretic type on mortality risk. The lack of significant interaction between diuretic type and HF phenotype (HFpEF, HFmrEF, and HFrEF) suggests that the comparable survival outcomes apply consistently across different forms of HF. The effect of loop diuretics on mortality has been investigated mainly in CHF, and the results are not directly comparable. Several studies have demonstrated a potential survival benefit associated with torsemide. The TORIC-HF (Torsemide in Congestive Heart Failure) trial found that patients receiving torsemide had lower total and cardiac one-year mortality than those receiving furosemide (2.2% vs. 4.5%; p <0.05) [8]. In the West Tokyo Heart Failure Registry (2006-2017), patients hospitalized with AHF were categorized according to the loop diuretic prescribed at discharge, either furosemide or torsemide/azosemide. After multivariable adjustment, the torsemide/azosemide group had a lower risk of the composite outcome (HR 0.80, 95% CI 0.66-0.96; p=0.017), including all-cause death (HR 0.73, 95% CI 0.54-0.99; p=0.044) and HF readmission (HR 0.81, 95% CI 0.66-0.99; p=0.038), compared with the furosemide group [9]. Conversely, the TRANSFORM-HF trial (Torsemide Comparison with Furosemide for Management of Heart Failure), which compared furosemide and torsemide in patients with CHF, as well as a post hoc analysis of the ASCEND-HF trial (Acute Study of Clinical Effectiveness of

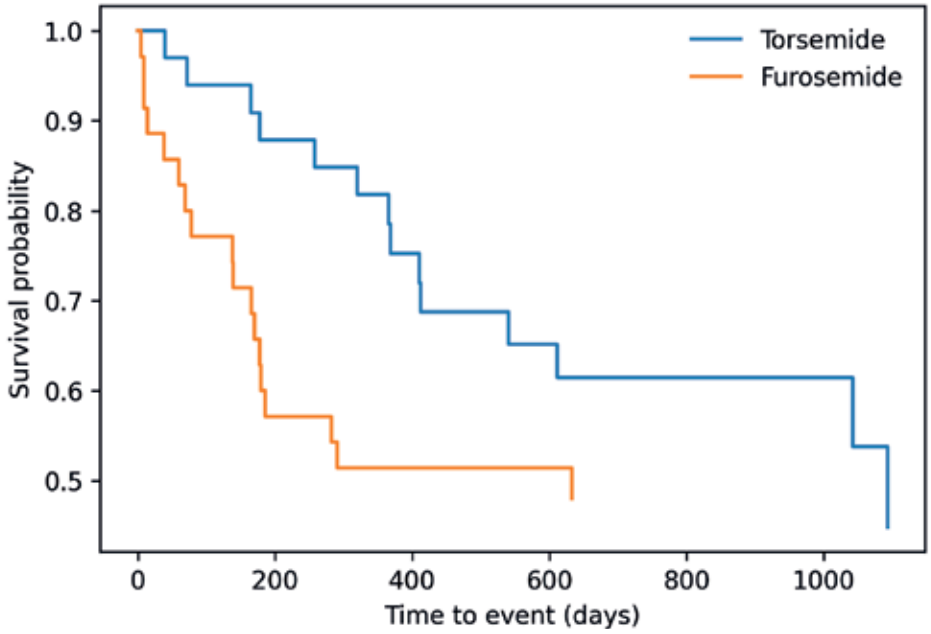

Fig. 2. Kaplan-Meier curves for survival

The Kaplan–Meier curves compare overall survival between patients treated with torsemide and those treated with furosemide. Survival probability is plotted against time to death (days). Both groups demonstrate the expected stepwise decline reflecting observed death events, with right-censoring appropriately incorporated into the risk sets. Deaths in the furosemide group occur earlier, beginning at approximately 600 days, whereas the torsemide group shows death events extending beyond 1,000 days. However, the survival curves do not show a clear or sustained separation, suggesting no meaningful difference in survival between the two treatment groups over the follow-up period.

Source: Own materials

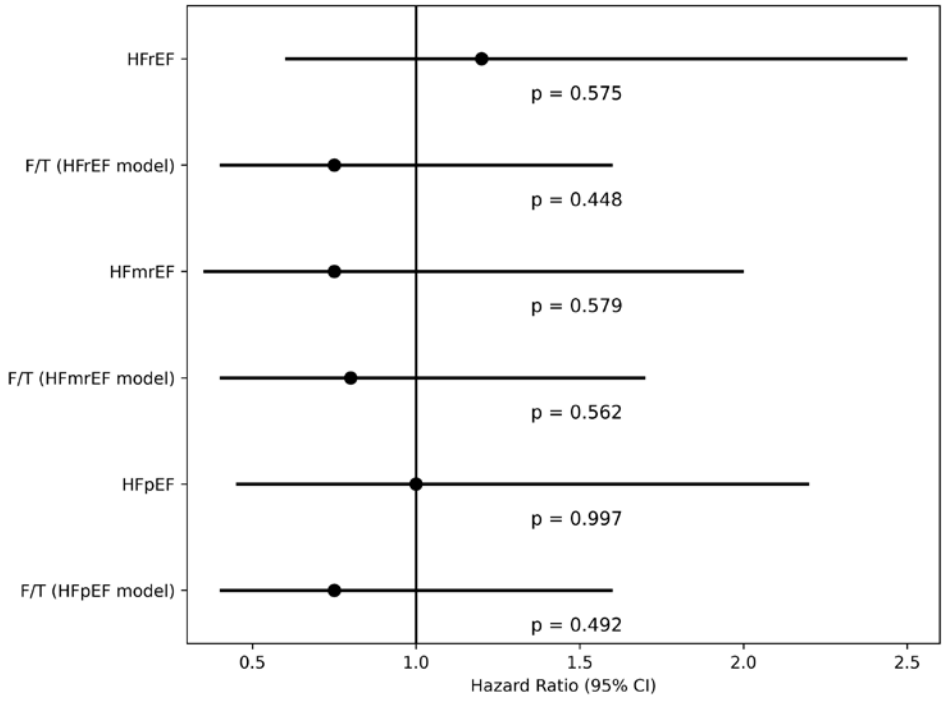

Fig. 3. Effects of diuretic type and heart failure phenotype on survival

Cox proportional hazards regression analysis demonstrated no significant association between loop diuretic type (furosemide vs torsemide) and mortality across models stratified by heart failure subtype (HFrEF, HFmrEF, and HFpEF) with hazard ratios near 1.0 and 95% CI crossing unity. Heart failure phenotypes entered as independent covariates were also not significantly associated with mortality risk.

Legend: F/T, furosemide/torsemide; CI, Confidence Interval; p, p-value; HFpEF, heart failure with preserved ejection fraction; HFmrEF, heart failure with mildly reduced ejection fraction; HFrEF, heart failure with reduced ejection fraction

Source: Own materials

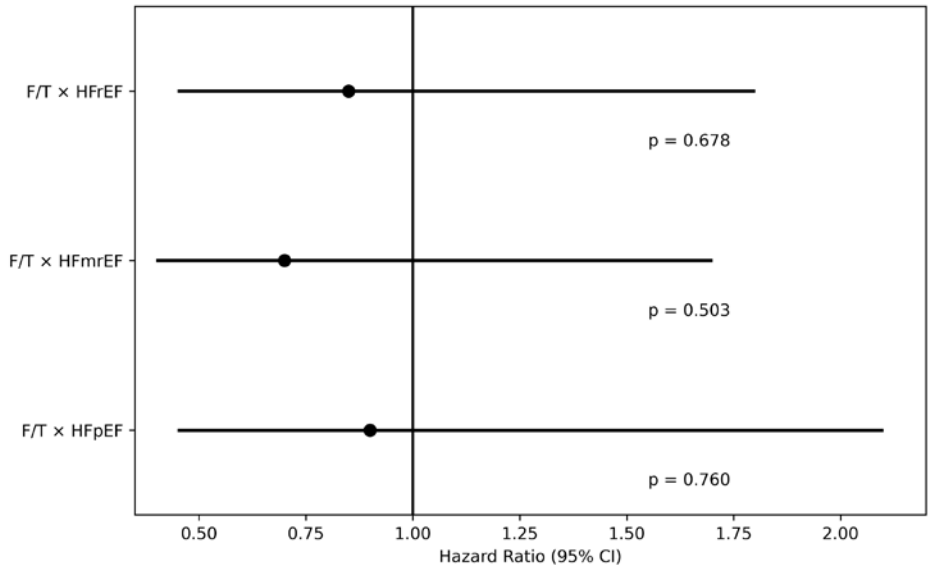

Fig. 4. The interactions between the phenotype of heart failure and type of loop diuretics on mortality risk
In Cox proportional hazards regression analyses, torsemide demonstrated a consistent numerical trend toward lower hazard compared with furosemide across heart failure phenotypes; however, all estimates did not reach statistical significance.
Legend: F/T, furosemide/torsemide; CI, Confidence Interval; p, p-value; HFpEF, heart failure with preserved ejection fraction; HFmrEF, heart failure with mildly reduced ejection fraction; HFrEF, heart failure with reduced ejection fraction

Source: Own materials

Nesiritide in Decompensated Heart Failure), suggested no significant differences in all-cause hospitalization and mortality, 30-day mortality, or rehospitalization [10, 11]. Another study comparing furosemide and torsemide after discharge in patients with HFpEF also found no significant reduction in all-cause mortality. Death occurred in 30.09% of patients in the furosemide group and 26.36% in the torsemide group (hazard ratio [HR] 0.81, 95% confidence interval [CI] 0.57-1.15; p=0.239) [12]. Meta-analysis of 4127 patients with HF from 10 randomized controlled trials including TRANSFORM-HF, showed that the torsemide group had a significantly lower number of hospitalizations (all-cause, cardiovascular-related, and HF-related) than the furosemide group, with no difference in all-cause mortality (risk ratio [RR] 1.02, 95% CI 0.91 to 1.15; p=0.70) [13]. The results of this study are consistent with several contemporary clinical investigations that failed to demonstrate a clear mortality benefit of torsemide over furosemide. However, the abovementioned studies are not completely suitable for comparison with intravenous loop diuretics used in AHF. Studies that specifically evaluate intravenous loop diuretics in this clinical context are scarce. These studies examined differences between the furosemide and torsemide groups with respect to fluid and sodium excretion, as well as overall clinical response related to the reduction of congestion and symptomatic improvement, and found no significant differences between the two groups [14-16]. We found no studies comparing mortality in AHF in furosemide and torsemide treated patients. The absence of in-hospital mortality in the torsemide group in our study, compared with four deaths in the furosemide group, represents a clinically noteworthy numerical trend, although statistical significance was not achieved. Regarding the duration of hospitalization, both groups had identical mean lengths of stay and comparable median durations. This suggests that, in the acute setting, the choice between furosemide and torsemide does not appear to influence short-term recovery trajectories. The European Society of Cardiology guidelines discuss the role of urine sodium measurement in the management of AHF. Specifically, spot urine sodium levels can be assessed to evaluate the diuretic response during treatment. A satisfactory diuretic response is defined as a urine sodium concentration greater than 50-70 mEq/L at 2 hours after diuretic administration, which can help guide subsequent treatment decisions [1]. Biegus et al. demonstrated that patients with AHF who received intravenous furosemide on admission and exhibited low or no increase in spot urine sodium concentration during the first 48 hours of hospitalization had a significantly higher risk of 1-year mortality [17]. The prognostic impact of natriuretic response on long-term mortality was further confirmed in an observational study including 175 patients treated at a tertiary center in the Netherlands [18]. These data suggest that spot urine sodium concentration is a predictor of adverse outcomes, independent of the specific loop diuretic used, which may explain why the differences in mortality and longer median survival observed between the groups in our study did not reach statistical significance.

The neutral results observed in our cohort reinforce the concept that diuretic response and clinical outcomes in AHF are likely driven by patient-specific factors, disease severity, and comorbid burden rather than the specific loop diuretic chosen. From a clinical perspective, these results suggest that both furosemide and torsemide can

be used interchangeably with respect to survival and hospitalization duration in AHF. Although torsemide was associated with numerically longer median survival and lower in-hospital mortality, these differences did not reach statistical significance and do not support the routine preferential use of torsemide over furosemide. These observations may reflect random variation rather than a true treatment effect and should therefore be considered hypothesis-generating, highlighting the need for larger, adequately powered randomized trials. Nevertheless, this observed trend aligns with prior pharmacological hypotheses suggesting that torsemide's higher bioavailability, longer half-life, and possible anti-fibrotic properties could confer theoretical advantages over furosemide. However, our findings indicate that such potential pharmacodynamic benefits did not translate into a demonstrable survival advantage in this cohort.

LIMITATIONS

Several limitations should be acknowledged. First, the study lacked sufficient statistical power to identify a significant difference in mortality between the two treatment groups, thereby increasing the likelihood of a type II error. Power calculations indicate that approximately 480 patients per group would be necessary to achieve 80% power. Given the limited sample size of our cohort, subgroup analyses other than phenotypes of HF, were not performed, although such analyses could potentially provide valuable insights into the clinical profiles of patients who respond more favorably to furosemide or torsemide. Second, a baseline imbalance in BMI was observed between the furosemide and torsemide groups (26.85 vs. 29.71 kg/m^2), showing a trend toward statistical significance (p=0.060). Similarly, some differences were observed between heart failure phenotypes (p=0.068). Given the limited sample size and low number of events, inclusion of additional covariates or propensity-based methods would have resulted in model overfitting and unstable estimates; therefore, we restricted multivariable adjustment to the most clinically relevant covariates. Third, the single-center design of the study may limit external validity and reduce the applicability of the results to broader patient populations. Furthermore, post-48-hour diuretic management and subsequent outpatient therapy were not standardized, reflecting real-world practice but introducing additional heterogeneity that may influence longer-term outcomes. Fourth, residual confounding cannot be excluded, including concomitant HF therapies. Finally, as this was not a randomized comparison, treatment allocation bias remains a possibility and may have affected the observed associations.

CONCLUSIONS

The study demonstrates no significant difference in long-term survival, in-hospital mortality, or length of hospital stay between furosemide and torsemide treatment in patients with AHF, irrespective of HF phenotype. Although numerically lower mortality and longer median survival were observed in the torsemide group, these differences did not reach statistical significance. Overall, the findings suggest that both loop diuretics demonstrate comparable short – and long-term clinical outcomes when used as initial intravenous therapy in AHF. Future studies should focus on randomized trials incorporating standardized in-hospital diuretic protocols and uniform post-discharge therapy, with particular attention to patient subgroups such as those with diuretic resistance, worsening renal function, or recurrent HF hospitalizations. The potential advantages of torsemide observed in some studies of CHF may become more evident in the AHF setting. The present study focused on the initial diuretic and natriuretic mechanisms of action of the evaluated drugs; therefore, potential benefits related to torsemide's metabolic or pleiotropic effects could not be demonstrated.

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CONFLICT OF INTEREST
The Authors declare no vonflict of interest

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ORIGINAL ARTICLE

Urinary tract infections in pregnant women from the Russian--Ukrainian military conflict regions: A multicenter study (2022-2025)

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ABSTRACT

Aim: To estimates of the prevalence rate of urinary tract infections (UTIs) in pregnant women from the Ukrainian-Russian military conflict regions and antimicrobial resistance of causing pathogens.

Materials and Methods: Retrospective multicenter cohort study was conducted from April, 2022 to May, 2025.The study population consisted of 2,576 pregnant women from the Ukrainian-Russian military conflict regions. Antibiotic susceptibility was done by the disc diffusion test as recommended by EUCAST guidelines.

Results: Among 2,576 pregnant women, 1,002 (38.9%) UTIs were observed. The most frequently reported UTI types were cystitis (48.4%) and asymptomatic bacteriuria (39%). Of all UTI cases, 14.6% were defined as acute pyelonephritis. The most common causative agents of UTIs were *Escherichia coli* (27.6%), *Klebsiella pneumoniae* (13.9%), *Proteus mirabilis* (11.2%), *Coagulase-negative staphylococci* (8.7%), *Enterococcus faecalis* (8.5%), *Enterobacter spp.* (7.2%), and *Pseudomonas aeruginosa* (6.6%). *Methicillin-resistance S. aureus* (MRSA), vancomycin resistance enterococci (VRE), and extended spectrum beta-lactamases (ESBL) production among Enterobacteriales was found observed in 11.3%, 9.1%, and 29.4% isolates, respectively. Carbapenem resistance was identified in 13.7% of P. aeruginosa strains.

Conclusions: This study findings demonstrate the high rate of UTIs in pregnant women from the Ukrainian-Russian military conflict regions and many cases are caused by pathogens that are resistant to antibiotics. Strategies for deterrence include optimal hygiene practices to minimize the risk of bacterial colonization and ascending infection.

KEYWORDS: Ukrainian-Russian military conflict regions, pregnant women, urinary tract infection, asymptomaticbacteriuria, cystitis, pyelonephritis, antimicrobial resistance

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INTRODUCTION

Urinary Tract Infections (UTIs) are one of the most common bacterial diseases in women during pregnancy period worldwide. According to the literature, a global estimated prevalence of UTIs in pregnant women of approximately 23.9% (ranging widely from 5% to over 70% depending on region) [1]. Is reported that in developed countries UTIs to affect between 10% [2, 3] and 24.3% [4] of pregnant women. UTIs during pregnancy represent a potentially serious complication, posing risks to both maternal and fetal health [5]. These infections can lead to complications such as pyelonephritis, preterm birth, low birth weight, and even maternal sepsis [6]. Therefore, prevention and control of urinary tract infections during pregnancy are important to protect the health of both the mother and the developing fetus.

According to the literature, UTIs in pregnancy can arise from a variety of causes, primarily attributed to anatomical

and physiological changes that occur during gestation. These changes often cause urine to become stagnant and make it easier for bacteria to move from the urethra into the bladder and potentially the kidneys [7]. Therefore, early detection and treatment of UTI during pregnancy are crucial to prevent complications that may affect the health of both the mother and the foetus.

In military conflict countries such as Ukraine, there is a high UTIs rate in pregnant women. According to the literature, the prevalence of UTIs in Ukraine was 29.5%. The most frequently reported UTI types were: 36.5% asymptomatic bacteriuria, 51,7% cystitis, and 11.8% pyelonephritis [8]. This work showed that many cases of UTIs are caused by pathogens that are resistant to antibiotics [8]. However, this study did not include pregnant women from regions of Ukraine affected by military action.

Current Ukrainian guidelines for management of UTIs in pregnant women recommend the use of antibiotics for treatment these infections. However, the high-level antimicrobial resistance is limiting antibiotic use for treatment of UTIs in Ukraine. To date, there is limited data describing the prevalence of UTIs in pregnant women from the Ukrainian-Russian military conflict regions and antimicrobial resistance of causing pathogens.

AIM

The aim of this study was to estimates of the prevalence rate of UTIs in pregnant women from the Ukrainian-Russian military conflict regions and antimicrobial resistance of causing pathogens.

MATERIALS AND METHODS

STUDY DESIGN AND PARTICIPANTS

We performed a retrospective multicenter cohort study was based on surveillance data for UTIs in pregnant women from the Ukrainian-Russian military conflict regions. (Kharkiv, Dnipro, Kherson, Zaporizhzhia, Odessa). A total of 2,576 pregnant women with and without symptoms of urinary tract infection (UTI) were included as a study subject 36 months period from April, 2022 to May, 2025. The selection criterion for the inclusion in this study was above 18 years and no past history related to any sexually transmitted diseases and immunocompromised status was noted.

DEFINITION

The classifications of UTIs in pregnancy include (a) lower urinary tract infections (Asymptomatic bacteriuria and Cystitis) and (b) upper urinary tract infection (Pyelonephritis) [7]. A UTIs in pregnancy is a bacterial infection of the urinary system (bladder, urethra, or kidneys) occurring during pregnancy. It is characterized by significant bacterial growth (≥105 CFU/mL) and ranges from asymptomatic bacteriuria (ASB) to acute cystitis or pyelonephritis. In this study type of UTIs in pregnant women includes asymptomatic bacteriuria (no symptoms but high bacteria count), cystitis (bladder infection), and pyelonephritis (kidney infection). UTIs in pregnant women is defined as the presence of at least 100,000 organisms per milliliter of urine in an asymptomatic patient, or as more than 100 organisms/mL of urine with accompanying pyuria in a symptomatic patient. A diagnosis of UTI should be supported by a positive culture for a uropathogen, particularly in patients with vague symptoms [9]. ASB is defined as the presence of more than 100,000 organisms/mL in two consecutive urine samples in the absence of declared symptoms. Cystitis involves only the lower urinary tract; it is characterized by inflammation of the bladder as a result of bacterial or nonbacterial causes. Acute pyelonephritis is characterized by fever, flank pain, and tenderness in addition to significant bacteriuria. Other symptoms may include nausea, vomiting, frequency, urgency, and dysuria. In this study two consecutive voided urine specimens with the same bacterial species isolated in quantitative count of >105 CFU/ml in pregnant women were considered to be positive for UTIs.

MICROBIOLOGICAL METHODS

In this study midstream urine samples from pregnant women were collected and processed following standard bacteriological tests. Urine of all patients was sampled and subjected to routine and microscopy examination and culture. Colony counts were performed, and confirmatory identification and antibiotic susceptibility tests were completed using a Vitek2 instrument system (BioMérieux, France). The significant bacteriuria was 105 CFU/ml was taken into consideration while confirmation as UTI. Bacterial isolates from urine samples were identified using standard microbiological techniques. Antimicrobial susceptibility testing was performed according to EUCAST guidelines at the private laboratories.

DATA COLLECTION

A standard data collection form was created to extract demographic and clinical data. Si this study clinical and microbial data were collected from medical records. Relevant information, including demographic data, baseline comorbidities, obstetrical characteristics, antimicrobial therapy regimens, urine culture results and antibiotic susceptibility testing data, was collected. In this work UTIs were diagnosed using midstream urine culture.

ETHICS

The study was conducted after getting a full approval by the research unit of The Zarifa Aliyeva International Center of Medical Science (Kyiv, Ukraine). Written informed consent for the study was obtained from the study participants. Confidentiality of results was kept and pregnant women's data were anonymised prior to the analysis.

STATISTICAL ANALYSIS

Statistical data was performed using Excel and SPSS 10.0 Statistical Software Package. Frequencies and percentages were reported for categorical variables. The prevalence of UTIs (asymptomatic bacteriuria, cystitis and pyelonephritis) was reported as the percentage of the total number of pregnant women. All data concerning associated risk factors were collected using structured questionnaires. Descriptive statistics and chi-square tests were used to analyze the data, and $p<0.05$ was considered to indicate statistical significance.

RESULTS

PREVALENCE OF UTIS

A total of 2,576 pregnant women from the Ukrainian-Russian military conflict regions were included as a study subject 36 months period (April, 2022 to May, 2025). During the study period, 1,002 of 2,576 pregnant women were found to have UTIs. The overall prevalence of UTIs in pregnant women from the Ukrainian-Russian military conflict regions was 38.9% [95% confidence interval (CI) 37.9-39.9%, P<0.001]. The UTIs cases among pregnant women from the Ukrainian-Russian military conflict regions varied significantly. In terms of these regions, fluctuations of the indicator values were observed of UTI in pregnant women – from the smallest in the Kharkiv and Odessa while higher percentages were reported in the Dnipro, Kherson and Zaporizhzhia regions of Ukraine.

The most frequently reported UTI types in pregnant women from the Ukrainian-Russian military conflict regions were 39% (95% CI 36.1-37.9) asymptomatic bacteriuria and 48,4% (95% CI 47.4-49.4) cystitis. Among all cases of UTIs, pyelonephritis was 14.6% (95% CI 13.9-15.3).

In all, 2,576 pregnant women from the Ukrainian-Russian military conflict regions with a mean age of 27.8 ± 6.9 years participated in the study. The majority among pregnant women, 60.5% (1,559), were aged 20 to 29 years, 74.5% (1,918) were self-employed, 52.0% (1,339) had basic education, 97.5% (2512) were married, and 81.0% (2,087) resided in an urban settlement (Table 1).

The majority, 48.0% (1,236) of all the study participants (pregnant women from the Ukrainian-Russian military conflict regions), reported for booking in the second trimester of their current pregnancy. The most, 91.5% (2,358) of the

Table 1. Characteristics of 2,675 pregnant women from the Ukrainian-Russian military conflict regions (2022-2025)

Variable	Total (n = 2,576)	UTI		P - value
		No (n = 1,574)	Yes (n = 1,002)	
	n (%)	n (%)	n (%)	
Age (years)				
Mean ± SD	27.8±6.9	27.9±6.8	27.7±5.51	
≥18	91 (3.5)	52 (57.1)	39 (42.9)	0.63
20-29	1559 (60.5)	979 (62.8)	580 (37.2)	
30-39	809 (31.4)	501 (61.9)	308 (38.1)	
≥ 40	117 (4.5)	39 (33.3)	78 (66.7)	
Occupation				
Salaried worker	181 (7.0)	129 (71.4)	52 (28.6)	0.76
Self-employed	1918 (74.5)	1159 (60.4)	759 (39.6)	
Unemployed	477 (18.5)	284 (59.5)	193 (40.5)	
Educational status				
Basic	1339 (52.0)	708 (52.9)	631 (47.1)	0.06
Secondary	748 (29.0)	554 (74.1)	194 (25.9)	
Tertiary	489 (19.0)	309 (63.2)	180 (36.8)	
Marital status				
Married	2512 (97.5)	1519 (60.5)	993 (39.5)	0.65
Single	64 (2.5)	52 (81.3)	12 (18.8)	
Place of residence				
Rural	489 (19.0)	233 (47.6)	256 (52.4)	0.06
Urban	2087 (81.0)	1339 (64.2)	748 (35.8)	
Trimester				
First	1198 (46.5)	811 (67.7)	387 (32.3)	0.15
Second	1236 (48.0)	696 (56.3)	540 (43.8)	
Third	142 (5.5)	64 (45.5)	78 (54.9)	
Dysuria				
Yes	218 (8.5)	103 (47.2)	115 (52.9)	0.30
No	2358 (91.5)	1470 (62.3)	888 (37.7)	

Table 1. Cont.

Frequency				
Yes	168 (6.5)	117 (69.6)	51 (30.8)	0.77
No	2408 (93.5)	1454 (60.4)	954 (39.6)	
Urgency				
Yes	64 (2.5)	38 (59.4)	26 (40.6)	1.00
No	2512 (97.5)	1533 (61.0)	979 (39.0)	
Lower abdominal pain				
Yes	451 (17.5)	283 (62.7)	168 (37.1)	0.85
No	2125 (82.5)	1288 (60.6)	837 (39.4)	

UTI, urinary tract infection
Source: compiled by the authors of this study

pregnant women did not experience any dysuria, 93.5% (2,408) had a normal frequency of urination, and 97.5% (2,512) had no urgency to urinate. Regarding pregnant women from the Ukrainian-Russian military conflict regions experiencing lower abdominal pain, most, 82.5% (2,125) did not experience any pain. There was no statistical association between the study variables and pregnant women obstetric and medical characteristics (Table 1).

RESPONSIBLE PATHOGENS AND ANTIMICROBIAL RESISTANCE

A total of 3295 isolates were recovered in 1.002 pregnant women from the Ukrainian-Russian military conflict regions with UTIs, of which 768 (23.3%), 2494 (75.7%), and 33 (1%) isolates were different Gram-positive cocci, and Gram-negative bacilli species, and Fungi (Candida albicans) respectively. In this study of all UTIs in pregnant women 87.8% were reported to be polymicrobial. The most common causative agents of UTIs in pregnant women from the Ukrainian-Russian military conflict regions was *Escherichia coli* (27.6%), *Klebsiella pneumoniae* (13.9%), *Proteus mirabilis* (11.2%), *Coagulase-negative staphylococci* (8.7%), *Enterococcus faecalis* (8.5%), Enterobacter spp. (7.2%), and *Pseudomonas aeruginosa* (6.6%) followed by *Streptococcus spp.* (4.2%), *Klebsiella oxytoca* (2.9%), *Citrobacter spp.* (2.8%), *Serratia*

Table 2. Distribution of microorganisms causing of Urinary Tract Infections (UTIs) in pregnant women from the Ukrainian-Russian military conflict regions (2022-2025)

Microorganisms	n	%
Gram-positive cocci	768	23.3
Enterococcus faecalis	279	8.5
Enterococcus faecium	27	0.8
Streptococcus spp.	138	4.2
CoNS	288	8.7
Staphylococcus aureus	36	1.1
Gram-negative bacilli	2494	75.7
Escherichia coli	911	27.6
Klebsiella pneumoniae	458	13.9
Klebsiella oxytoca	97	2.9
Enterobacter spp.	236	7.2
Proteus mirabilis	369	11.2
Serratia spp.	68	2.1
Citrobacter spp.	93	2.8
Pseudomonas aeruginosa	219	6.6
Acinetobacter spp.	43	1.3
Fungi	33	1.0
Candida albicans	33	1.0
Total	3295	100.0

CoNS, Coagulase-negative staphylococci
Source: compiled by the authors of this study

Table 3. Resistance profile of the main causative agents of UTIs in pregnant women from the Ukrainian--Russian military conflict regions (2022-2025)

Pathogen	Antibiotic	Total (n=1,957) %	Type of UTIs			P value
			ASB %	Cystitis %	Pyelonephritis %	
Escherichia coli	CRO	12.4	11.1	9.1	17.2	0.0081
	CIP	27.2	24.7	24.3	32.5	0.0637
	NOR	27.6	28.9	27.4	26.6	0.749
	TMP-SMX	28.4	25.4	26.7	33.0	0.0411
	FFM	2.3	2.3	2.2	2.3	0.975
	NIT	2.2	2.1	1.0	3.4	0.128
	AMC	32.3	23.5	31.5	38.8	<0.001
Klebsiella pneumoniae	CRO	10.2	7.5	7.2	15.5	0.0642
	CIP	19.7	15.5	15.0	28.6	0.0312
	NOR	15.3	17.8	15.0	13.2	0.565
	TMP-SMX	13.9	10.1	12.4	19.1	0.0631
	FFM	30.0	19.6	39.8	30.7	0.001
	NIT	66.6	70.1	63.5	66.1	0.547
	AMC	16.8	14.5	16.7	19.2	0.535
Proteus mirabilis	CRO	3.2	1.0	0.0	8.5	0.0061
	CIP	20.6	25.0	16.3	20.5	0.487
	NOR	19.9	25.3	15.9	18.6	0.378
	TMP-SMX	31.2	27.8	28.6	37.3	0.419
	FFM	20.5	28.6	19.6	13.2	0.154
	NIT	100.0	100.0	100.0	100.0	-
	AMC	6.9	7.4	4.9	8.3	0.654
Pseudomonas aeruginosa	CEF	12.3	14.8	17.0	8.0	0.173
	CIP	10.5	7.6	13.8	10.1	0.311
	PIP	14.1	16.7	15.4	10.2	0.378
	CAZ	10.2	11.8	10.9	8.0	0.614
	IMI	13.6	14.4	14.4	11.9	0.745

ASB, asymptomatic bacteriuria; UTIs, urinary tract infections.
CRO, Ceftriaxone; CIP, Ciprofloxacin; NOR, Norfloxacin; TMP-SMZ, trimethoprim-sulfamethoxazole; FFM, fosfomycin; NIT, Nitrofurantoin; AMC, Amoxicillin/clavulanic acid; CEF, cefepime; PIP, piperacillin-tazobactam; CAZ, ceftazidime; IMI, Imipenem
Source: compiled by the authors of this study

spp. (2.1%), *Acinetobacter spp.* (1.3%), and *Staphylococcus aureus* (1.1%) (Table 2).

In this study antimicrobial susceptibility tests (AST) were performed on a total of 3262 bacterial isolates (Gram-positive cocci and Gram-negative bacilli). In this study, many of isolates displayed a high resistance to antimicrobials, although there were some differences depending on the species and type of UTI (Table 3).

DISCUSSION

The aim of present study was to estimates of the prevalence rate of UTIs in pregnant women from the Ukrainian-Russian military conflict regions and antimicrobial resistance of major causing pathogens. The results presented in this study are based on multicentre retrospective surveillance data. According to the literature, many studies have already described resistance of responsible pathogens of UTIs in pregnant women, Ukrainian data are limited. This study expands upon the previous reports in Ukraine [4, 8] and is the first study to publish the prevalence of UTIs in pregnant women from the Ukrainian-Russian military conflict regions and antimicrobial resistance of responsible pathogens of UTIs.

This study identified a high prevalence of UTIs (38.9%) in pregnant women from the Ukrainian-Russian military conflict regions. The most frequently reported UTI types in pregnant women were 39% ASB and 48,4% Cystitis. Among all cases of UTIs, Pyelonephritis was 14.6%. A previous study in Ukraine conducted in 2020-2022 found that prevalence of UTIs among pregnant women from without Ukrainian-

Russian military conflict regions were 29.5%. Of these cases of UTIs, ASB, Cystitis, and Pyelonephritis was observed in 36.5%, 51,7%, and 11.8% in pregnant women, respectively [8]. According to the literature, overall global prevalence of UTIs (both symptomatic and asymptomatic) in pregnant women to be 23.9% [1]. Other studies report that prevalence of UTIs among pregnant women was 10% [2, 11] to 26% [12]. The prevalence of ASB and Cystitis occurs varied in 2% to 10% and 1% to 2% of pregnant women, respectively [7, 10]. Other studies report an UTI prevalence rate of ASB in pregnant women were 2-13% [13, 14] and acute cystitis in 1-4% [13, 15]. It is estimated, the prevalence of acute pyelonephritis in pregnant women were 0.5-4% [16].

In this study the most common causative agents of UTIs in pregnant women from the Ukrainian-Russian military conflict regions was *E. coli, K. pneumoniae, P. mirabilis, Coagulase-negative staphylococci* (CoNS), *E. faecalis, Enterobacter spp., and P. aeruginosa*. This is consistent with the results of previous studies conducted in Ukraine [4, 8] and other studies [17, 18]. In our study many of isolates displayed a high resistance to antimicrobials, although there were some differences depending on the species and type of UTI. *Methicillin-resistance S. aureus* (MRSA), *vancomycin resistance enterococci* (VRE), and *extended spectrum beta-lactamases* (ESBL) production among Enterobacteriales was observed in 11.3%, 9.1%, and 29.4% strains, respectively. ESBL production among *E. coli* isolates was higher than in K. pneumoniae. Resistance to third generation cephalosporins was observed in 12.4% *E. coli* and in 10.2% K. pneumoniae isolates, respectively. In this study carbapenem resistance was identified in 13.7% of P. aeruginosa strains, isolated from pregnant women from the Ukrainian-Russian military conflict regions with UTIs. Our findings correlate with various other studies [4, 8, 14, 17-19] where multidrug resistant uropathogens were isolated from pregnant women with UTIs.

The most common risk factors of UTIs in pregnant women was lack of healthcare facilities, lack of essential medication, lack of personnel and equipment. Medical clinics across the Ukrainian-Russian military conflict regions are depleted and under immense strain. This has led to a decline in the quality of healthcare services and main reason for the high rate of UTIs in pregnant women.

STRENGTHS AND LIMITATIONS

The main strength of this study was the first study to publish the prevalence of UTIs in pregnant women from the Ukrainian-Russian military conflict regions and antimicrobial resistance of responsible pathogens of UTIs. We believe our findings are a solid representation of the current prevalence of UTIs in pregnant women from the Ukrainian-Russian military conflict regions and antimicrobial resistance of major causing pathogens. Our data, together with other papers in the literature, complement the picture of antimicrobial resistance of major causing pathogens isolated from pregnant women. The limitations of this study include the retrospective nature of this analysis. The results were dependent on accurate and complete documentation in the medical records. The health status of our cohort may have been inadequately powered to detect significant findings and other associated risk factors for UTIs.

CONCLUSIONS

This study findings demonstrate the high rate (38.9%) of UTIs in pregnant women from the Ukrainian-Russian military conflict regions and many cases are caused by pathogens that are resistant to antibiotics. The most common risk factors of UTIs in pregnant women was lack of healthcare facilities, lack of essential medication, lack of personnel and equipment. Medical clinics across the Ukrainian-Russian military conflict regions are depleted and under immense strain. This has led to a decline in the quality of healthcare services and main reason for the high rate of UTIs in pregnant women. Untreated UTIs in pregnant women can lead to severe complications. Early detection and treatment of UTIs during pregnancy are crucial to prevent complications. Prevention of UTIs in women during pregnancy are essential to safeguard the health of both the mother and the fetus. Strategies for deterrence include optimal hygiene practices to minimize the risk of bacterial colonization and ascending infection. Preventive measures also involve screening for ASB during prenatal visits and promptly treating to symptomatic UTI. Preventive strategies and patient education, healthcare workers (physicians, advanced care practitioners, nurses, pharmacists, and other health professionals) can mitigate the prevalence of UTIs in pregnancy in women from the Ukrainian-Russian military conflict regions and promote optimal maternal and fetal outcomes.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CD38 genotype-dependent regulation of CD44 and TP53 links genetic variation to aggressive phenotype in chronic lymphocytic leukemia

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ABSTRACT

Aim: To estimate role of CD38 rs6449182 polymorphism in CLL risk and its effect on the expression of critical tumor suppressor TP53, the promoter of cell adhesion and migration, CD44, and the immune checkpoint molecule PD-L1.

Materials and Methods: This case-control study included 50 CLL patients and 50 healthy individuals, age- and sex-matched. CLL diagnosed by flow cytometry (CD5+/CD19+/CD23+) based on WHO criteria. Genotyping of the CD38 rs6449182 polymorphism was detected through TaqMan SNP Assay. mRNA expression levels of TP53, CD44, and PD-L1 were quantified using real-time RT-PCR .

Results: Mutant G-allele was more frequent in CLL patients than in controls (19.0% vs. 10.0%), although this difference was only marginally significant P=0.075. No statistically significant association was observed between specific CD38 genotypes and the regulatory status (upregulation/downregulation) of the target genes P>0.05, while individuals with GG genotype exhibited higher median fold changes for TP53 (FC=2.97) and CD44 (FC=2.00) than the wild-type, the presence of significant inter-individual variance and broad confidence intervals indicates that these genetic variants do not serve as definitive independent predictors of gene expression in this cohort.

Conclusions: results suggest that the CD38 rs6449182 polymorphism may, at least in part, be involved in CLL risk but that it does not lead to a significant genotype-specific regulatory effect on the fold change of CD44, TP53, or PD-L1 expression. Although CD38 remains an important prognostic biomarker, the absence of a genetic-phenotypic relationship found in this study illustrates the complexity of molecular regulation by CLL. Larger studies are needed to confirm these findings.

KEYWORDS: CD38, CD44, Chronic Lymphocytic Leukemia, Polymorphism, Gene Expression, TP53, PD-L1, Tumor Suppressor

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INTRODUCTION

Chronic lymphocytic leukemia (CLL) suffers from genomic heterogeneity, which stems from both genetic and epigenetic factors. Prognostic biomarkers include chromosomal abnormalities and immunoglobulin heavy chain variable area (IGHV) mutations in the populations. Across all regions examined for correlations between populations and cases of B-cell chronic lymphocytic leukemia (CLL), one IGHV allele was particularly common: IGHV1-69; all other alleles were nonspecific, except in several sub-regions, where a few rare cases occurred. IGHV alleles, including IGHV4-34 and IGHV1-69, are markedly expressed across populations [1-2]. In a recent study, 41 single-nucleotide polymorphisms (SNPs) were identified, some of which are located in genes encoding proteins associated with monoclonal B-cell lymphocytosis and progression to overt CLL [3]. These polymorphisms form the CLL polygenic risk score (CLL-PRS), which is a strong marker of disease risk and has a particularly high value in white populations [3]. In addition to a genetic predisposition to CLL, specific genetic alterations directly influence the aggressive nature of CLL. Driver cancer gene mutations in Tumor Protein p53 (TP53), and high Cluster of Differentiation 44 (CD44) expression at five years post-diagnosis indicate highly aggressive forms of disease, but these indications are undoubtedly different than those seen in unmutated IGHV CLL [4]. In contrast, the upregulation of Programmed Death-Ligand 1 (PD-L1) gives chronic lymphocytic leukemia (CLL) cells an escape route from immune surveillance by binding in a specific manner to PD-1 on T cells, so that these T lymphocytes, when inactivated and unpowered-up cells, are stopped under normal circumstances. [5]. The gene encoding Cluster of Differentiation 38 (CD38) has emerged as a key effector in CLL pathogenesis, not only encoding a cell-surface protein but also being susceptible to genetic polymorphisms. In particular, the CD38 rs6449182 polymorphism is a risk factor for CLL and an aggressive disease phenotype, largely because

of its association with increased CD38 expression [6], CD38 is also a collaborator in the oncogenic pathway network. It is also found as a component of the supramolecular complex with Matrix Metalloproteinase-9 (MMP-9) and CD44, which increases CLL cell migration and survival [7]. As a negative prognostic biomarker, it is an attractive candidate for new drugs, and several blockade strategies are currently being validated to improve patient outcomes [8]. However, the precise mechanistic relationship between the CD38 rs6449182 variant and the dysregulation of key oncogenic molecules such as CD44, TP53, and PD-L1 remains incompletely characterized. Thus, this study suggested evaluating the CD38 rs6449182 polymorphism for association with CLL risk and its influence on CD44, TP53, and PD-L1 gene expression.

AIM

The aim of this study is to estimate role of CD38 rs6449182 polymorphism in CLL risk and its effect on the expression of critical tumor suppressor TP53, the promoter of cell adhesion and migration, CD44, and the immune checkpoint molecule PD-L1

MATERIALS AND METHODS

STUDY DESIGN AND SAMPLE COLLECTION

The present case-control study was conducted at Mansoura University/ Oncology center (Egypt). It included 100 subjects between June and November 2024, comprising 50 patients with CLL and 50 matched healthy controls. The patient's diagnosis was confirmed in accordance with International Workshop CLL guidelines for flow cytometry-based diagnosis, demonstrating a characteristic immunophenotype (CD5+/CD19+/CD23+) [9].

ETHICAL CONSIDERATIONS

The study was carried out with official approval from the institutional review board (IRB) of Mansoura University, Faculty of Medicine (MDP. 24. 01. 141), on February 19, 2024. All procedures were performed in accordance with the ethical standards of the 1964 Declaration of Helsinki. All participants signed the consent form after receiving a detailed explanation of the study.

SAMPLE COLLECTION

Approximately 7 mL of venous blood was collected from each participant into EDTA tubes for subsequent DNA and total RNA extraction.

GENOMIC DNA ISOLATION AND QUALITY CONTROL

Genomic DNA was isolated from blood mononuclear cells using the QIAamp DNA Blood Mini Kit (Qiagen) according to the manufacturer's protocol. The concentration and purity of the isolated DNA were evaluated with a NanoDrop 2000 (Thermo Scientific). All samples used for subsequent analysis had an A260/A280 ratio between 1.7 and 1.9 and a minimum concentration of 50 ng/µL. DNA integrity was assessed by 1% agarose gel electrophoresis in 1X TBE buffer at 75V for one hour, which revealed clear high-molecular-weight bands without smearing (Fig. 1). Isolated DNA was stored at -80°C until genotyping.

GENOTYPING OF CD38 RS6449182

The CD38 rs6449182 (C>G) polymorphism was genotyped using a real-time PCR-based TaqMan SNP Genotyping assay. The following primer and probe sequences were used:

- F: 5'-CTCCGGCGGCCGCTTT-3'
- R: 5'-GGCATCGGTGCCAAGTGTT-3'
- VIC-labeled Probe (C allele): 5'-AGGAGCAGCTGGCCTT-3'
  FAM-labeled Probe (G allele): 5'-AGGAGCAGGTGGCCTTG-3'

The reaction mixture for each sample had a total volume of 10 µL, consisted of 20 ng of genomic DNA, 5 µL of TaqMan Master Mix (Applied Biosystems, USA), 0.5 µL of the custom probe mix, and nuclease-free water. The thermal cycling program was as follows: an initial denaturation at 95°C for 10 min, then 40 cycles of denaturation at 95°C for 15 sec and annealing/extension at 60°C for 1 min. Following amplification, genotypes were automatically determined by the real-time PCR system's software using an allelic discrimination plot, which differentiates homozygous C/C, heterozygous C/G, and homozygous G/G genotypes based on endpoint fluorescence (Fig. 2).

RNA EXTRACTION AND GENE EXPRESSION ANALYSIS BY QRT-PCR

Total RNA was isolated from whole blood using TRIzol reagent (Invitrogen, USA) according to the manufacturer's protocol. The purity and concentration were validated spectrophotometrically. Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using a Reverse Transcription System kit. Quantitative real-time PCR (qRT-PCR) was used to detect the mRNA expression levels of TP53, CD44, and PD-L1. The reaction mixture consists of SYBR Green Master Mix, gene-specific primers, and cDNA template. The GAPDH gene was amplified concurrently

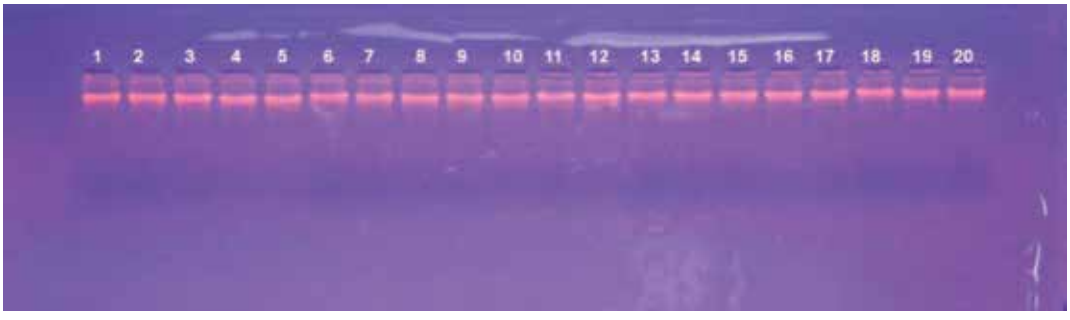

Fig. 1. Representative Agarose gel electrophoresis of genomic DNA. Lanes 1-10: DNA from healthy controls, Lanes 11-20: DNA from CLL patients

Source: Own materials

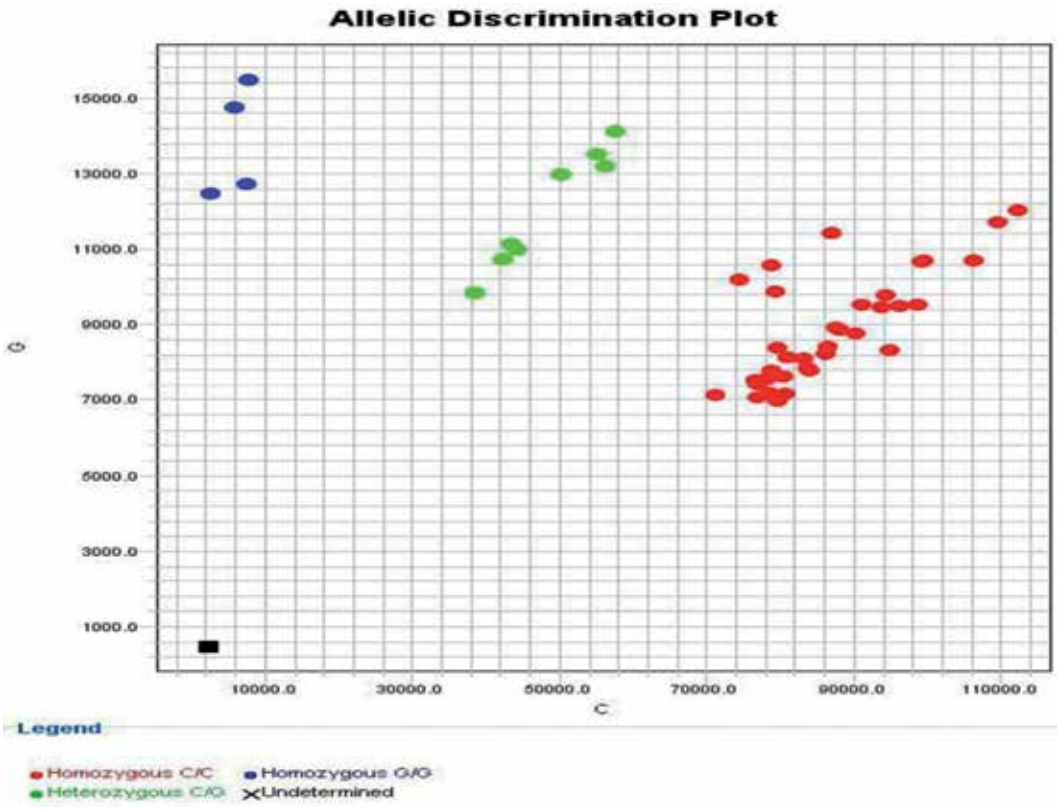

Fig. 2. Allelic discrimination plot for CD38 rs6449182 genotyping. The plot differentiates samples based on their fluorescence signals for the C (VIC-labeled) and G (FAM-labeled) alleles. Clusters are defined as: C/C (red), G/G (blue), C/G (green), and undetermined (black)

Source: Own materials

Table 1. Association of CD38 rs6449182 polymorphism with CLL risk

Group	Category	CLL Patients N(%)	Controls N(%)	OR (95% CI)	P-value
Genotypes	CC (Wild-type)	35 (70.0%)	41 (82.0%)	1.0 (Reference)	–
	CG(Heterozygous)	11 (22.0%)	8 (16.0%)	1.611 (0.583 – 4.450)	0.358
	GG (Mutant)	4 (8.0%)	1 (2.0%)	4.686 (0.500 -43.893)	0.176
Alleles	C (Wild-type)	81 (81.0%)	90 (90.0%)	1.0 (Reference)	–
	G (Mutant)	19 (19.0%)	10 (10.0%)	2.111 (0.928 – 4.805)	0.075

OR, Odds Ratio; CI, Confidence Interval. Statistical significance was set at p<0.05.

Source: Own materials

as an endogenous control. Amplification was performed using the following program: initial denaturation at 95°C for 10 min, followed by 40 cycles (95°C for 15 sec and 60°C for 1 min). The relative expression of each gene was evaluated by the 2^(-ΔΔCt) equation.

STATISTICAL ANALYSIS

Data analysis was conducted using IBM SPSS Statistics (version 27). The Kolmogorov-Smirnov and Shapiro-Wilk tests estimated the normality of continuous variables. Normally distributed data are represented as mean ± SD. While non-normally distributed data are presented as median and interquartile range (IQR). Categorical variables are reported as counts (n) and percentages (%). Between-group comparisons: Mann-Whitney U test for two independent groups. The Chi-Square test was used to detect associations between discrete variables. Findings presented as an odds ratio (OR) with a confidence interval of 95% (CI 95%). For all tests, a p-value of < 0.05 was considered statistically significant.

RESULTS

Association of CD38 rs6449182 polymorphism with CLL risk

The genotypes of the CD-38 rs6449182 and allele distributions show that the different types may be seen in Table 1 for the study groups. The wild-type CC genotype was taken as a reference. There was a higher frequency of the mutant G allele in CLL patients (19.0%) than in healthy people (10.0%). This led to an OR (95% CI: 0.928-4.805, p=0.075, non-statistically significant) for CLL risk, although it was not clear. At the genotype level, patients with homozygous mutant GG genotype represented 8.0% as against 2.0% of controls, giving a very high, although not statistically significant (OR=4.686, 95% CI: 0.500-43.893,

Table 2. Association of CD38 rs6449182 genotypes with CD44 expression in CLL patients

CD38 Genotype	CD44 UpregulatedN (%)	CD44 Downregulated (%)	OR (95% CI)	P-value
CC (Wild)	26 (70.3%)	9 (69.2%)	1.0(Reference)	–
CG	8 (21.6%)	3 (23.1%)	0.92 (0.21 – 4.08)	0.917
GG (Mutant)	3 (8.1%)	1 (7.7%)	1.04 (0.10 – 10.92)	0.974

**Odds ratio (OR) values are expressed with 95% confidence intervals (CI).*
The reference category for analysis was downregulated CD44 expression. Statistically significant at $p < 0.05$

Source: Own materials

p=0.176). The heterozygous CG genotype was also more common in patients (22.0 percent) than in healthy individuals (16.0 percent). The associated odds ratio from these data was not significant, with a value of 1.611 (95% CI: 0.583-4.450; p=0.358).

ASSOCIATION OF CD38 RS6449182 GENOTYPES WITH THE EXPRESSION OF CD44 IN CLL PATIENTS

The statistical analysis shows that CD44 expression levels (upregulated vs. downregulated) do not significantly correlate with the CD38 genotype. About 70% of the cases in both groups were of the wild-type genotype (CC). The computed P-values for the CG and GG genotypes were 0.917 and 0.974, respectively, both of which remain significantly above the conventional significance level of 0.05. Additionally, the 95% Confidence Intervals (CI) for CG (0.92) and GG (1.04) are noticeably wide and include the value of 1.0, indicating that these genetic variations do not function as significant predictors or risk factors for CD44 regulation in this study population. (Table 2)

ASSOCIATION OF CD38 RS6449182 GENOTYPES WITH TP53 EXPRESSION IN CLL PATIENTS

Variations and TP53 expression patterns. The homozygous mutant (GG) was totally absent in the TP53 downregulated subgroup, even though the wild-type (CC) allele was the most common genotype in both cohorts – an indeterminate or infinite Odds Ratio (OR). The results are not statistically significant, as indicated by the high P-values (0.605 and 0.556) and the remarkably wide Confidence Intervals (CI). These findings are likely impacted by the small sample size, which reduces the study's power to detect any possible biological relationship and upholds the null hypothesis (Table 3).

RELATIONSHIP OF CD38 RS6449182 GENOTYPES WITH PD-L1 EXPRESSION IN PATIENTS

The study of the CD38 genetic polymorphism in relation to PD-L1 expression patterns did not show a statistically significant association (Table 4). While the wild-type (CC) allele persisted at the baseline for comparison, the heterozygous (CG) variant gave an Odds Ratio (OR) of 2.52, which failed to reach significance at a P-value of 0.221. Likewise, the homozygous mutant (GG) reported an OR of 0.31 with a P-value of 0.322, reinforcing the lack of a discernible genetic effect. Since the 95% Confidence Intervals (CI) for both genotypes span the neutral value of 1.0, the results indicate that PD-L1 regulation occurs independently of the CD38 genotype in this analysis.

Table 3. Association of CD38 rs6449182 genotypes with TP53 expression in CLL patients

CD38 Genotype	TP53 Upregulated N (%)	TP53 Downregulated N (%)	OR (95% CI)	P-value
CC (Wild)	26 (66.7%)	9 (81.8%)	1.0 (Reference)	–
CG	9 (23.1%)	2 (18.2%)	1.56 (0.29 – 8.43)	0.605
GG (Mutant)	4 (10.3%)	0 (0.0%)	NA	0.556

**Odds ratio (OR) values are expressed with 95% confidence intervals (CI). The reference category for analysis was downregulated TP53 expression. Statistically significant at $p<0.05$. NA: Not applicable; OR could not be calculated due to a zero value in one group.*

Source: Own materials

Table 4: Association of CD38 rs6449182 genotypes with PDL-1 expression in CLL patients

CD38 Genotype	PDL-1 Upregulated N (%)	PDL-1 Downregulated N (%)	OR (95% CI)	P-value
CC (Wild)	18 (66.7%)	17 (73.9%)	1.0 (Reference)	–
CG	8 (29.6%)	3 (13.0%)	2.52 (0.58 – 10.93)	0.221
GG (Mutant)	1 (3.7%)	3 (13.0%)	0.31 (0.03 – 3.25)	0.322

The reference category was downregulated PDL-1 expression. Significance was set at $p<0.05$

Source: Own materials

Table 5. Distribution of CD38 rs6449182 genotypes by sex

Group	Sex	GG (Mutant)	CG	CC (wild)	Total	P-value
CLL Patients	Male	3 (9.7%)	8 (25.8%)	20 (64.5%)	31 (100%)	0.556
	Female	1 (5.3%)	3 (15.8%)	15 (78.9%)	19 (100%)	
Controls	Male	1 (3.2%)	7 (22.6%)	23 (74.2%)	31 (100%)	0.180
	Female	0 (0.0%)	1 (5.3%)	18 (94.7%)	19 (100%)	

Source: Own materials

Table 6. Gene expression fold changes by CD38 genotype

Gene	CD38 Genotype	Median (IQR)	FC vs. CC	P-value (vs. CC)
CD44	GG (Mutant)	9.13 (2.30 – 37.83)	2.00	0.331
	CG	7.65 (0.70 – 14.57)	1.68	0.367
	CC (Wild)	4.56 (0.98 – 10.50)	1.00	-
TP53	GG (Mutant)	8.42 (4.63 – 8.83)	2.97	0.229
	CG	5.18 (1.38 – 23.72)	1.83	0.511
	CC (Wild)	2.83 (0.76 – 13.67)	1.00	-
PDL-1	GG (Mutant)	0.33 (0.04 – 2.00)	0.31	0.121
	CG	2.71 (0.69 – 25.93)	2.51	0.168
	CC (Wild)	1.08 (0.49 – 2.91)	1.00	-

Data represented as median (interquartile range, IQR). FC vs. CC: Fold Change relative to CC genotype. P-value of Mann-Whitney U

Source: Own materials

DISTRIBUTION OF CD38 RS6449182 GENOTYPES BY SEX OF STUDY GROUPS

The distribution of CD38 rs6449182 genotypes was analyzed by sex in both the CLL and the control group (Table 5). While the GG and CG genotypes were more frequent in CLL patients than in controls for both males and females, these changes were not statistically significant (p=0.556 for males, p=0.180 for females).

GENE EXPRESSION FOLD CHANGES RELATIVE TO CD38 RS6449182 GENOTYPES

The median fold change (FC) in gene expression for CD44, TP53, and PD-L1 was compared across CD38 rs6449182 genotypes, using the CC genotype as the reference (Table 6). Although the GG genotype exhibited the highest median fold change for both CD44 (FC=2.00) and TP53 (FC=2.97), and the CG genotype showed the highest fold change for PD-L1 (FC=2.51), these differences were not statistically significant across all comparisons ($p > 0.05$)

DISCUSSION

This is a large-scale study of the CD38 rs6449182 polymorphism in CLL. The genetic distribution of this variant was determined, in addition to its regulatory effects regarding the three main gene products – CD44, TP53, PD-L1 – within the leukemic microenvironment. CD38, an enzyme important in the development of CLL, is a good prognostic marker. High CD38 expression is associated with the clinical characteristics and shorter survival times of bad patients [8]. Given the known clinical significance of CD38, a basic mechanistic question is whether particular genetic polymorphisms, for example, rs6449182, themselves can directly alter any measure of CD38 function and thus affect disease pathobiology. Several previous papers have described how SNPs in the CD38 gene probably change protein conformation, and in consequence can alter expression of association partners, notably CD44 [10]. In light of the above, the next question is whether the regulatory axis (CD38-CD44/TP53-PD-L1) may exert its effects at both immune and adhesive signalling levels in CLL. Our work in this connection is intended to plug this gap. We thereby hope to be able to draw a better statistical map of the genetics and associated illnesses than just taking the number of different symptoms. That being the case, our work has also found that mutant G allele was much more popular among CLL patients during the initiation stage than controls (19% versus 10%). Nevertheless, the data here are only at borderline significance (P=0.075). That points to some fine genetic influence which is as yet not an established risk factor in this population. Men and women did not differ in the distribution of genotypes (p=0.556, 0.180), suggesting that inheritance, transmission, and prevalence of these changes are not affected by gender in that patient population. Regarding molecular interactions, our findings indicate that the CC genotype at CD38 rs6449182 is not statistically associated with CD44 upregulation. Although approximately 70.3% of those with high CD44 mRNA levels were CC carriers, a similar percentage (69.2%) in the downregulated classes was also

observed, resulting in a non-significant P-value (P=0.917) and an OR of 0.92. This indicates that the CC genotype is not a significant predictor of high CD44 levels in this study. This is an important finding because CD44 is an essential cell adhesion molecule implicated in leukemic cell homing and migration [11-12]. Similarly, high CD44 levels are associated with progressive disease [13]. The BYK204165 regulation observed in this study seems independent of a particular form of CD38. No significant association was found between TP53 expression and CD38 genotypes. Despite this, the homozygous mutant (GG) appears to show the greater median fold change (FC=2.97), although it remains not statistically significant (P-value=0.229). TP53 is a key mediator of genomic stability in CLL, and its altered or mutated status is strongly associated with chemoresistance and poor clinical outcomes [14-15].

Some studies have researched new drugs that act on this road, for example, B-cell lymphoma 2 (BCL2) inhibitors and another one in reference to Bruton's Tyrosine Kinase (BTK) inhibitors [14].

Yet, research has not yet found any proof that this variant directly regulates levels of TP53mRNA. This implies that it is not this particular SNP in CD38 that causes TP53deregulation in CLL, but instead some direct mutations or a 17pdeletion [16]. Furthermore, the resolution of expression patterns forPD-L1 addresses previously enigmatic genetic effects. However, the fold change for the CG genotype was only 2.51, not reaching statistical significance (P=0.168). Large 95% CI around this estimate as well as a 1.0 in the middle of it indicate that regulation of PD-L1elsewhere does not change by CD38genotype [17] However, the lack of such an association suggests that other epigenetic changes and/or environmental factors in CLL immunosuppression are far more important than this particular polymorphism. Upregulations of CD44, TP53, and PD-L1 are often associated with more aggressive tumor behavior. and a higher chance of metastasis [8-13]. Information from the rs6449182-specified data collected group indicates that this genetic variant doesn't necessarily control or determine the expression level and distribution of gene patterns for these diseases. This huge difference in median and range of interindividual values suggests a highly intricate relationship between genetic factors, point mutations and changes in CLL pathogenesis. Different molecular pathways have been described that would cause CD44, TP53 and now even PD-L1 in chronic lymphocytic leukemia. These consist of epigenetic changes as promoter hypomethylation, activation of the NF-κB and STAT3 signaling pathways microenvironmental signals from stromal cells into interactions with sensitive malignant cells. And still further, TP53 expression can also be affected by genomic instability, 17p deletions, or stress-induced DNA damage responses; while PD-L1 expression might also often originate from inflammatory cytokines and related signal cascades, causing immune evasion within the leukemic niche [17].

But CD44 and TP53 continue to appear as promising targets for medicinal treatment in meta-analytic prognosis models [8] In our study, the CD38 rs6449182 polymorphism still did not shine. These findings underline the need for larger, multi-analyte profiling studies to provide a more comprehensive picture of the molecular landscape in CLL and better ways to treat this disease [18]. Breakthroughs in CLL by the development of therapy targeting these two pathways in anticancer are numerous on one hand, clinical trials were to complete for anti-CD44 monoclonal antibodies, and small molecule inhibitors that prevent adhesion mediated by CD44. Thus the prospect of this is that it may disrupt the homing signal of these leukemic cells within or their survival in bone marrow niches. While, researchers are developing inhibitors of mouse double minute 2 (MDM2) as well as new p53-reactivating small molecules designed to restore TP53 pathway function Those doctors may be able to finally overcome the resistance of high-risk CLL patient to chemotherapy, and give them a brighter prognosis at last [19].

CONCLUSIONS

In CLL, this research found that the CD38 rs6449182 allele is not the chief regulator of transcriptional activities of CD44, TP53, and PD-L1 transcriptional activities Therefore, the biological behavior of this fatal disease is determined by multiple factors particularly in phase I, mechanism is unlikely to play a prime role. One of these guidelines is that in prognosis, individual accounts should not be used alone. Instead, it is enough, compared with micro-environment and genetic polymorphisms for therapeutic prediction based mainly on the interactional complexity of factors Then, since this polymorphism does not matter for key therapeutic targets such as CD44 and TP53, when treatment strategies are being formulated according to those pathways, it makes sense to look directly at the expression levels of genes or chromosomal changes like 17p deletion And so in CLL: genetic risk may be more important than CD38 genotype Although the latter does indeed have marked adverse effects on an individual's outcome Another finding is whatsoever the cause, CLL will always emerge again These results mean a change from viewing a single factor as the answer, such as CD38, to using a range of signs in treatment networks and so mark the start of more precise diagnosis across all disease types – providing future clinical management with true personalization being conducted through molecular biology research.

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ORIGINAL ARTICLE

Histological changes in the structural organization of rat kidneys 3 days after administration of Leiurus macroctenus scorpion venom

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ABSTRACT

Aim: To investigate histological changes in the structural organization of rat kidneys 3 days after administration of Leiurus macroctenus scorpion venom.

Materials and Methods: The venom of Leiurus macroctenus, was administered to rats (0.5 ml of venom solution; 28.8 μg/ml; LD50=0.08 mg/kg). The study used 20 white male laboratory rats weighing 200 g (±10 g). The rats were divided into two groups: control group – 10 rats; experimental group – 10 rats. For microscopic examination, kidney samples from animals of all groups were taken. Histological samples of rat kidneys were stained with hematoxylin and eosin.

Results: Microscopically, on the 3rd day of the experiment after the introduction of Leiurus macroctenus scorpion venom, significant destructive-degenerative changes in all structural components of the kidney were detected. Most renal corpuscles are large with well-defined vascular glomeruli. The endothelium of the capillaries is thinned, destructively changed. Heterogeneity of changes in proximal and distal tubules is manifested.

Conclusions: The indirect effect of the Leiurus macroctenus scorpion venom led to the formation of voluminous inflammatory loci of leukocyte infiltrates in the interstitium and directly in the tubules and renal corpuscles on the 3rd day of the experiment. Significant alterative changes in the renal corpuscles were manifested by their deformation, atrophy, hyperemia and multiple hemorrhages. In the tubular system, pronounced hydropic and protein dystrophy were detected, and in their lumens, voluminous accumulations of cellular detritus were detected.

KEYWORDS: scorpions, kidneys, glomerulus, nephron, rats

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INTRODUCTION

Scorpion venom is a so-called arsenal of biological components, containing mainly peptides and proteins with various functions, in particular neurotoxins, phospholipases, mucoproteins, hyaluronidases, as well as nucleotides, biogenic amines, etc. Peptides from scorpion venom can be divided into two groups – peptides with disulfide bridges (DBPs) and peptides without disulfide bridges (NDBPs). The neurotoxic activity of scorpion venom is associated with the effect of DBPs, which demonstrate the ability to change the permeability of ion channels in excitable and non-excitable cells (blocking or reducing the action potential of ion channels). Depending on the effect on certain ion channels, toxins of sodium, potassium, calcium and chloride channels are distinguished [1, 2].

Sodium channel toxins have been experimentally shown to be the main cause of scorpion venom symptoms. These peptides have a structure of 58 to 76 amino acid residues stabilized by four disulfide bridges and are classified into α-toxins and β-toxins. α-toxins have the ability to prolong the depolarization time of the ion channel, while β-toxins contribute to a change in its activation threshold [3, 4].

DBPs that act on potassium ion channels have a wide variety of primary sequences, with 20-70 amino acid residues and a three-dimensional structure stabilized by three or four disulfide bridges. These peptides are classified according to their molecular weight and disulfide bridge structure into α-KTx, β-KTx, γ-KTx, and κ-KTx toxins [5].

Chloride and calcium channel toxins are currently less studied, but they play no less a role in the development of complications from scorpion stings. Chloride channel toxins (containing 30-40 amino acid residues) have a structural conformation stabilized by four disulfide bridges. The components of scorpion venom that act on calcium channels represent a heterogeneous family with wide variability in their primary amino acid sequence and molecular weight [6].

Scorpion NDBPs exhibit predominantly antimicrobial activity. They typically consist of 13-56 amino acid residues, possess hydrophobic properties, have a positive surface charge due to the presence of basic amino acids, and a helical structural conformation in environments that mimic the structure of biological membranes [7].

The biological effects of scorpion venom toxins are variable and are still under active investigation. The pathophysiology of renal injury following scorpion stings is poorly understood, but in vivo experiments in rats have demonstrated renal dysfunction following administration of Tityus stigmurus

venom. Infusion of 1.0 μg/mL of venom caused moderate renal tubular damage and mild glomerular damage. In addition, transient increases in perfusion pressure, renal vascular resistance, glomerular filtration rate, and renal excretion of Na+, K+, and Cl– were observed [8].

AIM

The aim of the study was to investigate histological changes in the structural organization of rat kidneys 3 days after administration of Leiurus macroctenus scorpion venom.

MATHERIALS AND METHODS

The venom of scorpions of the Buthidae family, genus Leiurus, species Leiurus macroctenus, was administered to rats once intramuscularly (0.5 ml of venom solution previously dissolved in saline solution; 28.8 μg/ml; LD50=0.08 mg/kg) [9].

The study used 20 white male laboratory rats weighing 200 g (±10 g), raised in the vivarium of the Educational and Scientific Center „Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv (Agreement on Scientific and Practical Cooperation between Taras Shevchenko National University of Kyiv, National Pirogov Memorial Medical University, Vinnytsya and I. Horbachevsky Ternopil National Medical University dated February 1, 2021). Rats were kept on a standard diet in an accredited vivarium in accordance with the „Standard Rules for the Arrangement, Equipment and Maintenance of Experimental Biological Clinics (Vivaria)". The experiments were conducted in accordance with the current regulatory documents regulating the organization of work with the use of experimental animals and compliance with the principles of the „European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [10]. Also, all interventions with animals were carried out in accordance with the Law of Ukraine No. 3447-IV of February 21, 2006 „On the Protection of Animals from Cruelty and Ethical Norms and Rules for Working with Laboratory Animals."

The rats selected for the experiment were divided into two groups: control group – 10 rats, no venom was administered, biological material was collected 3 days after the administration of saline solution; experimental group – 10 rats, histological material was collected 1 and 3 hours after the administration of the scorpion venom. Rats were euthanized by carbon dioxide inhalation. Kidneys of rats were isolated at 4 °C immediately after euthanasia.

For microscopic examination, kidney samples from animals of all groups were taken. The pieces were fixed in 10% formalin solution for 1 day. Then, the pieces were dehydrated in alcohols of increasing concentration and embedded in paraffin blocks. Histological samples of rat kidneys were stained with hematoxylin and eosin [11]. Histological slides were studied using a SEO SCAN light microscope and photodocumented using a Vision CCD Camera with an image output system from histological preparations.

RESULTS

Microscopically, on the 3rd day of the experiment after the introduction of Leiurus macroctenus scorpion venom, significant destructive-degenerative changes in all structural components of the kidney were detected. Alteration of the vascular and microcirculatory bed is noted, manifested by hyperemia, the formation of thrombi, and in extensive areas, damage to the vascular wall leads to hemorrhages. There are significant focal leukocyte infiltrations in the organ parenchyma.

The stroma of the kidney is destructively changed, collagen fibers in the dense connective tissue of the capsule are swollen, often lose their structure, significant swelling of amorphous substance is observed in the interstitium. The vessels of the microcirculatory bed have a disturbed wall structure, the endothelium is thinned, the basement membrane is not clear, and stagnant phenomena are observed.

In the cortical layer of the kidney, renal corpuscles of various shapes and sizes are found. Most renal corpuscles are large with well-defined vascular glomeruli, in the hemocapillaries of which stagnant phenomena are detected. The endothelium of the capillaries is thinned, destructively changed, endothelial cells with dense basophilic nuclei were detected. Mesangiocytes have irregularly shaped intensely basophilic, hyperchromic nuclei, which indicates their pyknosis. The intercellular substance of the mesangium is edematous, weakly oxyphilic. The urinary spaces in most renal corpuscles are narrow, almost absent or slit-like. Polymorphism of epithelial cells of the single-layered squamous epithelium of the parietal leaf of the glomerular capsule is noted, their nuclei are edematous or significantly compacted. Desquamation of epithelial cells into the urinary lumen is often detected. Podocytes in the visceral layer of the Shumlyansky-Bowman capsule have a damaged structure of cytopodia and cytopraeculum. Single renal corpuscles are characterized by small size, irregular shape with significantly compacted, wrinkled and destructive-degenerative changes (Fig. 1).

Heterogeneity of changes in proximal and distal tubules is manifested. Some epithelial cells are characterized by granular and hydropic dystrophy, pyknosis of nuclei. Thinning of the

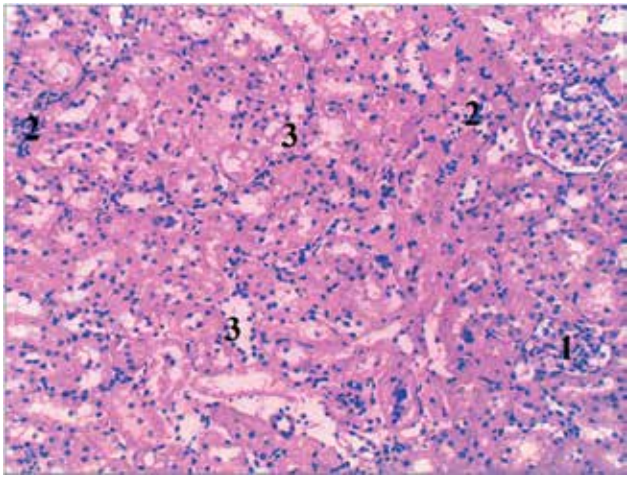

Fig. 1. Histological changes in the cortical layer of white rats kidney 3 days after the administration of the Leiurus macroctenus scorpion venom: 1 – atrophied renal corpuscle, 2 – histoleukocyte infiltrate, 3 – degeneration of the tubular apparatus. Staining with hematoxylin and eosin. x 100

Source: Own materials

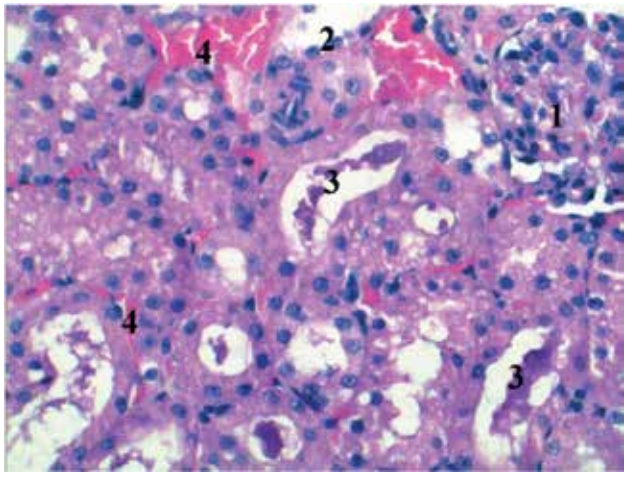

Fig. 2. Histological changes in the kidney of white rats 3 days after the administration of the Leiurus macroctenus scorpion venom: 1 – fragment of the renal corpuscle, 2 – destruction of the distal tubule, 3 – cellular detritus in the lumen of the tubule, 4 – blood-filled vessels. Staining with hematoxylin and eosin. x 100

Source: Own materials

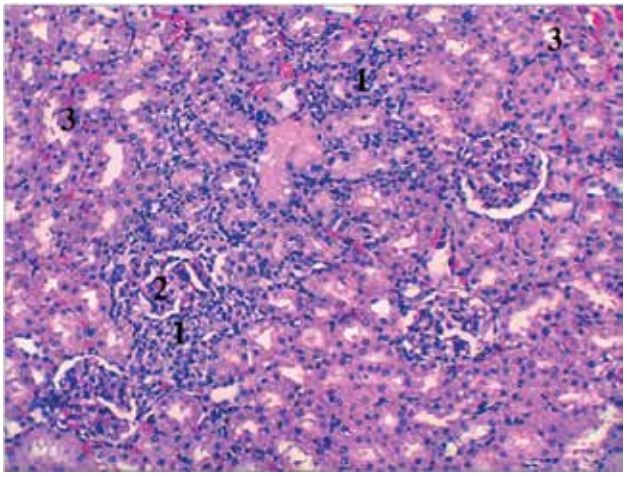

Fig. 3. Histological changes in the kidney of white rats 3 days after administration of the Leiurus macroctenus scorpion venom: 1 – histo-leukocyte infiltrate, 2 – renal corpuscle with slit-like urinary spaces, 3 – desquamation of epithelial cells of the distal tubules. Staining with hematoxylin and eosin. x 100

Source: Own materials

epithelium of the tubule wall is observed, desquamation of epithelial cells into the lumen of the tubules up to the exposure of the basement membrane. In the lumens of dilated distal tubules, significant protein-cellular, detrital accumulations are detected (Fig. 2). Many epithelial cells have a destroyed or significantly refined brush border. The nuclei of some epithelial cells do not have a clear karyolemma and are subject to lysis. Desquamation of epithelial cells into the lumen of the tubules is observed.

In the cortical layer of the kidneys of white rats at this period of the experiment, hemorrhages with hemolysis of erythrocytes are detected, which occur due to the direct toxic effect of the components of the venom on the vascular wall (Fig. 3). Also, foci of inflammatory reactions in the form of infiltrations of lymphocytes and macrophages are detected.

DISCUSSION

Scientific data indicate that the Hemiscorpius lepturus scorpion venom has a nephrotoxic effect, causing proteinuria and hematuria. Clinical data show that the venom of this scorpion is highly toxic, especially for children. However, the exact pathogenetic mechanisms of the toxic effect of the scorpion Hemiscorpius lepturus are still unclear. Induction of oxidative stress negatively affects mitochondrial homeostasis and ATP levels in the kidneys, reduces the activity of antioxidant defense, leads to an increase in the levels of reactive oxygen species (ROS), ultimately leading to disruption of the normal functioning of mitochondria [12]. Coenzyme Q10 deficiency (occurring under these conditions), as one of the main electron carriers in the mitochondrial respiratory chain, reduces the activity of mitochondrial complexes and reduces the formation of ATP, which is necessary for the modulation of the cells redox state. Such oxidative stress conditions lead to overexpression of genes involved in mitochondrial biogenesis, which increases energy demand. Increased activity of key metabolic sensors, such as AMPK (AMP activated protein kinase), may compensatorily regulate energy status in the kidney. High intracellular AMP/ATP ratio activates AMPK under metabolic stress to maintain energy homeostasis through fatty acid beta-oxidation, protein synthesis, ion transport, and induction of PGC-1α (γ-coactivator of receptor 1-alpha), a master regulator of cellular energy metabolism and mitochondrial biogenesis [13, 14].

Dizaji R. et al. suggest that naturally occurring toxins induce acute kidney injury through the action of PLA2, which is involved in hemolysis and cytotoxicity. It also plays a fundamental role in inflammation by activating arachidonic acid, which leads to the formation of eicosanoids (prostaglandins and leukotrienes). PLA2 also stimulates the hypothalamic-pituitary-adrenal axis to produce adrenocorticotropic hormone, corticosteroids, vasopressin, and acute phase proteins, causing local manifestations at the site of the bite, as well as hemodynamic changes [15].

It has been established that scorpion bites release a large amount of hemoproteins that enter the extracellular space, where hemoglobin oxidation occurs. In turn, after hemolysis, a high concentration of Fe3+ can be observed, releasing heme groups and, thus, accelerating the formation of free radicals that accumulate in the renal cortex, contributing to the toxic effect [16].

A thorough analysis of the scientific literature has demonstrated the involvement of macrophages in the development of acute kidney injury in scorpion stings. Thus, the level of MCP increases in the victim's body, which indicates the initiation of an inflammatory process in the organ and signals the risk of complications, since it directly affects the migration of macrophages, proliferation and differentiation of leukocytes in the epithelium of the human kidney tubules. MCP-1 stimulates the secretion of IL-6 and the intercellular expression of ICAM-1. In addition, by binding to chemokine receptors CC2 on the surface of podocytes, it can reduce the expression of both microRNA and nephrin

protein. The latter is involved in the formation of filtration gaps, so defects in this protein lead to the development of renal failure, disruption of the podocyte cytoskeleton, and disorders of the filtration process [17].

CONCLUSIONS

The indirect effect of the Leiurus macroctenus scorpion venom led to the formation of voluminous inflammatory loci of leukocyte infiltrates in the interstitium and directly in the tubules and renal corpuscles on the 3rd day of the experiment.

Significant alterative changes in the renal corpuscles were manifested by their deformation, atrophy, hyperemia and multiple hemorrhages. In the tubular system, pronounced hydropic and protein dystrophy were detected, and in their lumens, voluminous accumulations of cellular detritus were detected.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Stress, resilience and job satisfaction among emergency department healthcare workers in Greece

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ABSTRACT

Aim: This study aimed to assess levels of perceived stress, psychological resilience and job satisfaction among Emergency Department (ED) healthcare professionals in Attica, Greece, and examine the correlations among them.

Materials and Methods: A cross-sectional study was conducted in 12 public hospitals in Attica, Greece, using self-administered questionnaires: Perceived Stress Scale (PSS-10), Connor-Davidson Resilience Scale (CD-RISC-10), and Job Satisfaction Survey (JSS). Descriptive statistics, Pearson correlations, and multiple linear regression analyses were performed using SPSS v30.

Results: Participants reported moderate levels of perceived stress (M=17.72, SD=6.30) and psychological resilience (M=27.18, SD=6.01), while job satisfaction appeared variable, with an overall mean score indicating low to moderate satisfaction (M=113.10, SD=23.41). A significant negative correlation was found between perceived stress and job satisfaction ($r=-0.36$, $p < .001$), as well as between stress and resilience ($r=-0.53$, $p < .001$). A positive but weaker correlation was observed between resilience and job satisfaction ($r=0.14$, $p < .001$).

Conclusions: Psychological resilience plays a critical protective role against stress and positively influences job satisfaction among ED healthcare professionals. Resilience-enhancing interventions may reduce stress and improve satisfaction among ED staff.

KEYWORDS: mental well-being, work environment, healthcare professionals, emergency healthcare settings

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INTRODUCTION

Healthcare professionals in Emergency Departments (EDs) operate in high-stress environments characterized by time constraints, critical cases, and emotional intensity – factors linked to anxiety, burnout, and exhaustion [1, 2]. Psychological resilience has been identified as a key buffer against such stressors, promoting adaptability and sustaining mental well-being [3-4]. Studies show that greater resilience correlates with reduced anxiety and higher job satisfaction [5].

In Greece, despite mounting evidence of occupational stress in healthcare, limited research has examined how resilience, perceived stress, and job satisfaction interrelate – especially among ED staff. Existing studies mainly focus on stress and satisfaction, neglecting resilience and the unique pressures of ED work, including trauma exposure and shift-related fatigue [6, 7, 8]. This study addresses this gap by investigating these three constructs in ED professionals from Greece's largest health region, aiming to inform targeted interventions and improve staff well-being, retention, and patient care [9].

AIM

The primary aim of this study was to assess the levels of perceived stress, psychological resilience, and job satisfaction among healthcare professionals working in EDs in public hospitals in Attica, Greece.

A secondary aim was to examine the correlations among these three variables and investigate whether resilience mitigates the negative impact of stress on job satisfaction. Specifically, the following hypotheses were tested:

- H1: Perceived stress and resilience are significantly correlated.
- H2: Resilience is significantly correlated with job satisfaction.
- H3: Perceived stress is significantly correlated with job satisfaction.
- H4: Gender differences exist in all three variables.
- H5: Age-group differences exist in all three variables.

MATERIALS AND METHODS

This cross-sectional study was conducted in 12 public hospitals in Attica, Greece, targeting Emergency Department (ED) professionals, including physicians, nurses, and lab staff. Systematic random sampling ensured representativeness across roles and institutions. A total of 543 participants (67.8% women, 31.9% men) voluntarily completed anonymous, self-administered questionnaires between November 2024 and March 2025. Most were aged 45-54 years (29.5%). Data were collected confidentially via sealed envelopes.

Approvals for data collection were obtained from the scientific councils of the participating hospitals.

Three validated psychometric instruments were used in this study to assess perceived stress, psychological resilience, and job satisfaction. Perceived stress was measured using the 10-item version of the Perceived Stress Scale (PSS-10) [10], with official permission obtained through the Mapi Research Trust and additional approval from M.N. Adonis, who had previously translated the questionnaire into Greek for research purposes [11]. Participants responded on a 5-point Likert scale ranging from 0 ("never") to 4 ("very often"), with higher scores reflecting greater levels of perceived stress. Psychological resilience was assessed using the 10-item version of the Connor–Davidson Resilience Scale (CD-RISC-10) [12, 13], following written permission from the original developers of the instrument. The Greek validated version was used, as established by Galanis et al. [14]. Each item was rated on a 5-point scale from 0 ("not true at all") to 4 ("true nearly all the time"), with higher scores indicating greater resilience. Finally, Job satisfaction was evaluated using the Job Satisfaction Survey (JSS), developed by Spector [15], for which official permission for use was granted. The study employed the Greek validated version of the instrument by Tsounis and Sarafis [16], which has demonstrated satisfactory psychometric properties. Although the original scale includes nine dimensions, only the total job satisfaction score was utilized for the purposes of the present analysis.

Data were analyzed using IBM SPSS Statistics Version 30. Descriptive statistics summarized demographic data, while inferential tests were chosen according to data distribution and normality assumptions. Pearson's r assessed correlations between variables, and group differences were examined using independent samples t-tests or one-way ANOVA. Statistical significance was set at $p<.05$.

ETHICS

This work complies with the principles of the Declaration of Helsinki.

RESULTS

The descriptive statistics for the main variables are presented in Table 1. The average level of perceived stress (M=17.72, SD=6.30) indicates a moderate stress load, which is expected given the demanding and high-pressure nature of emergency healthcare environments. Psychological resilience showed a relatively higher mean (M=27.18, SD=6.01), suggesting that many participants possessed internal coping mechanisms that may mitigate the effects of chronic stress. Job satisfaction had an average score of 113.10 (SD=23.41), though with a wide range (13 to 199), reflecting considerable variation in work-related satisfaction among professionals.

Further analysis using Pearson's correlation coefficient (Table 2) revealed statistically significant relationships among the three variables. There was a strong negative correlation between perceived stress and psychological resilience ($r=-0.53$, $p<.001$), indicating that higher resilience is associated with lower stress levels. Similarly, perceived stress negatively correlated with job satisfaction ($r=-0.36$, $p<.001$), reflecting that individuals experiencing higher levels of stress are less satisfied with their jobs. Conversely, psychological resilience showed a weak but statistically significant positive correlation with job satisfaction ($r=0.14$, $p<.001$), suggesting a potential buffering effect of resilience on occupational wellbeing.

To examine gender differences, independent samples t-tests were conducted for perceived stress, psychological resilience, and job satisfaction. Levene's test for equality of variances was performed prior to each test. The assumption of homogeneity was met for perceived stress and psychological resilience, while it was violated for job satisfaction.

Table 1. Descriptive statistics of the main variables

Variable	Mean	SD	Min	Max	Skewness	Kurtosis
Perceived Stress	17.72	6.30	1	36	0.05	-0.46
Psychological Resilience	27.18	6.01	0	40	-0.37	0.41
Job Satisfaction	113.10	23.41	13	199	0.12	0.83

Source: compiled by the authors of this study

Table 2. Correlations between the three variables

	Perceived Stress	Psychological Resilience	Job Satisfaction
Perceived Stress	--		
Psychological Resilience	-.53***	--	
Job Satisfaction	-.36***	.14**	--

*Note: ** <0.01, ***< 0.001*

Source: compiled by the authors of this study

Gender differences were significant in two variables. Men reported lower perceived stress (M=15.89, SD=6.09) than women (M=18.58, SD=6.23), t (535)=-4.71, p < .001, and higher resilience (M=28.16, SD=6.04 vs. M=26.73, SD=5.95), t (538)=2.60, p=.010. No significant gender difference emerged in job satisfaction, though men scored slightly higher (M=115.10 vs. M=113.29), t (538)=0.89, p=.375.

To assess age differences, three one-way ANOVAs were conducted. Levene's test confirmed homogeneity of variances. No significant age effect was found on perceived stress, F (4, 538)=1.29, p=.273, η^2=.01. A significant effect of age was observed on resilience, F (4, 541)=5.46, p < .001, η^2=.04, with the 55+ group (M=29.65, SD=5.41) scoring higher than:

- <25 (M=24.88), p=.022
- 25-34 (M=26.19), p<.001
- 35-44 (M=26.80), p=.006
- 45-54 (M=27.42), p=.045

Job satisfaction also varied by age, F (4, 541)=2.56, p=.038, η^2=.02, but post hoc comparisons showed no significant differences.

Finally, subgroup analysis by professional role revealed varying levels of association. Among physicians, perceived stress and psychological resilience were strongly inversely correlated (r=-0.64, p<.001), while the relationship between stress and job satisfaction was moderate (r=-0.30, p<.001). Resilience and satisfaction were positively correlated (r=0.15, p<.05) (Table 3).

In nurses, perceived stress was moderately negatively associated with both resilience (r=-0.47, p<.001) and job satisfaction (r=-0.44, p<.001), while the correlation between resilience and satisfaction was slightly positive (r=0.23, p<.01) (Table 4).

Among clinical laboratory professionals, perceived stress showed significant negative correlations with both psychological resilience (r=-0.41, p<.001) and job satisfaction (r=-0.35, p<.001). However, the association between resilience and job satisfaction was not statistically significant (r=0.03, ns) (Table 5).

DISCUSSION

Taken together, the findings highlight the central role of psychological resilience as a protective factor in the high-stress environment of emergency healthcare. Individuals with greater resilience not only report lower stress levels but also tend to exhibit greater satisfaction with their work. As perceived stress increases, job satisfaction declines, suggesting a detrimental impact of stress on occupational wellbeing. These patterns support the hypothesis that

Table 3. Correlations among study variables for physicians

	PS	PR	JS
Perceived Stress (PS)	--		
Psychological Resilience (PR)	-.64***	--	
Job Satisfaction (JS)	-.30***	.15*	--

*Note: *<0.05, ***< 0.001*

Source: compiled by the authors of this study

Table 4. Correlations among study variables for nurses

	PS	**PR**	**JS**
Perceived Stress (PS)	--		
Psychological Resilience (PR)	-.47***	--	
Job Satisfaction (JS)	-.44***	.23**	--

*Note: ** <.01, ***< .001*

Source: compiled by the authors of this study

Table 5. Correlations among study variables for clinical laboratory personnel

	PS	**PR**	**JS**
Perceived Stress (PS)	--		
Psychological Resilience (PR)	-0.41***	--	
Job Satisfaction (JS)	-0.35***	0.03	--

*Note: ***<0.001*

Source: compiled by the authors of this study

resilience functions as a mediating variable and may serve as a target for future interventions aimed at improving mental health and retention among emergency healthcare workers.

PSYCHOLOGICAL RESILIENCE AS A PROTECTIVE FACTOR

Consistent with existing literature, psychological resilience emerged as a key predictor of occupational wellbeing. The significant inverse correlation between resilience and perceived stress (r=-0.53) aligns with earlier studies showing that resilient individuals are more adept at emotion regulation and cognitive reappraisal, which can reduce the subjective impact of stressors [17, 18]. Furthermore, although the correlation between resilience and job satisfaction was weaker (r=0.14), it was nonetheless significant, suggesting that resilience contributes positively to an individual's perception of their work environment and emotional rewards [19]. Resilience is often understood as the ability to recover from challenging situations, yet in healthcare settings it also encompasses the capacity to function effectively under sustained pressure, to remain empathetic, and to uphold constructive interpersonal relationships [20]. In the current study, older professionals (aged 55+) reported significantly higher resilience than their younger counterparts, possibly reflecting greater emotional maturity, cumulative experience, and developed coping strategies over time [21]. This finding emphasizes the need for resilience-building interventions targeted especially at early-career professionals, who may be more vulnerable to emotional exhaustion and burnout [22].

PERCEIVED STRESS AND, ITS DETRIMENTAL IMPACT

The finding that perceived stress negatively correlates with job satisfaction (r=-0.36) reinforces prior international evidence suggesting that chronic stress undermines motivation, engagement, and workplace wellbeing. ED professionals are often exposed to unpredictable shifts, critical incidents, and high mortality rates, all of which contribute to cumulative stress loads [23]. This persistent strain can lead to emotional exhaustion, reduced personal accomplishment, and withdrawal from meaningful patient interaction – a trajectory characteristic of burnout [24].In Greece, the effects of systemic under-resourcing and staff shortages may exacerbate these stressors, particularly in public EDs [25]. The present findings, showing moderate average stress levels, suggest an urgent need for policy-level support to mitigate occupational pressure, including staffing adjustments, psychological support systems, and resilience training programs.

JOB SATISFACTION: A COMPLEX CONSTRUCT

Job satisfaction was found to vary considerably among participants (SD=23.41), with some individuals reporting very low satisfaction scores, raising concerns about workplace morale and retention. While the correlation with resilience was relatively weak, its consistency across professional roles (especially among nurses: r=0.23, $p < .01$) suggests that resilience may be particularly impactful in professions that involve continuous patient interaction and emotional labor. Interestingly, gender differences in stress and resilience were observed, with women reporting significantly higher stress and lower resilience. This echoes previous findings indicating that female healthcare workers often bear disproportionate emotional burdens, including caregiving roles both at work and home [26]. However, job satisfaction did not significantly differ by gender, suggesting that while stress affects both genders, factors beyond emotional burden-such as professional recognition, autonomy, and support-may mediate satisfaction.

SUBGROUP ANALYSIS AND PROFESSIONAL ROLE DIFFERENCES

The differentiated findings across professional roles are particularly revealing. Among physicians, the correlation between resilience and stress was strongest (r=-0.64), suggesting that physicians may experience resilience as a core buffer in their high-responsibility roles. Nurses, on the other hand, showed stronger correlations between stress and job satisfaction (r=-0.44), reflecting perhaps a deeper emotional toll due to direct patient care, shift fatigue, and limited institutional voice [27]. Notably, clinical laboratory personnel showed no significant relationship between resilience and job satisfaction, indicating that the mechanisms linking resilience to satisfaction may depend on job content and interpersonal exposure.

IMPLICATIONS, LIMITATIONS, AND FUTURE DIRECTIONS

These findings have important implications for healthcare policy and workforce management. Incorporating resilience-building modules – especially targeting younger and newly employed staff – may enhance coping skills and reduce turnover. Evidence-based techniques like cognitive-behavioral interventions and mindfulness-based stress reduction (MBSR) have proven effective in fostering resilience [28, 29]. Moreover, the observed negative link between stress and job satisfaction highlights the need for institutional strategies to alleviate stress, including flexible schedules, protected breaks, and access to mental health services. Cultivating a team-oriented culture could also promote social support, a known resilience factor [30].

However, the study presents some limitations. The cross-sectional design precludes causal inferences, future longitudinal studies could better illuminate stress–resilience dynamics. Mixed-method approaches, including qualitative tools, could enrich future research. Additionally, the sample was restricted to public hospitals in Attica, limiting generalizability, comparative research in rural or private settings is needed. Lastly, the study used overall scores for resilience and job satisfaction, potentially overlooking relevant subdimensions (e.g., emotional exhaustion, autonomy, or perceived fairness), which merit further investigation.

CONCLUSIONS

The challenges faced by Emergency Department professionals cannot be addressed solely at the individual

level. While personal resilience plays a role, systemic conditions often shape the boundaries of what individuals can endure. Future efforts should focus not only on strengthening personal coping skills, but also on creating healthcare environments where resilience is not a requirement for survival, but a resource for growth and collaboration.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Immunohistochemical analysis and distribution of lymphocytes and Kupffer cells in the liver of rats with long-term experimental use of hemp seed oil

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ABSTRACT

Aim: To conduct histological and immunohistochemical analysis and distribution of lymphocytes and Kupffer cells in the liver of rats with long-term experimental use of hemp seed oil (HSO).

Materials and Methods: 26 sexually mature male rats (180-230 g, 5-7 months old) were divided into three groups: experimental (n=14, 0.5 ml/kg/day HSO orally for 10 weeks), control (n=6, 0.1 ml/kg/day HSO orally for 10 weeks), and intact (n=6). Histological and immunohistochemical (CD3, CD20, CD56, CD68) studies, alongside quantitative analysis of lymphocyte and macrophage distribution in hepatic lobules, were performed. Statistical significance was assessed using Mann-Whitney and Pearson tests, with $p<0.05$ considered significant.

Results: After 10 weeks of 0.5 ml/kg/day HSO, 71.43% of experimental rats developed mild fatty liver disease (Kleiner grade S1 steatosis), a significant difference from the control group ($p<0.0001$). No histological inflammation or necrotic changes in hepatocytes were observed. Small numbers of CD3 lymphocytes were present in portal tracts, without extending into or damaging the adjacent parenchyma. CD20 and NK resident lymphocytes were sparse. Aggregates of CD68-positive Kupffer cells were most common near liver lobule triads. The average number of Kupffer cells (5.79 ± 0.06 per 0.01 mm^2) in the experimental group significantly ($p<0.001$) exceeded the control by 1.49 times, suggesting hyperplasia of specialized macrophages and their increased role in liver immune function.

Conclusions: Ten weeks of experimental use indicates that hemp seed oil is safe to consume at a dose of 0.5 ml/kg/day.

KEYWORDS: liver, rats, histology, immunohistochemistry, CD3, CD20, CD56, CD68, hemp seed oil, safety

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INTRODUCTION

Hemp seed oil (Cannabis sativa L.), extracted by cold pressing, retains all the beneficial qualities of the product without heat and chemical processing, has significant nutritional and biological properties due to its unique composition, contains up to 90% unsaturated fatty acids, with polyunsaturated fatty acids accounting for 70-80% of this fraction [1, 2]. Hemp seed oil is rich in antioxidants, phospholipids, phytosterols, and essential minerals such as calcium, magnesium, sulfur, potassium, zinc, and vitamins [3]. Hemp seed oil is particularly valuable for its omega-6 and omega-3 acids. These important acids are rarely found in significant quantities in nature. The ideal Omega-6/Omega-3 ratio in hemp seed oil is 3:1, which meets human nutritional needs and complies with the recommendations of the European Food Safety Authority and food products for maintaining health and preventing chronic diseases [4, 5]. Hemp seed oil and hemp seeds have a preventive effect and support the cardiovascular, endocrine, and immune systems. Linoleic, linolenic, and arachidonic fatty acids prevent cholesterol deposits in the arteries, ensure healthy skin and hair, have a positive effect on the activity of the endocrine glands, and promote weight loss body by burning saturated fats [6].

Hemp seed oil is generally considered safe, but its effect on the liver may depend on the dosage and duration of use. E.M. Musa and co-authors (2012) studied the toxicological effects on the liver and side effects of hemp seed oil. They presented the results of their experimental study on white Wistar albino rats [7]. The experiment lasted 4 weeks, during which rats in three experimental groups were orally administered different doses of hemp seed oil. The rats in the first experimental group consumed 0.01 ml/kg/day, the rats in the second group consumed 0.1 ml/kg/day, and the rats in the third group consumed 1 ml/kg/day. After four weeks of the experiment, the liver enzymes ALT, AST, and LF were significantly elevated in all experimental groups of rats compared to the control group. In the group of rats that consumed hemp seed oil at a dose of 1 ml/kg/day (high dose), parenchymal fatty liver dystrophy (steatosis) was significantly pronounced, and histological examination of the liver showed widespread macro- and microvesicular steatosis and focal areas of hepatocyte necrosis [7].

The liver regulates homeostasis, including antigenic constancy. Cells of the immune system – lymphocytes, macrophages, Kupffer cells, and Ito cells – play an active role in this process [8]. Any impact on the liver or excessive oxidative stress with damage to cellular structures triggers

a response with the activation of resident macrophages and lymphocytes [9, 10]. Analysis experimental use of hemp seed oil, depending on the dose and duration, is important for establishing the safety of its use.

AIM

To conduct histological and immunohistochemical analysis and distribution of lymphocytes and Kupffer cells in the liver of rats with long-term experimental use of hemp seed oil.

MATERIALS AND METHODS

A series of experimental studies was conducted in a vivarium on 26 white sexually mature male rats weighing 180-230 g, aged 5-7 months at the start of the experiment, in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986), Council Directive 2010/63/ EU, Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruel Treatment," and after ethical approval by the Bioethics Committee at Danylo Halytsky Lviv National Medical University (Minutes No. 7 of August 29, 2022) [11, 12].

The rats were divided into three groups. The experimental group consisted of 14 animals, which were administered hemp seed oil orally once a day for 10 weeks at a dose of 0.5 ml/kg/day (average dose); the control group consisted of 6 animals, which were administered hemp seed oil orally once a day for 10 weeks at a dose of 0.1 ml/kg/day (minimum dose); the intact group consisted of 6 sexually mature white male rats. The dose for the experiment was calculated based on the recommended dose of 0.25 g/kg for an adult human and 35% oil content in hemp seeds using the equation: dose for animals (g/kg)=dose for humans (g/kg) × oil content in hemp seeds × Km for humans/Km for animals, where Km for an adult human was 37 and Km for a rat was 6 [13, 14]. The dose was 0.54 g/kg, and the oil density was 1.08 g/ml. Thus, 0.54 g/kg ÷ 1.08 g/ml=0.5 ml/kg.

The rats were kept in special cages in a ventilated room of a standard vivarium at a controlled temperature of 21±1°C and humidity of 50-55%, had a standard diet for laboratory animals and constant access to fresh water (ad libitum). Throughout the experiment, observations were made on feed intake and the general condition of the animals. The behavior of the rats and the condition of their fur were also assessed.

The material for research was collected at the end of the experiment after euthanasia and autopsy. The rats' livers were subjected to morphological examination. Pieces of liver tissue were fixed in 10% neutral buffered formalin, dehydrated in ascending concentrations of alcohol, and embedded in paraffin according to standard procedure, after which histological sections 5±1 μm thick were made from the paraffin blocks and stained with hematoxylin and eosin for general examination [15]. For a detailed study of the sinusoids of the liver lobules, a series of semi-thin sections 0.5-1 μm thick were prepared from epoxy blocks using a standard method, stained with methylene blue, and studied under a light microscope at a magnification of ×1000 (immersion) [16].

We studied the morphological features of the parenchymal and stromal-vascular compartments of the liver. The degree of hepatosis was determined according to Kleiner [17]. We also performed immunohistochemical studies on histological sections from paraffin blocks intended for standard morphological examination. Monoclonal antibodies were used for macrophages and Kupffer cells CD68 (Clone KP1, Dako), T-lymphocytes CD3 (Clone SP7, Thermo Fisher Scientific), B lymphocytes CD20 (Clone Ab-1, Thermo Fisher Scientific), NK lymphocytes CD56 (Clone 123C3, Dako). The studies were conducted in accordance with the manufacturer's protocol with the necessary controls and visualization using a detection system with diaminobenzidine chromogen [18, 19].

The results of immunohistochemical staining were analyzed and the location of lymphocytes and Kupffer cells in the portal tracts, near the central vein, and in the lobular sinusoids were semi-quantitatively assessed as follows: 0 (no cells); R (rare single cells present); 1+ (few); 2+ (moderate number); 3+ (many). For quantitative analysis we counted the Kupffer cells on an area of 0.01 mm2 of the histological specimen (100 μm×100 μm) in different areas of the liver lobules (near the portal tracts, around the central vein, and in the intermediate zone between the triad and the central vein). For general examination of histological preparations and microphotography, we used a Leica DM 2500 light microscope (Leica Microsystems GmbH, Germany) with a Leica DFC450 C digital camera (Germany) and Leica Application Suit Version 3.8 software.

Statistical processing of experimental research data was performed using Microsoft Office Excel. The reliability of the difference between group indicators was checked using the Mann-Whitney test, and relative indicators were checked using the Pearson test. The difference was considered statistically significant at a minimum significance level of $p<0.05$ [20].

RESULTS

Microscopically, the liver tissue of all study groups is represented by lobules, which are structural and functional units in the architecture of the liver. Histological analysis of liver samples from the experimental group of animals, which orally administered hemp seed oil at an average dose of 0.5 ml/kg/day, showed the following: hepatocytes formed rows of beams, the beam structure of the liver lobule was clearly preserved, and hepatocytes had a polygonal shape with rounded nuclei. The average diameter of hepatocytes was 21.15±2.43 μm and significantly exceeded the corresponding indicator in the control and intact groups of animals ($p<0.05$). In the fields of view, binuclear hepatocytes with a diameter of about 28.55 μm were diagnosed, but such hepatocytes were rare.

Light optical examination of histological sections of liver tissue revealed the presence of hepatocytes with signs of small-droplets and large-droplets fatty degeneration in the form of accumulations of optically empty vacuoles of

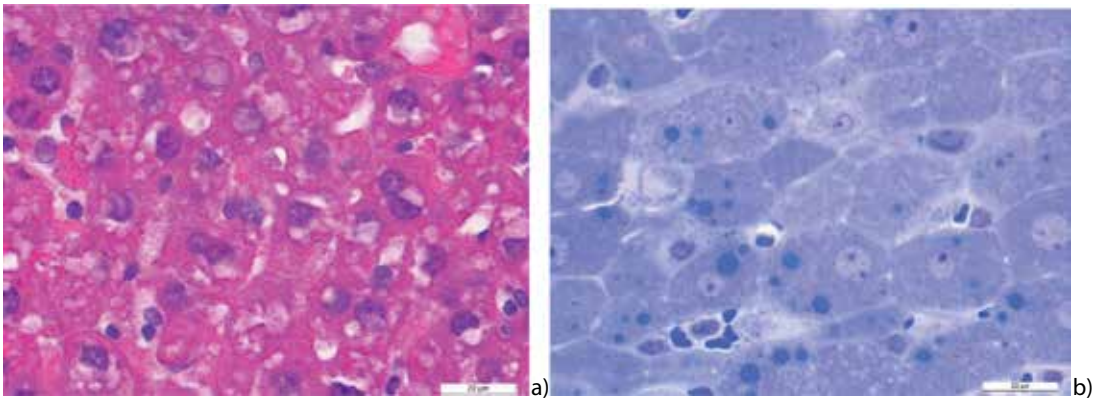

Fig. 1. Presence of hepatocytes with signs of small-droplet and large-droplet fatty degeneration, intermediate zone of the lobule, steatosis grade S1 according to Kleiner. Experimental group of hemp seed oil with a concentration of 0.5 ml/kg/day. Staining with hematoxylin and eosin (a). ×1000 (immersion); Semi-thin preparation (1 μm), staining with methylene blue (b). ×1000 (immersion)

Source: Own materials

various sizes in the cytoplasm of cells when stained with hematoxylin and eosin, swelling of the cytoplasm, and eccentric location of cell nuclei (Fig. 1a). Examination of semi-thin sections stained with methylene blue revealed the presence of rounded fat droplets in the cytoplasm of hepatocytes (Fig. 1b). These changes were more pronounced in hepatocytes at the periphery of the hepatic lobules and less pronounced in the centrilobular zone. There were no signs of protein dystrophy (hydropic or hyaline-droplet).

In 4 (28.57 [8.85-54.04] %) of 14 cases dust-like accumulation of neutral fats and microvesicular steatosis in <5% of liver cells were diagnosed, which was characteristic of Kleiner's S0 degree of hepatosis. In 10 (71.43 [45.96-91.15] %) of 14 cases, we diagnosed mild fatty dystrophy (Kleiner grade S1 steatosis) with small-drop and large-drop accumulation of neutral fats in 5-33% of hepatocytes and found a significant difference from the control group, $p(\chi 2)=0.00001$.

Light-optical examination of liver tissue samples from animals in the experimental group showed normal architecture and a small amount of connective tissue in the portal zone and central vein. T-lymphocytes (CD3-positive) were diagnosed in a small number (1+) in the portal tracts, did not extend beyond the hepatic limiting plate and did not destroy the hepatic limiting plate, did not spread into the adjacent parenchyma (Fig. 2 a). Single CD3-positive T-lymphocytes (R) were diagnosed near the central vein and in the adjacent sinusoids of the intermediate zone (Fig. 2 b).

B-lymphocytes and NK-lymphocytes (natural killers) were diagnosed in rare cases in different areas of the liver lobule (Fig. 3a, b).

Statistical analysis revealed a significant predominance of immunocompetent cells in the experimental series compared to the control and intact groups ($p<0.05$). Immunohistochemical typing revealed a significant predominance of expression of the CD3 T-lymphocyte population compared to the control ($p<0.05$) and a significant predominance of T-lymphocyte expression over

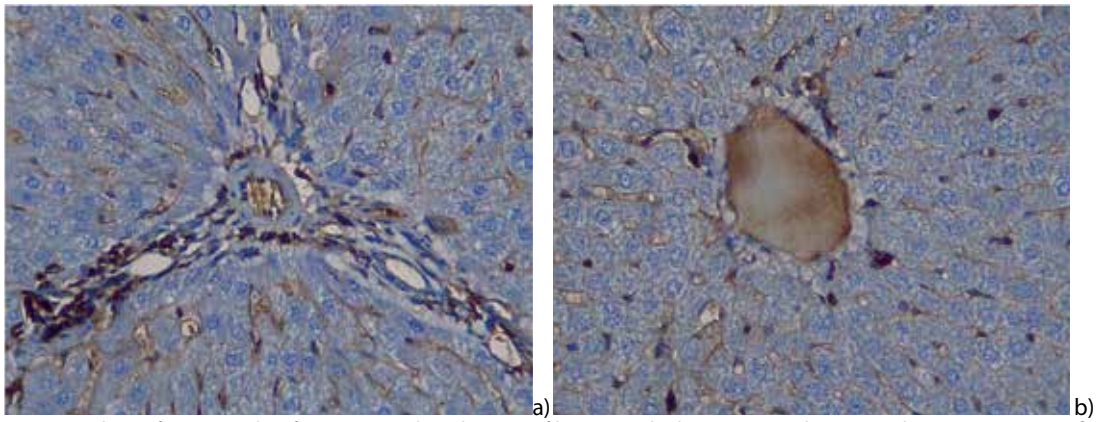

Fig. 2. Rat liver after 10 weeks of experimental application of hemp seed oil. Experimental group with a concentration of 0.5 ml/kg/day. Representative immunohistochemistry results for CD3. Liver triad (a). Central vein (b). Immunohistochemical staining for CD3 (Clone SP7, Thermo Fisher Scientific), ×400

Source: Own materials

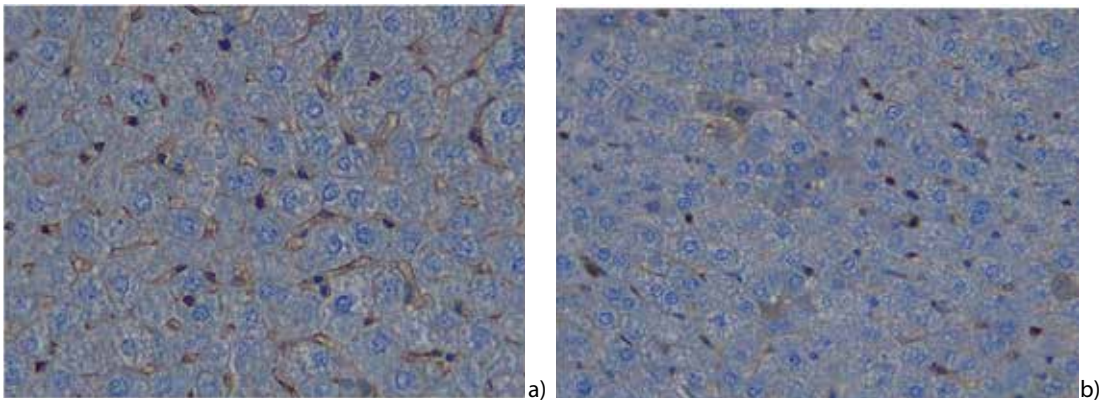

Fig. 3. Rat liver after 10 weeks of experimental application of hemp seed oil. Experimental group with a concentration of 0.5 ml/kg/day. Intermediate zone of the hepatic lobule. Representative immunohistochemistry results for CD20 (Clone Ab-1, Thermo Fisher Scientific) and CD56 (Clone 123C3, Dako). Presence single of B-lymphocyte in sinusoids (a), ×400. Presence single of NK-lymphocytes (natural killer cells) in sinusoids (b), ×400

Source: Own materials

B-lymphocyte expression ($p<0.05$). In quantitative terms, CD56-positive cells were the least numerous compared to other typed cells (R) ($p<0.05$). However, the predominant population was macrophages and Kupffer cells, which were mainly located in the periportal zone, in the lumen of the sinusoids, and least near the central vein.

CD68-positive Kupffer cell aggregates were most frequently diagnosed near the hepatic lobule triads (3+). Hypertrophy and hyperplasia of Kupffer cells were observed. Along with the sinusoids, there were fewer Kupffer cells (2+), they were enlarged, and very rarely, lipid vacuoles were visualized in their cytoplasm at high magnification under a microscope (Fig. 4).

The highest average number of Kupffer cells per 0.01 mm2 (100 μm × 100 μm) was determined in the hepatic triad zone and amounted to 5.79±0.06, which significantly ($p<0.001$) exceeded the corresponding indicator in the control group by 1.49 times. Also, in a comparative analysis, the average number of Kupffer cells exceeded the number in the intermediate zone (4.88±0.11) and around the central vein (2.82±0.12) by 1.49 and 2.82 times, respectively. As in the triad zone, in the intermediate zone of the lobule and around the central vein, the average CD68 cell counts significantly exceeded the corresponding values in the control group ($p< 0.05$), which is explained by the hyperplasia of specialized macrophages and their important role in liver immune function.

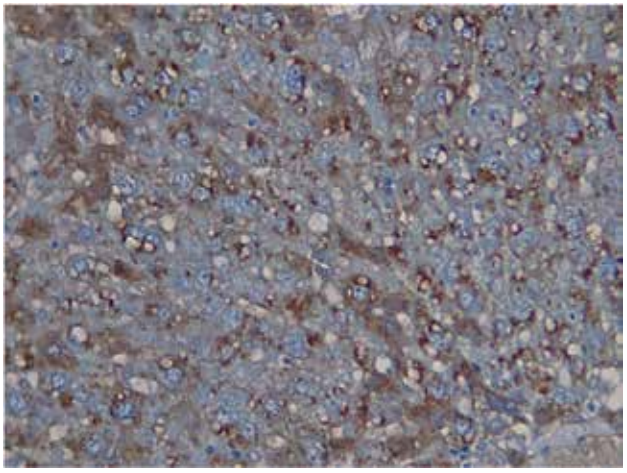

Fig. 4. Rat liver after 10 weeks of experimental application of hemp seed oil. Experimental group with a concentration of 0.5 ml/kg/day. Intermediate zone of the hepatic lobule. Representative immunohistochemistry results for CD68 (Clone KP1, Dako). Presence of Kupffer cells in sinusoids, ×400

Source: Own materials

Thus, morphological and immunohistochemical studies of the liver of rats after long-term experimental administration of hemp seed oil at a dose of 0.5 ml/kg/day (average dose) as a dietary supplement revealed the peculiarities of the histological organization of the parenchymal compartment and the distribution of lymphocytes and Kupffer cells in the classical liver lobule.

Light optical examination of histological sections of liver tissue revealed the presence of hepatocytes with signs of small-droplets and large-droplets fatty degeneration in the form of accumulation of optically empty vacuoles of various sizes in the cytoplasm of cells when stained with hematoxylin and eosin, cytoplasmic swelling, and eccentric cell nucleus location in the experimental group of animals after long-term use of hemp seed oil at a concentration of 0.5 ml/kg/day, which corresponded to the degree of hepatosis S1 according to Kleiner. The use of hemp seed oil at a dose of 0.1 ml/kg/day for 10 weeks did not lead to the development of steatosis.

Immunohistochemical analysis and distribution of lymphocytes and Kupffer cells in the liver of rats during long-term experimental use of hemp seed oil at a concentration of 0.5 ml/kg/day showed that T-lymphocytes (CD3-positive) were diagnosed in small numbers in the portal tracts, did not extend beyond the hepatic limiting plate and did not destroy the hepatic limiting plate, did not spread to the adjacent parenchyma. Single CD3-positive T lymphocytes were diagnosed near the central vein and in the adjacent sinusoids of the intermediate zone. B lymphocytes and NK

lymphocytes (natural killer cells) were single in different areas of the liver lobule.

Aggregates of CD68-positive Kupffer cells were most frequently diagnosed near the triads of the liver lobule. Hypertrophy and hyperplasia of Kupffer cells were determined. There were fewer Kupffer cells along the sinusoids, they were larger in size, and lipid vacuoles were very rarely visualized in their cytoplasm at high magnification under a microscope.

The highest average number of Kupffer cells per 0.01 mm2 (100 μm × 100 μm) was determined in the triad zone of the liver and amounted to 5.79±0.06, which significantly ($p<0.001$) exceeded the corresponding indicator in the control group by 1.49 times. Also, the average number of Kupffer cells in the liver triad zone in a comparative analysis significantly exceeded the number in the intermediate zone of 4.88±0.11 cells ($p<0.0001$) and around the central vein of 2.82±0.12 cells ($p<0.0001$), respectively. As in the triad zone, in the intermediate zone of the lobule and around the central vein, the average CD68 cell counts significantly exceeded the corresponding values in the control group, which is explained by the hyperplasia of specialized macrophages and their important role in the immune function of the liver.

DISCUSSION

Hemp seeds are characterized by exceptional nutritional value, containing significant amounts of high-quality proteins, minerals, dietary fiber, helpful lipids with a high content of polyunsaturated fatty acids, such as linoleic acid, alpha-linolenic acid, gamma-linolenic acid, and vitamins A, D, and E [21, 22]. Hemp seed oil contains polyunsaturated acids in an ideal omega 6 to omega 3 ratio of 3 to 1. This ratio of polyunsaturated fatty acids is optimal for human nutrition, useful for overall health and the functioning of the body's systems. However, according to P.M. Opyd et al. (2020), hemp seed oil contains a small amount of trans fatty acids, which inhibit the synthesis of polyunsaturated fatty acids and increase the risk of atherosclerosis in the blood vessels [22]. In an experimental study conducted by P.M. Opyd et al. (2020) on male Zucker rats with obesity and the addition of hemp seeds and hemp seed oil as a dietary supplement to their diet for 4 weeks, it was found that dietary supplements with ground hemp seeds were much more beneficial than oil, although both hemp seeds and hemp seed oil reduced plasma HDL cholesterol and triglyceride levels, reduced lipid peroxidation in plasma and the heart, but the antioxidant capacity of water-soluble compounds in blood plasma decreased under the influence of seeds, and acetylcholine-induced vasodilation decreased exclusively under the influence of hemp seeds [22].

In a recent 15-week experimental study, Hadil S. Subih and co-authors (2024) studied the effect of hemp seed oil on kidney and liver function and pro-inflammatory cytokines IL-2, IL-6, and TNF-α in rats with induced obesity. The authors found that hemp seed oil reduced levels of AST, ALT, uric acid, interleukins IL-2, and IL-6 in the group of rats with induced obesity. According to the authors, the dietary supplement of hemp seed oil in the diet of animals in the group with induced obesity indicates hepatoprotective properties, and the reduction in levels of pro-inflammatory cytokines IL-2 and IL-6 indicates promising anti-inflammatory effects [23]. The authors believe that further research is needed on the therapeutic potential of hemp seed oil, particularly for personalized nutrition and improving overall health indicators.

Jin A Lee et al. (2023) also conducted an 8-week experimental study to investigate the effect of cold-pressed hemp seed oil on reducing hypercholesterolemia. The authors hypothesized that hemp seed oil, with its optimal ratio of unsaturated fatty acids, may regulate lipid metabolism by inhibiting the synthesis of total cholesterol. Sprague-Dawley rats fed a high-cholesterol diet were orally administered hemp seed oil at different doses of 0.5 ml/kg and 1.0 ml/kg and simvastatin at a dose of 10 mg/kg. The authors found that LDL levels, which were elevated by a high-cholesterol diet, were significantly reduced by hemp seed oil and simvastatin. In the group of animals that received an oral dose of hemp seed oil at a dose of 0.5 ml/kg/day, a 26.7% reduction in LDL-C was recorded compared to the group of animals that received a high dose of hemp seed oil (1.0 ml/kg/day). The authors found that a low dose of hemp seed oil was more effective in reducing blood lipid-related factors than a high dose [24]. In our long-term study, a dose of hemp seed oil (0.5 ml/kg/day) led to mild hepatic steatosis (S1 according to Kleiner) and Kupffer cell hyperplasia, but no signs of steatohepatitis were found. In our opinion, long-term use of hemp seed oil at a dose of 0.5 ml/kg/day is undesirable, however, further studies are needed on the long-term effects of hemp seed oil on the liver, cardiovascular system, and kidneys in order to determine the therapeutic potential of hemp seed oil at various doses when used as a food or dietary supplement.

According to Nataly Martini (2021), studies on the addition of hemp seed oil remain inconclusive. Nataly Martini emphasizes that the hemp seed oil has been shown to lower HDL cholesterol and triglycerides in obese rats, the effect on the lipid profile is less clear in human studies. For example, in 36 children with hyperlipidemia, eight weeks of adding hemp seed oil significantly increased polyunsaturated fatty acid levels and the omega-3 index, but no changes in the lipid profile were observed. Also, in subjects with normolipidemia, no significant effect hemp seed oil on lipid parameters was observed over 12 weeks. Possibly, reasons for, these findings include individual differences in the absorption or metabolism of polyunsaturated fatty acids, as well as dosage requirements [2].

CONCLUSIONS

Based on the experimental study, it was found that long-term exposure (10 weeks) to hemp seed oil as a dietary supplement at a dose of 0.5 ml/kg/day caused mild fatty liver disease (Kleiner grade S1 steatosis) in 71.43% of cases with small and large droplets of neutral fat accumulation in 5-33% of hepatocytes, a significant difference from the control group (dose 0.1 ml/kg/day) was established, $p<0.0001$; no histological signs of inflammation or necrotic changes in hepatocytes were found.

The distribution of lymphocytes and Kupffer cells in the liver of rats during long-term experimental use of hemp seed oil at a dose of 0.5 ml/kg/day showed that CD3 lymphocytes were diagnosed in small numbers in the portal tracts, did not extend beyond the hepatic limiting plate and did not destroy the hepatic limiting plate, did not spread to the adjacent parenchyma. CD20 lymphocytes and NK lymphocytes were rare in different areas of the liver lobule. Aggregates of CD68-positive Kupffer cells were most frequently diagnosed near the triads of the liver lobule. The highest average number of Kupffer cells per 0.01 mm2 (100 μm × 100 μm) was 5.79±0.06 and significantly ($p<0.001$) exceeded the corresponding indicator of the control group by 1.49 times, which is explained by the hyperplasia of specialized macrophages and their important role in the immune function of the liver.

Experimental use over a period of 10 weeks indicates that hemp seed oil is safe to consume at a dose of 0.5 ml/kg/day, but further experimental studies are needed on the long-term effects of hemp seed oil for examining the morphological and morphometric characteristics of the vascular component of the rat liver under conditions of varying doses of hemp seed oil.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Evaluation of correlation of GDF11 and atherogenic factors in patients with diabetic dyslipidemia

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ABSTRACT

Aim: To explore link between circulating Growth Differentiation Factor 11 (GDF11) levels and atherogenic lipid parameters in individuals with diabetic dyslipidemia, and to evaluate whether GDF11 may serve as a potential biomarker to minimize atherosclerosis in this population.

Materials and Methods: A cross-sectional study was conducted at AL-Hassn Metabolism, Endocrine, and Diabetes Center in Kerbala, Iraq, February-April, 2025. A 172 participants with either type 1 or type 2 diabetes mellitus. Diabetic patients with dyslipidemia (n=90, 52 male and 38 female), with 4 having type 1 diabetes, 86 having type 2 diabetes, and without dyslipidemia (n=86: 36 male and 46 female), with 28 having type 1 diabetes and 54 having type 2 diabetes. Ages 20-70 years, serum level of GDF11 was determined using ELISA method. Atherogenic marker measures include midkine, NLRP3 inflammasome, PPARγ and oxidative stress. Additional data obtained included age, BMI, duration of diabetes, fasting glucose, and liver enzyme (ALT). Link between GDF11 and atherogenic markers was assessed using Spearman's correlation analysis to assess independent connections, multivariate regression analysis was performed, with adjustments for age, BMI, and glycemic control.

Results: diabetic individuals with dyslipidemia had considerably greater levels of all biomarkers tested (GDF11, SOD, NLRP3, MIDKINE, and PPARγ) than with diabetes alone. Diabetes and dyslipidemia are linked to increased oxidative stress (SOD), heightened inflammation (NLRP3, Midkine), and altered metabolic regulation (GDF11, ppary).

Conclusions: Diabetes with dyslipidemia linked to elevated levels of GDF11, SOD, NLRP3, midkine and PPARγ, indicating increased oxidative stress, inflammation, and metabolic dysregulation.

KEYWORDS: diabetes mellitus, dyslipidemia, growth differ nation factor, atherogenic factor

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INTRODUCTION

Growth Differentiation Factor 11 (GDF11) regulatory protein in the TGF-β superfamily, is emerging as a possible modulator of metabolic health. Its involvement in dyslipidemia in diabetic individuals, as well as its relationship with atherogenic variables, is still unknown. Diabetes mellitus (DM) is a chronic metabolic condition arising from intricate interactions between genetic and environmental variables, principally defined by sustained hyperglycemia and clinical manifestations include polyuria, dyslipidemia, and polyphagia [1]. Dyslipidemia denotes aberrant blood lipid concentrations, typically characterized by high triglycerides, total cholesterol, and LDL cholesterol, alongside diminished HDL cholesterol, and is acknowledged as a risk factor for cardiovascular disease [2]. Diabetic dyslipidemia is a specific lipid disorder commonly seen in type 2 diabetes, marked by diminished HDL cholesterol, elevated triglycerides, and a prevalence of tiny dense LDL particles [3]. Atherosclerosis is a persistent inflammatory arterial condition marked by the buildup of lipid-laden plaques, potentially resulting in stenosis or thrombosis, with increased LDL cholesterol as a primary risk factor [4]. Atherosclerosis can be diagnosed using new biomarkers such as Midkine, NLRP3 inflammasome, and Peroxisome Proliferator-Activated Receptor Gamma (PPARγ). Midkine is a growth factor that modulates cellular proliferation and inflammatory responses. In atherosclerosis, it encourages inflammatory cells and smooth muscle cells to build up in blood vessels, which promotes plaque formation [5]. The NLRP3 inflammasome, a key component of the innate immune system, has been shown to promote oxidative stress and endothelial dysfunction, thereby accelerating atherosclerosis development [6]. A nuclear receptor called Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) regulates genes linked to blood vessel function, inflammation, and the metabolism of fats and carbohydrates. By improving endothelial function, decreasing inflammation that causes plaque formation, and stabilizing plaques to halt disease progression and lower the risk of cardiovascular events, PPARγ helps prevent atherosclerosis [7]. Superoxide dismutase (SODs) are antioxidant enzymes that maintain redox homeostasis and protect against oxidative stress, processes central to vascular pathology [8]. Growth Differentiation Factor 11 (GDF11), a member of the TGF-β superfamily, is expressed in multiple tissues and has been implicated in diverse biological processes, including metabolism, aging,

and cardiovascular health [9]. Clinical studies suggest that reduced circulating GDF11/8 levels are associated with increased risks of myocardial infarction, stroke, heart failure, and all-cause mortality, supporting its potential role as a protective factor in cardiovascular disease [10].

AIM

The aim of this study is to explore link between circulating Growth Differentiation Factor 11 (GDF11) levels and atherogenic lipid parameters in individuals with diabetic dyslipidemia, and to evaluate whether GDF11 may serve as a potential biomarker to minimize atherosclerosis in this population.

MATERIALS AND METHODS

PARTICIPANTS AND STUDY DESIGN

From February to April 2025, a cross-sectional study was done at the Al-Hassan Metabolism, Endocrine, and Diabetes Center in Karbala, Iraq. The study involved 172 individuals diagnosed with type 1 and type 2 diabetes mellitus Participants were divided into two groups: diabetic patients with dyslipidemia (n = 90; 52 males and 38 females), including 4 individuals with type 1 diabetes and 86 with type 2 diabetes; and diabetic patients without dyslipidemia (n = 80; 36 males and 46 females), comprising 28 individuals with type 1 diabetes and 54 with type 2 diabetes. The participants' ages ranged from 20 to 70 years. A standardized questionnaire and medical records were used to gather sociodemographic and clinical information. The gathered parameters included age, sex, family history of diabetes, level of education, eating habits, level of physical activity, length of time with diabetes, medication history, height, and weight. To find out your body mass index (BMI), divide your weight in kilograms by your height in meters squared (kg/m^2). All subjects provided venous blood samples after an overnight fast. The serum was separated and analyzed to assess the following biochemical parameters: [insert specific laboratory tests undertaken, e.g., fasting blood glucose (FBG), growth differentiation factor 11 (GDF11)]. Midkine (MK), NOD-, LRR-, and pyrin domain-containing protein 3 inflammasome (NLRP3), Peroxisome Proliferator-Activated Receptor gamma (PPARγ), superoxide dismutase (SOD), complete blood count (CBC), and liver enzymes (AST, ALT) are included. Eligibility Requirements (Case Group): People with type 1 or type 2 diabetes mellitus who also have dyslipidemia. Patients were ready to give informed consent. Patients who are able to read, write, and speak Arabic well. Exclusion Criteria (Case Cohort): Patients who chose not to participate. People who have blood disorders. People who have chronic renal disease. People who have chronic liver illness. Women who are nursing or pregnant. People who have been told they have any kind of cancer. People with heart problems, such myocardial infarction or atrial fibrillation. People who have been diagnosed with thalassemia or an autoimmune disease.

MATERIALS

GDF11 kit, Midkine kit, NLRP3 inflammasome kit, PPARY kit, SOD kit (BT Lab Company, China), and liver enzyme kit.

STATISTICAL ANALYSIS

The statistical studies were performed on IBM SPSS 28. We used the Shapiro-Wilk test and Q-Q plots to check for normality. Non-normal continuous data were presented as median (IQR), whereas categorical data were expressed as frequencies and percentages. For continuous variables, we used the Mann-Whitney U test, and for categorical variables, we used Fisher's exact test. Correlations: Spearman's rank correlation evaluated the relationships between GDF11 and other continuous variables, establishing significance at $p<0.05$. Evaluation for diagnosis: ROC curve study assessed GDF11's capacity to distinguish between diabetes with and without dyslipidemia, providing AUC, 95% CI, cut-off values, sensitivity, and specificity. All tests were two-tailed, and a p-value of less than 0.05 was considered statistically significant.

ETHICAL APPROVAL

The research focused on the ethical tenets articulated in the Declaration of Helsinki. The method was carried out with the patient's verbal and informed consent prior to the collection of samples. The research methodology, participant data, and consent form were evaluated and sanctioned by a local ethics commission, as per document number MEC-64, in October 2023 to secure this approval.

RESULTS

The results start by Table 1, which relates different variables with Diabetes with and without Dyslipidemia and the associated p-value. In Table 1, the groups showed considerable similarity in terms of BMI, gender, domicile, education, dietary habits, and physical activity. Marked disparities were noted in age and occupation, with the dyslipidemia cohort being older and more frequently employed.

Table 2 indicates that individuals with diabetes and dyslipidemia are more commonly affected by type 2 diabetes, exhibit diminished dependence on insulin therapy, and display elevated ALT levels, which may indicate an increased risk of hepatic and metabolic complications. Although the durations of diabetes and glycemic control were similar between groups, dyslipidemia was associated with differing treatment regimens and increased liver enzyme levels, highlighting the need for comprehensive metabolic evaluation and surveillance in these patients.

In Table 3, diabetic individuals with dyslipidemia demonstrated markedly elevated levels of GDF11, SOD, NLRP3, Midkine, and PPARγ in comparison to their counterparts without dyslipidemia. The findings indicate that oxidative stress, inflammatory activation, and metabolic regulatory pathways are more pronounced in diabetic dyslipidemia, highlighting intricate linkages between lipid metabolism and systemic inflammation.

In Table 4, GDF11 levels exhibited a positive correlation with oxidative stress (SOD), inflammation (NLRP3, Midkine), and metabolic regulation (PPARγ), while demonstrating a negative correlation with BMI. This pattern indicates that GDF11 is involved in metabolic-inflammation interactions in diabetic dyslipidemia, likely representing a compensatory reaction to metabolic stress and inflammation.

Table 1. Sociodemographic characteristics of the study groups

Variable	Diabetes with Dyslipidemia	Diabetes without Dyslipidemia	p-value
Age	53 (48-57)	44 (20-56.5)	0.002
BMI	29.1 (27.7-31.9)	27.2 (24.5-32.3)	0.166
Gender (male)	52 (57.8%)	36 (43.9%)	0.093
Gender (female)	38 (42.2%)	46 (56.1%)	
Residence (urban)	85 (94.4%)	82 (100%)	0.06
Residence (rural)	5 (5.6%)	0 (0%)	
Education (illiterate)	44 (48.9%)	45 (54.9%)	0.73
Education (high school)	3 (3.3%)	3 (3.7%)	
Education (university)	43 (47.8%)	34 (41.5%)	
Occupation (no)	24 (26.7%)	56 (68.3%)	<0.001
Occupation (yes)	66 (73.3%)	26 (31.7%)	
Healthy diet (no)	66 (73.3%)	56 (68.3%)	0.504
Healthy diet (yes)	24 (26.7%)	26 (31.7%)	
Exercise (no)	72 (80%)	59 (72%)	0.282
Exercise (yes)	18 (20%)	23 (28%)	

Source: Own materials

Table 2. Diabetes-related clinical and metabolic parameter

Variable	Diabetes with Dyslipidemia	Diabetes without Dyslipidemia	p-value
Type (type 1)	4 (4.4%)	28 (34.1%)	<0.001
Type (type 2)	86 (95.6%)	54 (65.9%)	
DM Duration (years)	8 (5-15)	8 (3-15)	0.661
Insulin (no)	76 (84.4%)	48 (58.5%)	<0.001
Insulin (yes)	14 (15.6%)	34 (41.5%)	
Insulin duration (years)	2.5 (1-4.75)	7 (5.25-8.5)	<0.001
Oral DM Med (no)	14 (15.6%)	33 (40.2%)	<0.001
Oral DM Med (yes)	76 (84.4%)	49 (59.8%)	
Oral med duration (years)	6 (3-10)	9 (1-12)	0.596
FBG (mg/dL)	181.6 (172.7-303.5)	190 (139.2-250)	0.068
ALT (U/L)	20.4 (14-28.08)	14.1 (11.2-20.4)	<0.001

Source: Own materials

Table 3. Biomarker levels of GDF11, SOD, NLRP3, Midkine, and PPARγ in the study groups

Variable	Diabetes with dyslipidemia	Diabetes without Dyslipidemia	p-value
GDF 11 (ng/L)	705.26 (381.62-787.73)	338.05 (284.69-490.64)	<0.001
SOD (U/L)	286.55 (182.39-347.21)	177.51 (137.69-186.02)	<0.001
NLRP3 (pg/mL)	295.74 (162.65-434.95)	130.83 (112.56-155.77)	<0.001
Midkine Protein (pg/mL)	342.98 (262.39-410.69)	313.93 (280.2-336.46)	0.014
PPARγ (ng/L)	3367.68 (1854.53-5250.97)	1750.66 (1588.11-2003.45)	<0.001

Source: Own materials

Diabetes with dyslipidemia was designated as the positive group, and diabetes without dyslipidemia as the negative group. Table 5 GDF11 shows a moderate level of effectiveness in differentiating diabetic patients with dyslipidemia.

The chosen threshold of 339.317 ng/L has a high sensitivity and a moderate specificity, indicating that it is better able to identify dyslipidemia than to rule it out, Figure (1).

Diabetes with dyslipidemia was designated as the positive group, and diabetes without dyslipidemia as the negative

Table 4. Spearman's correlation between GDF11 levels and all studied parameters

Variable	Spearman Coefficient (r)	p-value
Age	0.168	0.028
BMI	-0.214	0.005
DM Duration (years)	0.120	0.116
Insulin Duration	0.231	0.114
Oral Med Duration (years)	0.107	0.226
FBG (mg/dL)	0.084	0.275
ALT (U/L)	0.021	0.787
GDF 11 (ng/L)	1.000	<0.001
SOD (U/L)	0.552	<0.001
NLRP3 (pg./mL)	0.537	<0.001
Midkine protein (pg/mL)	0.422	<0.001
PPARγ (ng/L)	0.460	<0.001

Source: Own materials

Table 5. ROC analysis of GDF11 levels for discriminating diabetic patients with dyslipidemia from those without dyslipidemia

Variable	Cut-off Value	Sensitivity	Specificity	AUC (95% CI)	p-value
GDF 11 (ng/L)	339.317	86.7%	51.2%	0.709 (0.632-0.786)	<0.001

Source: Own materials

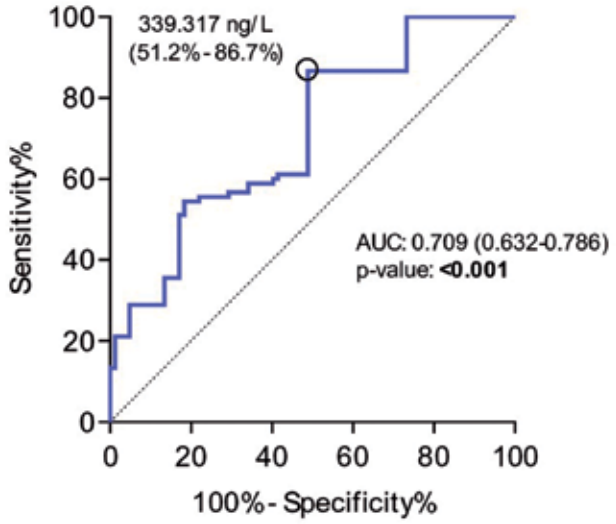

Fig 1. ROC analysis of GDF11 levels for discriminating diabetic patients with dyslipidemia from those without dyslipidemia

Source: Own materials

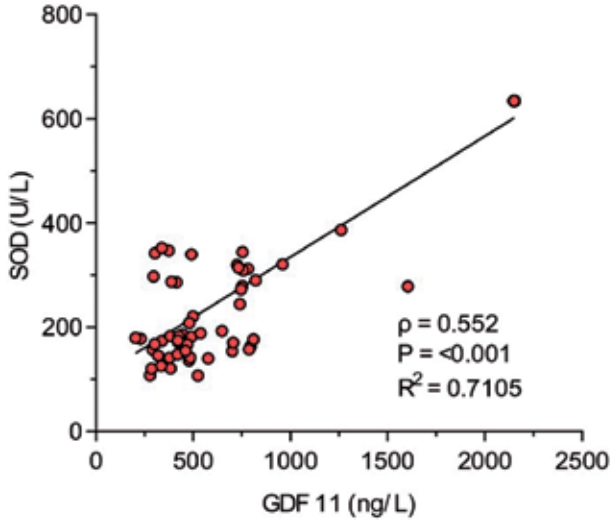

Fig. 2. Notable favorable correlation between GDF11 (ng/L) and SOD activity (U/L)

Source: Own materials

group. There is a noTable favorable correlation between GDF11 (ng/L) and SOD activity (U/L), indicating that an elevation in GDF11 concentration is associated with an increase in SOD activity, which suggests a possible regulatory or stimulatory influence of GDF11 on antioxidant enzymes, Figure (2).

There is a substantial positive connection between GDF11 and NLRP3 expression indicating that elevated GDF11 levels correspond with increased NLRP3 levels, Figure (3).

It is noticed that there is a significant positive correlation between GDF11 and PPARγ, suggesting that increased GDF11 levels are associated with elevated PPARγ expression, Figure (4).

Finally, no substantial association exists between GDF11 levels and ALT activity, indicating that GDF11 does not affect ALT in this sample, Figure (5).

Overall, dyslipidemia appears to exacerbate the biochemical and inflammatory characteristics of diabetes, potentially contributing to a higher risk of complications

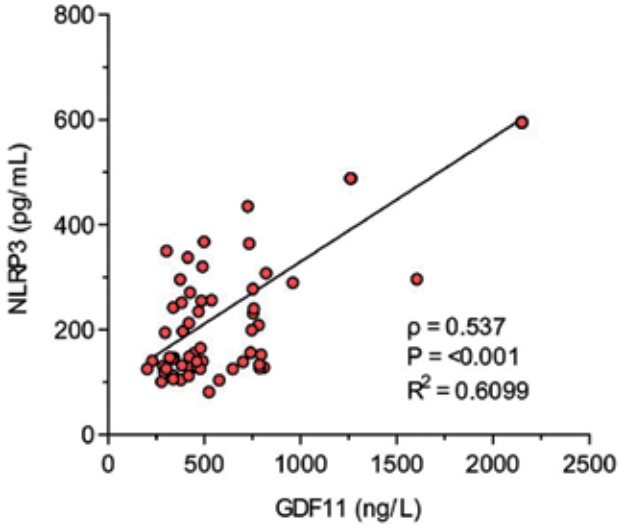

Fig. 3. A substantial positive connection between GDF11 and NLRP3
Source: Own materials

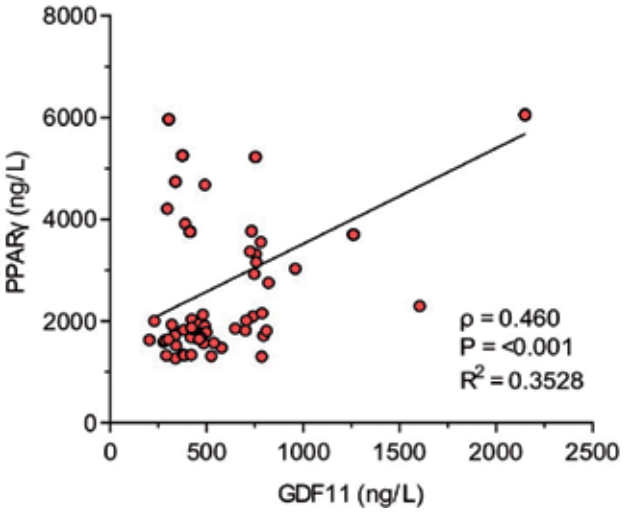

Fig. 4. A significant positive correlation between GDF11 and PPARγ
Source: Own materials

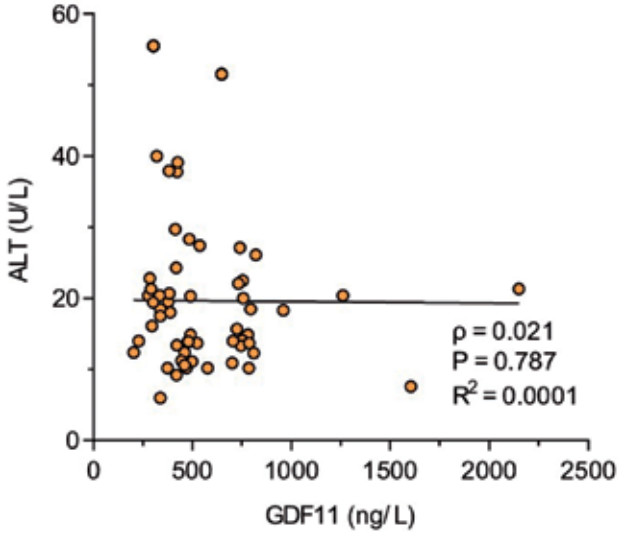

Fig. 5. Association exists between GDF11 levels and ALT activity
Source: Own materials

DISCUSSION

From results presented in Table (1) one can conclude that advanced age and occupational status are significantly associated with the prevalence of dyslipidemia in diabetic individuals. These findings correspond with contemporary research that recognizes age as a risk factor for metabolic complications in diabetes [11]. From Table (2) patients with dyslipidemia were primarily type 2 diabetics 95.6%, aligning with data that lipid abnormalities are characteristic of insulin-resistant type 2 diabetes rather than type 1 diabetes [12]. The reduced insulin utilization and abbreviated insulin duration in dyslipidemia patients indicate a heightened reliance on oral medications and less rigorous glycemic management, potentially aggravating lipid dysregulation [13]. Concurrently, ALT levels were markedly elevated in the dyslipidemia cohort, indicating potential hepatic steatosis and metabolic strain, a well-documented characteristic of diabetic dyslipidemia [14]. The data indicate that type 2 diabetes with dyslipidemia constitutes a metabolically compromised phenotype, marked by hepatic involvement and elevated cardiovascular risk. Table 3 indicates that MK levels were markedly elevated in patients with type 2 diabetes and dyslipidemia relative to those without dyslipidemia. The research also recognized MK as a possible prognostic biomarker for atherosclerosis in these patients [15] demonstrated that circulating GDF11 levels were inversely correlated with body mass index, waist circumference, and other metabolic parameters, implying its role in metabolic control [16]. Investigated the function of the NLRP3 inflammasome in diabetic retinopathy, indicating its participation in the chronic inflammation linked to diabetes [17]. Our observation that PPARγ levels are markedly elevated in diabetic individuals with dyslipidemia compared to those without may indicate compensatory upregulation or dysregulated expression of PPARγ in reaction to concurrent metabolic stress from glucose and lipid dysregulation. This may indicate a heightened demand for cholesterol management and anti-inflammatory modulation in individuals with dyslipidemia [18-19]. The results in Table (4), indicate that GDF11 levels exhibit a substantial positive link with SOD, NLRP3, Midkine, and PPARγ levels; however, a modest negative correlation with BMI was seen. The findings indicate that GDF11 may have a multifaceted regulatory role in metabolic and oxidative pathways in diabetic individuals.

CONCLUSIONS

Markedly heightened concentrations of GDF11, SOD, NLRP3, midkine, and PPARγ are associated with diabetes accompanied by dyslipidemia, signifying augmented oxidative stress, inflammation, and metabolic imbalance. In diabetic dyslipidemia, increased GDF11 may represent a compensatory response, highlighting its potential as a biomarker and therapeutic target. Elevated GDF11 could constitute a compensatory reaction, emphasizing its potential as a biomarker and therapeutic target in diabetic dyslipidemia.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Enterosorption therapy of chronic obstructive pulmonary disease against the background of endogenous intoxication

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ABSTRACT

Aim: improve the effectiveness of treatment for patients with COPD in the acute phase with clinical and laboratory manifestations of endogenous intoxication by including carbon or organosilicon sorbents in complex therapy.

Materials and Methods: To evaluate the effectiveness of enterosorption therapy in the complex treatment of patients with COPD in the acute phase with clinical and laboratory manifestations of endogenous intoxication, patients were divided into three groups according to severity. The first group consisted of 34 patients (21.9%) with mild bronchial obstruction (GOLD 1), the second group included 64 patients (41.3%) with moderate bronchial obstruction (GOLD 2), and the third group included 57 patients (36.8%) with severe bronchial obstruction (GOLD 3). The control group consisted of 20 healthy people aged 40 to 78 years. The following exclusion criteria were used: the presence of diabetes mellitus, acute coronary syndrome, previous myocardial infarction, rheumatological pathology, oncological and infectious diseases, acute inflammatory processes of any localization in patients.

Results: COPD in the exacerbation phase is accompanied by clinical and laboratory manifestations of endotoxicosis, more pronounced in patients with severe broncho-obstruction. This is manifested by an increase in the content of endogenous intoxication indicators in blood serum (molecules of medium weight by 84% and 126% ($p<0.001$) and erythrocyte intoxication index by 86% ($p<0.001$)). There is a direct correlation of mean strength between the severity of the disease and the level of MSM254 and MSM280 ($r=0.496$ and $r=0.557$, $p<0.01$) and weak strength – with an erythrocyte index of intoxication ($r=0.253$, $p<0.01$). COPD in the exacerbation phase is accompanied by the activation of free radical processes, most pronounced in patients with severe broncho-obstruction, manifested by a probable increase in malondialdehyde – by 116% ($p<0.001$) and a decrease in the activity of superoxide dismutase – by 59% ($p<0.01$) compared to the control. There is a direct mean strength correlation between severity and malondialdehyde levels ($r=0.486$, $p<0.01$) and inverse with serum superoxide dismutase ($r=-0.500$, $p<0.01$). MDA content in blood serum increases, SOD activity decreases with increasing age of patients.

Conclusions: COPD exacerbation accompanied by clinical and laboratory manifestations of endogenous intoxication, namely increasing concentrations of malondialdehyde and superoxide dismutase activity decrease depending on the severity and age of bronchial patients. With the progression of the disease also increases the content of interleukin 1β and 10, tumor necrosis factor α, circulating immune complexes, immunoglobulin E and reduced lysozyme activity in serum, in proportion to the increase in the severity of the disease. The use in treatment of 10-day course enterosorbtsiynoyi therapy using carbon (karbolaynu) or silicone (enterosgel) enterosorbent in treatment of patients with COPD exacerbation is accompanied by a decrease in clinical and laboratory manifestations of endotoxemia, such as cough, shortness of breath, discharge of phlegm, weakness and significant reduction of the average molecular weight and erythrocyte intoxication index, malondialdehyde, interleukins, immunoglobulin E and circulating immune complexes and increased activity of SOD and content of lysozyme in serum.

KEYWORDS: respiratory tract, metabolic disorders, oxidative stress, chronic obstructive pulmonary disease, endogenous intoxication, therapy

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is one of the most common and severe progressive diseases of the respiratory system. COPD is one of the leading causes of death worldwide, which determines the significant economic and social importance of this pathology [1]. Today, this disease ranks fourth in the global "rating" of the main causes of death. In recent decades, there has been a steady increase in the incidence of the disease due to environmental pollution, the spread of smoking, and an aging population. According to the WHO's grim forecast, by 2030 COPD will rank fifth in terms of incidence and third in terms of mortality among diseases, after ischemic heart disease, cerebrovascular disease, accidents, and depression [2, 3]. In Ukraine, respiratory diseases remain the most common pathology in the structure of morbidity among the population. In particular, mortality from COPD is 86.3 per 100,000 population, which is 66 times higher than the corresponding figure for bronchial asthma.

Endogenous intoxication (EI) plays an important role in the pathogenesis of many diseases. It is associated with the accumulation of excess metabolites of normal and pathological metabolism and the waste products of various pathogenic microorganisms in the tissues and biological fluids of the body [4]. Endogenous intoxication syndrome (EIS) accompanies every chronic disease, aggravating the clinical picture of the disease and worsening the prognosis. The clinical manifestations of the EI symptom complex are

nonspecific and are characterized by general weakness, sleep and appetite disorders, myalgia, and headache. If left uncorrected, they lead to the depletion of the body's morphofunctional capabilities, impaired immune responses, and profound changes in homeostasis, which can lead to the breakdown of compensatory mechanisms [5].

In EI, the main focus is on treating the underlying disease that caused the development of this syndrome (poisoning, burns, hepato- and nephropathy, pancreatitis, respiratory diseases, etc.). Methods of efferent detoxification therapy, which include enterosorption, have always been of great importance in the complex of therapeutic measures, as they contributed to a noticeable reduction in the severity of EI and the prevention of multiple organ damage [6, 7].

The basis for the treatment of COPD is pharmacotherapy. At the current level of development, drug therapy for COPD is designed to prevent and control symptoms, reduce the frequency and severity of exacerbations, improve health and exercise tolerance, and quality of life of the patient. Therefore, particular attention, especially in geriatric practice, should be paid to low-toxicity, highly effective drugs that are convenient and easy to use, such as enterosorbents.

AIM

To improve the effectiveness of treatment for patients with COPD in the acute phase with clinical and laboratory manifestations of endogenous intoxication by including carbon or organosilicon sorbents in complex therapy.

MATERIALS AND METHODS

At the First Therapeutic Department of Ternopil Municipal Hospital No. 2, 155 patients (average age 63.4±1.0 years) were examined. Among them were 101 men (65%) and 54 women (35%). To evaluate the effectiveness of enterosorption therapy in the complex treatment of patients with COPD in the acute phase with clinical and laboratory manifestations of endogenous intoxication, patients were divided into three groups according to severity. The first group consisted of 34 patients (21.9%) with mild bronchial obstruction (GOLD 1), the second group included 64 patients (41.3%) with moderate bronchial obstruction (GOLD 2), and the third group included 57 patients (36.8%) with severe bronchial obstruction (GOLD 3). The control group consisted of 20 healthy people aged 40 to 78 years. The following exclusion criteria were used: the presence of diabetes mellitus, acute coronary syndrome, previous myocardial infarction, rheumatological pathology, oncological and infectious diseases, acute inflammatory processes of any localization in patients.

All patients were divided into three groups based on the treatment they received. Fifty-one patients (33%) with COPD received only basic therapy (BT), 52 patients (33.5%) in addition to BT, received the organosilicon enterosorbent enterosgel in the form of a gel orally, 1 tablespoon 3 times a day for 10 days, 2 hours before or 2 hours after meals and medication. The next 52 patients (33.5%), in addition to BT, additionally received a fourth-generation carbon enterosorbent, Carboline, in the form of small granules orally, 1 teaspoon 3 times a day for 10 days, 2 hours before or 2 hours after meals and medication.

To evaluate the effectiveness of enterosorbent therapy in the complex treatment of patients with COPD in the acute phase with clinical and laboratory manifestations of endogenous intoxication, patients were divided into two age groups. Group I consisted of 90 middle-aged and elderly patients aged 40-59 years (of whom 30 received BT, 30 received BT and enterosgel, and 30 received BT and carboline). and the second group consisted of 64 elderly and senile patients over 60 years of age (of whom 21 patients received BT, 22 received BT and enterosgel, and 21 received BT and carboline). The control group consisted of 20 healthy people of comparable age and gender.

The diagnosis of COPD was made based on standardized diagnostic criteria based on clinical symptoms, medical history, physical examination, and confirmed by spirometry data. To assess clinical symptoms in patients with COPD, the Modified Dyspnea Severity Scale approved by the Medical Research Council and the COPD assessment test were used. To confirm the diagnosis of COPD, post-bronchodilator FEV1/FVC and FEV1 measurements were performed to assess the severity of COPD, the degree of reversibility of airflow limitation, and to predict the response to long-term use of bronchodilators or glucocorticosteroids.

To detect endogenous intoxication (EI) in patients, serum levels of MSM254 and MSM280 were determined. The erythrocyte intoxication index (EII) was determined using the Togaybaev method. The intensity of LPO processes was assessed by the content of malondialdehyde (MDA) in serum, which was determined by reaction with thiobarbituric acid. To study antioxidant protection (AOP), the activity of superoxide dismutase (SOD) was investigated. The levels of IL-1β, TNF-α, and IL-10 were determined by immunoenzymatic assay. The number of circulating immune complexes (CIC) was determined by precipitation in 10% polyethylene glycol with a molecular weight of 6 KD, followed by photometry. The activity of serum lysozyme was determined by a turbidimetric method of quantitative lysozyme determination. The content of total Ig E was studied using an enzyme-linked immunosorbent assay.

The reliability of differences between groups was assessed using the nonparametric Wilcoxon U test (Mann–Whitney). When conducting a correlation analysis, the parametric correlation method was used to determine Pearson's linear correlation coefficient (r), followed by verification of the reliability of the result using Student's criterion.

RESULTS

All patients were undergoing treatment during the exacerbation of the disease. Among the clinical symptoms, all patients had a cough: mostly in the morning with mild bronchial obstruction, in the morning and at night with moderate bronchial obstruction, and constant with severe bronchial obstruction. The degree of dyspnea increased with the progression of COPD. One hundred thirty patients (80%) reported sputum production in varying amounts in the morning. Physical examination revealed a change in

the shape of the chest (barrel-shaped) in 93 (57%) patients. 140 patients (86%) had tachypnea, in 126 (77%) percussion revealed a boxy lung sound, and auscultation revealed weakened breathing, prolonged exhalation, scattered multiple dry rales, of which 54 (33%) had fine-bubble wet rales in the lower basal sections. In 37 patients (23%), the percussion sound was clear pulmonary, and auscultation revealed hard breathing and isolated low-pitched dry rales. All patients complained of general weakness, lethargy, headache, sleep and appetite disturbances. According to computer spirography and pneumotachometry, all patients had obstructive or mixed external respiratory dysfunction, which increased with the progression of COPD. In 23% of patients, there was mild ventilatory failure, in 40% – moderate, and in 37% – severe. The reversibility test for bronchial obstruction showed a 4-10% increase in FEV1 under the action of a bronchodilator, indicating the presence of irreversible bronchial obstruction.

Radiographically, all patients showed changes in the bronchopulmonary pattern: in 110 patients (67%) – basal pneumofibrosis, in 57 (35%) – diffuse, in 163 (100%) – enlarged, heavy roots, in 135 (83%) – pulmonary emphysema.

In patients of all study groups, there was a significant increase in the levels of MSM254, MSM280, and EII compared to the control group. EII increased with increasing age of patients. In mature patients, it increased by 30%, in middle-aged patients – by 76%, in elderly patients – by 91%, and in older patients – by 106% compared to the control group. All indicators are significant ($p<0.001$). The use of BT improved the condition of patients and reduced the level of intoxication, which manifested itself in a decrease in the serum levels of MCM254, MCM280, and EII.

During hospitalization, a progressive increase in MDA was noted. The amount of SOD in all groups of patients decreased in inverse proportion to the severity of the disease. After BT, MDA levels decreased and SOD activity increased. With age, serum MDA levels increased compared to the control group in all age groups of the subjects. In mature age, this indicator tended to increase and rose by 26% compared to the control. In middle age, the increase was 96%, in elderly age – 110%, and in senile age – 107%.

When comparing patients from different age groups, it was found that changes in SOD levels were most pronounced in the elderly, decreasing by 44% compared to the control group, by 25% in the elderly, by 24% in middle-aged patients, and by 17% in mature patients.

During hospitalization, an increase in pro-inflammatory cytokines IL-1β and TNF-α and pro-inflammatory cytokine IL-10 in blood plasma was noted. The changes were significant in all groups.

When using BT, IL-1β levels decreased by 43% in group I, by 41% in group II, and by 33% in group III. IL-10 levels decreased by 15% in group I, by 20% in group II, and by 36% in group III. The concentration of TNF-α in the blood serum of COPD patients after BT decreased in group I by 17%, in group II by 29%, and in group III by 24% compared to the baseline level. All the results obtained differed significantly from the control group. Hyperproduction of proinflammatory cytokines IL-1β and TNF-α and an increase in anti-inflammatory IL-10 were observed in all age groups of patients. Thus, in mature age, IL-1β increased significantly by 5.4 times, in middle age by 12.1 times, in elderly age by 18.3 times, and in senile age by 22.9 times compared to the control. The concentration of TNF-α in middle-aged patients was 1.8 times higher than the control values, in middle-aged patients – 2.9 times higher, and in elderly patients – 4.2 times higher. In elderly patients, the indicator increased 5.6 times. The greatest increase in IL-10 was observed in middle-aged patients (5.1 times).

Before treatment, patients showed an increase in CEC levels and Ig E levels in blood serum and a decrease in lysozyme levels.

In patients after BT, the difference between CEC, Ig E, and lysozyme levels and the control decreased, although it was not significant. The largest increase in CEC levels was observed in the serum of elderly patients (2.1 times).

As a result of BT, clinical manifestations of endotoxicosis decreased in patients. The frequency and severity of cough and shortness of breath decreased. There was a tendency to decrease the number of patients complaining of sputum discharge. Under the influence of BT, there was a significant decrease in complaints of general weakness. In blood serum, the levels of MSM254, MSM280, EII, MDA, cytokines (IL-1β, TNF-α, IL-10), CIK, and Ig E decreased, while the levels of lysozyme and SOD increased.

The use of enterosorbents together with BT in COPD contributed to a reduction in the clinical manifestations of endotoxicosis. Better dynamics of indicators were observed in patients with mild bronchial obstruction and in mature age. When Enterosgel was added to BT, a decrease in MSM content and an increase in erythrocyte resistance to toxic effects were noted. The content of MSM254 in patients of group I decreased by 14%, the level of MSM280 by 15%, and EII by 27%. In group II, the level of MSM254 decreased by 22%, MSM280 by 28%, and EII by 31%. In group III, the content of MSM254 in blood serum decreased by 23%, MSM280 by 31%, and EII by 23%. The concentration of MDA in the blood serum of patients in group I decreased by 16%, in group II by 33%, and in group III by 30%. SOD activity increased in the examined patients in group I by 11%, in group II by 19%, and in group III by 29%.

There was a decrease in the level of IL-1β in blood serum in patients of the I group by 61%, in the II and III groups – by 57 and 56%, respectively. The content of IL-10 in the I group decreased by 19%, in II – by 29%, in III – by 45%. The level of TNF-α in the I group decreased by 21%, in II and III – by 42 and 41%, respectively. Serum CIC decreased by 24% in Group I, 19% – in II, and 25% in III compared to baseline. There was an increase in serum lysozyme activity by 28% in Group I ($p<0.01$), by 21% – in II ($p<0.05$), and by 67% in III ($p<0.001$). Patients' serum Ig E content decreased by 29% in Group I ($p<0.001$), 30% – in II ($p<0.01$) and by 21% – in III ($p<0.05$).

After joining BT carboline, the content of MSM254 in the blood serum of patients of group I decreased by 17%, MSM280 – by 19%, EII – by 37%. In the II group, the

level of MSM254 decreased by 26%, MSM280 – by 36%, EII – by 37%. In group III, the content of MSM254 in blood serum decreased by 31%, MSM280 – by 35%, EII – by 28%. The level of MDA decreased by 19%, II – by 46%, III – by 36%. SOD activity increased in Group I by 16%, II – by 23%, III – by 37%. Therefore, after the combined use of BT and carboline, patients of the I and II groups showed an approximation of the content of MDA and the activity of SOD to the control indicators, as evidenced by an unreliable difference between them.

After the combination of BT with carboline, there was a decrease in the content of IL-1β in the I group by 72%, in the II group – by 65%, and in the III – by 68%. The IL-10 level decreased in Group I by 38%, in II – by 47%, and in III – by 61%. The level of TNF-α also decreased by 43% in Group I, by 54% – in II and by 62% – in III. When compared with the control, the greatest increase in indicators took place in the I group, which was evidenced by the lack of a significant difference between all the studied indicators of the cytokine link with the control group. The number of CIC decreased in group I by 27%, in II – by 25%, in III – by 36%, compared to pre-treatment data. The level of Ig E decreased in Group I by 35%, in II – by 37%, in III – by 29%. Lysozyme activity increased by 35% in Group I, by 34% – in II and by 92% in III compared to baseline. When comparing the obtained results with the control group, it was established that all indicators of immunological status in the III group and the level of Ig E in the II group were significantly different from the control values.

Our observations indicate that the use of BT improved the condition of patients and reduced the level of intoxication in all groups of different ages, as evidenced by the following dynamics of EI indicators: the content of MSM254 and MSM280 in the blood serum of patients of the I age group after the use of BT decreased by 9% and 20%, respectively, compared to the initial level, and EII – by 19%. Similar dynamics were noted in the II age group. The level of MSM254 decreased after BT by 9%, MSM280 – by 16%, EII – by 19%. When comparing the obtained indicators after the use of BT with the control group, it was noted that they decreased in the direction of normalization. After BT, the level of MDA in the I age group decreased by 15%, and in the II – by 21%. SOD activity increased by 18% and 8% respectively. Compared to the control group, a significant difference was maintained between it and the indicators of age groups, which indicates insufficient effectiveness of BT.

Depending on the age of the patients, the level of indicators of cytokine and immunological status in the blood serum changed. When using BT, the amount of IL-1β significantly decreased by 23 and 42%, IL-10 – by 32 and 10%, and the concentration of TNF-α by 8 and 33%, respectively, in the I and II age groups. Comparing the obtained results, it was established that all of them are reliably different from the control group. Compared to the initial level, the content of CIC decreased by 12% in the I age group and by 17% – in the II. The amount of Ig E decreased by 8 and 19%, respectively. When analyzing the activity of lysozyme in the blood serum of patients with COPD, it can be noted its increase by 25% in the I age group and by 12% – in the II. All of them significantly differed from the control, except for the activity of lysozyme in the 1st age group.

After joining the BT enterosgel, the content of MSM254 in patients of the I age group decreased by 15%, MSM280 – by 24%, EII – by 25%. In the II age group, the level of MSM254 decreased by 28%, MSM280 – by 31%, and EII – by 31%. When comparing the obtained results with the control ones, it can be noted that there was a decrease in the difference between them, especially in the II age group.

Joining BT carboline led to a decrease in the content of MSM254 in the I age group by 19%, MSM280 – by 29%, EII – by 30%. In the II age group, the level of MSM254 decreased by 28%, and MSM280 and EII – by 37%. When comparing the obtained results with the control ones, it can be noted that there was a decrease in the significant difference between them, especially in the 1st age group.

After combining BT with enterosgel, the content of MDA in the I age group decreased by 22%, and in the II – by 37%. SOD activity increased by 23% and 16% respectively. Compared to the control group, a significant difference was maintained between its indicators and the level of MDA in the I and II age groups. After the treatment, a decrease in the difference between the obtained indicators and the control group was noted, which may indicate an improvement in the condition of the patients, although only the activity of SOD in patients of the 1st age group returned to normal. With additional use of carboline against the background of BT, the concentration of MDA decreased by 32% in the I age group and by 39% in the II. Serum SOD activity increased by 29 and 15%, respectively. However, the indicators did not reach the reference level and a significant difference with them was maintained.

The use of enterosgel against the background of BT led to a decrease in the level of IL-1β in blood serum in patients of the I age group by 49%, in II – by 62%. The concentration of IL-10 decreased by 44 and 16%, respectively. The use of enterosgel against the background of BT led to a decrease in the level of TNF-α in blood serum in patients by 29% in I and 48% – in II age groups. When comparing the obtained data with the control, it was established that all indicators were reliably different from the control group.

When carboline carbon sorbent was included in BT, the level of IL-1β and IL-10 in the blood serum of patients of the I age group decreased equally by 59%, and II – by 64 and 17%, respectively. The concentration of TNF-α in blood serum in patients decreased by 42% in I and 52% – in II age groups. The combination of BT with enterosgel resulted in a decrease in both CIC and Ig E content in both subgroups. The content of CIC decreased by 22 and 23%, and the amount of Ig E – by 26 and 27% in the I and II age groups, respectively. The amount of lysozyme in the blood serum of patients increased by 44% in the I age group and by 21% – in the II. The changes were likely. When BT was combined with carboline, the level of CIC decreased in the I age group by 23%, in II – by 31% compared to the initial data, the concentration of Ig E – by 27 and 37%,

respectively. Lysozyme content, by contrast, increased in the I age group – by 50% compared to 29% in II ($p<0.01$).

The analysis of the results of laboratory tests of blood serum of the examined patients by the method of parametric correlation revealed a correlation of average strength between MSM254 and MSM280 and indicators of the POL system, cytokine system and immunological status after BT treatment. With all indicators, it was direct and only with SOD inverted. A correlation of weak strength was established with lysozyme and with EII. After BT was performed, correlations between endotoxicosis and EII markers were maintained in patients of the I age group. Most of them were of medium strength and positive direction. In the II age group, the strength and credibility of these links is significantly reduced. This indicates that the treatment carried out more reduced endotoxicosis in elderly and senile patients. During the correlation analysis of the therapeutic effectiveness of enterosorbents in the complex therapy of patients of different ages, it was established that the use of enterosorption therapy improved the condition of patients and reduced the level of intoxication, which was manifested by a decrease in the serum level of MSM254 and MSM280, EII, MDA, cytokines (IL-1β, TNF-α , IL-10), CIC and Ig E and an increase in the levels of lysozyme and SOD. The parametric correlation method revealed a positive correlation between EII and other indicators of endotoxicosis in different age groups. Treatment of COPD patients in the exacerbation phase of BT in combination with carboline led to the emergence of new correlations between almost all indicators of different links of endotoxicosis, which had a direct medium strength character. Between some of them, the correlation dependence was close. For example, between IL-1β and Ig E ($r=0.702$, $p<0.001$), TNF-α and MSM280 $r=0.716$, $p<0.001$), IL-10 and EII ($r=0.733$, $p<0.001$). After the enterosgel treatment, the correlations between SOD, Ig E, CIC and MSM became weak and unreliable. But these connections strengthened and became directly proportional to the average strength with lysozyme and EII. Strong positive correlations between MSM and IL-10 were also observed. Positive medium strength correlations of EII with other indicators such as MDA ($r=0.638$, $p<0.001$), lysozyme ($r=0.371$, $p<0.01$), IL-1β ($r=0.631$, $p<0.001$), IL-10 ($r=0.641$, $p<0.001$), TNF-α ($r=0.639$, $p<0.001$) were noted.

DISCUSSION

According to the scientific publishers, the pathophysiology of irreversible obstruction of the respiratory tract in patients with COPD is a chronic inflammatory process, which is accompanied by a significant increase in the activity of the processes of the biological membranes of cells and is closely related to the clinical features of the disease [8, 9].

It is known that exo- and endogenous intoxication plays a significant role in the pathogenesis of many diseases. Endogenous intoxication syndrome is a complex multicomponent process due to the pathological biological activity of any endogenous products or dysfunction of natural detoxification and biotransformation systems [4, 9]. In view of this, chronic endotoxicosis is now considered as the basis for the emergence and progression of chronic diseases of the internal organs and, accordingly, as an important integral criterion for the severity of their course.

According to a number of authors, the main etiological factor of the disease is smoking, which was confirmed by our research. In 90 patients (55%) the disease developed on the background of prolonged smoking, of which 73 (81%) were male. Calculating the packets/years index in smokers, they found that it was higher in patients with severe bronchial obstruction compared to patients with bronchial obstruction of mild and moderate severity. Other causes of COPD were harmful conditions of production and frequent acute diseases of the respiratory system in history. The severity of the COPD clinic varied depending on the duration of the disease and the severity of its course. All the patients we observed were treated during the exacerbation of the disease.

General manifestations of endotoxicosis may have different origin, it is this syndrome that is the result of poisoning of the body as the final products of metabolism and intermediate compounds that accumulate in excessive amounts of damage by detoxification systems [10, 11]. The degree of severity of endotoxemia is an important and objective criterion for assessing the condition of the body under the conditions of accumulation of end products of metabolism of proteins, lipids and other substances, which occurs when catabolic processes are increased. Endogenous intoxication syndrome not only accompanies acute and chronic pathology, but also in itself is an important factor in their pathogenesis due to the formation of a large number of biologically active substances, tissue destruction products, active proteolysis, lipid hydrocytes and proteins, which significantly determines.

The current concept of the pathogenesis of COPD considers it as an environmentally indirect chronic inflammatory disease with the predominant lesions of the distal respiratory tract, the lung parenchyma and the formation of emphysema, which results in bronchial obstruction, which does not exclude the possibility of developing exogenous intoxication syndrome. In order to check this assumption, the dynamics of EI in the serum of patients, in particular the content of MSM and EII, were evaluated. The increase in these indicators is a significant proof of the presence of endogenous intoxication syndrome in patients with COPD, which is due to the insufficiency of the body of detoxification of the body, resulting in disruption of exogenous toxins and the accumulation of intermediate metabolism [2, 8, 12].

To assess the condition of cell membranes, the erythrocyte membrane permeability test was performed, which is one of the criteria of the effect of EI on the plasma membrane. Violation of the integrity of the erythrocyte membrane, changes in the properties of lipid bichand and the conformation of proteins under the influence of toxic substances changes the functional ability of erythrocytes to bind various compounds that underlies the definition of EII.

Thus, it was confirmed that COPD is accompanied by pronounced endotoxicosis, as evidenced by a number of clinical symptoms, an increase in MSM254, MSM280 and

EII in serum. The formation of excess MSM in the body and their distribution in biological fluids is caused by impaired balance between the processes of anabolism and catabolism towards the latter. This leads to the defeat of toxic metabolites of the respective organs and impaired detoxifying function of the liver and excretory function of the kidneys. In addition, MSMs promote erythrocyte hemolysis, inhibit glucose formation in them, reduce DNA and globin synthesis in erythroblasts, disrupt hydrocarbon metabolism, inhibit protein synthesis, damage hepatocytes, disintegrate lymphocyte function [12, 13].

Scientific publications contain results that indicate that any pathology activates processes, which leads to the accumulation of toxic substances (endotoxins) in the body. Increasing in the serum the content of products of the floor, as well as increasing the activity of enzymes of detoxification of active forms of oxygen are nonspecific tests of endotoxicosis. Lipid decay products (aldehydes, dialdehydes, epoxides) have a detrimental effect on various structures of the cell membrane, proteins, nucleic acids, therefore, are endopathogen [13].

According to modern scientific literature, the pathophysiology of irreversible obstruction of the respiratory tract in patients with COPD is a chronic inflammatory process, which is accompanied by a significant increase in the activity of the processes of paellalogical membranes of cells and is closely related to the clinical features of the disease [4, 8]. One of the most common biomarkers is MDA, which can be estimated by the intensity of Bro in the tissue under study and the degree of damage. It should be borne in mind that, unlike free radicals, it is a stable metabolite of non -radical destruction of polyunsaturated fatty acids, which makes it one of the main biomarkers of the floor [14, 15].

As a result of the studies, it was found that the long recurrent course of the disease is accompanied by the depletion of the protective mechanisms of the body and impaired processes of the floor.

The basis of treatment of already formed COPD is pharmacotherapy. In recent years, research has shown that it is difficult for many patients to fully follow the treatment of COPD [16]. Particular attention should be paid to low -toxic high -performance drugs, convenient and easy to use enterosorbents.

Scientific publications have a lot of data that indicate that the high efficacy of enterosorption therapy justifies the feasibility of its use in various pathological conditions, which underlies the syndrome EI, which is accompanied by the accumulation of toxic substances of endogenous and/or exogenous origin in the blood. Among the various enterosorbents, special attention is paid to silicon – enterosgel [17, 18]. Numerous studies indicate its high efficiency, safety and adsorption ability to bind and eliminate only toxic metabolites and pathogenic microflora. Hepatoprotective and antioxidant effect of the drug is established. Particular attention is paid to carbon enterosorbents, first of all, granular, namely carbon enterosorbent IV carboline generation. Carbon enterosorbents are able to absorb and excrete endo and exotoxins from the body, fix the pathogens of bacterial and viral nature on their surface with subsequent purification of the pathological cell and the body.

Summarizing the results of the study, we can note that the use of enterosgel and carboline, together with basic COPD therapy, helped to reduce clinical manifestations of endotoxicosis, which was manifested by improving the condition of patients and reducing the number of complaints. When combining basic therapy with enterosorbents, there was a significant cough regression. The number of patients who complained of shortness of breath, sputum, tachypnoe and tachycardia also decreased. The best dynamics of indicators were observed in patients with adulthood and with mild bronchial obstruction. Explain changes in endotoxicosis indicators can be explained by the fact that Enterosgel and carboline adsorb in the intestine toxic metabolites, have hepatoprotective and antioxidant effects, significantly improve cellular and humoral immunity, reduce the level of icing, stabilization.

The use of enterosorbents reduces the manifestations of endotoxicosis, improves the condition in the system of the floor-aoz, the links of the immune system, which promotes compensation of secondary immunodeficiency, reduces the titer of autoantibodies and the content of the interest in serum. In general, it is noted faster to eliminate the signs of EI.

CONCLUSIONS

COPD exacerbation accompanied by clinical and laboratory manifestations of endogenous intoxication, namely increasing concentrations of malondialdehyde and superoxide dismutase activity decrease depending on the severity and age of bronchial patients. With the progression of the disease also increases the content of interleukin 1β and 10, tumor necrosis factor α, circulating immune complexes, immunoglobulin E and reduced lysozyme activity in serum, in proportion to the increase in the severity of the disease. The use in treatment of 10-day course enterosorbtsiynoyi therapy using carbon (karbolaynu) or silicone (enterosgel) enterosorbent in treatment of patients with COPD exacerbation is accompanied by a decrease in clinical and laboratory manifestations of endotoxemia, such as cough, shortness of breath, discharge of phlegm, weakness and significant reduction of the average molecular weight and erythrocyte intoxication index, malondialdehyde, interleukins, immunoglobulin E and circulating immune complexes and increased activity of SOD and content of lysozyme in serum.

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CONFLICT OF INTEREST

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ORIGINAL ARTICLE

Effective interventions used by preschool teachers to develop executive functions in preschoolers with attention difficulties

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ABSTRACT

Aim: This study explores the interventions used by preschool teachers to develop executive functions in preschoolers with attention difficulties. It aims to provide a comprehensive understanding of effective interventions for fostering executive function development, as viewed from the perspectives of preschool teachers.

Materials and Methods: A qualitative study was conducted through semi-structured interviews with thirty preschool teachers. Thematic analysis was employed to examine the interventions proposed by teachers for enhancing executive functions in preschoolers with concentration difficulties. The results were subsequently discussed in relation to the existing literature.

Results: The results indicate that motor games and interest-driven activities are the most effective in developing executive functions in children with attention difficulties. Teachers suggested that successful interventions often incorporate rhythm and movement, aligning with children's interests.

Conclusions: Early and effective interventions aimed at developing executive functions in preschoolers at risk of ADHD can delay the onset of symptoms and enhance academic success. Teachers emphasise that motor games and activities based on children's interests are key strategies for improving concentration and self-regulation; however, further research is required to validate these findings.

KEY WORDS: ADHD, Preschoolers, Interventions, Executive Functions, Preschool Teachers

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INTRODUCTION

Executive Functions (EFs) and self-regulation are essential cognitive processes that influence success across various life domains, including education, work, and social relationships, by enabling individuals to manage novel and complex situations [1]. These functions begin to develop in infancy, grow rapidly during preschool years, reach maturity in late adolescence, and decline in later adulthood [2]. During preschool, the core components of executive function (working memory, cognitive flexibility, and inhibitory control) undergo significant development [3]. Deficits in these areas are linked to a higher risk of developing Attention Deficit Hyperactivity Disorder (ADHD) [4], making early interventions vital [5].

Targeted early interventions for preschoolers have been shown to enhance executive functions (EFs), delay or reduce symptoms of ADHD, and improve long-term developmental outcomes, potentially decreasing the need for formal diagnosis and associated healthcare costs [6]. Effective strategies commonly include imaginative play, storytelling, music, motor games, and quiet activities [7]. Additionally, approaches such as digital tools, mindfulness practices, martial arts, and aerobic exercise are also beneficial, particularly when aligned with children's interests [8]. Recent research further supports the effectiveness of interventions that integrate physical activity, structured play, mindfulness, and educational technologies to strengthen executive functions (EFs) in preschoolers exhibiting ADHD symptoms [9,10]. In academic contexts, teachers have also utilised storytelling, rhymes, music, and visual aids to promote attention and self-regulation [11], underscoring the value of creative and multimodal approaches in early childhood education.

Play remains a powerful medium for cognitive and emotional development. It aids in managing behavioural challenges while supporting the growth of executive function skills [12, 13]. Imaginative play is particularly effective in promoting inhibitory control and is associated with improved learning outcomes in children with developmental difficulties [14]. Storytelling – especially dramatised or sequential – has also been shown to improve emotional literacy and attention regulation [15].

Physical activity supports both mental health and the development of executive functions, particularly when combined with cognitive challenges [16]. Calming routines, such as yoga, encourage controlled movement and foster self-regulation strategies [17, 18]. Music-based interventions have been linked to improvements in self-regulation and inhibitory control, particularly for children with ADHD [19]. Additionally, arts-based activities, including painting and group cultural experiences, enhance metacognitive and behavioural skills [20]. While digital tools such as educational

robotics show promise [21], passive screen time may hinder self-regulation [22], highlighting the importance of guided, interactive media use [23].

The literature highlights the critical role of executive functions in social development and the emergence of psychopathology, particularly among children with ADHD and externalising behaviours, where deficits in EFs are closely linked to symptom manifestation. Interventions targeting EFs in the preschool years have proven effective, particularly in reducing attention-related difficulties. Numerous studies and reviews have highlighted the success of diverse approaches – including play, storytelling, physical activity, music, art, and technology – in enhancing executive functions, even among children without diagnosed ADHD. However, a gap remains concerning preschool teachers' perspectives on which interventions they find most effective for children with attention difficulties. This study aims to address this gap by exploring the perceived effectiveness of interventions proposed by the Centre on the Developing Child at Harvard University [7], as well as those involving digital tools [10], robotics [21], art-based methods [20], and child-directed activities [8].

AIM

This study explores the interventions used by preschool teachers to develop executive functions in preschoolers with attention difficulties.

It aims to provide a comprehensive understanding of effective interventions for fostering executive function development, as viewed from the perspectives of preschool teachers.

MATERIALS AND METHODS

This qualitative study explored kindergarten teachers' perspectives on the most effective interventions for children with concentration difficulties in the classroom. The sample comprised thirty active teachers from Greece and England. Using convenience sampling, the researcher, who is also a kindergarten teacher, initially contacted colleagues, who then referred others. After obtaining ethical approval, participants received detailed information and consent forms via email. Semi-structured interviews, informed by a literature review and pilot-tested with five teachers, were conducted in person or by phone between March 30 and May 15, 2024.

Data were analysed through thematic analysis, following the steps outlined by Braun and Clarke [24]: transcription, familiarisation, coding, theme development, and presentation. This method was chosen for its suitability in examining subjective experiences, consistent with Tongs' [11] study conducted in Hong Kong.

To ensure validity and reliability, the researcher reviewed interview transcripts multiple times, conducted participant validation, and sought peer review for consistency. Ethical protocols were strictly followed; participants were informed of their rights, provided with consent forms, and assured of confidentiality. Names were anonymised, data were encrypted, and all printed materials would be destroyed after ten years. Participants were offered access to psychological support if needed and referred to ADHD Hellas [25] for additional resources. Interviews were arranged flexibly, including phone or video conferencing, to ensure participants' comfort and informed consent.

ETHICS

This work complies with the principles of the Declaration of Helsinki.

RESULTS

Participants highlighted the increasing challenge of managing preschoolers with concentration difficulties, noting a significant rise in such cases over the past five years. Although each child demonstrates unique characteristics, common patterns emerged. These children exhibit strong cognitive potential but struggle to maintain attention on tasks, activities, or play. Their efforts are frequently left incomplete, and they often display restlessness, impulsiveness, disorganisation, and a higher tendency for accidents. Additionally, some show aggressive or rude behaviour, have difficulty respecting boundaries, and appear either withdrawn or overly dominant in peer interactions. Individualised support or one-on-one instruction often leads to improved concentration, particularly when activities align with the child's interests or involve novelty.

One participant stated: „They often fail to consider the consequences of their actions before responding, acting impulsively. Some may also exhibit aggressive or rude behaviour towards other children or toys. They find it challenging to adhere to rules" (P.18).

The data analysis provided insights into the research question regarding which EF interventions preschool teachers perceive as most effective for children with concentration difficulties. Teachers prioritised motor games and interest-based activities, followed by music, new technologies, art, imaginative play, storytelling, quiet activities, and traditional games.

MOTOR GAMES

Motor games were frequently described as highly engaging and beneficial for sustaining attention. Their dynamic nature, including rhythmic movement, encourages prolonged participation and better adherence to the rules. Teachers noted that even when children deviate from rules during motor play, it is less disruptive, given the overall flow of the activity. The integration of cognitive tasks into motor games was also emphasised as a strategy to enhance learning outcomes. Calming motor exercises, such as yoga, were recommended to support attention during subsequent structured tasks.

A participant reported: „They thoroughly enjoy motor games, as they incorporate music and a change of rhythm whenever they are in motion. They can engage in these activities independently and pursue them with great enthusiasm. I believe it alleviates their tension and benefits them if a calming motor activity follows afterwards" (P.2).

ACTIVITIES BASED ON CHILDREN'S INTERESTS

Interventions derived from children's interests were ranked as the second most effective strategy. These activities reportedly lead to high levels of focus, cooperation, and motivation among children with concentration difficulties. Teachers strongly supported this approach, advocating for curriculum designs that are rooted in student interests. However, they expressed concern regarding the limitations imposed by mandated, pre-structured programmes from the Ministry of Education and the need to prepare children for more rigid pedagogical approaches in primary school.

A teacher noted: „When something originates from the student and his interests or from the interests of his classmates, it effortlessly captures their attention. This is the most effective method. Children become focused, can collaborate with their peers, and feel the need to share their interests with others" (P.28).

MUSIC

Music was seen as an effective tool for improving attention and reducing impulsivity. Rhythmic patterns were particularly beneficial, offering both emotional regulation and opportunities for social interaction. Children preferred engaging with musical instruments and rhythm-based games over more traditional musical learning, such as singing. Movement-based music activities – like the „lava dance" or „freeze dance" – were also noted to enhance spatial awareness and focus.

One participant highlighted: „I have noticed that it calms them down magically. They enter a state of ecstasy. It's not just the singing; the playing of instruments also has this effect... When there is a movement within the music, it aids them significantly, allowing them to concentrate" (P.15).

NEW TECHNOLOGY

Participants acknowledged the advantages of using interactive technologies to promote cognitive engagement and attention. Activities such as virtual museum visits, coding with Bee-Bots, and interactive whiteboard games were noted for their effectiveness. However, teachers cautioned against excessive screen time, particularly passive use such as video viewing. They emphasised the importance of setting clear boundaries and suggested implementing „technology contracts" to manage transitions and expectations.

Teachers pointed out: „Yes, I use new technology more often, and I engage in activities that stimulate thinking... I need to remind them of the rule from the outset: a contract is required" (P.6).

„I don't use it. The kindergarten where I work in England is free from new technologies; the children have no contact with screens or any other technological devices" (P.10).

ART ACTIVITIES

Opinions on art activities were mixed. Some teachers found that children with attention difficulties struggled to complete crafts or lacked attention to detail. Others felt that when art tasks matched the child's interests, they became more focused and collaborative. Free painting – particularly with tempera and watercolours – was highlighted as especially effective, especially when children could paint standing at an easel, combining movement with creativity.

Several teachers emphasised: „In crafts, they may not fully grasp what they need to do at first... They appreciate the freedom to move, stand, and create using their imagination" (P.12).

„I believe that all our actions should stem from the children and their artistic expression..." (P.30).

IMAGINATIVE PLAY

Both free and structured imaginative play were reported to significantly aid children with concentration difficulties. During free play, children often display high levels of engagement and focus. However, challenges arose when these children attempted to control peer interactions or became upset when peers did not conform to their imagined scenarios. Structured imaginative play offered opportunities to embed cognitive goals and assess individual needs early in the school year.

One teacher stated: „The imaginary play certainly aids them as it captivates their interest... They want to take on the roles they envision and define the game accordingly. They do not readily accept others' opinions..." (P.12).

STORYTELLING

Teachers observed a decline in children's ability to attend during storytelling sessions, which they attributed to increased screen exposure. Interactive techniques – such as using expressive voice, puppets, and related props – were more effective than traditional or digital storytelling methods. Teachers also used concise stories with imagery and questions to maintain interest and promote comprehension.

Participants reported: „A few years ago, they listened to a fairy tale that they enjoyed... In recent years, with the rise of screens, the fairy tale has become increasingly meaningless..." (P.22).

„In England, we utilise story bags filled with hero dolls and objects... whenever their hero is mentioned, they stand up and act out the roles assigned to them" (P.10).

QUIET ACTIVITIES

Quiet activities, such as board games, cooking, and experiments, were used to enhance EF. However, children with attention difficulties often struggle to remain engaged in turn-based games. Cooking and experiments were found to be more successful, as they enabled children to manage impulses and sustain attention.

Teachers pointed out: „We incorporate board games into the children's daily schedules... although children with ADHD may struggle... these activities are beneficial for their growth" (P.17).

„Preschoolers with concentration difficulties... exhibit greater motivation and engage more actively in cooking and experiments..." (P.3).

TRADITIONAL GAMES

Traditional games combine movement and music to support EF development and cultural learning. While

some teachers exclude these games, others integrate them primarily during outdoor activities. Children with concentration difficulties often struggle with rule-following in such settings, but with teacher support, these games can foster socialisation and self-regulation.

One participant stated: „In the garden, we favour traditional games because the children enjoy them. However, preschoolers with concentration difficulties often struggle... A teacher can assist in integrating these children by reminding them of the rules and alleviating their tension" (P.6).

DISCUSSION

The findings of this study confirm and extend prior literature on interventions that effectively develop EFs in preschoolers with attention difficulties. Teachers identified motor activities and child-driven tasks as particularly beneficial, echoing evidence from Diamond & Lee [3] and Diamond [8], who emphasise intrinsic motivation as a catalyst for EF development.

The central role of movement-based interventions is consistent with research showing that physical activity enhances attention, working memory, and inhibitory control [16]. Teachers' emphasis on calming follow-up routines, such as yoga, aligns with Barenberg et al. [17] and Benzing et al. [18], who advocate for structured movement combined with regulation strategies.

Interest-led learning remains underutilised due to educational policy constraints, despite its proven value [3]. The challenge of balancing standardised curricula with personalised instruction is a key issue raised by participants.

Music and imaginative play, though often underestimated in structured learning contexts, emerged as powerful tools for EF support. These findings are reinforced by Antonietti et al. [19] and Thibodeau et al. [14], who demonstrate how these interventions foster self-regulation and cognitive flexibility.

The use of technology showed potential but highlighted tensions between interactive and passive media. Teachers advocated for tools like Bee-Bots, consistent with Di Lieto et al. [21] and Geronti [10], while raising concerns similar to those of Cliff et al. [22] regarding unregulated screen time at home.

Art and storytelling were more nuanced: while some children disengaged during crafts or passive story formats, interactive, interest-based approaches facilitated focus and creative expression. These findings align with those of Andersen et al. [20] and White & Carlson [15], underscoring the need for flexibility and teacher creativity.

Lastly, traditional games offer untapped potential for fostering EFs in group contexts, especially when rules are scaffolded by educators, as seen in Amani et al. [12] and Healey & Healey [13].

This study adds a qualitative dimension to the existing literature by foregrounding the voices of preschool teachers and their practical strategies. The findings emphasise that effective EF interventions must be adaptive, child-centred, and balanced with broader curriculum requirements.

CONCLUSIONS

This study highlights that kindergarten teachers consider motor games and activities aligned with children's interests as the most effective interventions to support preschoolers with concentration difficulties and enhance EFs. Engaging, rhythmic movement combined with cognitive challenges fosters sustained attention and self-regulation. Meanwhile, music, imaginative play, storytelling, and hands-on activities, such as cooking, further support EF development. However, external pressures, such as mandated curricula, may limit teachers' ability to tailor interventions to children's interests fully. The findings underscore the need for interventions that strike a balance between structure and flexibility, suggesting that active, participatory methods are most beneficial. Given the qualitative nature and sample limitations of this research, further quantitative studies involving diverse educator perspectives are recommended to expand understanding and improve early intervention strategies.

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CONFLICT OF INTEREST
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ORIGINAL ARTICLE

Adaptive swimming as a means of physical and sports rehabilitation for people with musculoskeletal disorders

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ABSTRACT

Aim: To investigate the impact of adaptive swimming training sessions on the psycho-emotional and functional state of individuals with musculoskeletal disorders.
Materials and Methods: The research was conducted in 2025, with 32 male service members aged 26-58 who underwent rehabilitation for musculoskeletal injuries over 6 weeks. The research used a combination of theoretical, empirical, and mathematical statistical methods. The psycho-emotional state was assessed using the Wessman-Ricks technique, and the functional state was assessed using a 100 m swimming test and the duration of heart rate recovery after exercise.
Results: Data analysis showed that adaptive swimming training sessions have a pronounced rehabilitative effect: they normalize the psycho-emotional state, reduce stress levels, and increase the body's adaptive capabilities. The integral indicator of emotional state after 6 weeks of training increased by 1.15 points ($p \leq 0.001$) and reached a good level, reflecting the restoration of emotional balance. Functional indicators also improved: the time to cover a distance of 100 meters was reduced by 51 seconds ($p \leq 0.001$); the duration of heart rate recovery was reduced by 3.65 beats per minute ($p \leq 0.001$).
Conclusions: It has been established that adaptive swimming training sessions contribute to improving the psycho-emotional state and functional capabilities of individuals with musculoskeletal disorders. A significant improvement in the integral indicators of the cardiovascular system's emotional and functional states has been observed.

KEYWORDS: adaptive swimming, adaptive sports, service members, health, musculoskeletal system, psycho-emotional state, functional state, physical education and sports rehabilitation

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INTRODUCTION

The modern needs for rehabilitation of people with musculoskeletal disorders and the development of scientifically based approaches to restoring the body's functional capabilities necessitate the active introduction of adaptive physical culture and sports. In this regard, adaptive swimming occupies a special place, as it offers significant potential for health, rehabilitation, and social integration thanks to the unique properties of the aquatic environment [1-3].

Adaptive swimming is used in both medical and rehabilitation practice and in the adaptive sports system, thereby improving the quality of life of people with various nosologies, in particular musculoskeletal disorders [4, 5]. At the same time, this type of motor activity is considered a form of physical education and sports in the aquatic environment, specially adapted to the functional capabilities of people with musculoskeletal disorders, taking into account the nature, degree, and location of the damage. Its key features include individualized physical activity, modification of movement techniques, the use of special aids (belts, noodles, boards, floats), and a combination of health, rehabilitation, and sports activities [6-9].

The health and rehabilitation effects of adaptive swimming are mainly due to the properties of the aquatic environment, which creates specific conditions for the motor activity of people with musculoskeletal disorders [10-12]. These include: reduction of the effects of gravity, which makes it possible to perform exercises without excessive strain and helps to reduce pain; optimization of axial load (thanks to the buoyancy of water, the load on the musculoskeletal system is reduced, which is extremely important in cases of impaired support and gait); water resistance, which ensures uniform load on muscle groups without the risk of overload; hydrostatic pressure, which has a positive effect on blood circulation, lymphatic drainage, and the functional state of the cardiovascular system; thermal effect of water, which promotes muscle relaxation and pain reduction [13-15].

The relevance of adaptive swimming in the rehabilitation of people with musculoskeletal disorders has sparked growing scientific interest, reflected in a significant number

of contemporary domestic and international studies. A review of the scientific literature shows that the problem of rehabilitation of people with musculoskeletal disorders is relevant in most countries worldwide, as the effectiveness of such measures directly affects the level of social integration, psycho-emotional state, and physical health of this category of the population [2, 4].

In modern scientific works, adaptive swimming is considered an essential component of comprehensive medical and physical rehabilitation programs, which contributes to the restoration of motor functions, reduction of contractures, improving coordination, normalizing muscle tone, and forming compensatory motor skills in people with musculoskeletal disorders, particularly after spinal injuries and amputations [5]. Scientists [16-18] note that training sessions in the aquatic environment activate the cardiovascular and respiratory systems, strengthen the muscle corset, reduce muscle hypertonicity, and create a relaxing effect that helps reduce the impact of stress.

At the same time, several studies emphasize the need for further scientific justification of methodological approaches to organizing adaptive swimming training sessions, taking into account the nosological specificity and functional state of persons undergoing rehabilitation [2, 19]. Despite the growing number of scientific works on the use of adaptive physical culture in the rehabilitation of people with musculoskeletal disorders, the results of these studies remain fragmentary. Most studies focus primarily on the physiological aspects of motor function recovery. At the same time, the impact of adaptive swimming training sessions on the psycho-emotional state, motivation for physical activity, and overall well-being of individuals with musculoskeletal disorders is insufficiently covered [20, 21]. In addition, existing studies do not always take a comprehensive approach to assessing the effectiveness of adaptive swimming, failing to integrate analyses of individuals' functional and psycho-emotional states in the rehabilitation process.

Modern concepts of rehabilitation emphasize the need for a systematic impact on the human body, encompassing not only the restoration of motor functions but also the improvement of the psycho-emotional state, the development of positive motivation for physical activity, and an increase in the overall quality of life [1, 6, 22]. In view of this, adaptive swimming is considered a promising means of physical and sports rehabilitation, capable of providing a comprehensive positive effect through a combination of physical, psycho-emotional, and social factors. At the same time, this necessitates further scientific research aimed at a detailed study of the impact of adaptive swimming training sessions on the bodies of people with musculoskeletal disorders, taking into account both functional and psycho-emotional indicators.

AIM

The aim is to investigate the impact of adaptive swimming training sessions on the psycho-emotional and functional state of individuals with musculoskeletal disorders.

MATERIALS AND METHODS

PARTICIPANTS

The research, conducted in 2025, involved 32 service members aged 26 to 58 who were undergoing rehabilitation after musculoskeletal injuries (traumatic and functional disorders of the spine and joints). Based on the decisions of a multidisciplinary rehabilitation team, these individuals were recommended to undergo physical and sports rehabilitation, including adaptive sports, particularly adaptive swimming.

Rehabilitation measures were carried out at rehabilitation centers in Zhytomyr, and adaptive swimming training sessions were conducted at the "Avangard" sports complex (25 m indoor pool) three times a week for 45 minutes. The results were processed and analyzed at the Department of Physical Education and Sports Rehabilitation of the S. P. Koroliov Zhytomyr Military Institute (ZMI). All training sessions were conducted under medical supervision and with the participation of rehabilitation specialists. The criteria for inclusion of participants in the research were determined as follows: male service members who had participated in combat operations and suffered injuries and damage to the musculoskeletal system (spinal injuries) were included in the research; after treatment, the service members were prescribed adaptive swimming as a means of physical and sports rehabilitation; absence of diseases and contraindications due to health conditions (chronic infectious diseases, etc.) to adaptive swimming training sessions. The exclusion criteria were discontinuation of training sessions due to health conditions or other reasons, and the service members' own desire to withdraw from the research at any time. All participants were informed of the research objectives, after which they provided written consent to participate solely for scientific purposes.

RESEARCH METHODS

The research involved the use of a combination of theoretical, empirical, and mathematical statistical methods. Theoretical methods included analysis and generalization of literary sources. Analysis and generalization of literary sources were used to conduct an analytical review of scientific sources on the outlined range of issues (27 sources from PubMed, Scopus, Web of Science, and Index Copernicus were analyzed).

Empirical methods included methods for assessing the psycho-emotional and functional state of service members. The dynamics of the participants' psycho-emotional state were assessed using the Wessman-Ricks emotional state self-assessment technique, and their functional state was assessed using a swimming test in a pool (100 meters) and analysis of heart rate (HR) recovery 1 minute after the end of the exercise. The testing was conducted twice: at the beginning of the participants' stay at the training base and after 6 weeks of systematic adaptive swimming training sessions.

The level of emotional state was determined based on the results of a survey using an electronic form (Google Forms), in which participants selected the statements that most accurately reflected their current psycho-emotional state. The analysis was carried out using the following

scales: "Calmness – Anxiety," "Energy – Fatigue," "Elation – Depression," and "Self-confidence – Helplessness," each rated on a ten-point scale. The formula determined the integral indicator of emotional state (ES): $ES = (I_1 + I_2 + I_3 + I_4) / 4$, where I_1–I_4 are individual indicators on the corresponding scales. ES values within the range of 8-10 points were interpreted as very good, 6-7 points as good, 4-5 points as poor, and 1-3 points as bad.

To assess functional fitness (functional endurance, aerobic working capacity, and cardiovascular system adaptation capabilities), a 100 m swimming test and analysis of HR dynamics 1 minute after the end of the exercise were used. The testing was conducted in an indoor 25 m pool at a water temperature of 27-30°C, which provided safe and comfortable conditions for people with musculoskeletal disorders. Before the test, participants performed a standard five-minute preparatory phase, which included light water movements and breathing exercises. During the test, any method of movement in the water was allowed, taking into account the nature of the injury and the individual's functional capabilities; short-term slowdowns or stops were allowed without leaving the water or stopping the timer. If necessary, buoyancy aids (boards, noodles, belts) were used, and their use was recorded and maintained unchanged during retesting. Retesting was conducted under the same conditions after the completion of the six-week program. Heart rate was recorded using a Garmin Forerunner 165 sports watch and a Garmin HRM-Pro Plus chest strap, which ensured accurate determination of maximum heart rate at the finish and 1 minute after recovery. A reduction in the time taken to cover the distance and a decrease in the duration of heart rate recovery were considered positive changes in functional status.

STATISTICAL METHODS

The methods of mathematical statistics were used to process the data obtained. The reliability of the difference between the indicators was determined using the Student's t-test. The results were presented as Mean±m, where Mean is the arithmetic mean, m is the standard error. The reliability of the difference was set at $p<0.05$. All statistical analyses were performed using STATISTICA 6.1 software package (number AGAR909E415822FA), adapted for medical and biological research.

ETHICS

The procedure for organizing the study and the topic of the article were previously agreed with the Committee on compliance with Academic Integrity and Ethics of the ZMI. Also this study followed the regulations of the World Medical Association Declaration of Helsinki. Informed consent was received from all participants who took part in this study.

FRAMEWORK

This scientific article was carried out according to the plan of the research work of the National Academy of Internal Affairs for 2020-2026 „Psychological, pedagogical and sociological support of law enforcement officers» (state registration number 0113U008196).

RESULTS

Adaptive swimming programs were developed by specialists in physical and sports rehabilitation, taking into account the type and level of spinal (cervical, thoracic, lumbar) and joint injuries, the degree of damage, individual functional capabilities, and motor experience of the persons undergoing rehabilitation. The rehabilitation process was implemented in stages, including functional recovery, training, and adaptation, which ensured a gradual and safe increase in motor activity. The functional recovery stage (1 week) focused on adapting to the aquatic environment, developing proper breathing techniques, reducing muscle tension, and mastering basic water movements. The training and adaptation stage (5 weeks) involved consolidating restored motor functions, increasing physical fitness, and adapting the body to systematic, measured training loads in the aquatic environment.

Depending on the type and degree of injury, the program included a set of corrective exercises aimed at stretching, decompression, and stabilization of the spine (stretching with support on the edge, with a noodle or a float; maintaining a horizontal position, balance exercises, smooth rolls, sliding with body axis control, etc.). Symmetrical and asymmetrical swimming exercises were used during the training sessions. Symmetrical exercises ensured an even distribution of load and the formation of rational movement patterns. In contrast, asymmetrical exercises were aimed at correcting muscle imbalance, activating deep stabilizing muscles, and improving neuromuscular control of movements. Performing exercises in the aquatic environment reduced axial load on the spine and joints, thereby increasing the safety and effectiveness of the rehabilitation. The selection of exercises, their duration, and intensity were determined individually, using support devices (noodles, boards, special floats) to provide additional relief for the spine.

The results of the research show that six weeks of adaptive swimming training sessions have a pronounced rehabilitative effect, manifested in normalization of the psycho-emotional state, reduced stress levels, and increased adaptive capabilities. The dynamics of most indicators are characterized by a change from a poor to a good level, which is one of the key criteria for the effectiveness of rehabilitation programs.

In particular, the increase in the integral ES indicator from 5.83±0.13 to 6.98±0.10 points (p±0.001) reflects the restoration of emotional balance and a decrease in manifestations of maladjustment. From a rehabilitation perspective, this indicates a reduction in psycho-emotional stress and activation of internal regulatory mechanisms, which is a necessary prerequisite for further physical and mental recovery (Table 1).

An improvement in the "Calmness-Anxiety" indicator to 6.59±0.11 points (p±0.01) indicates a pronounced anxiolytic effect of adaptive swimming. Training sessions in the aquatic environment, combining rhythmic movements, controlled breathing, and sensory relaxation, help reduce anxiety, which is one of the basic tasks of psycho-emotional rehabilitation. A significant increase in the "Energy – Fatigue" indicator

Table 1. Dynamics of indicators of the psycho-emotional and functional state of service members with musculoskeletal disorders (n = 32)

Researched indicators	Research stages	Mean±m	Significance of the difference	
			t	p
Psycho-emotional state indicators, points				
Calmness – Anxiety	initial	5.91±0.18	3.22	p≤0.01
	final	6.59±0.11		
Energy – Fatigue	initial	5.75±0.15	4.87	p≤0.001
	final	6.75±0.14		
Elation – Depression	initial	5.84±0.20	5.37	p≤0.001
	final	7.25±0.17		
Self-confidence – Helplessness	initial	5.81±0.15	5.03	p≤0.001
	final	7.19±0.23		
Integral indicator of emotional state	initial	5.83±0.13	7.01	p≤0.001
	final	6.98±0.10		
Functional state indicators				
100 m swimming test, s	initial	310.34±5.53	7.50	p≤0.001
	final	259.44±3.94		
HR recovery time 1 minute after the end of the exercise, bpm	initial	22.44±0.80	3.85	p≤0.001
	final	26.09±0.51		

Legend: Mean – arithmetic mean; m – standard error; t – Student's t-test value; p – p-value
Source: compiled by the authors of this study

from 5.75±0.15 to 7.50±0.14 points (p±0.001) indicates the restoration of the body's functional reserves. In terms of rehabilitation, this can be interpreted as a reduction in chronic fatigue symptoms, increased tolerance to physical exertion, and improved overall functional status. Positive dynamics on the "Elation – Depression" scale (up to 7.25±0.17 points; p±0.001) reflect the normalization of the emotional background and an increase in the motivational component. This is of significant importance for the rehabilitation process, as increased elation contributes to greater individual involvement in recovery activities and to the formation of a stable commitment to systematic exercise.

Particularly significant in the rehabilitation aspect is the reliable increase in the indicator on the "Self-confidence – Helplessness" scale to 7.19±0.23 points (p<0.001), which is considered good. This indicates the restoration of psychological self-regulation, a decrease in feelings of helplessness, and the formation of an internal resource for overcoming difficulties, which is a critically important factor for successful social and professional reintegration.

Thus, the improvement in the psycho-emotional state created favorable conditions for the implementation of physiological adaptations caused by systematic motor activity in the aquatic environment. In this regard, it is advisable to analyze changes in the cardiovascular system's functional state, which reflect the effectiveness of the next stage of the experiment.

During the research, the results of a 100-meter swimming test were analyzed, during which the maximum heart rate was recorded immediately after completing the distance, as well as its recovery indicators 1 minute after the load. At the initial stage, the average time to cover 100 meters was 5 min 10.34 s (310.34±5.53 s), indicating reduced functional endurance and limited cardiovascular adaptive capabilities in individuals with musculoskeletal disorders. After 6 weeks of adaptive swimming training sessions, a statistically significant improvement in results was observed – the average time to cover the distance was reduced to 4 min 19.44 s (259.44±3.94 s) (p±0.001). The observed positive dynamics indicate a significant increase in functional endurance, driven by the combined effects of the aquatic environment and systematic training. The reduction in the time taken to cover the distance can be explained by improved coordination and efficiency of movement patterns in water, reduced pain and muscle tension due to the unloading effect of water, increased aerobic capacity of the cardiovascular and respiratory systems, and increased tolerance to physical exertion. The results confirm the pronounced rehabilitative effect of adaptive swimming, manifested in increased overall functional readiness and greater opportunities for people with musculoskeletal disorders to participate in everyday physical activity.

Analysis of heart rate recovery 1 minute after exercise also showed positive changes. At the initial stage, the HR reduction was 22.44±0.80 bpm, whereas after completing the training sessions, it increased to 26.09±0.51 bpm, indicating accelerated heart rate recovery. The improvement in HR recovery rate

indicates the activation of parasympathetic regulatory mechanisms, increased cardiac efficiency, and growth in the body's adaptive reserves. In terms of rehabilitation, this is an essential indicator of reduced cardiovascular stress from physical activity and increased safety during motor activity in individuals with musculoskeletal disorders.

DISCUSSION

Modern rehabilitation approaches to the recovery of individuals with musculoskeletal disorders are based on comprehensive measures, among which adaptive swimming plays a key role. Its effectiveness stems from the unique properties of the aquatic environment, which provide a combination of therapeutic, restorative, and psycho-correctional effects [2, 23].

The results of the research are consistent with scientific literature and confirm the positive impact of adaptive swimming on participants' psycho-emotional state [5, 24, 25]. A significant improvement in the integral ES indicator, along with positive dynamics on selected scales, indicates a reduction in psycho-emotional tension, increased confidence in one's own abilities, and the formation of internal resources for self-regulation. This is crucial for the effectiveness of the rehabilitation process and further social adaptation.

Along with psycho-emotional changes, a significant improvement in aerobic performance was recorded. The reduction in time taken to cover 100 meters after 6 weeks of training indicates increased functional endurance, improved motor skills, and greater tolerance to physical exertion. The uniform involvement of muscle groups can explain these changes, reduced axial load on the joints, and improved coordination of movements in the aquatic environment. The positive dynamics of heart rate recovery 1 minute after exercise indicate an increase in the cardiovascular system's efficiency and the body's adaptive reserves. Regarding rehabilitation, this confirms the feasibility and safety of the physical exercises performed [26].

The results obtained confirm the advisability of using adaptive swimming as an effective means of comprehensive physical and sports rehabilitation and confirm the conclusions of many scientists [3, 4, 17, 27] regarding the need to individualize programs taking into account the nosology, functional state, and psycho-emotional characteristics of persons undergoing rehabilitation.

CONCLUSIONS

It has been established that adaptive swimming training sessions have a pronounced rehabilitative effect in individuals with musculoskeletal disorders, manifested in comprehensive improvements in psycho-emotional state and functional capabilities of the body. It has been proven that systematic adaptive swimming training sessions contribute to the normalization of the psycho-emotional state, reduction of anxiety and fatigue, increased confidence in one's own abilities, and motivation to actively participate in the rehabilitation process. A statistically significant increase in functional endurance has been found, as confirmed by a reduction in the time taken to swim a distance of 100 meters ($p \pm 0.001$). Adaptive swimming had a positive effect on the functional state of the cardiovascular system, as evidenced by accelerated heart rate recovery after physical exertion and increased cardiac efficiency ($p \pm 0.001$).

PROSPECTS FOR FURTHER RESEARCH

We see prospects for further research into the effectiveness of adaptive swimming, taking into account different nosological groups, the duration of rehabilitation programs, and their combination with other physical therapy methods.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Impact of sarcoidosis on clinical outcomes in patients with dilated cardiomyopathy: A propensity-matched analysis from the TriNetX network

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ABSTRACT

Aim: Our study aims to compare the outcomes in patients with dilated cardiomyopathy (DCM) who have sarcoidosis and who do not have sarcoidosis.

Materials and Methods: We used data from the TriNetX US collaborative network database, including DCM patients with and without sarcoidosis between Aug 1, 2015 and Aug 1, 2025. Propensity score matching was done to minimize differences in baseline characteristics. A total of 4,514 patients in each cohort (sarcoidosis vs. non-sarcoidosis) were studied after propensity score matching. The primary outcome was ventricular assist device (VAD) placement, and the secondary outcomes were ventricular arrhythmia, hospitalization or ED visits, acute kidney injury, and stroke.

Results: The incidence of VAD insertion was more frequent in the sarcoidosis group (2.2% vs. 1.3%; OR: 1.676, 95% CI: 1.210-2.321; P<0.001). Secondary outcomes also showed significant differences, with higher rates of ventricular arrhythmia (OR: 2.082, 95% CI: 1.833-2.364; P<0.001), acute kidney injury (OR: 1.305, 95% CI: 1.157-1.473; P<0.001), and hospitalization/ED visits (OR: 1.159, 95% CI: 1.003-1.340; P<0.001) in the sarcoidosis cohort. Other arrhythmia events were more frequent (OR: 2.517, 95% CI: 2.425-2.613; P<0.001). The risk of composite stroke did not differ significantly between groups (OR: 1.132, 95% CI: 0.957-1.339; P=0.146).

Conclusions: Sarcoidosis in DCM was associated with higher risk of VAD insertion and most secondary outcomes, except stroke, indicating worse prognosis and need for closer monitoring and targeted management.

KEYWORDS: dilated cardiomyopathy, sarcoidosis, ventricular assist device

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INTRODUCTION

Sarcoidosis is a systemic granulomatous disease that can involve multiple organ systems. Although sarcoidosis most frequently involves the lungs, extrapulmonary forms are a major cause of morbidity and mortality. These may include involvement of the skin, eyes, lymph nodes, liver, spleen, and heart, among other organs.

Cardiac involvement, referred to as cardiac sarcoidosis, is estimated to occur in approximately 20-25% of patients, most of whom are asymptomatic (clinically silent). Clinically manifest cardiac involvement is observed in only about 5% of patients with pulmonary or systemic sarcoidosis [1, 2]. Of note, cardiac involvement can occur without evidence of disease in other organs [3].

Cardiac sarcoidosis may manifest as conduction abnormalities, ventricular arrhythmias, and/or heart failure [4]. Heart failure is less common than arrhythmia at initial presentation and represents the first clinical manifestation in fewer than 20% of cases. Patients with sarcoidosis have a higher 10-year risk of heart failure compared with the general population (3.18% versus 1.72%) [5].

Despite the well-recognized association between sarcoidosis and dilated cardiomyopathy (DCM) development, the literature is limited regarding outcomes in patients with DCM and concomitant sarcoidosis compared to those with DCM alone. This study aims to address this gap by evaluating the impact of sarcoidosis on clinical outcomes in patients with DCM. Specifically, it investigates the need for mechanical circulatory support via ventricular assist device (VAD) implantation, as well as the incidence of ventricular arrhythmias, acute kidney injury (AKI), all-cause hospitalization or emergency department visits, and cerebrovascular accidents (CVA).

AIM

Our study aims to address a knowledge gap by comparing the outcomes in patients with dilated cardiomyopathy (DCM) who have sarcoidosis and who do not have sarcoidosis. Our

primary outcomes are VAD placement incidence. Secondary outcomes are ventricular arrhythmia, acute kidney injury, hospitalization/ED visit and stroke.

MATERIALS AND METHODS

DATA SOURCE

This study was conducted using the TriNetX research network, a federated database providing access to electronic health records (EHRs) from 147 healthcare organizations (HCOs). A total of 86 providers responded with patients. The TriNetX platform aggregates deidentified patient data, ensuring compliance with HIPAA deidentification standards.

PATIENT POPULATION

We conducted a retrospective observational multicentric cohort study including adult patients (≥18 years old) with dilated cardiomyopathy, categorized into two groups depending if they have sarcoidosis or not. Cohort 1 (sarcoidosis group) included patients with dilated cardiomyopathy who have sarcoidosis, while Cohort 2 (non-sarcoidosis group) included patients with dilated cardiomyopathy who do not have sarcoidosis. Dilated cardiomyopathy and sarcoidosis diagnosis was identified using ICD-10 codes. Additional details on cohort identification and study window definitions, including the relevant ICD-10, RxNorm, and Current Procedural Terminology codes, are provided in the Supplemental Appendix.

STUDY ENDPOINTS

The index event was defined as the first recorded diagnosis of sarcoidosis for patients in the sarcoidosis cohort, identified based on ICD-10 codes including D86.0 (Sarcoidosis), D86.9 (unspecified sarcoidosis), occurring between August 1, 2015, and August 1, 2025. Dilated cardiomyopathy was identified based on ICD-10 codes including I42.0 and other related subcodes in the appendix.

The primary outcome of interest was incidence of ventricular assisted device placement. Secondary outcomes included ventricular arrhythmia, acute kidney injury, all-cause hospitalization or emergency department (ED) visits, and occurrence of stroke. The Supplemental Appendix elaborates on outcome definitions and ICD-10 codes.

STATISTICAL ANALYSIS

Continuous variables are presented as mean ± standard deviation (SD), whereas categorical variables are presented as number (percentage), as appropriate. Baseline characteristics were compared between the sarcoidosis and non-sarcoidosis groups using independent samples Student's t-tests for continuous variables and chi-square tests for categorical variables. To mitigate baseline differences between cohorts, 1:1 propensity score matching was performed using greedy nearest neighbor matching with a caliper of 0.1 times the pooled SD of the linear propensity scores. Covariate balance between cohorts before and after propensity score matching was assessed using standardized mean differences (SMDs) rather than hypothesis testing, as SMDs are independent of sample size and provide a more appropriate measure of balance in matched observational studies. An SMD of <0.1 was considered indicative of adequate balance. P-values were reported for unmatched cohorts for descriptive purposes only and were not used to assess post-matching balance. Variables included in the matching process were age, sex, race, comorbidities (hypertension, heart disease, hyperlipidemia, diabetes, ischemic heart disease, chronic kidney disease, diseases of liver, cerebrovascular diseases. asthma, chronic obstructive pulmonary disease and tobacco use), medication use (antiarrhythmic, antihypertensive, ACEI, ARBs, antilipemic, diuretics, beta-blockers, digoxine, antineoplastic, antimetabolite and glucocorticoids). The standardized mean difference represents the difference between the means of 2 groups in terms of SD units, assessing balance in measured variables in the sample weighted by the inverse probability of treatment. The variables were selected based on their potential impact on overall and cardiovascular outcomes.

After propensity score matching (PSM), adjusted outcomes were compared between cohorts using hazard ratios (HRs) and 95% confidence intervals (CIs) derived from Cox proportional hazards regression models. Kaplan–Meier survival analysis was used to assess time-to-event outcomes, with differences between cohorts evaluated using the log-rank test. A P-value<0.05 was considered statistically significant. All statistical analyses were conducted using integrated R (The R Foundation) within the TriNetX platform. Odds ratios (ORs) were used to compare cumulative incidence of outcomes during the follow-up period, whereas hazard ratios (HRs) were derived from Cox proportional hazards models to evaluate time-to-event outcomes. HRs were considered the primary effect measure for outcomes with a temporal component.

ETHICS APPROVAL

This study was conducted using de-identified data from the TriNetX research network. In accordance with U.S. federal regulations, studies using only de-identified data are not considered human subjects research and are exempt from institutional review board (IRB) approval. TriNetX, LLC has received a waiver from the Western IRB and complies with the Health Insurance Portability and Accountability Act (HIPAA), with de-identification confirmed through a qualified expert determination as defined in Section §164.514(b)(1) of the HIPAA Privacy Rule.

RESULTS

STUDY POPULATION

This retrospective cohort study identified a total of 279,146 patients with a diagnosis of dilated cardiomyopathy. Among them, 4,516 patients with sarcoidosis, and 274,630 patients without sarcoidosis. After applying 1:1 propensity score matching (PSM) to balance baseline characteristics, 4,514 patients were included in each cohort (sarcoidosis and non-sarcoidosis groups) for the final analysis (Fig. 1).

PATIENT CHARACTERISTICS

The baseline characteristics of the study cohorts, before and after propensity score matching (PSM) are shown in Table 1, P-values are not used for balance assessment post-

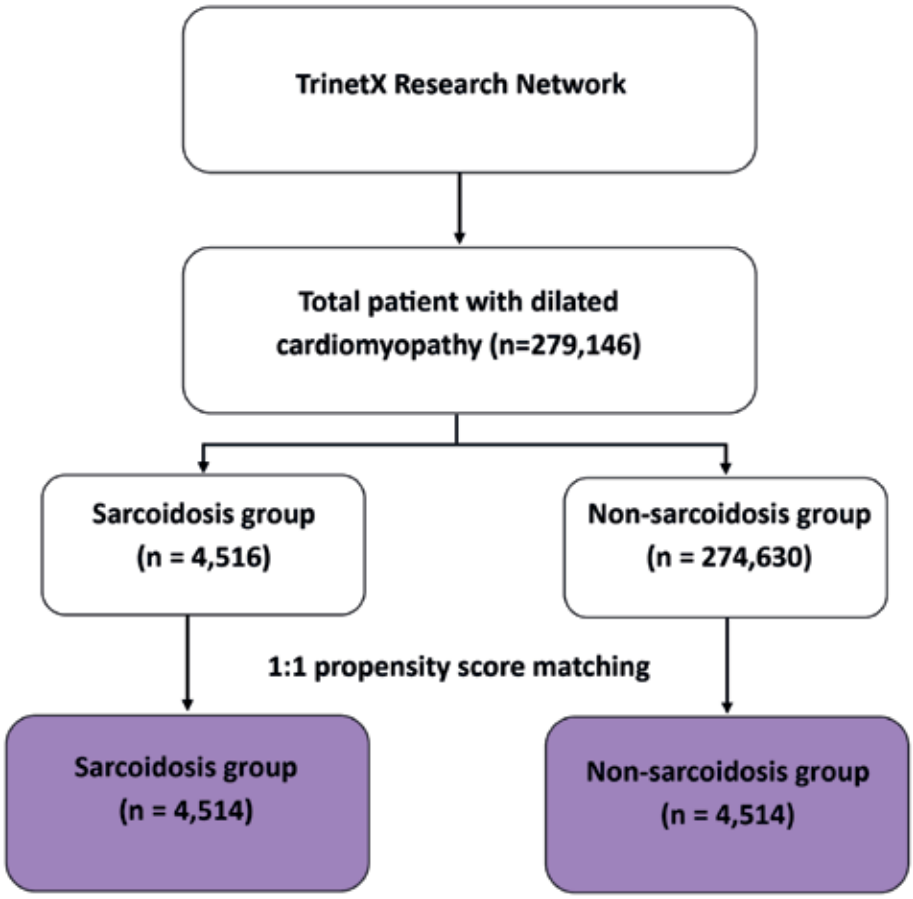

Fig. 1. Flow diagram of patient selection and cohort derivation following propensity score matching

Source: Own materials

PSM. In the unmatched cohort, patients with sarcoidosis were slightly younger at index (mean age: 59.4±12.9 years vs. 63.3±15.6 years, P<0.001) compared to those without sarcoidosis. The proportion of male patients was slightly lower in the sarcoidosis group (60.9%% vs. 64.9%%, P<0.001), also the sarcoidosis group had a higher proportion of female patients (39.0%% vs. 35.1%, P<0.001). Regarding racial distribution, the sarcoidosis group was more likely to be black (37.9% vs.17.1%, P<0.001) compared to the non-sarcoidosis group, while other racial differences were minor.

Before matching, the sarcoidosis group exhibited a higher prevalence of hypertension (74.5% vs. 72.2%, P<0.001), asthma (18.3% vs. 10.8%, P<0.001), chronic obstructive pulmonary disease (17.5% vs. 16.6%, P<0.001), diabetes mellitus 36.5% vs. 33.1%, P<0.001), overweight and obesity (37.2% vs. 19.2%, P<0.001), hyperlipidemia (57.7% vs. 55.1%, P<0.001), disease of liver (16.3% vs. 12.7%, P<0.001) and chronic kidney disease (30.5%% vs. 27.7%, P<0.001). Conversely, tobacco use (5.6% vs. 5.0%, P<0.001). The prevalence of ischemic heart disease (52.6% vs. 52.4%, P<0.001), cerebrovascular disease (16.4% vs. 16.2%, P<0.001) were nearly the same between the two groups.

After propensity score matching (PSM), the two cohorts were well balanced across key baseline characteristics, including age, sex, race, cardiovascular disease risk factors, comorbidities, and medication use with standardized mean differences (SMDs)<0.1 for most variables, indicating a well-matched cohort, P -values are not reported or interpreted for matched comparisons. The final matched cohort consisted of 4,514 patients in the norepinephrine group and 4,514 patients in the phenylephrine group.

Primary outcome: Incidence of ventricular assisted device implantation

After matching, during the follow-up period, a total of 98 patients (2.2%) in the sarcoidosis cohort had VAD placement, compared to 59 patients (1.3%) in the non-sarcoidosis cohort. Having sarcoidosis and dilated cardiomyopathy was associated with a significantly higher risk of VAD placement with hazard ratio (HR) for VAD placement was 1.609 (95% CI: 1.165-2.222; P<0.001), indicating that patients with sarcoidosis had approximately a 61% higher risk of VAD placement relative to those without sarcoidosis. The proportional hazards assumption was not violated (P=0.593) (Table 2, Fig. 2).

After matching, the secondary outcomes demonstrated significant differences between the sarcoidosis group and the nonsarcoidosis group. Ventricular arrhythmia events were more frequently reported in the sarcoidosis group, with a hazard ratio of 1.885 (95% CI:1.678,-2.117, P<0.001). Acute kidney injury was also more frequently reported in the sarcoidosis group, with a hazard ratio of 1.231 (95% CI:1.103-1.373, P<0.001). Hospitalization or emergency department (ED) visits were more frequent in the sarcoidosis group, with a hazard ratio of 1.186 (95% CI:1.069-1.317, P<0.001).

Table 1. Baseline characteristics of patients in sarcoidosis and non-sarcoidosis groups before and after propensity score matching (PSM) with SMDs

	Before PSM			After PSM		
	Before Matching (Sarcoidosis group, n=170,555)	Before Matching (Non-sarcoidosis group, n=309,793)	Standardized Difference	After Matching (Sarcoidosis group, n=138,051)	After Matching (Non-sarcoidosis group, n=138,051)	Standardized Mean Difference (SMDs)
Demographics						
current age (Mean ± SD)	64.5±12.7	68.2±15.3	0.261	64.5±12.7	64.7±16.2	0.007
Age at index (Mean ± SD)	59.4±12.9	63.3±15.6	0.268	59.4±12.9	59.5±16.4	0.006
Female n (%)	1,763 (39.0%)	96,360 (35.1%)	0.082	1,762 (39.0%)	1,741 (38.6%)	0.010
Malen; n (%)	2,751 (60.9%)	178,149 (64.9%)	0.082	2,750 (60.9%)	2,772 (61.4%)	0.010
White (%)	2,359 (52.2%)	167,158 (60.9%)	0.175	2,359 (52.3%)	2,372 (52.5%)	0.006
Black or African American; n (%)	1,713 (37.9%)	47,069 (17.1%)	0.479	1,711 (37.9%)	1,723 (38.2%)	0.005
Asian; n (%)	65 (1.4%)	13,828 (5.0%)	0.204	65 (1.4%)	74 (1.6%)	0.016
Comorbid conditions						
Hypertension	3,363(74.5%)	198,396 (72.2%)	0.050	3,361 (74.5%)	3,307 (73.3%)	0.027
Dyslipidemia	2,604 (57.7%)	151,418 (55.1%)	0.051	2,602 (57.6%)	2,518 (55.8%)	0.038
Chronic kidney disease	1,376 (30.5%)	107,693(27.7%)	76,181	1,374 (30.4%)	1,332 (29.5%)	0.020
Ischemic heart diseases	2,375 (52.6%)	143,963 (52.4%)	0.003	2,374 (52.6%)	2,290 (50.7%)	0.037
Overweight and obesity	1,679 (37.2%)	77,313 (28.2%)	0.193	1,677 (37.2%)	1,633 (36.2%)	0.020
Diabetes mellitus	1,648 (36.5%)	90,861 (33.1%)	0.072	1,646 (36.5%)	1,594 (35.3%)	0.024
Asthma	827 (18.3%)	29,767 (10.8%)	0.213	825 (18.3%)	798 (17.7%)	0.016
Chronic obstructive pulmonary disease	790 (17.5%)	45,490 (16.6%)	0.025	790 (17.5%%)	728 (16.1%)	0.037
Cerebrovascular diseases	740 (16.4%)	44,472 (16.2%)	0.005	740 (16.4%)	761 (16.9%)	0.012
Tobacco use	226 (5.0%)	15,499 (5.6%)	0.028	226 (5.0%)	229 (5.1%)	0.003
Diseases of liver	735(16.3%)	34,881(12.7%)	0.102	733(16.2)	688(15.2)	0.027
Medication use						
Antiarrhythmic	2,560 (56.7%)	132,954 (48.4%)	0.166	2,558 (56.7%)	2,578 (57.1%)	0.009
Beta-blockers	3,172 (70.2%)	178,631 (65.0%)	0.111	3,170 (70.2%)	3,139 (69.5%)	0.015
Antihypertensive	1,304(28.9%)	63,825(23.2%)	0.129	1,303(28.9%)	1,321(29.3%)	0.009
Digoxin	415 (9.2%)	28,140 (10.2%)	0.036	415 (9.2%)	401 (8.9%)	0.011
Diuretics	2,944 (65.2%)	162,125 (59.0%)	0.127	2,942 (65.2%)	2,900 (64.2%)	0.019
Antilipemic agents	2,313 (51.2%)	132,561 (48.3%)	0.059	2,312 (51.2%)	2,287 (50.7%)	0.011
Previous use of ACEI	1,871 (41.4%)	102,325 (37.3%)	0.085	1,869 (41.4%)	1,809 (40.1%)	0.027
Previous use of ARBs	1,778 (39.4%)	85,612 (31.2%)	0.172	1,777 (39.4%)	1,780 (39.4%)	0.001
Antineoplastic--Antimetabolite	403 (8.9%)	6,288 (2.3%)	0.291	401 (8.9%)	381 (8.4%)	0.016
Glucocorticoids	2,532 (56.1%)	115,924 (42.2%)	0.280	2,530 (56.0%)	2,528 (56.0%)	0.001

Source: Own materials

Table 2. Primary and secondary clinical outcomes: sarcoidosis vs. non-sarcoidosis in dilated cardiomyopathy patients

Outcome	Risk of Event, n (%) (sarcoidosis group)	Risk of Event, n (%) (non-sarcoidosis group)	Hazard Ratio (95% CI)	P-Value
Primary Outcome				
Ventricular assisted device placement	98 (2.2%)	59 (1.3%)	1.609 (1.165-2.222)	< 0.001
Secondary Outcome				
Ventricular Arrhythmia	732 (22.0%)	463 (11.9%)	1.885 (1.678-2.117)	< 0.001
Hospitalization Or ED Visits	651 (40.0%)	542 (36.5%)	1.186 (1.069-1.317)	< 0.001
Acute Kidney Injury	721(20.6%)	583 (16.6%)	1.231 (1.103-1.373)	< 0.001
Stroke	314 (8.1%)	279 (7.2%)	1.084 (0.923-1.274)	= 0.658

Source: Own materials

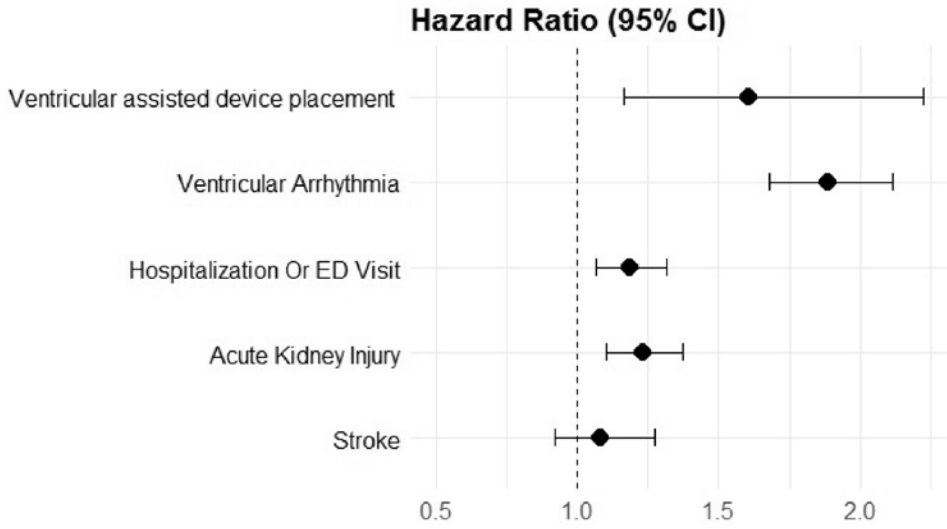

Fig. 2. Hazard ratio for the primary and secondary outcomes

Source: Own materials

The risk of composite stroke was statically insignificant between the 2 groups with a hazard ratio of 1.084 (95% CI: 0.923-1.274, P =0.658) (Table 2, Fig. 2).

DISCUSSION

In this retrospective cohort study, we found that patients with dilated cardiomyopathy (DCM) and concomitant sarcoidosis had a significantly greater risk of requiring ventricular assist device (VAD) placement compared with those with DCM alone. Specifically, sarcoidosis was associated with a hazard ratio of 1.61 for VAD placement, corresponding to a 61% higher relative risk compared with non-sarcoidosis patients. In addition, ventricular arrhythmias occurred more frequently in sarcoidosis patients with odds ratio of 2.082, suggesting that the arrhythmogenic burden may contribute to worsening heart failure, earlier clinical decompensation, and ultimately the need for mechanical circulatory support.

These results are consistent with previous observations that cardiac sarcoidosis (CS) is associated with an increased risk of atrioventricular conduction abnormalities, ventricular arrhythmias, and progression to advanced heart failure [1]. In a large retrospective cohort study conducted in California, sarcoidosis was identified in 0.09% of residents and emerged as the strongest predictor of adverse cardiac outcomes. Specifically, CS was associated with markedly elevated risks of heart failure (hazard ratio [HR] 11.2; 95% CI, 10.7-11.7), atrioventricular block (HR 117.7; 95% CI, 103.3-134.0), and ventricular tachycardia (HR 26.1; 95% CI, 24.2-28.1) [6]. Notably, approximately 22% (95% CI, 18-26%) of the association between sarcoidosis and mortality was explained by the presence of one or more of these cardiovascular complications. Other cohort study showed that patients with cardiac sarcoidosis are more prone to advanced cardiac replacement therapy (VAD placement, heart transplantation) [10]. These findings align with our study, where increased arrhythmic burden and progressive heart failure were key contributors to the higher likelihood of LVAD placement in patients with CS.

The progression of cardiac sarcoidosis (CS) to advanced or end-stage heart failure is primarily driven by granulomatous inflammation, conduction system abnormalities, and adverse ventricular remodeling. The hallmark pathological process

begins with the infiltration of noncaseating granulomas into the myocardium, which initiates localized inflammation, interstitial edema, and myocyte injury [4, 7]. Over time, this inflammatory process evolves into scarring and fibrosis, leading to irreversible loss of viable myocardium. The combination of reduced contractile reserve, diastolic stiffness, and electrical conduction disturbances contributes to both systolic and diastolic dysfunction, as well as a heightened risk of malignant ventricular arrhythmias, since scar tissue is a well-recognized substrate for reentrant electrical activity [5, 8, 9]. These overlapping mechanisms - myocardial injury, fibrosis, conduction abnormalities, and adverse remodeling - culminate in progressive pump failure and explain why CS patients experience disproportionately high rates of ventricular assist device (VAD) implantation, transplantation, and adverse cardiac outcomes.

Clinically, these findings indicate that sarcoidosis in the context of dilated cardiomyopathy (DCM) defines a distinct subgroup of patients at markedly higher risk for early decompensation and progression to advanced, refractory heart failure. Recognition of sarcoidosis as a prognostic marker in DCM should prompt clinicians to adopt more vigilant surveillance strategies and to consider earlier referral to advanced heart failure and transplant programs. Furthermore, the increased arrhythmic burden highlights the need for proactive rhythm monitoring, individualized device therapy, and timely consideration of implantable cardioverter-defibrillators or cardiac resynchronization therapy when appropriate. Taken together, these insights support a more proactive, multidisciplinary approach aimed at mitigating disease progression and improving survival in sarcoidosis-associated cardiomyopathy.

VAD implantation represents a clinically meaningful endpoint reflecting progression to advanced heart failure; however, it is also influenced by several non-biological factors, including referral patterns, institutional practice variability, and access to specialized advanced heart failure and transplant centers [11]. As such, VAD placement may not exclusively reflect underlying disease severity. In the context of this observational analysis, VAD implantation should therefore be interpreted as an associative outcome indicative of transition to advanced heart failure care rather than a direct surrogate for myocardial dysfunction alone [12]. This distinction is particularly relevant in large real-world databases, where practice variation and healthcare access may contribute to observed differences in VAD utilization between patient populations.

Our study also showed a significantly increased incidence of AKI between patients in the sarcoidosis group. Which can be attributed to renal involvement in sarcoidosis, as recent findings have revealed that approximately one-third of cardiac sarcoidosis patients have renal involvement [13]. Also, we found that patients with sarcoidosis had an increased risk of hospitalization or emergency department visits, which is also consistent with the findings of previous research [14]. which can be explained by increased frequency of arrhythmia and worsening outcome for sarcoidosis patients. Contrarily, our study found no statistically significant risk difference in the stroke between patients with and without sarcoidosis. Given the lack of definite evidence in the current literature discussing the association between the cardiac sarcoidosis and stroke, more studies are required to investigate this potential association.

Future research should aim to validate our findings through prospective, multicenter studies with standardized diagnostic and management criteria, as well as longer-term follow-up to more accurately define survival, arrhythmic outcomes, and transplant needs. In conclusion, our study highlights the importance of recognizing sarcoidosis in patients with dilated cardiomyopathy, as this subgroup carries a significantly higher risk of LVAD placement and adverse outcomes. These findings emphasize the need for early identification, proactive monitoring, and timely referral to advanced heart failure and transplant programs to optimize patient care.

LIMITATIONS OF THE STUDY

This study should be interpreted in the context of its limitations as it has notable limitations. First, the retrospective design makes it inherently vulnerable to missing data, incomplete records, and selection bias. The relatively small sample size further limits statistical power and reduces the generalizability of the findings to broader populations. In addition, the diagnosis of sarcoidosis was based on International Classification of Diseases (ICD) codes, which carries the risk of misclassification or coding errors and biases common in real-world databases. A major limitation of this study is the inability to distinguish cardiac sarcoidosis from systemic sarcoidosis without cardiac involvement, as diagnoses were based exclusively on ICD-10 codes. Consequently, some patients classified as having sarcoidosis may not have had direct myocardial involvement, potentially attenuating or biasing observed associations. Furthermore, Important clinical variables such as left ventricular ejection fraction, New York Heart Association functional class, cardiac imaging findings (e.g., cardiac MRI or FDG-PET), inflammatory activity, and intensity or duration of immunosuppressive therapy were unavailable in TriNetX. These unmeasured factors may substantially influence outcomes and introduce residual confounding despite propensity score matching. Finally, the lack of systematic long-term follow-up data, particularly on VAD placement and arrhythmic events, precluded a comprehensive assessment of prognosis. Despite the use of propensity score matching (PSM) to minimize confounding bias, the possibility of residual confounding remains.

CONCLUSIONS

Our study demonstrates an association between sarcoidosis and a higher incidence of adverse cardiac outcomes, including ventricular assist device (VAD) placement and ventricular arrhythmias, among patients with dilated cardiomyopathy compared with those without sarcoidosis. These findings suggest that sarcoidosis may identify a subgroup of patients with DCM who experience a greater burden of adverse cardiac events; however, causal inferences cannot be drawn from this observational analysis. Given the limited existing literature examining outcomes in patients with DCM and

concomitant sarcoidosis, our results should be considered hypothesis-generating. Further prospective studies with detailed phenotyping and standardized management approaches are needed to better define risk stratification, prognostic implications, and optimal monitoring strategies in this population.

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AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of this study are available through the TriNetX research network but are subject to licensing restrictions. Access to TriNetX data can be obtained upon reasonable request and with permission from TriNetX, LLC.

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Enhancing cognitive engagement in medical students through problem-based histology instruction: Implications for competency-oriented physician training

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ABSTRACT

Aim: This study aimed to verify experimentally the effectiveness of a problem-search approach for teaching Histology in enhancing the level of cognitive engagement among first-year medical students enrolled in specialty I2 "Medicine," as well as to substantiate the feasibility of its implementation in the educational process of future physician training.

Materials and Methods: The study was conducted during the 2024-2025 academic year and consisted of three stages (summative, formative, and final) using a pedagogical quasi-experimental design. A total of 756 undergraduate students enrolled in specialty I2 "Medicine" participated and were divided into a control group (n=352) and an experimental group (n=354). The level of cognitive engagement was assessed before and after the formative stage using B. K. Pashnev's questionnaire. The experimental group was taught using a combination of problem-based learning, the case method, heuristic dialogue, small-group work, project-based learning, and digital interactive technologies.

Results: At the summative stage, no statistically significant differences between the groups were identified. After the implementation of the problem-inquiry teaching methodology, the experimental group demonstrated a significant increase in the proportion of medical students with an advanced level of cognitive engagement (from 32.5% to 70.1%) compared with the control group (χ^2=38.369; $p<0.05$).

Conclusions: The proposed methodology for teaching Histology to future physicians, grounded in a problem-search approach, is an effective means of enhancing medical students' cognitive engagement and may be recommended for implementation in higher medical education practice.

KEYWORDS: higher medical education, teaching method

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INTRODUCTION

Cognitive engagement is regarded as a key component of learning activity among medical students, reflecting the direction of their intellectual efforts toward deep processing of educational material through the use of self-regulated learning strategies. Cognitive engagement is defined as the emotional-intellectual work of students involving the active application of volitional effort, the investment of time, and other relevant resources in learning tasks and mental activities [1-3]. Numerous studies have demonstrated that the effectiveness of student engagement influences academic achievement [3], life satisfaction [4], and the prevention of dropout due to academic failure [5, 6]. In medical education, this construct acquires particular importance, as it is associated with the development of critical thinking, analytical information processing, knowledge integration, and the ability to solve professionally oriented problems, extending beyond formal participation in the educational process. Cognitive engagement is conceptually distinct but closely interrelated with behavioral and emotional engagement, forming the foundation of deep learning and the professional competence of future physicians [7, 8].

Despite the growing scientific interest in student engagement, its cognitive dimension remains methodologically challenging to assess. The predominance of self-report instruments and indirect behavioral indicators limits the ability to fully capture the depth of students' intellectual activity and highlights the need to develop pedagogical approaches aimed at its purposeful enhancement [7].

While reviewing existing studies, we identified a lack of data on the impact of active teaching methods in basic morphological disciplines on the level of cognitive engagement among medical students. In this context, the present study substantiates the feasibility of implementing a problem-inquiry approach in teaching Histology, which integrates problem-based learning, the case method, heuristic dialogue, small-group work, and digital interactive tools. The application of this methodology is considered an effective means of enhancing medical students' cognitive engagement by fostering intrinsic learning motivation, developing analytical and synthetic thinking, and promoting the independent application of knowledge in educational and professional contexts.

AIM

This study aimed to verify experimentally the effectiveness of a problem-search approach for teaching Histology in

enhancing the level of cognitive engagement among first-year medical students enrolled in specialty I2 "Medicine," as well as to substantiate the feasibility of its implementation in the educational process of future physician training.

MATERIALS AND METHODS

The study investigating the impact of modern pedagogical strategies applied in teaching Histology as part of the discipline "Histology, Cytology, and Embryology" on the level of students' cognitive engagement was conducted during the 2024-2025 academic year and consisted of three stages through the application of multiple methods:

Stage 1. Summative:

- search for empirical studies and systematic reviews in scientometric databases relevant to the research topic;
- development of the study design;
- selection of survey instruments;
- administration of questionnaires;
- development of a methodological support framework for teaching the discipline based on a problem-inquiry approach;

Stage 2. Formative:

- implementation of a set of pedagogical conditions aimed at developing students' self-educational competence;
- post-experimental questionnaire survey;

Stage 3. Final:

- mathematical and statistical processing of the obtained results;
- evaluation and generalization of the study findings.

INSTRUMENTS

During the study, quantitative indicators (level in points) of students' cognitive engagement were determined. To assess students' cognitive engagement (SCE) at the first stage of studying Histology as part of the discipline "Histology, Cytology, and Embryology," an adapted version of B. K. Pashnev's questionnaire was administered to first-year undergraduate students enrolled in specialty I2 "Medicine" at Bogomolets National Medical University [9]. The survey was conducted anonymously, and students indicated a code-identifier on the questionnaire.

The questionnaire consisted of two groups of items: 42 items aimed at assessing cognitive engagement and 10 items designed to measure response insincerity or social desirability. Individual responses were compared with an answer key, and one point was awarded for each match. The results were evaluated in points and categorized into established levels: satisfactory (1), medium (2), and advanced (3), according to the scoring key. If six or more responses matched the insincerity scale key, the results were considered invalid [9]. At the summative stage of the pedagogical experiment, the questionnaire also included general items: (1) gender, (2) age, and (3) year of study. For the convenience of data processing, respondents' answers were entered into MS Excel spreadsheets.

The study of cognitive engagement involved 756 first-year undergraduate students enrolled in specialty I2 "Medicine" at Bogomolets National Medical University (352 in the control group [CG] and 354 in experimental group [EG]). Each group was randomly formed from 25 academic groups. Among the respondents, females predominated (CG: n=266; EG: n=270), while 170 participants were male (CG: n=86; EG: n=84). The mean age of the students was 17.7 years.

In CG, Histology teaching was conducted using traditional methods, with a predominance of reproductive learning approaches based on conscious memorization and reproduction of knowledge. According to the curriculum, lectures were delivered online, and practical classes were conducted in classroom settings [10]. During practical sessions, students completed tests on the LIKAR_NMU online platform, participated in frontal questioning, studied histological slides, and produced drawings in practical class protocols [10].

In EG, teaching of Histology as part of the discipline "Histology, Cytology, and Embryology" was implemented based on the problem-search approach while preserving the approved procedure of lectures and practical classes according to the curriculum. Instead of frontal questioning focused on reproducing theoretical material from textbooks, the following methods were actively applied: problem-based learning (PBL), heuristic dialogue, brainstorming, the case method, visualization of educational material, small-group work, interactive methods using digital technologies, and project-based learning.

To confirm or refute the effectiveness of the developed methodology for teaching Histology based on a problem-search approach, the obtained results of students' cognitive engagement (SCE) in the EG and CG were analyzed after the summative stage (SCE1) and the formative stage (SCE2) of the experiment. Respondents' answers were entered into MS Excel spreadsheets according to the code-identifiers used in the anonymous questionnaires.

DATA ANALYSIS

Statistical data processing was performed using the EZR software statistical package and MS Excel. Students' responses were entered, processed, and partially visualized in tables and charts generated in MS Excel.

To determine statistically significant differences in levels of students' cognitive engagement, statistical hypothesis testing was applied, and the null (H0) and alternative (H1) hypotheses were formulated. H0: statistically significant differences between the two distributions are not present. H1: statistically significant differences between the two distributions are present. The Shapiro-Wilk test was applied to assess the normality of data distribution, and the skewness and kurtosis values were calculated. Accordingly, the nonparametric Pearson's χ^2 test was used to test the null and alternative hypotheses and to compare two empirical distributions of the same attribute. The analyzed samples were random and independent, with independent observations within each sample. The measurement scale was nominal with three categories (levels): satisfactory, medium, and advanced.

At the formative stage of the study the SCE2 level was assessed for the same number of participants as at the

summative stage: 352 students in the CG and 354 students in the EG. The results of the student survey conducted to assess SCE2 formation at the end of the formative stage were compared with the scoring keys and differentiated according to the established levels: satisfactory, medium, and advanced. To identify statistically significant differences in SCE levels, statistical hypothesis testing was applied, and the null (H0) and alternative (H1) hypotheses were formulated. H0 states: the probabilities of random assignment of EG and CG students to each of the t categories (t=1, 2, …, C, where C=4 for both samples) are equal, and the higher SCE levels in the EG are explained by random factors. H1: $p1i \neq p2i$ for at least one category; therefore, the higher SCE level in the EG is explained by the implementation of the developed problem-search Histology teaching methodology. As in the previous stage, the Shapiro-Wilk test was used to assess data normality, and skewness and kurtosis values were calculated. Consequently, Pearson's nonparametric χ^2 test was applied to compare the two empirical distributions of the same attribute.

ETHICS

According to institutional regulations, ethical approval was not required for anonymous educational research. Nevertheless, the study was conducted in accordance with international ethical standards, including the Declaration of Helsinki. All participating students provided written consent for the publication of the results. Students were informed about the study and participated in the survey voluntarily.

RESULTS

In both the CG and EG, according to the Shapiro-Wilk test (W), the distributions of SCE1 level outcomes deviated from normality (Table 1), as $p<\alpha$ ($\alpha=0.05$). In this case, the p-value was lower than the α level; therefore, the null hypothesis of normal distribution was rejected, and the alternative hypothesis was accepted. It should be noted that a distribution is considered normal when the skewness and kurtosis coefficients are equal to zero.

The obtained results confirmed the absence of statistically significant differences between CG and EG at the summative stage, indicating the representativeness and balance of the baseline conditions. This ensures the objectivity and reliability of the subsequent evaluation of the effectiveness of the developed methodology for teaching Histology based on a problem-based, inquiry-oriented approach during the formative stage of the experiment.

Comparative analysis showed that at the summative stage of the pedagogical experiment, a satisfactory level was observed in 5.4% of students in CG and 2.3% of students in EG; a medium level was demonstrated by 64.2% of students in CG and 65.3% of students in EG; an advanced level was identified in 30.4% of students in CG and 32.5% of students in EG (Table 2).

Based on the χ^2 distribution table for degrees of freedom $\nu=(k-1)(C-1)=(2-1)(3-1)=2$ at a significance level of $\alpha=0.05$, the critical value was $\chi^2cr=6$. The results of SCE1 levels assessment in the CG and EG were analyzed, and χ^2emp values were calculated using the EZR statistical package (Table 2). Comparing the calculated $\chi^2emp=4.819$ with $\chi^2cr=6$ the following inequality is obtained: $\chi^2emp<\chi^2cr$, with $p>\alpha$. Thus, at the experiment summative stage, no statistically significant differences in SCE1 levels between the CG and EG were identified at $\alpha=0.05$, providing grounds for accepting the null hypothesis (H0).

These data confirmed the absence of statistically significant differences between the CG and EG at the summative stage of the pedagogical experiment and, accordingly, indicate that the baseline conditions were representative and balanced, thus ensuring the objectivity and reliability of the subsequent evaluation of the effectiveness of the developed problem-inquiry-based methodology for teaching Histology at the formative stage of the experiment.

The formative stage aimed to test the hypothesis regarding the effectiveness of the problem-search methodology for Histology teaching. The results of the student survey conducted to assess SCE formation at the end of the formative stage (SCE2) are presented in Table 2 and demonstrated the following distribution: a satisfactory level was identified in 2.3% of students in CG and 0.6% of students in EG; a medium level was confirmed in 50.3% of CG and 29.4% of EG students; an advanced level was achieved by 47.4% of CG and 70.1% of EG students (Fig. 1).

According to the χ^2 distribution table for degrees of freedom $\nu=(k-1)(C-1)=(2-1)(3-1)=2$ at $\alpha=0.05$, the critical value was $\chi^2cr=6$. The results of the SCE2 level assessment in the CG and EG were analyzed, and χ^2emp values were calculated using the EZR statistical package. Analysis of SCE2 levels after completion of the formative stage of the

Table 1. Analysis of data distribution normality in CG and EG at the summative and formative stages of the pedagogical experiment

Group	W	p	Skewness	Kurtosis
	SCE1	SCE1	SCE1	SCE1
CG	0.99	0.01	0.04	-0.06
EG	0.99	0.03	0.19	-0.21
Group	SCE2	SCE2	SCE2	SCE2
CG	0.98	0.00008	0.27	-0.66
EG	0.97	0.00001	-0.48	-0.33

Source: compiled by the authors of this study

Table 2. Distribution of students' cognitive engagement levels in CG and EG at the summative and formative stages of the pedagogical experiment

Level	SCE1				SCE2			
	CG (n=352)	EG (n=354)	χ2emp	p	CG (n=352)	EG (n=354)	χ2emp	p
Satisfactory	19	8			8	2		
Medium	226	231	4.819	>0.05	177	104	38.369	<0.05
Advanced	107	115			167	248		
Total	706		χ^2cr=6	α=0.05	706		χ^2cr=6	α=0.05
Me (QI-QIII)	21 (16-24)	20 (16-24)	-		23 (18.5-29.7)	26.4 (21.8-30.2)	-	
SD	5.5	5.4			7.26	5.92		

Notes: χ^2emp – Chi-square empirical value; χ^2cr – Chi-square critical value; Me – median; QI-QIII – interquartile range; SD – standard deviation
Source: compiled by the authors of this study

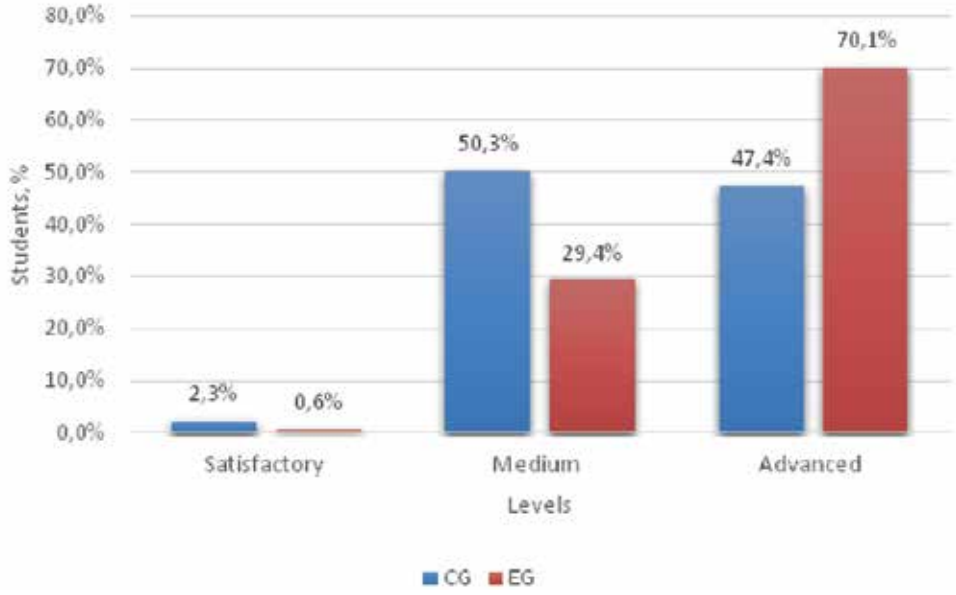

Fig. 1. Distribution of students' cognitive engagement levels (SCE2) in the control group (CG) and experimental group (EG) at the formative stage of the pedagogical experiment

Source: Own materials

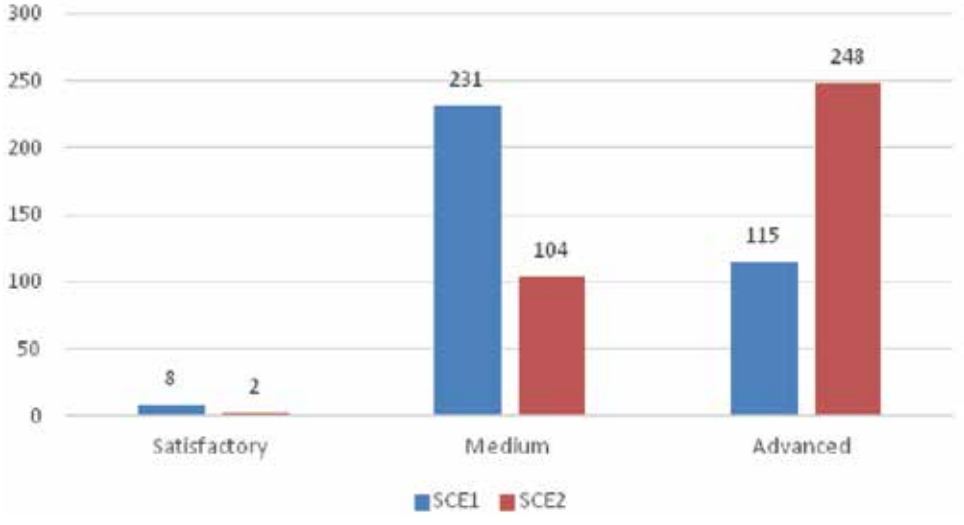

Fig. 2. Dynamics of changes in students' cognitive engagement levels (SCE) in the experimental group (EG) before (SCE1) and after (SCE2) the formative stage of the pedagogical experiment

Source: Own materials

pedagogical experiment revealed substantial differences between the CG and EG, allowing confirmation of their statistical significance ($\nu=2$, $\alpha=0.05$, $\chi^2cr=6$; $\chi^2emp=38.369$; $\chi^2emp>\chi^2cr$; $p<\alpha$) following the application of the developed methodology (Table 2).

The performed statistical processing of the pedagogical experiment data made it possible to establish that medical students' cognitive engagement levels improved as a result of implementing the problem-search methodology for teaching Histology (Fig. 2).

DISCUSSION

Contemporary concepts of medical education emphasize not only the acquisition of professional competencies but also the purposeful development of students' cognitive engagement, critical thinking, and capacity for lifelong learning [7]. Within this paradigm, learning-related cognitive engagement is conceptualized as an active and goal-oriented process in which students assume responsibility for knowledge construction through meaningful interaction with educational content, peers, and instructors [7, 8, 11, 12]. This approach aligns with the demands of modern medicine, which requires not merely the reproduction of information but also the ability to analyze, interpret, and apply knowledge under conditions of clinical uncertainty.

Problem-based learning (PBL) [13, 14], case-based learning (CBL) [15], and other active teaching strategies have attracted considerable attention as effective tools for enhancing cognitive engagement in medical education [16–18]. Systematic reviews of the literature demonstrate that these approaches promote deeper understanding of learning material, the development of critical thinking, and increased learner autonomy compared with traditional (reproductive) instruction [14, 19]. Their educational value is largely attributable to their focus on stimulating critical reasoning, hypothesis generation, and the development of interpretative skills, which are fundamental to the preclinical training of physicians [20].

An important aspect highlighted in contemporary research is the transformation of the teacher's role in the context of active pedagogical strategies [13]. The instructor ceases to function as the primary source of information and instead assumes the role of a learning facilitator who creates cognitively challenging educational environments, guides students' inquiry processes, and supports reflective activity [21]. This approach is consistent with the principles of competency-based medical education and strengthens subject-subject interaction, which is essential for activating cognitive engagement and learning motivation of future doctors [22]. Existing empirical evidence indicates that PBL and active learning methods influence students' cognitive engagement through several key mechanisms:

Promotion of self-directed learning – students assume responsibility for identifying, processing, and applying knowledge, thereby deepening intellectual engagement [13, 15, 23].

Contextualization of learning – authentic problems and clinical cases increase the relevance of educational content and enhance intrinsic motivation [14].

Development of social interaction and reflection – collaborative tasks and group discussions support the formation of metacognitive and critical skills [18].

Enhanced knowledge transfer and application – active learning methods stimulate the use of knowledge in novel situations, which is a defining feature of high-level cognitive engagement [14].

These mechanisms are consistent with contemporary conceptualizations of student cognitive engagement as a combination of strategic effort, deep cognitive processing, and emotional investment in the learning process.

The application of cognitively rich didactic models in teaching basic morphological disciplines, particularly Histology, is especially relevant [20]. These subjects require students to interpret complex structural and developmental processes, integrate visuospatial information, and establish links between theoretical knowledge and clinical contexts. Research indicates that passive instructional formats do not ensure sustained and deep learning in such disciplines, whereas problem-based and active learning approaches significantly improve conceptual understanding and the development of clinical reasoning in future physicians [14, 16, 24-26].

Analysis of the results of the present study confirms the effectiveness of implementing a problem-search-based methodology for teaching Histology and demonstrates a statistically significant increase in students' cognitive engagement levels. In particular, an increase was observed in the proportion of students exhibiting an advanced level of cognitive engagement, which represents the highest stage of its development and characterizes the future physician's capacity for creative cognition, intellectual initiative, and independent knowledge construction [11, 12]. At this level, students are actively involved in research activities, capable of problem formulation, hypothesis generation, selection of appropriate verification methods, and interpretation of results. In professional practice, this creative level manifests in the ability to identify non-standard solutions to complex clinical situations, integrate interdisciplinary knowledge, critically evaluate scientific information, and implement innovations, thereby ensuring readiness to work under conditions of uncertainty and continuous professional development [27]. The present study also demonstrates that the methods applied in the experimental group reduced the proportion of students with low and medium levels of cognitive engagement by facilitating their transition to higher levels.

At the same time, despite the positive outcomes, certain limitations should be acknowledged. The implementation of PBL and active learning methods requires substantial resources, targeted faculty development, and comprehensive curricular revision, which may constrain their widespread adoption. In addition, evidence regarding the isolated impact of these approaches on traditional indicators of knowledge acquisition remains heterogeneous, underscoring the need for further research into optimal models for integrating active learning with conventional teaching methods and discipline-specific content.

CONCLUSIONS

The results of this study substantiate the need to modernize traditional approaches to teaching morphological disciplines through the implementation of a problem-inquiry-based approach, which is consistent with contemporary requirements of competency-based medical education. The application of this approach in Histology teaching creates conditions for the development of sustained learning motivation, cognitive autonomy, and prerequisites for the formation of clinical thinking among medical students.

The practical significance of this study lies in substantiating the universality of the problem-search approach for modernizing the educational process in higher medical education institutions and for preparing medical students for professional practice in the context of rapid scientific and technological change.

Further research should focus on evaluating the long-term effects of the proposed methodology on academic performance, clinical reasoning, and the professional readiness of future physicians.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Variants of the structure of the urinary system organs in human fetuses

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ABSTRACT

Aim: To find out the features of individual anatomical variability of the urinary system organs in the fetal period of human ontogenesis.

Materials and Methods: 102 human fetuses with a parieto-calcaneal length (PCL) of 160.0-480.0 mm (4-10 months of gestation) were studied. A complex of morphological research methods was applied, which included anthropometry, morphometry, injection of blood vessels with dyes for visualization of blood vessels of macrospecimens, preparation of macrospecimens, and statistical analysis.

Results: Our material revealed a number of variants of the shape and structure of the ureters, in particular, doubling of the renal pelvis and ureters – 4 observations. The reasons for this may be impaired morphogenesis of the metanephros at the end of the 6th – beginning of the 7th week of intrauterine development. Bends of the proximal part of the ureter, megaureter were observed in 2 cases, polymegacolix, megacolix – in 2 cases. Aberrant renal vessels were detected in 8 specimens. The reasons for their occurrence may be impaired angiogenesis at the end of the 6th week of prenatal development.

Conclusions: 1. Variants of the structure and topography, as well as malformations of the upper urinary tract, were detected in 15.7% of 102 studied fetal specimens. 2. The ratio of detected variants and anomalies of the structure and topography in female fetuses prevailed over those in male fetuses in the ratio of 5:3. 3. Extreme forms of anatomical variability of the structure of renal vessels and their syntopy with the renal pelvis and ureter in all observations were a potential danger of vasorenal conflicts, which would subsequently lead to hydronephrotic transformation of the kidney.

KEYWORDS: urinary system, anatomy, anatomical variability, congenital malformations, kidney, ureter, renal arteries, renal veins, retroperitoneal space, prenatal development, fetus

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INTRODUCTION

One of the main tasks of anatomy is the study of anatomical variability of organs and systems of the human body, the elucidation of its embryonic causes [1, 2]. In this regard, embryological studies are of great importance, since structural variants and possible congenital defects, in particular of the urinary system, are initiated in the early period of human prenatal development [3-7]. Comprehensive information about individual, sex and age related, constitutional anatomical variability, the time and etiology of its occurrence in the intrauterine period of development will allow to improve the interpretation of data from diagnostic medical imaging of fetal development, to draw a clear line between norm and pathology [8-10]. The organs of the urinary system stand out among other systems of the human body with a large number of structural variants [11]. The reasons for this are not fully understood, but it can be assumed that the later appearance of metanephros compared to other organs, the multiplicity of sources of rudiments of the urinary and related reproductive systems, as well as the syntopic influence of adjacent structures explain the high percentage of variability and even malformations of the urinary system organs [12-14]. Therefore, morphological studies of the variant anatomy of the human urinary system organs in the early period of ontogenesis have important theoretical and practical significance and will allow us to clarify the etiology of their occurrence.

AIM

The aim of the research was to find out the features of individual anatomical variability of the urinary system organs in the fetal period of human ontogenesis.

MATERIALS AND METHODS

We studied 102 human fetuses with a parieto-calcaneal length (PCL) of 160.0-480.0 mm (4-10 months of gestation). We used a complex of morphological research methods, which included anthropometry, morphometry, injection of vessels of macrospecimens, preparation of macrospecimens, and statistical analysis.

The studies were performed in compliance with the basic provisions of ICH GCP (1996), the Council of Europe Convention on Human Rights and Biomedicine (04.04.1997), the Declaration of Helsinki of the World Medical Association on the Ethical Principles of Conducting Scientific Medical Research Involving Human Subjects (1964-2008), EEC Directive No. 609 (dated 24.11.1986), and orders of the Ministry of Health of Ukraine.

RESULTS

Our material revealed a number of variants of the shape and structure of the ureters. Despite the fact that in human fetuses the abdominal part of the ureter is a direct continuation of the renal pelvis, located on the psoas major muscle and somewhat flattened in the anterior-posterior direction, in two fetuses (male 200.0 mm and female 210.0 mm PCL) the upper part of the right ureter had two bends in the frontal plane (forward and backward) after leaving the renal pelvis (Fig. 1). And although it is known that the right kidney is usually located lower than the left, we believe that the bends of the ureter may be associated with temporary faster growth rates.

The bends of the ureters caused by nephroptosis or the outlet of the renal arteries at an acute angle from the aorta can impede the outflow of urine, as can the compression of the ureters by aberrant renal vessels. Thus, in 4 cases we observed the intersection of the right ureter with the common iliac artery at the point of its branching into the external and internal, in 4 cases – the intersection of the ureter with the external iliac artery. In 2 observations, the left ureter crossed the common iliac artery, in 3 – the point of branching of the common iliac artery and in 5 – the external iliac artery. At the level of contact with the iliac vessels, the ureters form an arch, the convexity of which is directed anteriorly and medially. We believe that under certain syntopic circumstances, anatomical prerequisites may arise for the expansion of the renal pelvis and the possible development of congenital hydronephrosis as a result of compression of the pyelo-ureteral segment, for example, by the anomalous lower left renal artery (Fig. 2), which passes behind it.

The skeletal level of the kidneys significantly affects the topography of the elements of the renal hilum and the shape of the ureters. We found a high location of the organ

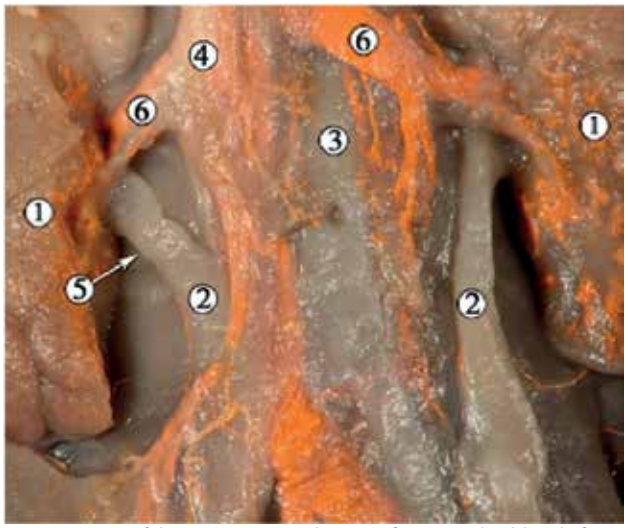

Fig. 1. Organs of the retroperitoneal space of a 4-month-old male fetus 200.0 mm TPD. Injection of dye into veins. Macroscopic specimen. Magn. x2.5. 1 – kidneys; 2 – ureters; 3 – aorta; 4 – inferior vena cava; 5 – bends of the ureter; 6 – renal veins.
Source: Own materials

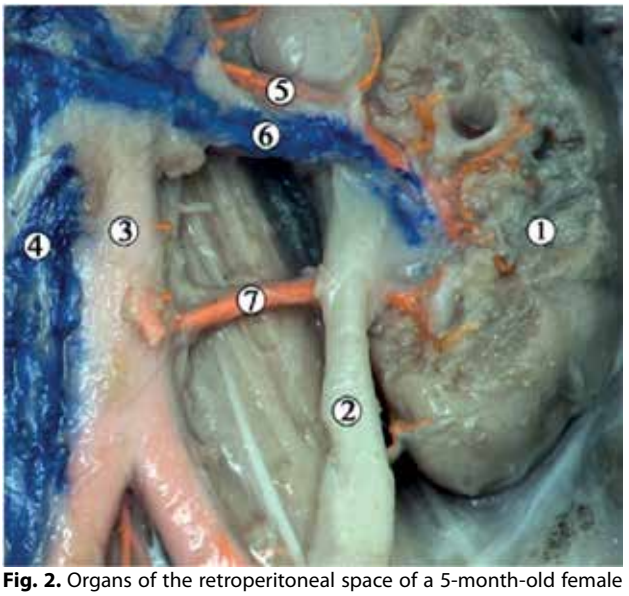

Fig. 2. Organs of the retroperitoneal space of a 5-month-old female fetus 230.0 mm PCL. Injection of dye into arteries. Macroscopic specimen. Magn. x3.5. 1 – kidney; 2 – ureter; 3 – aorta; 4 – inferior vena cava; 5 – renal artery; 6 – renal vein; 7 – accessory inferior left renal artery.
Source: Own materials

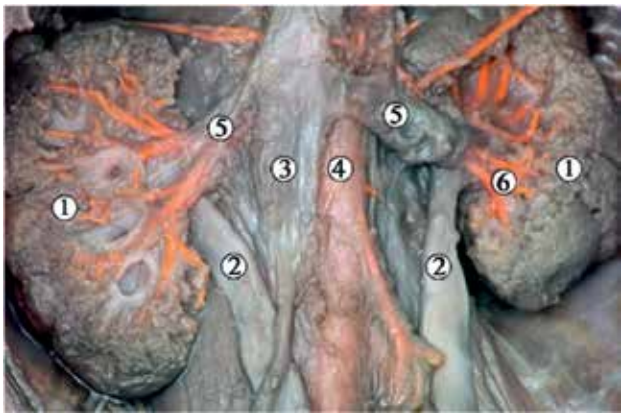

Fig. 3. Organs of the retroperitoneal space of a 6-month-old female fetus 260.0 mm PCL. Injection of dye into arteries. Macroscopic specimen. Magn. x4. 1 – kidneys; 2 – ureters; 3 – inferior vena cava; 4 – aorta; 5 – renal veins; 6 – segmental renal arteries.
Source: Own materials

on the left in a fetus with a gestational age of 260.0 mm (Fig. 3). The kidney corresponded to the level from the XI thoracic to the gap between the II and III lumbar vertebrae. Due to such a high position of the kidney, the left ureter departed from the renal hilum almost at a right angle. In two observations, the left kidney was lower than the right, and in one fetus with a gestational age of 320.0 mm, both kidneys were lowered (the right kidney occupied the level from II to V, the left - from I to IV lumbar vertebrae).

In a 7-month-old human female fetus with a 210.0 mm PCL (Fig. 4), an aberrant right inferior renal artery was found, which branches off from the abdominal aorta at the level of the inferior mesenteric artery and went to the lower end of the kidney behind the ureter. In this case, such a syntopy of the renal vessel and ureter could lead to compression of the latter, and in the future, this uretero-vasal conflict

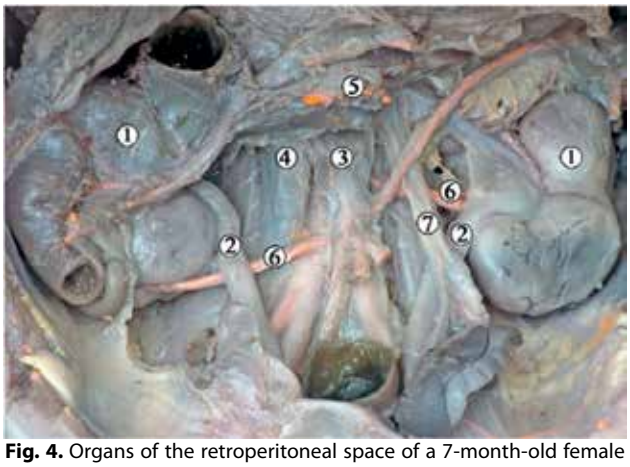

Fig. 4. Organs of the retroperitoneal space of a 7-month-old female fetus 210.0 mm PCL. Arteries are filled with a mixture based on lead red lead and protacrylic, veins – with gelatin and blue aniline dye. Macroscopic specimen. Magn. x3.5. 1 – kidneys; 2 – ureters; 3 – aorta; 4 – inferior vena cava; 5 – ileocolonic vessels; 6 – aberrant renal arteries; 7 – left testicular vessels.

Source: Own materials

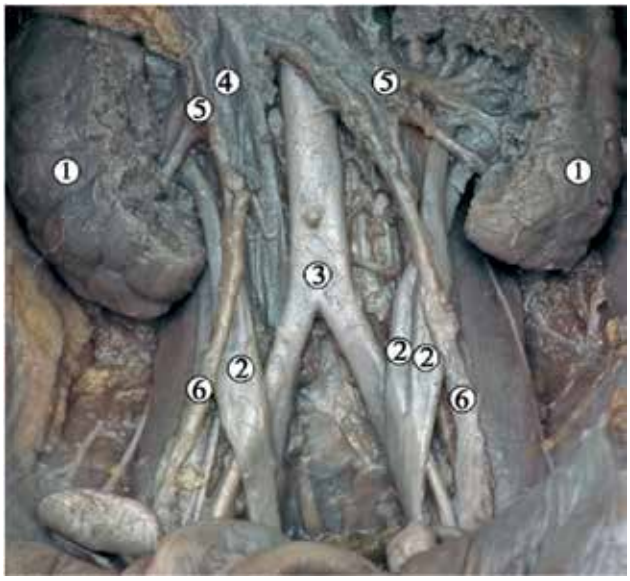

Fig. 5. Organs of the retroperitoneal space of a 6-month-old male fetus 270.0 mm PCL. Macroscopic specimen. Magn. x3,0. 1 – kidneys; 2 – ureters; 3 – aorta; 4 – inferior vena cava; 5 – renal veins; 6 – testicular veins.

Source: Own materials

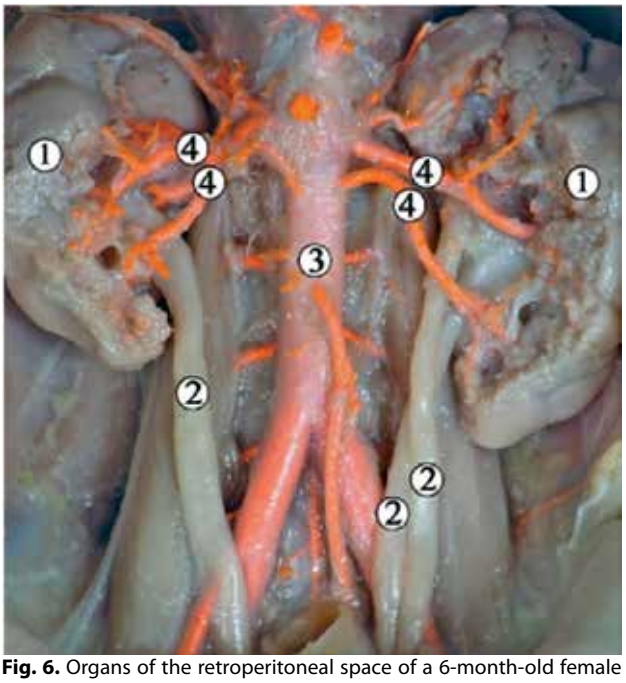

Fig. 6. Organs of the retroperitoneal space of a 6-month-old female fetus 260.0 mm PCL. Injection of dye into arteries. Macroscopic specimen. Magn. x3.0. 1 – kidneys; 2 – ureters; 3 – aorta; 4 – renal arteries; 5 – accessory renal arteries.

Source: Own materials

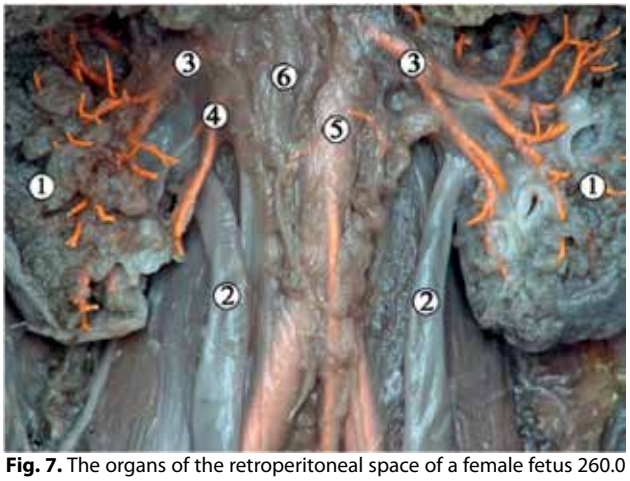

Fig. 7. The organs of the retroperitoneal space of a female fetus 260.0 mm PCL. Injection of dye into arteries. Macroscopic specimen. Magn. x3.5. 1 – kidneys; 2 – pyeloureteral segments; 3 – inferior vena cava; 4 – aorta; 5 – renal arteries; 6 – additional right inferior renal artery; 7 – ureteric branch of the additional renal artery; 8 – psoas major muscles.

Source: Own materials

could cause pyeloectasia. An additional renal artery was found on the left, which branches off from the place of aortic bifurcation at a right angle to the hilum of the kidney behind the pelvis. In our opinion, the features of the syntopy of the renal and ovarian vessels with the left pelviureteral segment could cause left-sided hydronephrosis.

In two fetuses, we found duplication of the ureters and anomalies of the renal vessels. Thus, in a male fetus with a PCL of 270.0 mm (Fig. 5), there was duplication of the left renal pelvis and the abdominal part of the ureter, in front of which an aberrant inferior renal vein passed.

In a female fetus with a TPD of 260.0 mm (Fig. 7), complete duplication of the renal pelvis and ureters on the left and additional renal arteries on both sides were found, which run in front of the ureteral segments to the lower edge of the renal sinus. In this fetus, the left kidney is significantly larger than the right, its lower end is located at the level of the V lumbar vertebra, and the renal pelvis is dilated. It is likely that compression of the ureters by an aberrant artery in front could cause the development of congenital hydronephrotic transformation.

Accessory renal arteries were found in two specimens of female fetuses with a PCL of 260.0 mm (Fig. 7) and 270.0

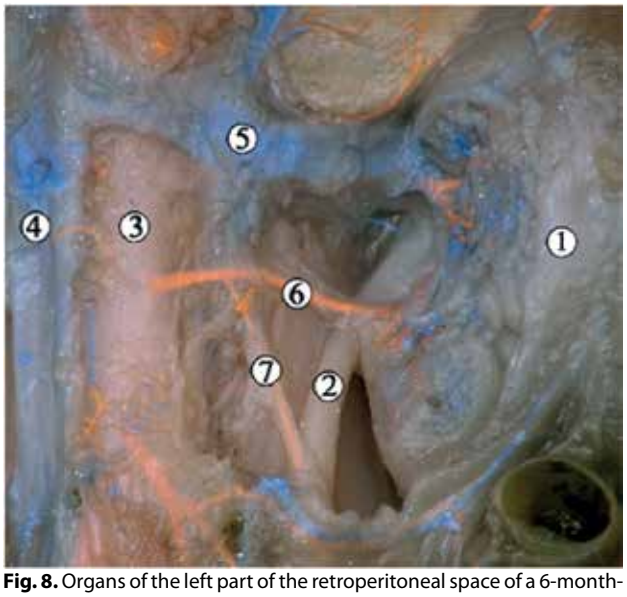

Fig. 8. Organs of the left part of the retroperitoneal space of a 6-month-old female fetus 270.0 mm PCL. The arteries are filled with a mixture based on red lead, the veins – with barium and blue aniline dye. Macroscopic specimen. Magn. x4.5. 1 – kidney; 2 – ureter; 3 – aorta; 4 – inferior vena cava; 5 – left renal vein; 6 – additional left inferior renal artery; 7 – left ovarian artery.

Source: Own materials

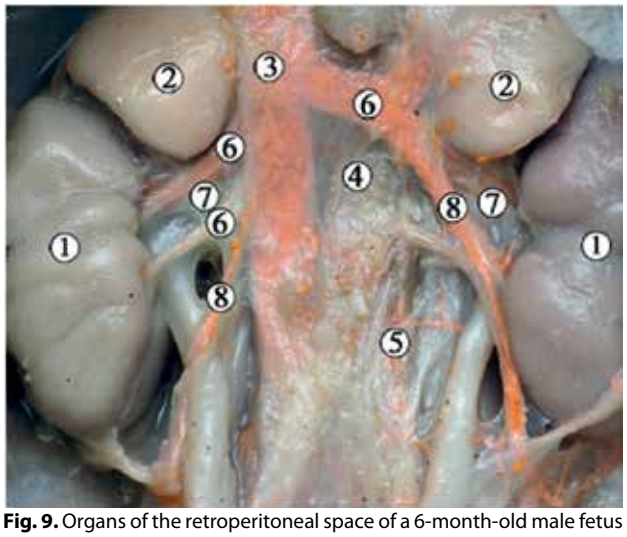

Fig. 9. Organs of the retroperitoneal space of a 6-month-old male fetus 300.0 mm PCL. Injection of dye into veins. Macroscopic specimen. Magn. x2.5. 1 – kidneys; 2 – adrenal glands; 3 – inferior vena cava; 4 – aorta; 5 – connection of branches of ureteral veins with veins of the peritoneum; 6 – renal veins; 7 – renal arteries; 8 – testicular veins.

Source: Own materials

mm (Fig. 8). In a female fetus with a PCL of 270 mm (Fig. 9), the left accessory renal artery extended from the aorta to the renal hilum almost at a right angle, passed in front of the PUS and gave off the ureteral branch. The left ovarian artery flowed into the anomalous renal artery.

Multiple renal veins were found in the specimens of 4 male fetuses – 270.0, 290.0 (Fig. 9), 300.0 and 350.0 mm PCL. In all observations, on the left, two renal veins separately flowed into the inferior vena cava, and on the right, they merged beyond the renal hilum. Thus, the lower branches of the renal veins always passed in front of the renal pelvis and the beginning of the ureter, and could impede the outflow of urine from the renal pelvis due to its compression.

In an 8-month-old male fetus with a PCL of 370.0 mm, a doubling of the renal pelvis and ureters, polymegacolix, and megaureter were detected (Fig. 10). The calyces of the upper segments of the right kidney are significantly dilated, the renal pelvis is bifurcated. The proximal part of the right upper ureter is significantly elongated, as a result of which it forms four bends, starting from the level of the lower end of the kidney and before entering the bladder, it is located in front of the lower ureter. It is likely that the indicated morphological changes in the renal pelvis and ureters in this case led to hydronephrotic transformation of the renal parenchyma, which could later, at birth, cause Fraley syndrome. The main etiological factor of this congenital pathology is a violation of intrapelvic pressure due to numerous bends and obstruction of the ureter.

In a 7-month-old male fetus with a 350.0 mm PCL, right-sided nephroptosis was found, and an additional right renal artery (Fig. 11) arose from the aorta 0.8 cm above its bifurcation, below the inferior mesenteric artery, and directed to the lower end of the right kidney. It extended anteriorly to the inferior vena cava and posteriorly to the right ureter. Due to compression between the dorsal surface of the lower end of the kidney and the additional renal

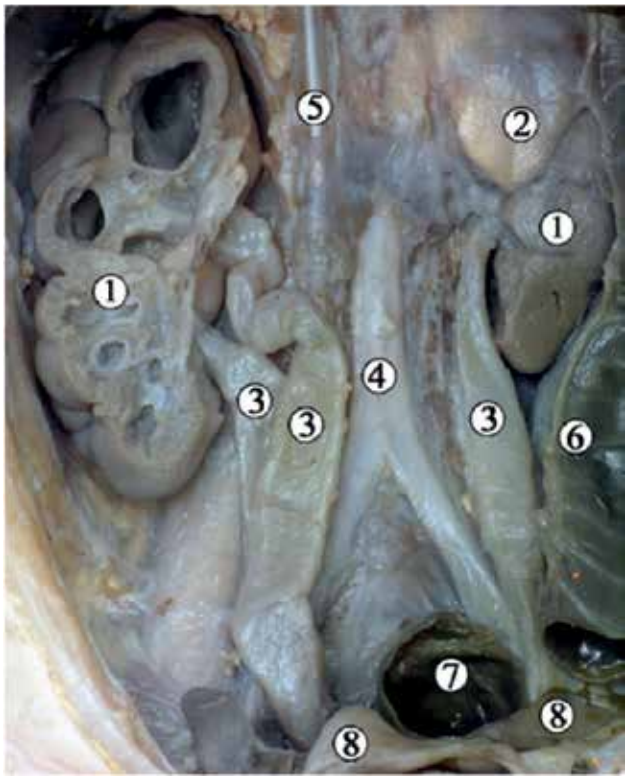

Fig. 10. Organs of the retroperitoneal space of an 8-month-old male fetus 370.0 mm PCL. Macroscopic specimen. Magn. x1.5. 1 – kidneys; 2 – left adrenal gland; 3 – ureters; 4 – aorta; 5 – inferior vena cava with an inserted catheter; 6 – descending colon; 7 – rectum; 8 – testicles.

Source: Own materials

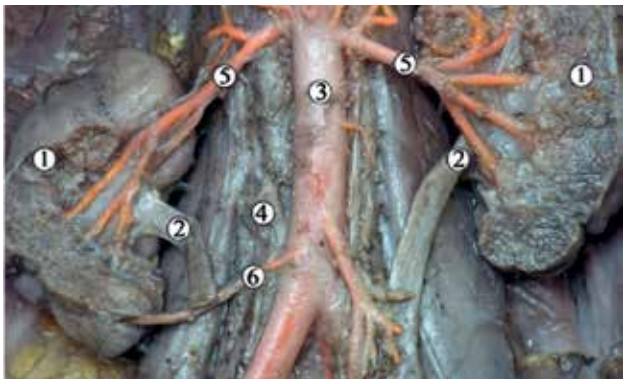

Fig. 11. Organs of the retroperitoneal space of a 7-month-old male fetus 350.0 mm PCL. Injection of dye into arteries. Macroscopic specimen. Magn. x1.5. 1 – kidneys; 2 – ureters; 3 – aorta; 4 – inferior vena cava; 5 – renal arteries; 6 – additional right inferior renal artery.

Source: Own materials

of the upper urinary tract, for example, nephroptosis, aberrant renal vessels, duplication of ureters, can cause severe urodynamic disorders [14].

We have investigated and described preparations with extreme forms of anatomical variability of the human urinary system, which illustrate certain embryological factors and the time of possible occurrence of structural variants and congenital defects of the human urinary system, which has also been the subject of study by other researchers [1, 15, 16].

Analyzing the etiology and time of occurrence of variants and anomalies of the structure of the ureters, it should be noted that it is in the second half of the 6th week of intrauterine development that the rudiment of the ureter and the renal calyceal system appears in the form of a dichotomous protrusion of the wall of the blind end of the

Table 1. Identified variants and malformations of the upper urinary tract in human fetuses

Structural variant or congenital defect	Male	Female	Total
- doubling of the pelvis and ureters	2	2	4
- bends of the proximal part of the ureter, megaureter	1	1	2
- polymegacolix, megacolix	1	1	2
- aberrant renal vessels	2	6	8
Total observations:	6	10	16

Source: Own materials

artery, the right ureteral segment was curved ventrally. A branch to the proximal segment of the ureter begins from the anastomosis between the additional renal artery and the lower branch of the right renal artery.

Thus, structural variants and malformations of the upper urinary tract were detected in 14 of the 102 specimens examined, which amounted to 15.7% (Table 1). By sex, their ratio is 3:5, that is, a greater number of them was detected in female fetuses (10), and a smaller number (6) was detected in male fetuses.

In our opinion, the main role in the occurrence of congenital hydronephrosis belongs to malformations of the upper urinary tract and vasoureteral conflicts, in which close syntopic connections of the renal pelvis and proximal part of the ureter with abnormal vessels can cause urodynamic disorders.

DISCUSSION

The study of extreme forms of anatomical variability of the urinary system organs is very relevant for understanding the etiology and timing of the appearance of structural variants and the occurrence of possible congenital defects. Their early detection during diagnostic and screening studies of the fetus will contribute to the prevention and timely surgical correction of possible congenital defects [6]. It should be noted that even minor anatomical variants

ureteral diverticulum in the cranial and caudal directions. At the end of the 6th week of intrauterine development, the number of diverticulum generations increases, and at the beginning of the 7th week of intrauterine development, the renal calyceal system consists of two or three renal pelvises of oval or triangular shape, surrounded by a strip of mesenchyme. It is during this period of prenatal development that variants of the structure and anomalies of the shape and number of renal pelvises and ureters can arise. At about the same time (end of the 6th week of gestation), the renal arteries emerge as extra-organ blood vessels of the metanephros, which soon connect with the intra-organ blood vessels. It is during this period of prenatal development that vascular anomalies of the kidney can occur. We agree that the widespread variations in renal blood supply reflect changes in its vascularization during embryonic and early fetal life [17, 18]. Like P. Sasikala et al. [19], S. Covantev et al. [20], K.C. Sudikshya et al. [21], we can argue that variants and anomalies of the renal vessels and kidneys are interrelated.

Regarding the causes of nephroptosis, the time of occurrence of this defect is the 9th week of gestation, since it is during this period that the renal hilum and renal pelvis reach their definitive skeletal location. In the further development of a person, other factors of nephroptosis also arise, even after birth [22, 23].

CONCLUSIONS

1. Variants of the structure and topography, as well as malformations of the upper urinary tract, were detected in 15.7% of 102 studied fetal specimens.
2. The ratio of detected variants and anomalies of the structure and topography in female fetuses prevailed over those in male fetuses in the ratio of 5:3.
3. Extreme forms of anatomical variability of the structure of renal vessels and their syntopy with the renal pelvis and ureter in all observations were a potential danger of vasorenal conflicts, which would subsequently lead to hydronephrotic transformation of the kidney.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Comparative effectiveness of atorvastatin monotherapy *vs.* atorvastatin plus bempedoic acid combination in patients with coronary artery disease: A multicenter observational study

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ABSTRACT

Aim: To evaluate the impact of adding bempedoic acid to atorvastatin therapy on cardiovascular outcomes, including major adverse cardiovascular events (MACE), cardiac arrest, all-cause hospitalization, and myopathy, in patients with established coronary artery disease

Materials and Methods: We conducted a retrospective cohort study using the TriNetX global health research network. Patients with established CAD were categorized based on their antilipemic therapy into two cohorts: atorvastatin monotherapy and atorvastatin plus bempedoic acid. Propensity score matching was employed to balance baseline characteristics between the cohorts. The primary outcome was the occurrence of MACE, while secondary outcomes included cardiac arrest events, all-cause hospitalization, and incidence of myopathy.

Results: After matching, 6,549 patients were included in each cohort. MACE occurred more frequently in the atorvastatin group (5.7%) compared to the combination therapy group (3.2%), with a hazard ratio of 1.606 (95% CI: 1.302-1.980, P<0.001). Cardiac arrest and all-cause hospitalizations were also higher in the atorvastatin group, with hazard ratios of 1.628 (95% CI: 1.041-2.544, P<0.001) and 1.418 (95% CI: 1.177-1.710, P<0.001), respectively. No significant difference in myopathy was observed (HR 0.915, 95% CI: 0.741-1.129).

Conclusions: Adding bempedoic acid to atorvastatin therapy appears to confer a protective effect in CAD patients by significantly reducing MACE, cardiac arrest, and hospitalizations without increasing myopathy risk. Prospective studies are warranted to confirm these findings.

KEYWORDS: bempedoic acid, atorvastatin, statin intolerance, coronary artery disease, low-density lipoprotein cholesterol

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LIST OF ABBREVIATIONS

ASCVD – Atherosclerotic Cardiovascular Disease
LDL – Low-Density Lipoprotein
EHR – Electronic Health Records
HCO – Healthcare Organizations
HIPAA – Health Insurance Portability and Accountability Act
ICD-10 – International Classification of Diseases, Tenth Edition
RxNorm – A standardized nomenclature for clinical drugs
MACE – Major Adverse Cardiovascular Events
MI – Myocardial Infarction
SD – Standard Deviation
PSM – Propensity Score Matching
ACE – Angiotensin-Converting Enzyme
β-blockers - Beta-Blockers
LDL-C – Low-Density Lipoprotein Cholesterol
CI – Confidence Interval
RCT – Randomized Controlled Trial
OR – Odds Ratio
HR – Hazard Ratio
CI – Confidence Interval
CAD – Coronary Artery Disease
CLEAR – Cholesterol Lowering via Bempedoic acid, an Evaluation of Cardiovascular Outcomes
MACE – Major Adverse Cardiovascular Events
SMD – Standardized Mean Differences

INTRODUCTION

Atherosclerotic Cardiovascular Disease (ASCVD) is one of the leading causes of death and disability worldwide, which places a significant strain on the healthcare systems worldwide. Interventions to control the risk factors associated with atherosclerosis have been an active research topic in the past decades [1].

Given that many risk factors are nonmodifiable like age and genetic makeup, research efforts have mainly targeted

modifiable factors [2]. Of these modifiable risk factors, reduction of low-density lipoprotein (LDL) has been a primary therapeutic target for most of the interventions and the medications. The introduction of statins was a breakthrough in this regard, with reflected improvements in mortality and morbidity [3].

Statins continue to hold their ground today as a cornerstone in the prevention of Atherosclerotic disease [3]. However, a substantial percentage of patients with ASCVD have not achieved established targets for low-density lipoprotein cholesterol levels with statins alone, which can represent inadequate lipid-lowering response with high intensity of statin or intolerance to higher doses due to dose-related adverse effects [4]. In such cases, the addition of non-statin lipid-lowering agents represents an appropriate therapeutic strategy. Available options include ezetimibe, proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, and bempedoic acid [5-7].

The combination of statin plus bempedoic acid can reduce LDL-C more than statin therapy alone; however, the evidence in support of a clinical benefit is less robust than for ezetimibe and PCSK9 inhibitors [8], because bempedoic acid has mainly been tested as monotherapy for statin-intolerant patients [7].

With growing emphasis to aggressively lower LDL-C levels among high-risk patients, there has been considerable interest in the comparative effectiveness of statin monotherapy versus combination therapy with statins and bempedoic acid. This comparison is particularly relevant to patients who do not achieve lipid goals with statins alone or who are unable to tolerate high-intensity statin therapy. Accordingly, this study compares cardiovascular outcomes, and safety profiles between patients treated with statins alone and those receiving combined statin and bempedoic acid therapy.

AIM

The purpose of this study is to compare the cardiovascular outcomes of patients with established coronary artery disease who received atorvastatin or atorvastatin with bempedoic acid. The primary endpoint of the study was the occurrence of major adverse cardiovascular events (MI and stroke). Secondary outcomes were cardiac arrests, all-cause hospitalization, and the development of myopathy.

MATERIALS AND METHODS

DATA SELECTION

This study was conducted using the TriNetX research network, a federated database providing access to electronic health records (EHRs) from 168 healthcare organizations (HCOs). A total of 144 providers responded with patients. The TriNetX platform aggregates deidentified patient data, ensuring compliance with HIPAA deidentification standards (§164.514(a)).

PATIENT POPULATION

We performed a multicenter, retrospective observational cohort study involving patients with established coronary artery disease. Participants were grouped according to the lipid-lowering therapy received: atorvastatin alone or atorvastatin in combination with bempedoic acid. Cohort 1 comprised patients treated with atorvastatin who had no prior exposure to bempedoic acid, whereas Cohort 2 included patients receiving concomitant atorvastatin and bempedoic acid therapy. The index date was defined as the initiation of lipid-lowering treatment and was identified using ICD-10 and RxNorm codes, specifically 83367 for atorvastatin and 2282403 for bempedoic acid. This index date marked the start of a one-year follow-up period during which study outcomes were assessed. Established coronary artery disease was identified using ICD-10 diagnostic codes. Comprehensive details regarding cohort construction, and the ICD-10, RxNorm, and Current Procedural Terminology codes employed are provided in the Supplemental Appendix.

STATISTICAL ANALYSIS

Continuous variables are summarized as means with standard deviations (SD), while categorical variables are reported as counts and percentages, as appropriate. Baseline characteristics between the atorvastatin-only group and the atorvastatin plus bempedoic acid group were compared using independent-sample Student's t tests for continuous variables and chi-square tests for categorical variables. To address baseline imbalances between the two cohorts, 1:1 propensity score matching was conducted using a greedy nearest-neighbor approach with a caliper width equal to 0.1 of the pooled SD of the linear propensity score. Variables incorporated into the matching model included demographic factors (age, sex, and race), comorbid conditions (hypertension, hyperlipidemia, diabetes mellitus, chronic kidney disease, cardiomyopathy, heart failure, pulmonary disease, obesity, cerebrovascular disease, and nicotine dependence), concomitant medications (antiarrhythmic agents, ACE inhibitors, angiotensin receptor blockers, β-blockers, diuretics, empagliflozin, dapagliflozin, and canagliflozin), and laboratory parameters (LDL cholesterol, hemoglobin A1C, urea, and creatinine). Covariate balance was assessed using standardized mean differences, expressed in SD units, with smaller values indicating improved balance between groups. Variables were selected for inclusion based on their potential influence on cardiovascular and overall clinical outcomes.

The outcomes after PSM were calculated for the matched cohorts by hazards ratios with their 95% CIs estimated from Cox proportional hazards models. Time-to-event outcomes were described using Kaplan–Meier survival curves; differences between groups were tested with the log-rank test. For all tests, the threshold for statistical significance was a two-sided P value<0.05. All analyses were run using integrated R software [The R Foundation] within the TriNetX platform.

ETHICS APPROVAL

This study was conducted using de-identified data from the TriNetX research network. In accordance with U.S.

Table 1. Baseline characteristics of patients in atorvastatin and atorvastatin plus bempedoic acid groups before and after propensity score matching (PSM)

	Before PSM			After PSM		
	Before Matching (Atorvastatin group, n= 3,071,930)	Before Matching (Atorvastatin plus bempedoic acid group, n= 6,550)	Standardized Difference	After Matching (Atorvastatin group, n=6,549)	After Matching (Atorvastatin plus bempedoic acid group, n=6,549)	Standardized Difference
Demographics						
Age at Index (Mean ± SD)	67.4±11.9	67.7±9.9	0.029	67.7±10.9	67.7±9.9	0.003
Female [%]	1,145,788 (37.3%)	3,315 (50.6%)	0.271	3,352 (51.2%)	3,314 (50.6%)	0.012
Male (%)	1,924,366 (62.6%)	3,223 (49.2%)	0.273	3,179 (48.5%)	3,223 (49.2%)	0.013
White [%]	2,186,916 (71.2%)	5,165 (78.9%)	0.178	5,253 (80.2%)	5,164 (78.9%)	0.034
Black or African American [%]	397,611 (12.9%)	630 (9.6%)	0.105	620 (9.5%)	630 (9.6%)	0.005
Comorbid conditions						
Hypertension	1,894,354 (61.7%)	5,458 (83.3%)	0.500	5,392 (82.3%)	5,458 (83.3%)	0.027
Dyslipidemia	1,671,331 (54.4%)	6,021 (91.1%)	0.935	5,995 (91.5%)	6,020 (91.9%)	0.014
Cardiomyopathy	206,998 (6.7%)	724 (11.1%)	0.046	644 (9.8%)	724 (11.1%)	0.040
Diabetes mellitus	943,652 (30.7%)	2,420 (36.9%)	0.132	2,243 (34.2%)	2,419 (36.9%)	0.056
Overweight, obesity and other hyperalimentation	584,708 (19.0%)	2,631 (40.2%)	0.476	2,612 (39.9%)	2,631 (40.2%)	0.006
Diseases of the respiratory system	1,303,009 (42.4%)	4,418 (67.5%)	0.520	4,508 (68.8%)	4,417 (67.4%)	0.030
Heart failure	557,749 (18.2%)	1,737 (26.5%)	0.202	1,590 (24.3%)	1,737 (26.5%)	0.052
Cerebrovascular diseases	468,619 (15.3%)	1,883 (28.7%)	0.330	1,841 (28.1%)	1,883 (28.8%)	0.014
Nicotine dependence	468,644 (15.3%)	1,254 (19.1%)	0.103	1,184 (18.1%)	1,254 (19.1%)	0.027
Chronic kidney disease	492,629 (16.0%)	1,399 (21.4%)	0.137	1,295 (19.8%)	1,399 (21.4%)	0.039
Medication use						
Antiarrhythmics	1,070,707 (34.9%)	4,738 (72.3%)	0.811	4,810 (73.4%)	4,737 (72.3%)	0.025
ACE inhibitors	714,802 (23.3%)	2,677 (40.9%)	0.384	2,590 (39.5%)	2,676 (40.9%)	0.027
Angiotensin II inhibitor	475,717 (15.5%)	2,869 (43.8%)	0.652	2,776 (42.4%)	2,868 (43.8%)	0.028
Beta-blockers	1,220,589 (39.7%)	5,073 (77.5%)	0.829	5,023 (76.7%)	5,072 (77.4%)	0.018
Diuretics	931,807 (30.3%)	3,521 (53.8%)	0.488	3,511 (53.6%)	3,521 (53.8%)	0.003
Empagliflozin	39,576 (1.3%)	755 (11.5%)	0.428	653 (10.0%)	754 (11.5%)	0.050
Dapagliflozin	22,044 (0.7%)	383 (5.8%)	0.291	275 (4.2%)	382 (5.8%)	0.075
Canagliflozin	12,766 (0.4%)	101 (1.5%)	0.115	76 (1.2%)	100 (1.5%)	0.032
Laboratory						
LDL cholesterol, [md/dL]	97.7±41.4	112.5±45.5	0.340	96.6±40.7	112.5±45.5	0.369
Hemoglobin A1c [%]	6.6±1.8	6.2±1.2	0.288	6.4±1.6	6.2±1.2	0.135
Blood urea nitrogen [mg/dL]	20.7±13.0	18.3±8.5	0.216	19.6±11.2	18.3±8.5	0.132
Creatinine [mg/dL]	1.4±4.2	1.0±1.8	0.108	1.2±2.9	1.0±1.8	0.059

Source : Own materials

federal regulations, studies using only de-identified data are not considered human subjects research and are exempt from institutional review board (IRB) approval. TriNetX, LLC has received a waiver from the Western IRB and complies with the Health Insurance Portability and Accountability Act (HIPAA), with de-identification confirmed through a qualified expert determination as defined in Section §164.514(b)(1) of the HIPAA Privacy Rule.

RESULTS

STUDY POPULATION

This retrospective cohort study identified a total of 3,078,480 patients with established coronary artery disease who are receiving atorvastatin or atorvastatin plus bempedoic acid. Among them, 3,071,930 patients received atorvastatin, and 6,550 patients received atorvastatin plus bempedoic acid (Fig. 1A). After applying 1:1 propensity score matching (PSM) to balance baseline characteristics, 6,549 patients were included in each cohort (atorvastatin and atorvastatin plus bempedoic acid groups) for the final analysis (Fig. 1B).

PATIENT CHARACTERISTICS

The baseline characteristics of the study cohorts, before and after propensity score matching (PSM), are shown in Table 1. In the unmatched cohort, age at index was comparable between patients receiving atorvastatin and patient receiving atorvastatin plus bempedoic acid were (mean age: 67.4±11.9 years *vs.* 67.7±9.9 years, P=0.03). The proportion of male patients was slightly higher in the Atorvastatin group (62.6% *vs.* 49.2%, P<0.001), while the atorvastatin plus bempedoic acid group had a higher proportion of female patients (50.6% *vs.* 37.3%, P<0.001). Regarding racial distribution, the atorvastatin plus bempedoic acid group was more likely to be White (78.9% *vs.* 71.2%) compared to the atorvastatin group (Fig. 2).

Before matching, the atorvastatin plus bempedoic acid patient group exhibited a higher prevalence of cardiovascular risk factors, including cardiomyopathy (11.1% *vs.* 6.7%, P<0.001), cerebrovascular disease (28.7% *vs.* 15.3%, P<0.001), hypertension (83.3% *vs.* 61.7%, P<0.001), hyperlipidemia (91.9% *vs.* 54.4%, P<0.001), diabetes mellitus (36.9% *vs.* 30.7%, P<0.001), nicotine dependence (19.1% *vs.* 15.3%, P<0.001), Heart failure (26.5% *vs.* 18.2%, P<0.001), overweight and obesity (40.2% *vs.* 19.0%, P<0.001), pulmonary disease (67.5% *vs.* 42.4%, P<0.001), and chronic kidney disease (21.4% *vs.* 16.0%, P<0.001).

Covariate balance between the atorvastatin and atorvastatin plus bempedoic acid cohorts was assessed before and after propensity score matching using standardized mean differences (SMDs). Balance was visually evaluated with a Love plot (Fig. 2), which displays the SMDs for all baseline covariates before and after matching. An absolute SMD <0.1 was considered indicative of adequate covariate balance. The final matched cohort consisted of 6,549 patients in the atorvastatin group and 6,549 patients in the atorvastatin plus bempedoic group.

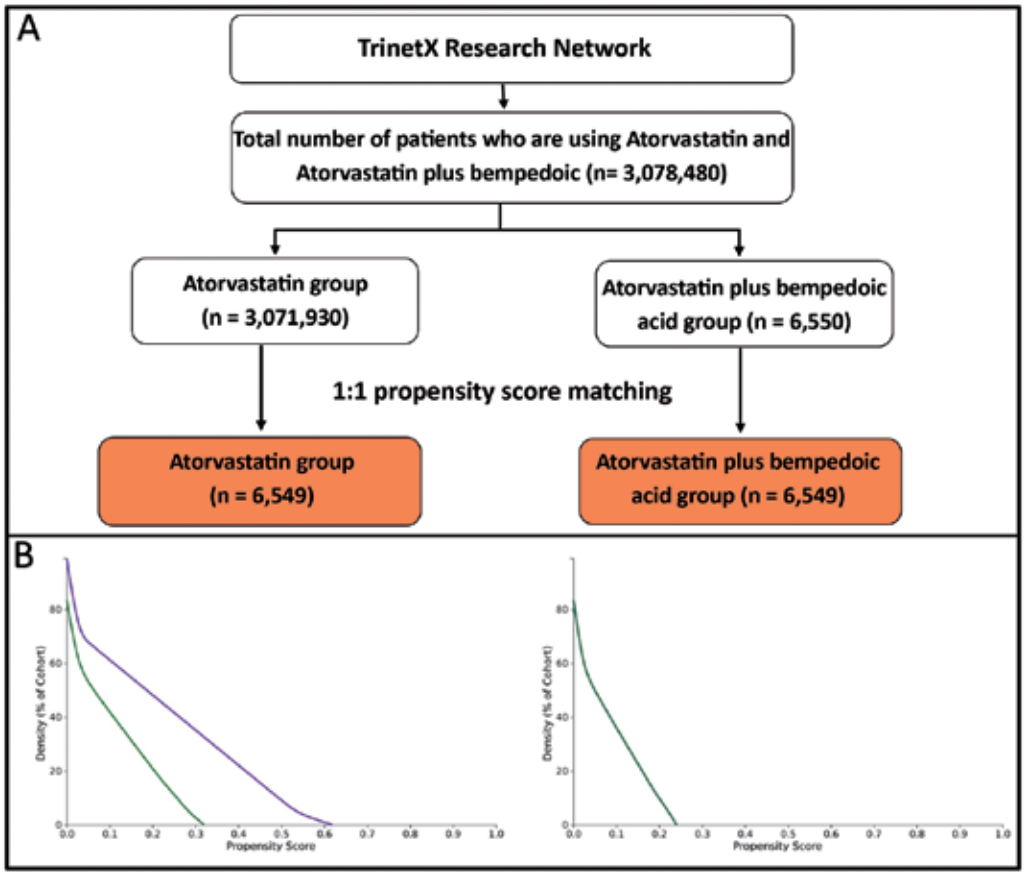

Fig. 1. A: Flow Diagram of Patient Selection and Cohort Derivation Following Propensity Score Matching. B: Propensity score density distributions before and after weighting

Source : Own materials

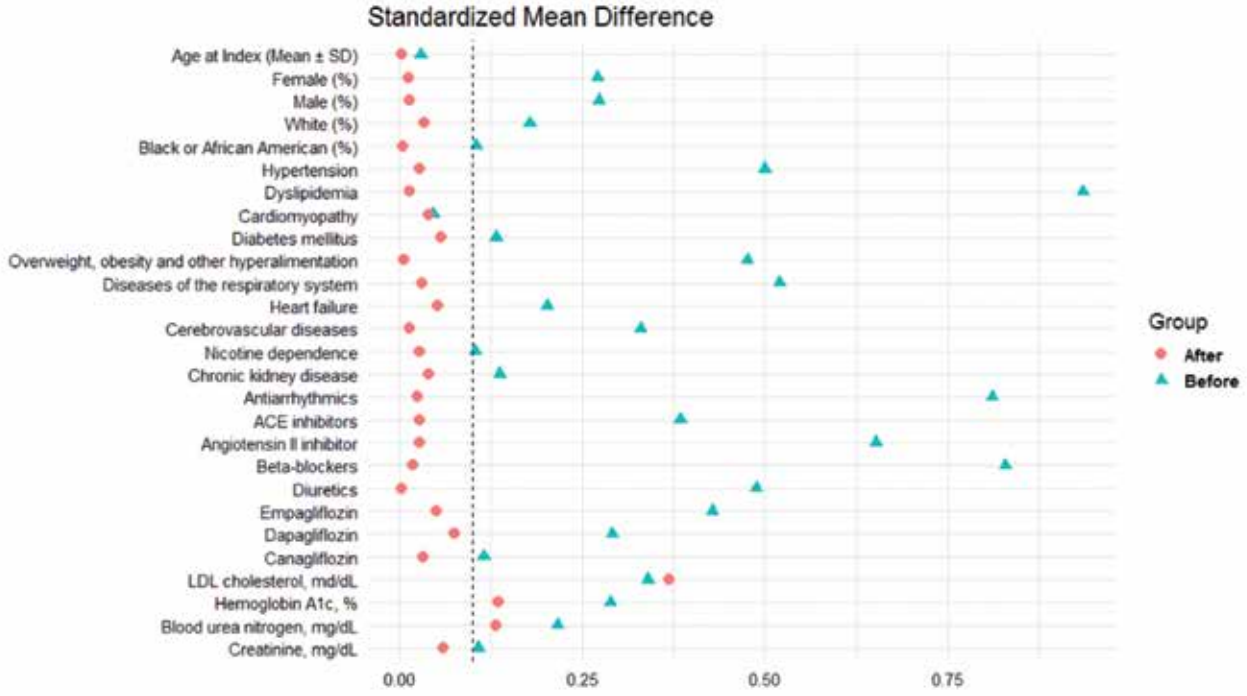

Fig. 2. Baseline characteristics and cardiovascular risk factor prevalence in Atorvastatint and Atorvastatin plus bempedoic acid groups before and after propensity score matching.

Source : Own materials

Table 2. Primary and secondary clinical outcomes: atorvastatin *vs.* atorvastatin plus bempedoic acid groups

Outcome	Risk of Event [%] (Atorvastatin group)	Risk of Event [%] (Atorvastatin plus bempedoic acid group)	Hazard ratio [95% CI]	P value
Primary Outcome				
Major adverse cardiovascular events (MI, stroke)	243 (5.7%)	137 (3.2%)	1.606 (1.302–1.980)	P < 0.001
Secondary Outcome				
Cardiac arrest events	54 (0.8%)	30 (0.5%)	1.628 (1.041–2.544)	P < 0.001
All-Cause Hospitalization	252 (13.9%)	196 (9.0%)	1.418 (1.177–1.710)	P < 0.001
Incidence of myopathy	189 (3.4%)	160 (3.3%)	0.915 (0.741–1.129)	P < 0.001
Values are n (%).				

Source : Own materials

Primary outcome: Major adverse cardiovascular events (MI, stroke)

After matching, during the follow-up period, a total of 243 patients (5.7%) in the atorvastatin cohort experienced Major adverse cardiovascular events, compared to 137 patients (3.2%) in the atorvastatin plus bempedoic acid cohort. Receiving atorvastatin alone was associated with a significantly higher risk of major adverse cardiovascular events with a hazard ratio of 1.606 (95% CI: 1.302-1.980, P<0.001) (Table 2, Fig. 3).

Secondary outcome: Cardiac arrest events, all cause hospitalization and incidence of myopathy.

After matching, the secondary outcomes demonstrated significant differences between the atorvastatin group and the atorvastatin plus bempedoic acid group. Cardiac arrest events were more frequently reported in the atorvastatin group, with a hazard ratio of 1.628 (95% CI: 1.041-2.544, P<0.001). All cause hospitalizations were more commonly reported in the atorvastatin group, with a hazard ratio of 1.418 (95% CI: 1.177-1.710, P<0.001). Between the two groups there was no difference in the incident of myopathy with a hazard ratio of 0.915 (95% CI: 0.741-1.129) (Table 2, Fig. 3).

DISCUSSION

In this large, real-world, propensity score matched cohort study of patients with established coronary artery disease receiving LDL-lowering therapy for secondary prevention,

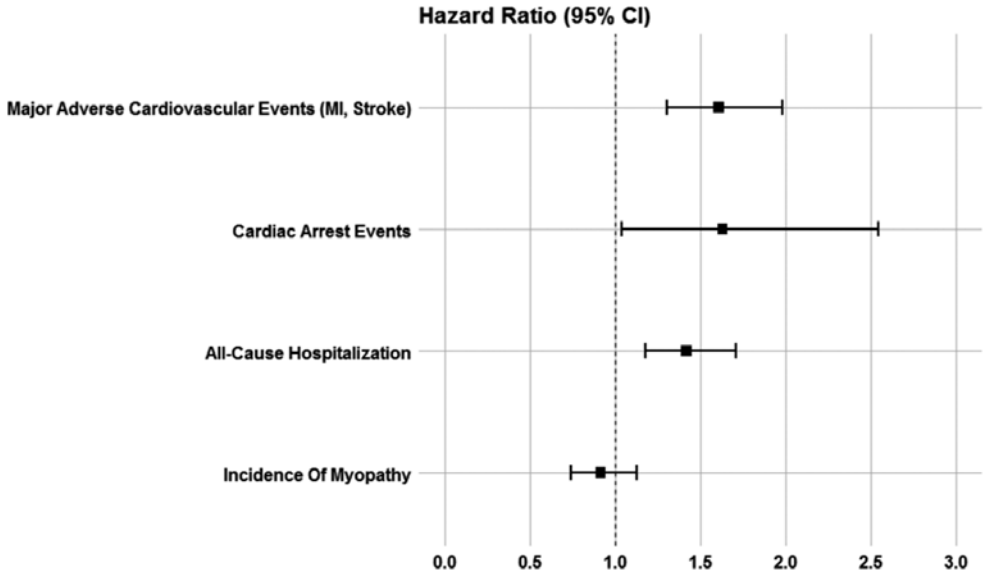

Fig. 3. Primary and secondary outcomes: hazard ratio during follow-up between propensity score-matched patient cohorts

Source : Own materials

treatment with combination therapy of bempedoic acid and a atorvastatin was associated with a lower incidence of major adverse cardiovascular events (MACE), defined as a composite of myocardial infarction and stroke, lower incidence of cardiac arrest events and decrease in all cause hospitalization compared with atorvastatin therapy alone over 1 year of follow-up. On the other hand, there is no difference in the incidence of myopathy between the two groups.

Previous randomized clinical trials evaluating bempedoic acid included similar patient populations but were not adequately powered to detect differences in cardiovascular outcomes. The CLEAR Wisdom trial demonstrated that the addition of bempedoic acid to maximally tolerated statin therapy significantly reduced low-density lipoprotein cholesterol levels; however, cardiovascular outcomes were not the primary endpoint [9]. Similarly, the CLEAR Harmony trial, which enrolled statin-intolerant patients and those receiving maximally tolerated statin therapy, was not powered to detect differences in cardiovascular events [10].

Our findings are consistent with prior evidence from randomized trials and meta-analyses. A meta-analysis of six randomized clinical trials demonstrated a significant reduction in MACE with bempedoic acid, driven primarily by a 24% reduction in myocardial infarction risk [11]. Another meta-analysis including eleven randomized clinical trials showed reductions in MACE, myocardial infarction, and unstable angina [12]. However, these trials enrolled heterogeneous populations, including statin-intolerant patients, individuals on low-dose statins, and those receiving therapy for both primary and secondary prevention. In contrast, our study focused specifically on patients with established coronary artery disease receiving secondary preventive therapy, enhancing the clinical relevance of our findings to this population.

The CLEAR Outcomes trial further demonstrated that bempedoic acid was associated with a reduction in major adverse cardiovascular events, including significant reductions in myocardial infarction and coronary revascularization, though the effect on stroke did not reach statistical significance [7]. Our real-world findings extend these results by demonstrating similar benefits in a secondary prevention population treated with combination therapy.

The effect of bempedoic acid on cardiac arrest events has not been definitively established in major randomized trials. In the present study, combination therapy was associated with a lower incidence of cardiac arrest events compared with atorvastatin alone. This observation may reflect downstream effects of reduced ischemic events; however, given the observational nature of the study, this finding should be interpreted cautiously and considered hypothesis-generating.

All-cause hospitalization has not been systematically evaluated as an endpoint in prior bempedoic acid trials. The CLEAR Outcomes trial specifically examined hospitalization for unstable angina, which occurred in 1.3% of bempedoic acid patients versus 2.0% of placebo patients which represents a 34% relative risk reduction for this specific type of cardiovascular hospitalization [7]. In this study, combination therapy was associated with a lower rate of all-cause hospitalization compared with atorvastatin alone. While major cardiovascular outcome trials have reported reductions in specific cardiovascular hospitalizations, such as unstable angina, our findings suggest that the reduction in ischemic events observed with combination therapy may translate into fewer hospitalizations overall. Nevertheless, this association cannot establish causality and warrants further investigation in prospective studies.

Regarding safety, no significant difference in myopathy was observed between the two groups. This finding is consistent with the pharmacologic mechanism of bempedoic acid, which is activated in the liver and not in skeletal muscle [13]. Prior trials have shown mixed results regarding

muscle-related adverse events [7, 10], however, our findings provide reassuring real-world evidence of comparable muscle safety in patients receiving combination therapy.

LIMITATIONS OF THE STUDY

Our study has limitations like other observational retrospective studies. It cannot establish causality. Despite the fact that propensity score matching helped reduce confounding, unmeasured factors such as physical activity, health care accessibility and socioeconomic status may still have influenced the results. Additionally, our data analysis did not include information about the dose and the frequency of atorvastatin and bempedoic acid. Therefore, our findings cannot establish a dose response relationship. Furthermore, atorvastatin or bempedoic acid use was diagnosed based on International Classification of Diseases (ICD) codes, which carries the risk of misclassification or coding errors and biases common in real-world databases..

In regard to these limitations, our study has notable strengths. It is considered one of the first studies to compare atorvastatin users to atorvastatin plus bempedoic acid users using a large, real-world dataset spanning diverse healthcare settings, thereby investigating the relationship between use and cardiovascular outcomes across a diverse, multicenter patient population. We employed rigorous propensity score matching to enhance comparability between the atorvastatin and atorvastatin plus bempedoic acid cohorts across a wide range of demographic and clinical variables. Also, our methodology allowed exploration of potential associations between atorvastatin or atorvastatin plus bempedoic acid exposure and cardiovascular outcomes within a large, diverse patient population.

CONCLUSIONS

Our findings suggest that the addition of bempedoic acid to atorvastatin therapy may confer a protective effect in patients with coronary artery disease, as it significantly decreased risk of MACE, all cause hospitalization and cardiac arrest events. To date, the existing literature includes only a limited number of studies investigating the association and clinical outcomes of combined bempedoic acid and atorvastatin therapy in patients with coronary artery disease. More prospective studies and clinical trials are needed to investigate the potential benefits of this combination therapy in this high-risk population.

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AVAILABILITY OF DATA AND MATERIAL

The data supporting the findings of this study are available through the TriNetX research network but are subject to licensing restrictions. Access to TriNetX data can be obtained upon reasonable request and with permission from TriNetX, LLC.

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Research management skills in medical PHD education: A cross-sectional study from Ukraine

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ABSTRACT

Aim: To characterise Ukrainian PhD students' research management skills, the difficulties experienced in Ukrainian medical PhD education, and to provide insights into potential directions for improving medical education.

Materials and Methods: The authors conducted a cross-sectional study using a convenience sample. In 2025, the authors surveyed 118 medical PhD students of Bogomolets National Medical University to evaluate their research management competence and attitudes towards it. The authors offered a 17-item questionnaire on research management. To process the data, the authors used descriptive and thematic analysis.

Results: This study shows that medical PhD students at Bogomolets National Medical University generally recognise the importance of research management skills for successful doctoral training. However, attitudes towards these skills remain fragmented, and recognition of their value does not translate into confidence or effective practice. The findings reveal significant gaps in strategic planning, adaptability to change, bureaucratic communication, self-discipline, and the ethical use of digital tools such as artificial intelligence. Research management effectiveness relies mainly on external control and personal self-regulation rather than structured curricular support.

Conclusions: The research management skills of Ukrainian medical PhD students should be conceptualised not as their individual psychological capacity but as a competence shaped by educational design, mentoring structures, ethical training, and career support. The results support the need for curricular integration of research management as a structured competence area, including training in planning, adaptability, communication, academic integrity, and the responsible use of digital tools.

KEYWORDS: management competence, Medical PhDs, medical education

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INTRODUCTION

The development and effective management of research skills make up a core component of doctoral education in medicine, as they directly influence the quality of the final output. Medical PhD programmes are expected to equip students with competences that support academic leadership, interdisciplinary collaboration, and continuous professional development, where research management skills play a crucial role. They include skills in research design, data collection and analysis, academic writing, ethical conduct, project management, and dissemination of results. Effective management of these skills implies the ability of medical PhD students to plan, monitor, evaluate, and adapt their research activities within complex academic and clinical environments. However, existing studies indicate that many PhD students experience difficulties in structuring and implementing their research trajectories [1].

In recent years, Ukrainian higher education has been undergoing significant transformation, related to the adoption of new PhD training programmes and numerous external stressors, including the COVID-19 pandemic and war [2]. At the same time, the importance of research management competence, which defines the success of doctoral studies, remains underscored.

AIM

The aim of the paper is to define Ukrainian PhD students' research management skills, the difficulties experienced in Ukrainian medical PhD education, and to provide insights into potential directions for improving medical education.

MATERIALS AND METHODS

The authors conducted a cross-sectional study using a convenience sample, reviewing postgraduate education standards and analyzing the PhD curricula and syllabuses, in order to define the place of research management.

In 2025, the authors surveyed medical PhD students of Bogomolets National Medical University (BNMU) to evaluate their research management competence and attitudes towards it. The survey questions were composed by the authors following a series of consultations with relevant academicians. Overall, 118 respondents participated in the study, 66 females and 52 males. The authors offered them a 17-item questionnaire on research management, with closed-ended questions and three open-ended questions.

The medical PhD students were asked about their gender, age, specialty, significance of professional knowledge and skills on a 5-point scale, significance of soft skills on a 5-point scale, significance of communication in research

on a 5-point scale, significance of adherence to deontology in research on a 5-point scale, significance of management and leadership skills on a 5-point scale, and related issues, such as self-assessment of the success of their research on a 5-point scale, productive communication in research on a 5-point scale, adherence to deontology rules on a 5-point scale, success of research management on a 5-point scale, and their future plans of making a career abroad upon defending the thesis. They were also asked open-ended questions about how significant research management is to them, their weakest points in research management, and the factors that improve research management competence.

To process the data, the authors used descriptive analysis; data were presented as frequencies and percentages. An open-ended question on the significance of soft skills was analyzed using the qualitative description method. Respondents' quotes were grouped into categories through contextual analysis, with the number of respondents supporting each idea denoted.

ETHICS

The study protocol was approved by BNMU ethical committee, which granted a waiver of informed consent. The medical PhDs were invited to participate in the questioning through the Google Classroom, notified that the participation is voluntary and anonymous. Their informed consent was expressed in the Google Form. The study was conducted according to the laws and regulations in Ukraine. The survey design adhered to the CHERRIES checklist guidelines. The authors did not discuss the results with the respondents.

FRAMEWORK

The study was conducted within the framework of the BNMU research project «Effective education technologies in postgraduate education» (state registration: 0124U001087; term: 2024-2026).

RESULTS

A total of 118 PhD students participated in the study. Of these, 66 (55.9%) were female and 52 (44.1%) were male. Most respondents were under 30 years of age (n=66, 55.9%), followed by those aged 30–40 years (n=30, 25.4%), 40–50 years (n=12, 10.2%), and over 50 years (n=10, 8.5%). Participants represented doctoral programmes in Medicine (n=74, 62.7%), Dentistry (n=28, 23.7%), Pharmacy (n=10, 8.4%), Medical Management and Education (n=6, 5%).

The analysis of the closed-ended questions is presented in Table 1. Clinical and professional knowledge was perceived

Table 1. Self-assessment of the research management competence and its components by medical PhD students of BNMU

Question	Overall (n=118, % from n)
What is your gender?	
- Female	66 (55.9%)
- Male	52 (44.1%)
- Another	0
What is your age:	
- under 30	66 (55.9%)
- 30-40	30 (25.4%)
- 40-50	12 (10.2%)
- over 50	10 (8.5%)
How significant for you are the clinical skills? (from 1 to 5, where 1 - insignificant, 5 -very significant)	
5	90 (76.3%)
4	20 (16.9%)
3	8 (6.8%)
2	0
1	0
How significant for you are the soft skills? (from 1 to 5, where 1 - insignificant, 5 -very significant)	
5	76 (64.4%)
4	38 (32.2%)
3	4 (3.4%)
2	0
1	0
How significant for you are the communication skills? (from 1 to 5, where 1 - insignificant, 5 -very significant)	
5	84 (71.2%)
4	24 (20.3%)
3	10 (8.5%)
2	0
1	0

Tabela 1. Cont.

How significant for you are the ethical skills? (from 1 to 5, where 1 - insignificant, 5 -very significant)	
5	74 (62.7%)
4	22 (18.6%)
3	16 (13.6%)
2	4 (3.4%)
1	2 (1.7%)
How significant for you are the management skills (from 1 to 5, where 1 - insignificant, 5 -very significant)	
5	84 (71.2%)
4	30 (25.4%)
3	4 (3.4%)
2	0
1	0
Evaluate by the 5-point scale how successful your study is:	
5	16 (13.6%)
4	36 (30.5%)
3	48 (40.7%)
2	14 (11.9%)
1	4 (3.4%)
Evaluate by the 5-point scale how successful you communicate regarding the study:	
5	
4	34 (28.8%)
3	48 (40.7%)
2	28 (23.7%)
1	6 (5.1%)
	2 (1.7%)
Evaluate by the 5-point scale well you keep to ethical principles in your study:	
5	
4	72 (61.0%)
3	28 (23.7%)
2	18 (15.3%)
1	0
	0
Evaluate by the 5-point scale the success of your research project:	
5	
4	20 (16.9%)
3	54 (45.8%)
2	32 (27.1%)
1	8 (6.8%)
	4 (3.4%)
Are you planning to continue research work after the thesis?	
- Yes	60 (50.8%)
- No	18 (15.3%)
- It's hard to say	40 (33.9%)
Are you planning to take up a leadership position after defending the thesis??	
- Yes	
- No	68 (57.6%)
- It's hard to say	14 (11.9%)
	34 (28.8%)
Are you planning immigration after defending the thesis?	
- Yes	10 (8.5%)
-No	78 (66.1%)
- It's hard to say	30 (25.4%)

Source: compiled by the authors of this study

as highly relevant to managing research activities by the majority of respondents: 76.3% (n=90) rated it as very significant, 16.9% (n=20) as significant, and 6.8% (n=8) as moderately significant. Soft skills were also rated highly for research management success, with 64.4% (n=76) indicating they were very significant, 32.2% (n=38) significant, and 3.4% (n=4) moderately significant; none of the respondents considered soft skills insignificant.

Communicative skills in research management were rated as very important by 71.2% of respondents (n=84), important by 20.3% (n=24), and moderately important by 8.5% (n=10). Perceptions of deontological skills in research management showed greater variability. While 62.7% (n=74) rated them as very important and 18.6% (n=22) as important, 13.6% (n=16) considered them moderately important, 3.4% (n=4) unimportant, and 1.7% (n=2) completely unimportant. Research management skills themselves were considered very significant by 71.2% of respondents (n=84), significant by 25.4% (n=30), and moderately significant by 3.4% (n=4); these results are somewhat lower than those from the open-ended questions.

Self-assessment of doctoral study progress indicated that 13.6% of respondents (n=16) rated their studies as very successful, 30.5% (n=36) as successful, and 40.7% (n=48) as moderately successful.

Regarding self-assessment of their dissertation management, 16.9% of respondents (n=20) reported very successful progress, 45.8% (n=54) successful progress, and 27.1% (n=32) average progress. Poor or very poor management of the dissertation process was reported by 10.2% of participants (n=12).

In terms of career intentions, 15.3% of respondents (n=18) stated that they planned to end their research career after defending their thesis, while 33.9% (n=40) were uncertain. Leadership or managerial career aspirations were reported by 57.6% of respondents (n=68), whereas 13.6% (n=16) did not plan further career advancement, and 28.8% (n=34) were undecided. Additionally, 8.5% of respondents (n=10) reported a clear intention to work abroad (Table 1).

The open responses on the significance of research management were grouped into positive, negative, and undecided attitudes towards research management; the open responses on the missing management qualities were grouped into leadership, planning, communication, ethical, and practical qualities; and the open responses on possible improvement were grouped into self-discipline, studying research management, good mentorship, control, and "no suggestions."

The analysis of the open responses, presented in Table 2, showed that attitudes toward research management skills varied: a positive attitude was reported by 39.0% of participants (n=46). In contrast, 21.2% of respondents (n=25) reported that research management skills are not useful.

Self-assessment of insufficient research management skills revealed several areas of concern. Insufficient leadership-related skills were reported by a minority of respondents, while communication-related difficulties were reported more frequently. Insufficient communication with other researchers was indicated by 11.9% of respondents (n=14). Difficulties related to bureaucratic communication were reported by 22.0% of respondents (n=26).

With regard to academic integrity skills, insufficient knowledge of deontological principles was reported by 5.9% of respondents (n=7). In contrast, insufficient knowledge of the use of artificial intelligence was reported by 26.3% of respondents (n=31). Practical skills related to self-regulation were also affected: insufficient self-discipline was reported by 23.7% of respondents (n=28), and insufficient self-monitoring by 16.1% of respondents (n=19).

Control from the PhD department, including regular reporting requirements, was identified as a determining factor by 61.0% of respondents (n=72). Control from supervisors was reported by 18.6% (n=22), and deadlines defined by the Ministry by 7.6% (n=9).

Assistance and supervision were also frequently reported: supervision and collaboration with a mentor or supervisor were identified by 39.0% of respondents (n=46), and collaboration with peers by 15.3% (n=18).

Self-control skills were identified as a key determinant of research management effectiveness by 54.2% of participants (n=64), while self-regulation skills and strong discipline were reported by 12.7% (n=15). Finally, 20.3% of respondents (n=24) reported no factors determining research management effectiveness (Table 2).

DISCUSSION

This study offers empirical evidence on how Ukrainian PhD students in medical and health-related fields perceive research management skills. The findings demonstrate that respondents strongly acknowledge the importance of research management, clinical expertise, soft skills, and communication for successful activity, which aligns with several European studies [1].

Thus, the high valuation of research management by Ukrainian students reflects a broader global shift toward competency-based doctoral education. Similar trends have been reported in studies highlighting communication, leadership, and organisational skills as essential outcomes of doctoral education rather than optional additions [3, 4]. In Ukraine, this shift may be accelerated by the need for adaptability, rapid decision-making, and coordination.

At the same time, the presence of negative and absent attitudes, which account for almost half of the responses, indicates fragmentation in perceptions. The high percentage of respondents unable to define their attitude may reflect insufficient explicit integration of research management within doctoral curricula, rather than assumed competence.

Despite recognising the importance of research management competencies, a substantial proportion of respondents reported only moderate or low success in managing their doctoral studies. Approximately 15% of respondents reported poor or very poor overall doctoral progress, and more than 10% indicated difficulties in managing their dissertation process.

This discrepancy echoes findings from qualitative research, showing that stress, anxiety, and uncertainty

Table 2. Open-ended questions on attitude to research management

Attitude to research management skills as a discipline	
The answer cluster defined by analysis	Phrases related to the subgroup and the alphanumerical numbers of respondents
Positive attitude	They are necessary (n=46; 39.0%) They can help in writing thesis and completing the work (n=21; 17.8%)
Negative attitude	They are not useful (n=25; 21.2%) They limited useful (n=18; 15.3%)
Absent attitude	I don't know. I can't define (n=31; 26.3%)
Self-assessment of the research management skills insufficient in a respondent	
The answer cluster defined by analysis	Phrases related to the subgroup and the alphanumerical numbers of respondents
Leader skills	Assertiveness in difficult situation (n=13; 11.0%) Persuasion skills (n=7; 5.9%)
Communication skills	Communication with other researchers (n=14; 11.9%) Communication with the seniors. teachers. advisor (n=3; 2.5%) Communication skills related to bureaucracy (n= 26; 22.0%)
Strategy and planning skills	Planning the thesis structure (n=17; 14.4%) Planning the sequence of activities (n=11; 9.3%) Ability to adapt the planning to changes (n=46; 39.0%)
Academic integrity skills	Knowledge on plagiarism check and deontology (n=7;5.9%) Knowledge on the AI use in thesis (n=31; 26.3%)
Practice skills. self-discipline	Self-discipline (n=28; 23.7%) Self-monitoring (n=19; 16.1%)
Factors which determine the research management effectiveness	
The answer cluster defined by analysis	Phrases related to the subgroup and the alphanumerical numbers of respondents
External control	Control from PhD department. necessity to report every term (n=72; 61.0%) Control from supervisor (n=22; 18.6%) Deadlines defined by Ministry (n=9; 7.6%)
Assistance and supervision	Supervision and collaboration with a mentor or supervisor (n=46; 39.0%) Collaboration with peers (n=18; 15.3%)
Self-development of the person	Self-control skills (n=64; 54.2%) Self-regulation skills and strong discipline (n=15; 12.7%)
Curriculum components	Information meetings with PhD administration (n=11; 9.3%) Studying the courses on research structure (n=24; 20.3%)
No factors	Negative answer (n=24; 20.3%)

Source: compiled by the authors of this study

impair PhD students' sense of control and effectiveness [5]. Current war-related disruptions in Ukraine, such as air raid alarms, power outages, displacement, and increased clinical workloads, likely intensify these stressors, with burnout and emotional exhaustion as the most exhausting factors [6-8].

One of the most significant difficulties leading to poor success in research management was reported by respondents as challenges in strategic planning and adaptability. The communication component in research management emerged as a relatively strong domain in self-assessment, which may reflect increased reliance on communication during crisis conditions. Prior research has shown that the communication competence of medical PhD students is shaped by individual factors such as motivation, self-regulation, and language proficiency [9], as well as by structured educational interventions. Systematic reviews consistently demonstrate that communication skills can be effectively taught through targeted pedagogical approaches [10, 11]. Nevertheless, the presence of respondents reporting poor communicative success reinforces the need for structured, longitudinal communication training. Numerous respondents noted communication-related challenges in relation to bureaucratic communication, which may reflect the complexity of administrative procedures. Relatively fewer respondents reported difficulties in communication with supervisors or advisors, which may indicate either effective supervisory relationships or, alternatively, underreporting due to hierarchical academic culture.

While most respondents rated ethical principles as important, approximately one fifth assigned them low importance, yet nearly all rated their own adherence as good or excellent. This divergence mirrors earlier research on publication ethics and professional conduct among PhD students, which has identified social desirability bias and limited formal ethics training as contributing factors to

inflated self-assessments [12]. In medical education, this gap underscores the need for systematic deontological education. Studies on conflict management further support the value of explicit training in ethical and conflict-resolution skills [13]. More than one quarter reported limited understanding of AI use in thesis preparation. This suggests a rapidly emerging competence gap, and the lack of structured guidance on ethical and effective AI use may create uncertainty and risk among doctoral candidates.

Overall, the analysis of factors influencing research management effectiveness demonstrates a strong reliance on external control mechanisms, institutional monitoring, and reporting requirements. While such structures may support accountability, they also indicate a predominantly extrinsic model of research management. In contrast, self-development factors, especially self-control skills, were also highly valued, highlighting a dual dependence on both external regulation and internal discipline. Notably, a considerable proportion of respondents reported no determining factors for research management effectiveness. This absence of perceived support structures may point to disengagement, limited awareness of resources, or inadequate communication between administration and PhD students.

Career intention data from this study raise additional concerns. Nearly half of Ukrainian respondents were uncertain about their career prospects. Similar patterns have been reported internationally, where lack of structured mentoring and career planning contributes to attrition from academic pathways [14, 15]. At the same time, more than half of respondents expressed aspirations toward leadership roles, which aligns with foreign studies [16, 17]. These findings highlight the growing importance of mentoring, reflective practice, and career development support in graduate education [18, 19].

The intention to work abroad after completing their studies reflects broader global mobility trends but carries particular significance in the Ukrainian context. While international experience may enhance professional development, sustained outmigration of medical PhD graduates weakens national healthcare and academic systems. Strategic leadership training for researchers has been shown to support retention and institutional resilience [20].

The results support the need for explicit curricular integration of research management as a structured competence area, including training in planning, adaptability, communication, academic integrity, and responsible use of digital tools. International evidence increasingly supports doctoral training models that integrate communication, ethics, management, and leadership as core curricular elements [20, 21]. In war-affected contexts, such integration may be essential for sustaining doctoral engagement, preventing burnout, and preparing future leaders capable of contributing to healthcare system recovery.

LIMITATIONS

This study was conducted exclusively among PhD students of BNMU, which limits the generalisability of the findings to other institutions. The study is based on self-reported data, which may be subject to subjective bias and social desirability, particularly in ethically sensitive areas. Future longitudinal and multi-institutional studies are recommended to explore the development of research management skills throughout doctoral training.

CONCLUSIONS

This study shows that Ukrainian medical PhD students generally recognise the importance of research management skills. Attitudes toward these skills remain fragmented, and recognition of their value does not consistently translate into confidence or effective practice. The findings reveal significant gaps in strategic planning, adaptability to change, bureaucratic communication, self-discipline, and ethics. Research management effectiveness is perceived to rely mainly on external control and personal self-regulation, indicating limited development of autonomous research management competence.

Uncertainty about academic career continuation, alongside aspirations for leadership roles, highlights the need for integrated mentoring, career guidance, and leadership training within medical PhD programmes. Overall, the results support the explicit integration of research management as a core, structured competence in medical doctoral education.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Resilience as a factor of psychological stability of modern police officers in crisis situations of their professional activities

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ABSTRACT

Aim: To investigate the psychological characteristics of resilience among modern police officers in wartime crises.

Materials and Methods: The research, which lasted from 2023 to 2025, involved 120 employees of the National Police of Ukraine: experimental group (EG, n=60) – police officers performing their duties in combat conditions; control group (CG, n=60) – police officers whose activities took place in relatively safe conditions. The resilience of police officers was assessed using three methods: the Connor-Davidson Resilience Scale, the Wagnild and Young Resilience Scale, and the Posttraumatic Growth Inventory.

Results: It has been established that resilience in police officers not only provides a basic ability to cope with the negative consequences of traumatic experiences, but also acts as a mechanism that helps transform these experiences into a source of development. It has been determined that police officers' resilience in combat conditions serves a protective function, helping maintain stability and minimize the destructive effects of stress. Overcoming extreme situations in such an environment contributes to rethinking life values, the formation of new meanings, and spiritual growth, thereby enhancing the ability to adapt.

Conclusions: It has been shown that, under relatively safe conditions, resilience contributes to the internal restructuring of a police officer's personality and promotes personal growth. However, in combat situations, it tends to become a resource for preservation, minimizing the impact of traumatic events but not stimulating development through posttraumatic growth. This underscores the need for a comprehensive approach to developing resilience among police officers, whose work involves high levels of danger and psychological stress.

KEYWORDS: resilience, psychological stability, police officer, crisis conditions, professional activities, martial law

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INTRODUCTION

Maintaining psychological stability, the ability to recover quickly from shocks, and the willingness to continue performing service duties in conditions of constant danger are becoming not only professional requirements but also existential necessities [1, 2]. The current transformation processes associated with the full-scale war in Ukraine have placed police officers at the epicenter of extremely complex social and psychological challenges [3, 4]. In this situation, resilience becomes particularly important, as it determines not only a police officer's endurance in the face of crisis events but also their ability to transform traumatic experiences into a resource for development and effective functioning in an extreme environment [5].

At the same time, it should be noted that some researchers rightly emphasize that resilience cannot be reduced to "overcoming" in the narrow sense; instead, it appears as the ability of an individual or social system to build a whole life in objectively difficult conditions [6]. This emphasis on the constructive and even creative dimension of resilience opens up the prospect of understanding it as a potentially productive process capable of transforming negative experiences into a foundation for development [7]. In this context, we believe the distinction between resilience and psychological recovery is crucial. Recovery involves a return to the previous level of functioning after a psychotraumatic event, which is often accompanied by symptoms of depression or post-traumatic stress disorder and requires the long-term mobilization of resources [8-10]. Resilience, on the other hand, is the ability to function effectively in the face of stress, preventing destructive consequences and, at times, even using the crisis as a point of growth [5].

The professional activities of law enforcement officers during wartime are particularly challenging due to the combination of constant risk to life and health and the need to comply with regulatory and legal requirements. Hence, the requirements for psychological readiness are increasing: police officers must maintain a high level of emotional stability, sociability, and the ability to interact constructively with people of different ages, social status, and professional experience [11]. Psychological tension is exacerbated by the need to make decisions in conditions of uncertainty, limited information, and time constraints, while ensuring the legality and moral justification of

every action in wartime [2]. According to experts [12], being in a constant state of heightened psychological readiness is often accompanied by a high risk of trauma and mental disorders. Extreme conditions contribute to the development of latent forms of post-traumatic reactions, manifested through anxiety, exacerbation of character accentuations, uncertainty in professional actions, and increased aggression in interactions with the environment [13, 14]. The intensity and duration of stressors determine the speed and severity of post-traumatic stress disorder manifestation. It is in the context of these threats that the need for purposeful self-control and management of one's own emotional states becomes apparent. This substantiates the importance of resilience as a multidimensional phenomenon that underpins the professional stability of police officers in high-risk conditions and emphasizes the need to develop psychological resources and implement organizational measures that foster the maintenance of psycho-emotional balance and prevent professional burnout. According to experts [15, 16], resilience not only helps police officers overcome the adverse effects of stress on their personalities but also actively produces positive outcomes, contributing to personal and professional development, well-being, and effectiveness in critical conditions. The social component of resilience, including the ability to interact effectively with colleagues and citizens, is an additional resource for law enforcement officers to overcome stressful situations.

AIM

The aim is to investigate the psychological characteristics of resilience among modern police officers in wartime crises.

MATERIALS AND METHODS

PARTICIPANTS

The research, which lasted from 2023 to 2025, involved 120 employees of the National Police of Ukraine: experimental group (EG, n=60) – police officers performing their service duties in combat conditions; control group (CG, n=60) – police officers whose work took place in conditions of relative safety. The age range of participants was 23 to 42 years, corresponding to the active phase of their professional activities, when already acquired competencies and the ability to develop resilience further are combined. Length of service was not taken into account, but this allowed both young professionals who are just developing their own adaptation mechanisms and experienced police officers who have been in a high-stress environment for a long time to be included. The criteria for including respondents were as follows: research participants are police officers performing their service duties; no contraindications to mental or physical health. The exclusion criterion was the respondents' desire to withdraw from the research at any time.

STAGES OF THE RESEARCH

The research organization followed a precise sequence of stages, ensuring scientific validity and internal logic. The preparatory stage consisted of defining the methodological principles, clarifying the object and subject of the research, formulating the tasks, and selecting valid psychological diagnostic tools. It was at this stage that an analysis of scientific sources was conducted, which enabled the identification of contemporary conceptual interpretations of resilience and the parameters most relevant to studying the psychological peculiarities of police officers in wartime conditions. The main stage of the research was conducted from 2023 to 2025 at the Kharkiv National University of Internal Affairs (KhNUIA, Kharkiv, Ukraine), where police officers attended advanced training. At this stage of the research, the resilience indicators of the EG and the CG police officers were analyzed. The final stage was the generalization, statistical processing, and interpretation of the results obtained. Thus, the organization of the research was based on the principles of gradualism and systematicity, which ensured a complete reproduction of the empirical picture of resilience in a professional police environment characterized by high levels of risk and stress.

RESEARCH METHODS

Theoretical analysis and generalization of literature, psychological methods, statistical methods. The method of theoretical analysis and generalization of literature was used to study scientific sources on the topic of research (25 sources from MedLine, Scopus and Web of Science databases were studied).

Psychological methods involved the use of three psycho-diagnostic techniques: the Connor-Davidson Resilience Scale, the Wagnild and Young Resilience Scale, and the Posttraumatic Growth Inventory. The primary tool in our research was the Connor-Davidson Resilience Scale (CD-RISC), which allows us to assess overall psychological stability, the ability to cope effectively with stress, and the ability to mobilize internal resources and overcome difficult life circumstances. This research used the ten-point version of the scale (CD-RISC-10), a shortened version of the original 25-point version, which has been tested in numerous studies for validity and reliability [17]. The essence of the scale is to assess a set of characteristics that cover the cognitive, emotional-volitional, and behavioral components of resilience. It measures the ability to adapt, maintain clarity of thought, stay focused under stress, resist negative emotions, and mobilize resources to overcome difficulties. Ten statements are rated on a five-point Likert scale from 0 ("not at all true") to 4 ("almost always true"), yielding a total score from 0 to 40, with higher scores reflecting greater resilience. The use of the Connor-Davidson Resilience Scale in this research is methodologically justified, as it not only provides a quantitative measure of resilience but also enables comparative analysis with parameters such as anxiety, aggression, and coping strategies. In combination with other psychological diagnostic techniques, this technique allows us to develop a comprehensive understanding of resilience among police officers in highly stressful conditions. It provides a reliable basis for the further development of effective psychological support programs.

To provide an in-depth analysis of police officers' professional resilience and identify its specific components, the Wagnild and Young Resilience Scale [18] was used. This technique was chosen to assess the level of personal resilience in conditions of high psycho-emotional stress, characteristic of police work during wartime. It allows for the assessment of specific components of resilience, in particular the ability to change, self-control, self-confidence, positive thinking, and social support. The original 25-question version of the scale was used for the research. Each statement was rated by the respondent on a 7-point scale, where 1 corresponds to "strongly disagree" and 7 to "strongly agree." The total score was determined as the sum of all responses, and the results were interpreted according to the following criteria: less than 65 points – low level of resilience, 65-81 points – sufficient level, and above 81 points – high level.

The next step in the research was the application of the Posttraumatic Growth Inventory (PTGI), which allows studying positive changes in personality after traumatic experiences [19]. PTGI occupies a key place in modern research aimed at assessing positive personality transformations after traumatic events. This technique allows for the quantitative measurement of the phenomenon of posttraumatic growth, which the authors consider to be a new level of development of internal resources as a result of overcoming an extreme experience. The essence of the technique is based on the idea that trauma can not only disrupt habitual life orientations but also stimulate the activation of internal potential, the formation of renewed sensory structures, and increased personality adaptability in crisis conditions. The PTGI conceptual model covers five key aspects of growth: rethinking interpersonal relationships, openness to new opportunities, a deeper appreciation of life, the development of personal strength, and the transformation of the spiritual sphere. The questionnaire procedure involves respondents evaluating a series of statements representing these dimensions on a seven-point scale from "did not happen at all" to "happened to a considerable extent." The phenomenon of posttraumatic growth is differentiated and closely related to individual readiness for transformation, which is determined by the stage of life. This approach allows us to record participants' subjective perceptions of the depth of positive changes after a traumatic experience. Hence, integrating PTGI into the set of techniques used provides a comprehensive assessment of the resource potential of a police officer's personality, combining quantitative accuracy with the depth of qualitative interpretation.

STATISTICAL METHODS

Statistical methods were used to process the data obtained. To achieve the research aim, we used a selective linear correlation coefficient (Pearson's correlation coefficient). Pearson's correlation coefficient (r) is an indicator of linear dependence between two variables, which takes values from -1 to +1 inclusive. It is equal to the sum of the products of the deviations divided by the product of their standard deviations. A value of +1 means that the dependence between the variables is linear, and all points of the function lie on a straight line that reflects the growth of one variable as the other grows. With correlation coefficient values from 0 to +1, the correlation is positive (positive relationship). A value of -1 means that all points lie on a line that reflects the decrease of one variable as the other increases. With correlation coefficient values from -1 to 0, the correlation is negative (negative relationship). If the correlation coefficient is 0, there is no linear correlation between the variables. If r=-0.09 – 0.0 or 0.0 – 0.09, there is no correlation; if r=-0.3 – -0.1 or 0.1 – 0.3, the correlation is low (weak relationship); if r=-0.5 – -0.3 or 0.3 – 0.5, the correlation is average (moderate relationship); if r=-0.7 – -0.5 or 0.5 – 0.7, the correlation is above average (noticeable relationship); if r=-0.9 – -0.7 or 0.7 – 0.9, the correlation is high (close relationship); if r=-1.0 – -0.9 or 0.9 – 1.0, the correlation is very high (strong relationship); if r=-1.0 or 1.0, the nature of the relationship is considered functional. All statistical analyses were performed using SPSS software, version 10.0, adapted for medical and biological research.

ETHICS

During the research, the principles of academic validity were observed: participation was voluntary, results were anonymous, and psychological support was provided for participants with elevated anxiety or distress. The initial survey of respondents for inclusion in a specific group was conducted using Google Forms, enabling a standardized procedure while accounting for participants' characteristics. The procedure for organizing the study and the topic of the article were previously agreed with the Committee on compliance with Academic Integrity and Ethics of the KhNUIA. Also this study followed the regulations of the World Medical Association Declaration of Helsinki. Informed consent was received from all participants.

FRAMEWORK

This scientific article was carried out according to the plan of the research work of the Lviv State University of Internal Affairs for 2020-2026„Psychological, pedagogical and sociological support of law enforcement officers» (state registration number 0113U008196).

RESULTS

The analysis of correlation relationships between resilience indicators on various psycho-diagnostic scales made it possible to more clearly define the specifics of the psychological resilience of police officers in wartime conditions. Table 1 shows that in the EG (police officers working in combat conditions), a statistically significant positive correlation was found between the overall results on the Connor-Davidson Resilience Scale (CD-RISC) and the Wagnild and Young Resilience Scale (r=0.326; p=0.001). This coefficient indicates a moderate correlation, confirming the convergent validity of the two techniques, i.e., their ability to reflect a familiar psychological construct to some extent. At the same time, this correlation is not high enough to consider the instruments interchangeable, as each reflects different structural aspects of resilience. The CD-RISC focuses on the

procedural aspects of psychological resilience, such as the ability to recover, respond flexibly, and overcome stressors in high-risk situations. The Wagnild and Young Resilience Scale, on the other hand, is based on the idea of resilience as a relatively stable resource that includes optimism, belief in one's own abilities, and an inner philosophy of life. The significant correlation between the indicators of both scales among police officers operating in a war environment can be interpreted as evidence that, in an extreme environment, different aspects of resilience become more integrated and manifest in an interrelated form.

However, another trend can be observed in the CG: the correlation between the scales was weak and statistically insignificant (r=0.172; p=0.061). This indicates that in the absence of critically high psycho-emotional stress, neither instrument reflects a common semantic plane with equal clarity. In addition, in conditions of relative safety, the Connor-Davidson Resilience Scale may be more sensitive to situational adaptive manifestations. In contrast, the Wagnild and Young Resilience Scale reflects more stable attitudes that are less likely to be actualized in normal circumstances. That is why the correlation proved to be statistically insignificant, which does not negate the validity of the techniques but indicates the need for careful interpretation of the results outside extreme contexts.

The comparative analysis of data from both groups of respondents allows us to conclude that, in situations of military risk, resilience emerges as a holistic and multidimensional phenomenon in which internal resources and procedural mechanisms function in an interconnected manner. That is why the correlation between the methodologies among the group of police officers involved in wartime activities is statistically significant. This highlights the need for a comprehensive approach to assessing resilience, combining multiple instruments and accounting for the context of the individual's functioning, especially in the police profession, where psychological stability is a key factor in effectiveness and mental health preservation.

The results of our research also allowed us to identify statistically significant positive correlations between the overall level of resilience on the Connor-Davidson Resilience Scale and all aspects of posttraumatic growth (Table 2).

As shown in Table 2, in the EG (police officers working in conditions of potential danger from combat operations), there are statistically significant positive correlations between the overall level of resilience on the Connor-Davidson Resilience Scale and all aspects of posttraumatic growth. The most pronounced correlation is with the indicator of personal strengthening (r=0.208, p=0.023), which indicates a tendency to transform traumatic experiences into an internal resource that enhances feelings of confidence, competence, and psychological stability. This shows that for police officers directly involved in military operations, the ability to remain psychologically resilient is not only a defense mechanism but also the basis for qualitative personal change. The identified link between resilience and spiritual-existential transformations (r=0.197, p=0.031) is also essential. This suggests that overcoming extreme situations in a military environment contributes to rethinking life values, the formation of new meanings, and spiritual growth, thereby enhancing the ability to adapt. Equally indicative are the results regarding relationships with others (r=0.191, p=0.036), new opportunities (r=0.187, p=0.040), and appreciation of life (r=0.189, p=0.038). All these correlations indicate that resilience creates conditions for a constructive rethinking of traumatic experiences, which manifests itself in both the interpersonal and the personal-existential spheres. In fact, it is the combination of inner endurance and the ability to find meaning in difficult events that forms the basis for police officers to maintain psychological integrity in wartime conditions.

The situation is sharply contrasting in the CG of respondents – police officers whose activities take place in relatively safe conditions. None of the aspects of posttraumatic growth shows a statistically significant relationship with the level of resilience. The correlation coefficients fluctuate around zero, indicating the absence of a systematic relationship between these constructs in respondents not involved in combat operations. For example, the correlation between resilience and appreciation of life is only r=–0.023 (p=0.800), and between resilience and relationships with others, r=0.007 (p=0.943). This gap between the groups suggests that it is the extreme conditions associated with military events that actualize the latent potential of resilience as a resource for posttraumatic growth. In other words, under normal life circumstances, psychological stability primarily serves to maintain balance but does not necessarily become a driver of personal transformation.

The results of the correlation analysis presented in Table 3 reveal the peculiarities of the relationship between resilience, as measured by the Wagnild and Young Resilience Scale, and posttraumatic growth, as measured by the PTGI, in two contexts: among police officers directly involved in combat operations and among their colleagues who were not.

Table 1. Correlation relationship between resilience indicators on the Connor-Davidson Resilience Scale and the Wagnild and Young Resilience Scale in police officers of the EG (n = 60) and the CG (n = 60)

Wagnild and Young Resilience Scale	Connor-Davidson Resilience Scale	
	EG	CG
Overall level	r=0.326	r=0.172
	p=0.001	p=0.061

Legend: r – Pearson correlation coefficient value, p – statistical significance, critical correlation coefficient for p<0.05 is 0.179

Source: compiled by the authors of this study

Table 2. Correlation relationship between resilience indicators (according to the Connor-Davidson Resilience Scale) and posttraumatic growth in police officers of the EG (n = 60) and the CG (n = 60)

Posttraumatic growth scale	Connor-Davidson Resilience Scale	
	EG	CG
Relationships with others	r=0.191	r=0.007
	p=0.036	p=0.943
New opportunities	r=0.187	r=-0.017
	p=0.040	p=0.856
Personal strengthening	r=0.208	r=0.012
	p=0.023	p=0.895
Spiritual and existential changes	r=0.197	r=0.006
	p=0.031	p=0.946
Appreciation of life	r=0.189	r=-0.023
	p=0.191	p=0.007

Legend: r – Pearson correlation coefficient value, p – statistical significance, critical correlation coefficient for p<0.05 is 0.179

Source: compiled by the authors of this study

Table 3. Correlation relationship between resilience indicators (according to the Wagnild and Young Resilience Scale) and posttraumatic growth in police officers of the EG (n = 60) and the CG (n = 60)

Posttraumatic growth scale	Wagnild and Young Resilience Scale	
	EG	CG
Relationships with others	r=-0.055	r=0.176
	p=0.551	p=0.054
New opportunities	r=-0.063	r=0.151
	p=0.491	p=0.099
Personal strengthening	r=-0.063	r=0.157
	p=0.497	p=0.088
Spiritual and existential changes	r=-0.066	r=0.165
	p=0.474	p=0.072
Appreciation of life	r=-0.051	r=0.160
	p=0.582	p=0.081

Legend: r – Pearson correlation coefficient value, p – statistical significance, critical correlation coefficient for p<0.05 is 0.179

Source: compiled by the authors of this study

These data are indicative because they demonstrate how psychological resilience operates differently in situations of extreme risk than in relatively safe conditions.

From this, it can be concluded that none of the correlations in the EG were statistically significant, and all coefficients had a weak negative manifestation. This means that an increase in resilience, as measured by the Wagnild and Young Resilience Scale, is not associated with the strengthening of any aspect of posttraumatic growth, but, on the contrary, may even slightly reduce its expression. This result can be explained by the fact that resilience in the combat conditions of police work primarily serves a protective function, aimed at maintaining stability and minimizing the destructive effects of stress. Accordingly, individuals with high resilience in wartime may be less prone to experiencing transformational changes that are interpreted in civilian settings as "growth after trauma." In other words, resilience in the context of war is seen not so much as a source of development as a stabilizing mechanism that protects the individual from the need to undergo radical internal restructuring.

In contrast, other patterns can be observed in the CG. Although no statistically significant results were found here either, all correlation coefficients are positive and borderline significant, particularly for forms of posttraumatic growth associated with personal strengthening (r=0.157, p=0.088) and spiritual changes (r=0.165, p=0.072). This indicates that in conditions of relative safety, the resilience

of police officers is not only a factor in maintaining internal balance, but also a factor that opens up opportunities for a more profound processing of traumatic experiences. In the CG, police officers with higher levels of resilience tend to experience positive changes in their attitudes towards life, interpersonal relationships, and their own worldview.

DISCUSSION

Scientists [2, 3] note that irregular working hours, the need to resolve conflicts quickly, the processing of large volumes of documents, and interaction with people who have suffered trauma or loss have a constant psychological impact on modern police officers. In these conditions, stress resilience, the ability to regulate emotions, and maintain concentration even in critical situations are key [11, 20]. However, the socio-psychological context of martial law emphasizes that effective interaction with the population and colleagues, as well as compliance with the law, is complicated by many stressors; therefore, the development of professional stress resilience must be systematic. Thus, the socio-psychological peculiarities of police activities during wartime are formed as an integrative system of professional, emotional, and cognitive characteristics that determine effective functioning in high-risk, constantly stressful conditions. Therefore, it can be argued that in high-risk professions, including policing, professional activity can be a source of trauma.

At the same time, the uniqueness of the situation lies in the fact that the police officer simultaneously plays the role of a human rights defender, peacemaker, and, in certain circumstances, a direct participant in hostilities, which requires a high level of emotional stability, cognitive flexibility, and the ability to adapt quickly [13, 14]. Hence, the ability to model the development of events, predict citizens' behavior, and assess the potential consequences of decisions is a critical component of professional competence, as any mistake can have significant legal, social, and psychological impacts.

According to scientists [17, 21, 22], even under the most extreme developmental conditions, a person can maintain adaptability; therefore, researchers' attention should focus, among other things, on the mechanisms that enable modern police officers to overcome potential difficulties. Against this background, individual characteristics and behavioral strategies that interact with the body's biological systems to modulate responses to stress and traumatic events are essential. All of this, together with social support, provides a resource for overcoming difficulties and strengthens overall resilience. The psychological and behavioral dimensions of police resilience emphasize the importance of viewing it not only as a personality trait but also as a dynamic process of adaptation [23]. However, in our opinion, resilience encompasses not only the ability of law enforcement officers to adapt to internal and external circumstances but also the ability to recover from stressful or traumatic factors and maintain functioning under challenging conditions. Hence, resilience can act as an integrative mechanism that combines the physical, psychological, emotional, and social resources of a police officer's personality. However, as our results show, internal resilience becomes the basis for positive transformations, turning traumatic experiences into a resource for development.

As scientists rightly point out [24, 25], resilience is inextricably linked to the psyche's ability to recover from adverse conditions, and the key task of its study is to identify the mechanisms that allow modern police officers not only to withstand challenges but also to become psychologically stronger after experiencing crisis events. Our research confirms this: resilience, integrating various personality traits of modern police officers, ensures greater flexibility in coping and contributes to the formation of new meanings in life under extreme challenges. In turn, our data confirm the need for a comprehensive approach to the research and development of resilience in representatives of the police profession, whose activities are associated with a high level of danger and psychological stress.

CONCLUSIONS

It has been established that for police officers who are in the zone of military stressors, resilience not only provides the basic ability to withstand the negative consequences of traumatic experiences, but also acts as a mechanism that helps transform these experiences into a source of development. This confirms the idea that in wartime, it is the combination of inner endurance and the ability to rethink events that forms the basis for psychological adaptation and personal growth. In contrast, in conditions of relative safety, the phenomenon is contextually determined: resilience realizes its potential as a factor of posttraumatic growth only in specific and extreme socio-psychological conditions.

The comparison of data from the two groups of respondents demonstrates a fundamentally different nature of resilience. While in safe conditions it contributes to internal restructuring, which leads to personal growth, in combat conditions it rather serves as a resource for preservation, minimizing the impact of traumatic events but not stimulating posttraumatic growth. This ambivalence explains the contradictory nature of resilience: in situations of extreme danger, it acts as a "psychological bulletproof vest" that protects against destructive influences, yet can also limit the potential for personal transformation.

The results indicate the need to distinguish between the stabilizing and developmental aspects of police officer resilience, as this distinction significantly affects approaches to psychological support for law enforcement officers in conditions of martial law. In a stable environment, its growth may be directly related to processes of personal and spiritual development. At the same time, in a constant-threat situation, the main goal becomes maintaining mental integrity, even at the cost of delaying internal growth.

PROSPECTS FOR FURTHER RESEARCH

It is planned to investigate the impact of resilience on the performance indicators of police officers in a state of martial law.

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ORIGINAL ARTICLE

Administrative-legal assurance of the reliability and ethics of medical decisions in wartime

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ABSTRACT

Aim: This study aims to provide a comprehensive analysis of the administrative-legal assurance of the reliability and ethics of medical decision-making in wartime conditions, combining normative analysis with empirical data from a frontline Ukrainian hospital.

Materials and Methods: The normative framework includes international standards of the World Health Organization, the International Committee of the Red Cross, professional medical associations, the European Charter of Patients' Rights (2002), European Union legislation, and the national healthcare and wartime legislation of Ukraine. The empirical component comprised a cross-sectional anonymous survey conducted between May and September 2025 among 40 healthcare workers at a rural hospital located near the active frontline in the Dnipropetrovsk region. The questionnaire assessed decision-making complexity, perceived probability of errors, stress impact, resource availability, preparedness, and willingness to report incidents.

Results: Wartime conditions significantly increase the perceived complexity of clinical decision-making, particularly in resource allocation, evacuation prioritization, and surgical interventions. Respondents reported elevated stress levels and increased risk of errors, primarily associated with limited resources, disrupted logistics, and administrative constraints. The reliability and ethical integrity of medical decisions were found to depend on the availability of updated clinical protocols, state monitoring mechanisms, organized evacuation systems, and guaranteed minimum healthcare services.

Conclusions: Effective administrative-legal support is a prerequisite for ensuring both the reliability and ethical soundness of medical decisions in wartime. Strengthening institutional safeguards, resource allocation mechanisms, and clear regulatory guidance is essential to maintain medical standards under extreme conditions.

KEYWORDS: public interest, ethical standards, public administration

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INTRODUCTION

In wartime conditions, medical decision-making acquires a somewhat different meaning than in peacetime, as decisions are made under altered circumstances, including resource shortages, risks to healthcare workers, and restrictions on movement (mobility). These factors may affect not only the adoption of such decisions, but also their reliability, validity, and compliance with ethical standards.

The Ukrainian experience is one of the few contemporary examples of a healthcare system functioning during a large-scale war in Europe. According to the Ministry of Health of Ukraine, as of 1 August 2025, 2419 healthcare infrastructure facilities have been damaged or destroyed as a result of hostilities, including hospitals, outpatient clinics, primary care centers, and emergency medical stations. Numerous attacks on medical facilities, ambulance teams, and medical transport have been recorded, posing a direct threat to the lives of both patients and healthcare personnel [1].

In a joint statement, United Nations International Children's Emergency Fund (UNICEF), United Nations Population Fund (UNFPA), and World Health Organization (WHO) called for an immediate end to attacks on healthcare facilities, emphasising that such attacks "kill and cause severe injuries to patients and health workers, destroy vital health infrastructure, and undermine the population's ability to access essential care" [2]. These circumstances create an unprecedented context for medical decision-making, which often simultaneously has clinical, ethical, and administrative-legal dimensions [3, 4]. Under such conditions, the study of administrative-legal mechanisms ensuring the reliability and ethical soundness of medical decision-making during wartime becomes particularly important.

AIM

The aim of the article is to provide a comprehensive analysis of administrative-legal assurance for the reliability and ethics of medical decisions in wartime, combining regulatory analysis with an empirical survey of healthcare workers in a frontline Ukrainian hospital.

MATERIALS AND METHODS

The normative basis of the study consists of international standards of WHO, UNICEF, UNFPA, The International Committee of the Red Cross (ICRC), professional medical associations, the European Charter of Patients' Rights (2002),

European Union (EU) legislation, as well as the national legislation of Ukraine in the healthcare and legislation governing wartime conditions.

EMPIRICAL STUDY DESIGN

The empirical part of the study included a cross-sectional survey of healthcare workers at a small rural hospital located near the active front line in the Dnipropetrovsk region of Ukraine. The survey was conducted during May - September 2025 and aimed to assess how wartime conditions affect the reliability and ethical soundness of medical decision-making. The study didn't require ethics committee approval as it was an anonymous survey of healthcare workers regarding their professional experience.

PARTICIPANTS

A total of 40 healthcare workers participated in the survey, representing all categories of hospital personnel: doctors (n=8; 20%), nurses (n=20; 50%), paramedics (n=6; 15%), and administrative/other personnel (n=6; 15%). The average age of participants was (mean [M] ± standard deviation [SD]) 38.5±9.2 years (range: 24-58 years). The sample included 28 women (70%) and 12 men (30%), reflecting the gender distribution typical of healthcare institutions in Ukraine. The median length of professional experience was 7.5 years (interquartile range [IQR]: 4.0-12.0 years), with mean experience of 8.2±5.3 years.

DATA COLLECTION INSTRUMENT

Data were collected using an anonymous paper-based questionnaire developed specifically for this study. The questionnaire consisted of the following blocks:

1. Demographic information: age, gender, professional category, years of work experience.
2. Subjective complexity of decision-making: assessed on a scale from 0 to 10 for six types of medical decisions (resource allocation, evacuation prioritization, surgical decisions, documentation, ethical decisions, and diagnostics)
3. Self-assessed probability of errors: estimated as a percentage (0-100%) for each decision type
4. Impact of stress on performance: categorized as "high impact" or "low/moderate impact" for each decision type
5. Assessment of resource availability and level of preparedness for high-risk conditions: rated on a 5-point Likert scale (1=very low, 5=very high)
6. Willingness to report incidents: rated on a 5-point Likert scale (1=very unwilling, 5=very willing)
7. Open-ended question: "What are the major challenges you face in medical decision-making under wartime conditions?"

The questionnaire was pilot-tested with 5 healthcare workers from another facility and revised based on their feedback before final administration.

THE NORMATIVE FRAMEWORK AND EMPIRICAL DATA ANALYSIS

Descriptive statistics were used to analyze the data. Continuous variables with normal distribution were presented as M±SD, while non-normally distributed variables were presented as Me (IQR). Categorical variables were presented as frequencies and percentages. Dialectical, hermeneutic, comparative, analytical, synthetic and systemic analysis methods were also used for the theoretical and legal analysis of the normative framework.

ETHICS

Study was conducted in accordance with provisions of Helsinki Declaration of World Medical Association, Council of Europe Convention on Human Rights and Biomedicine, and Ukrainian legislation. Aggregated statistical data without disclosure of personal information were used.

FRAMEWORK

The study was performed within framework of initiative research work «Expert-diagnostic system for objectification of forensic medical examination of traumatic brain injury» (state registration number 0123U101528; term 2023-2026).

RESULTS

A medical decision is the result of professional medical judgment formed within state-defined administrative and legal procedures. It is subject to verification for accuracy, must be based on evidence and compliance with ethical standards, and, especially in wartime, is made in accordance with the principle of the maximum possible medical care under the specific circumstances.

The term "medical decision" is not explicitly defined in the legislation of most countries. A comparative legal analysis of the regulatory acts of the EU and Ukraine shows that its meaning depends on the legal constructs used by the legislator. Such constructs include informed consent, clinical protocols, standard of care, medical intervention, methods of treatment, patient rights, quality of care, etc. [5, 6].

It is appropriate to view a medical decision not as an abstract category but as a set of different types of decisions that a medical personal makes within the clinical process and that have distinct legal significance. The content of each type of decision is expressed through its form and consequences, namely:

1. Medical conclusion as the formal result of the decision. This is a legally significant document that officially records the outcome of the doctor's professional judgment (diagnosis, assessment of condition, need for treatment, prognosis, etc.). A medical conclusion is created according to a defined administrative procedure and is subject to control, verification, or expert review (which may include military medical, forensic medical, and other expert evaluations). in treatment tactics, granting disability status, etc.).
2. Medical intervention, which logically constitutes the legal consequence of the decision. These are actions or a set of actions implementing the medical decision (treatment, surgical intervention, administration of medicinal products or medical devices, resuscitation measures, etc.).
3. Patient management tactics. This represents the clinical content of the medical decision, reflecting the

prioritization or sequencing of actions, resource needs, and compliance with treatment standards. This category is linked to clinical protocols that determine the minimum requirements for diagnosis and therapy and should generally comply with evidence-based medicine. In wartime, however, a "clinical exception" regime may arise. triage, which prioritizes patients with the highest chance of survival given the available resources.

4. Professional medical judgment, which carries ethical and deontological content and represents the internal intellectual-ethical process through which medical personnel evaluate data, consider risks, interpret treatment protocols, and combine them with clinical experience and professional ethical standards. Professional judgment includes determining the reliability of clinical information under conditions of incompleteness while maintaining maximum adherence to ethical standards [7].

Administrative and legal mechanisms for ensuring the reliability and ethical soundness of medical decisions in wartime include: a) establishing unified rules of evidence-based medicine; b) regulating the use of clinical protocols; c) implementing standardized triage procedures; d) ensuring safe working conditions for healthcare personnel; e) maintaining independent ethics committees; f) guaranteeing the professional independence of healthcare workers and protecting them from liability for decisions made within the principle of the maximum possible medical care, among others [8].

The empirical survey revealed significant challenges in medical decision-making under wartime conditions. Table 1 presents the indicators of complexity, risk, and stress associated with different types of medical decisions.

As shown in Table 1, documentation (7.8±1.5), resource allocation (7.6±1.4), and ethical decisions (7.4±1.6) were perceived as the most complex types of decisions. These decision types also showed high self-reported error probabilities (21.8±8.2%, 21.3±7.9%, and 20.7±8.5%, respectively) and high percentages of respondents reporting significant stress impact (74%, 71%, and 59%, respectively).

The survey respondents reported low levels of resource availability (2.6±0.8 out of 5.0) and specialized training for work under high-risk conditions (2.9±0.9 out of 5.0). Willingness to report incidents was rated at 3.0±1.1 out of 5.0, indicating partial openness and the presence of barriers in internal quality control.

The empirical findings made it possible to identify the most vulnerable segments of medical decision-making in frontline healthcare facilities. These include documentation management, allocation of limited resources, and surgical and ethical decisions. These areas demonstrate the highest levels of complexity, combined with workload intensity, time deficits, and substantial stress.

The empirical basis of the study forms the foundation for further legal analysis of ways to mitigate the risks that arise in the work of frontline healthcare facilities. These measures include improving internal regulations, clinical protocols, control procedures, disciplinary mechanisms, accountability systems, and rules governing documentation and the circulation of personal data. The actual effectiveness of such elements in healthcare facilities located near active combat zones remains insufficiently explored [9].

DISCUSSION

Clinical decision-making combines a doctors' professional experience, the best available clinical evidence, and the individual needs and values of the patient [5]. Ensuring the reliability and ethical soundness of medical decisions in wartime conditions is based on a complex framework of international humanitarian law [6], acts of international organizations, and national administrative and legal mechanisms. At the international level, the Geneva Conventions (I-IV) of 1949 and the Additional Protocols of 1977 establish the obligation of states to guarantee non-discriminatory access to medical care, triage based solely on medical need, legal protection of medical personnel making decisions in good faith under extreme conditions, and the application of the standard of best possible care under the circumstances, that is, the best possible assistance given available resources.

The EU has developed an advanced regulatory system that guarantees patient rights and sets standards for accessibility,

Table 1. Indicators of complexity, risk, and stress in decision-making (n=40)

Decision Type	Mean Difficulty (0-10 scale)	Mean Self-reported Error Probability (%)	% Reporting „High stress impact"
Documentation	7.8±1.5	21.8±8.2	74.0%
Resource allocation	7.6±1.4	21.3±7.9	71.0%
Ethical decisions	7.4±1.6	20.7±8.5	59.0%
Surgical decisions	7.0±1.7	19.6±7.3	68.0%
Diagnostics	6.6±1.5	18.5±6.8	48.0%
Evacuation prioritization	6.2±1.6	17.4±7.1	45.0%

Note: Difficulty scores range from 0 (not difficult at all) to 10 (extremely difficult). Error probability represents respondents' subjective assessment of the likelihood of making an error in each type of decision

Source: compiled by the authors of this study

informed decision-making, voluntariness, and quality of medical care. The European Charter of Patients' Rights (2002) [10] enshrines rights essential for medical decision-making, including the right to accurate information, the right to free and informed consent or refusal of treatment, the right to confidentiality, and the right to access quality care consistent with standards and clinical protocols.

An important ethical and administrative guideline is the document Ethical Principles in Health Care in Times of Armed Conflict, adopted by the World Medical Association (WMA), the International Committee of Military Medicine (ICMM), the International Council of Nurses (ICN), and the International Pharmaceutical Federation (FIP) [11]. The document outlines a common denominator of ethical principles for health care applicable during armed conflict and other emergencies. These principles must be incorporated into administrative regulations and guidelines issued by national health authorities. In particular, a doctor acting within their discretionary authority should not be subject to administrative or criminal liability for failing to provide a "full" standard of care if decisions were made in good faith and with consideration of resource limitations. It remains essential to uphold the principles of humanity, non-discrimination, respect for patient dignity, the prohibition of torture, confidentiality, and equitable access to medical care [12].

WHO conducts regular monitoring of health systems in armed conflict zones. As of 2025, WHO's attention has been largely focused on Ukraine. According to Health Resources and Services Availability Monitoring System (HeRAMS), since 24 February 2022, 826 healthcare facilities in Ukraine have sustained infrastructure damage; in 88% of cases, the damage was conflict-related. Among undamaged or partially damaged facilities, 4% lost some degree of functionality. The main factors influencing facility functionality (and thus medical decision-making) include staffing shortages (51%), security issues (31%), and damage to facilities (26%). Sexual and reproductive health services, as well as maternal and newborn care, remain available in 87% of facilities. The highest levels of inaccessibility or partial accessibility of maternal and newborn services were recorded in Donetsk (49% available) and Kherson (46% available) regions. Regarding noncommunicable diseases, 9% of facilities reported service unavailability, with staffing shortages being the main barrier (30%) [13]. The situation is worse in Kherson region, where 40% of facilities reported unavailability of services [14].

CONCLUSIONS

1. The study demonstrates that the reliability and ethical soundness of medical decisions in wartime depend on a dual system of safeguards. Normative analysis shows that international humanitarian standards, national administrative regulation, and institutional governance mechanisms create the legal framework for decision-making. At the practical level, reliability is determined by the functioning of monitoring systems, availability of resources, staffing stability, evacuation logistics, and clear wartime protocols, while ethical integrity is supported by guarantees of minimum services, nondiscrimination, and continuity of care. The analysis confirms that without adequate administrative and legal support, physicians cannot realistically ensure either reliable or ethically sound decision-making in conflict settings.
2. The empirical survey confirms the analytical findings and shows that wartime conditions substantially increase decision-making risks. Respondents reported the highest vulnerability in documentation, resource allocation, and time-critical clinical decisions, where stress, safety risks, and workload pressures directly affect performance. These results indicate that legal regulation must move beyond declarative guarantees toward operational administrative tools, including adaptive protocols, simplified documentation standards for emergency conditions, stronger internal monitoring systems, and protected reporting procedures.

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ORIGINAL ARTICLE

Features of macrophage marker CD68 expression in the colonic mucosa of patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis

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ABSTRACT

Aim: The aim of the study was to determine the features of macrophage marker CD68 expression in the colonic mucosa of patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis.

Materials and Methods: The study material comprised colonic mucosal biopsies obtained from 12 healthy individuals (group 1), biopsies of the colonic mucosa from the diverticular orifice region of 34 patients with symptomatic uncomplicated diverticular disease (group 2), and 26 patients with acute uncomplicated diverticulitis (group 3). Immunohistochemical study was performed using a monoclonal antibody to CD68. CD68 expression was assessed by counting the absolute number of immunopositive cells in the field of view of the microscope ×400. In immunopositive cells, the degree of expression of the monoclonal antibody to CD68 was assessed by determining the brightness coefficient.

Results: Comparative analysis of the obtained results revealed an increased number of macrophages in the colonic mucosa of patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis, with the greatest increase observed in acute uncomplicated diverticulitis. The brightness coefficient decreased in patients with symptomatic uncomplicated diverticular disease and, more markedly, in acute uncomplicated diverticulitis, indicating increased expression of the studied monoclonal antibody by macrophages. In these patients, macrophages acquired elongated and irregular shapes and increased in size.

Conclusions: The mucous membrane of the colon in patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis is characterized by a higher content of macrophages, mainly in the lamina propria, which are characterized by increased morphofunctional activity. The latter manifests itself in increased expression of the monoclonal antibody to CD68 by macrophages, an increase in the size of $CD68^+$-cells, and their acquisition of an elongated or irregular shape. The quantitative and qualitative changes in macrophages in the colonic mucosa identified by the authors are more pronounced in patients with acute uncomplicated diverticulitis compared to patients with symptomatic uncomplicated diverticular disease.

KEYWORDS: macrophage, colonic mucosa, morphology, CD68, symptomatic uncomplicated diverticular disease, acute uncomplicated diverticulitis

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INTRODUCTION

Macrophages are the most abundant immune cells in the wall of the large intestine [1]. It has been shown that during the antenatal and intranatal periods, as well as immediately after birth, embryonic-derived macrophages are present in the large intestinal wall. Postnatally, with increasing age, embryonic-derived macrophages are gradually replaced by monocyte-derived macrophages [2].

Macrophages in the wall of the large intestine perform complex and diverse functions. They maintain tissue homeostasis by engulfing and destroying microorganisms that have penetrated the intestinal wall and by clearing it of dead cells. Macrophages can also capture and endocytose antigens and subsequently present them to T and B lymphocytes, thereby participating in the initiation of adaptive immune responses [3]. Macrophages produce numerous cytokines, chemokines, complement components, and enzymes that induce and regulate inflammatory processes [2, 3]. Macrophages also contribute to angiogenesis in the large intestinal wall, regulate intestinal secretion, and are involved in the repair of the damaged epithelial layer [1]. Macrophages influence colonic motility through interactions with smooth muscle cells and enteric neurons [4].

Macrophages are functionally plastic cells capable of changing their phenotype in response to microenvironmental conditions [2]. Generally, macrophages are classified into classically activated, proinflammatory, M1 macrophages and alternatively activated, anti-inflammatory, M2 macrophages [5-7]. Under physiological conditions, M1 macrophages exert bacteriostatic and cytostatic functions with antigen presentation, whereas M2 macrophages are responsible for tissue regeneration and repair. Changes in

the morphofunctional state of macrophages and disruption of the balance between their phenotypes may lead to the development of inflammation in the colon. Recent studies have demonstrated the significance of inflammation in the onset, progression, and outcomes of numerous colonic diseases, which constitute a substantial health burden for both women and men [8].

Diverticular disease is a significant global burden on healthcare systems, which is characterized by the formation of solitary or multiple hernia-like protrusions of the mucosal and submucosal layers through defects in the muscular layer of the colon [9, 10].

Diverticular disease is characterized by a range of clinical manifestations from uncomplicated symptoms to acute complications requiring surgery [11]. This pathology remains asymptomatic in the majority of people. About 20-25% of those people will develop symptoms without complications, experiencing the so-called «symptomatic uncomplicated diverticular disease», while about 5% of them will develop diverticulitis (uncomplicated or complicated) [12, 13]. The mechanisms underlying the development of diverticular disease, its progression, and the occurrence of complications remain the subject of extensive research. Most authors emphasize the importance of the traumatic, ischemic, and inflammatory concepts in the pathogenesis of diverticular disease [12]. Macrophages may play a significant role in the development of inflammation in the colonic wall in diverticular disease, as they are known to regulate the functional and metabolic activity of this typical pathological process. However, the available literature on the role of macrophages in the pathogenesis of inflammation in diverticular disease remains controversial. Moreover, there is a lack of published data regarding the evaluation of macrophages within the inflammatory cellular infiltrate of the colon in patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis.

AIM

The aim of the study was to determine the features of macrophage marker CD68 expression in the colonic mucosa of patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis.

MATERIALS AND METHODS

The study material comprised colonic mucosal biopsies obtained from 12 healthy individuals (group 1), biopsies of the colonic mucosa from the diverticular orifice region of 34 patients with symptomatic uncomplicated diverticular disease (group 2), and 26 patients with acute uncomplicated diverticulitis (group 3). In patients of group 3, biopsies were obtained 8 weeks after an episode of acute diverticulitis. Patients in groups 2 and 3 were treated at the Center of Gastroenterology and Endocrinology of Feofaniya Clinical Hospital of the State Administration of Affairs during the period of 2019-2022.

The collection of material for group 1 was carried out during autopsies at the Department of Pathology of the Municipal Non-Profit Enterprise of the Kyiv Regional Council «Kyiv Regional Clinical Hospital». Autopsy findings and histological examination of the autopsy material revealed no pathology of the gastrointestinal tract.

Colonic mucosal biopsies were fixed in 10% neutral buffered formalin (pH 7.4) for 24-48 hours. After fixation, the materials were processed using a standard protocol in an Excelsior AS apparatus (Thermo Fisher Scientific, UK) and subsequently embedded in paraffin blocks using a HistoStar embedding system (Thermo Fisher Scientific, UK). Serial histological sections 2-3 μm thick were cut from the paraffin blocks on a rotary microtome (HM 325, Thermo Scientific Scientific, UK). Sections were stained with hematoxylin and eosin.

Immunohistochemical study was performed on Super Frost Plus adhesive slides (Menzel, Germany). The Master Polymer Plus Detection system (Peroxidase, chromogenic DAB) (Master Diagnostica, Spain) was used, citrate buffer (pH 6.0) and EDTA buffer (pH 8.0) were used for high-temperature processing of antigen epitopes. Immunohistochemical study was performed using a monoclonal antibody to CD68 (clone KP1, Master Diagnostica, Spain) (a marker of macrophage cells). Microscopic examinations were performed using Carl Zeiss PrimoStar microscope with a built-in color digital camera Axiocam 208 color (Carl Zeiss, Germany).

CD68 expression in the colonic mucosa was assessed by counting the absolute number of immunopositive cells in the field of view of the microscope ×400. In immunopositive cells, the degree of expression of the monoclonal antibody to CD68 was assessed by determining the brightness coefficient in the Lab color model using the computer program «Analysis of color properties of raster images» [14].

The data in groups 1-3 was statistically processed using the PAST program (version 4.15, Natural History Museum, University of Oslo, Norway). The mean values of the indicators in the groups were compared using Student's t-test and Mann-Whitney U-test. Differences in indicators were considered significant at $p<0.05$. A scatter plot was used to illustrate the results obtained. An approximation model was constructed for the obtained indicators using Microsoft Excel 2024.

RESULTS

In group 1, on survey microscopy of the specimens, a focal-diffuse moderately expressed cellular infiltration was observed in the lamina propria of the mucous membrane between connective tissue fibers, in the lamina muscularis mucosae between connective and muscle fibers. The infiltration predominated in the lamina propria of the mucous membrane and was represented by fibroblasts, lymphocytes, plasma cells, eosinophils, and $CD68^+$-macrophages. The absolute number of $CD68^+$-macrophages was 21.5±0.73. Macrophages were characterized by round and elongated shapes, small size, and light-brown cytoplasmic staining (Fig. 1). The brightness coefficient in $CD68^+$-cells was 0.62±0.012. The immune cells identified within the cellular infiltrate are known to mediate immune defense mechanisms.

In group 2, focal cellular infiltration was detected in the epithelial layer of the colonic mucosa, whereas

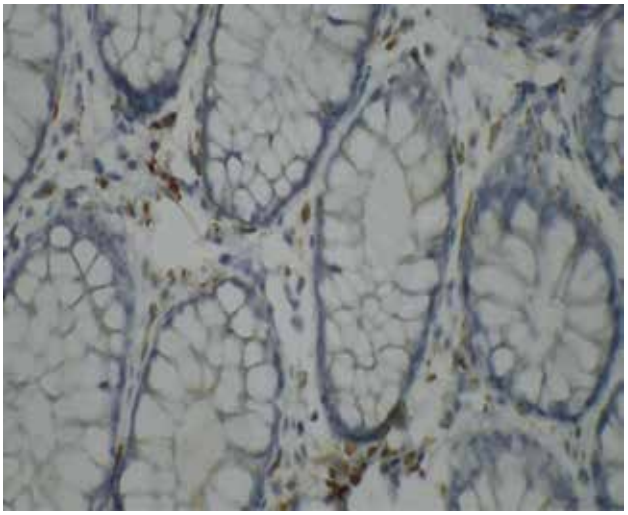

Fig. 1. Group 1. Macrophages in the lamina propria of the colonic mucosa. Immunohistochemical reaction with monoclonal antibody to CD68, × 400.
Source: Own materials

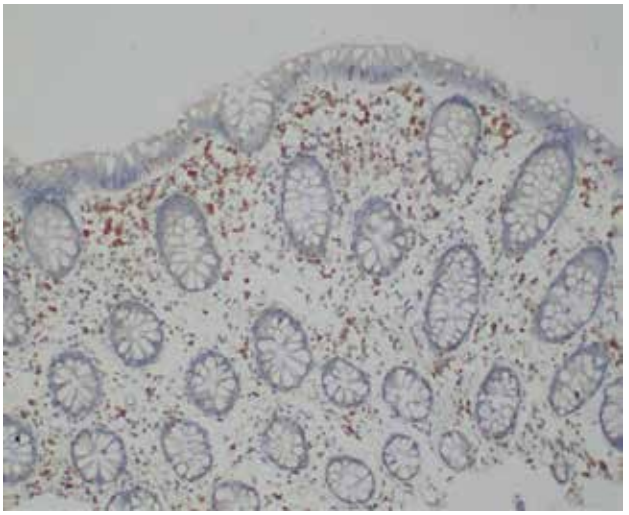

Fig. 2. Group 2. Macrophages as a part of the polymorphic cellular infiltration in the colonic mucosa. Immunohistochemical reaction with monoclonal antibody to CD68, × 400.
Source: Own materials

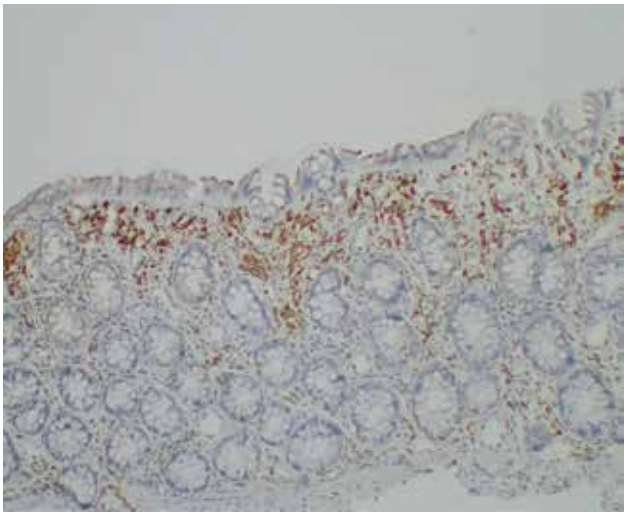

Fig. 3. Group 3. CD68+-cells in the mucous membrane of the colon. Immunohistochemical reaction with monoclonal antibody to CD68, × 400.
Source: Own materials

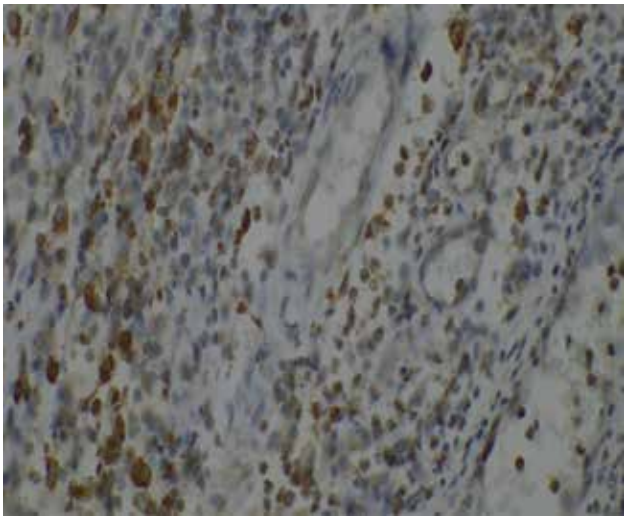

Fig. 4. Group 3. CD68+-cells in granulation tissue. Immunohistochemical reaction with monoclonal antibody to CD68, × 400.
Source: Own materials

diffuse cellular infiltration was observed in the lamina propria of the mucous membrane and lamina muscularis mucosae. The degree of cellular infiltration was maximal in the lamina propria compared with other layers of the mucosa. Compared with group 1, the cellular infiltration was more pronounced and consisted of similar cell types. In this group, macrophages expressing the monoclonal antibody to CD68 were larger than in group 1 and exhibited round, elongated, or irregular shapes with dark-brown cytoplasmic staining (Fig. 2). Compared with group 1, the absolute number of macrophages (48.9±1.17) increased ($p<0.05$), whereas the brightness coefficient in $CD68^+$-cells (0.53±0.010) decreased ($p<0.05$).

In group 3, polymorphic cellular infiltration of the colonic mucosa was observed in the epithelial layer, lamina propria of the mucous membrane and lamina muscularis mucosae. The infiltration was focal in the epithelium, diffuse in the lamina propria and muscularis mucosae, with the greatest severity in the lamina propria. The cellular infiltrate consisted of neutrophils, eosinophils, plasma cells, lymphocytes, fibroblasts, and macrophages. The degree of cellular infiltration was markedly greater than in groups 1 and 2.

In three cases of this group, granulation tissue of varying maturity was observed in some visual fields, filling erosive-ulcerative defects and characterized by different proportions of fibrous, vascular, and cellular components. The latter consisted of neutrophils, eosinophils, plasma cells, lymphocytes, fibroblasts and macrophages. Macrophages localized in the layers of the mucous membrane (Fig. 3) and in the granulation tissue (Fig. 4) were enlarged,

exhibited elongated and irregular shapes, and expressed the monoclonal antibody to CD68, as evidenced by intense dark-brown cytoplasmic staining.

The absolute number of macrophages was increased ($p<0.05$) compared to groups 1 and 2 and amounted to (68.4±1.33). The brightness coefficient in $CD68^+$-cells was (0.45±0.010), which indicated its decrease ($p<0.05$) compared to groups 1 and 2.

DISCUSSION

The study conducted by the authors revealed the peculiarities of the expression of the macrophage cell marker – monoclonal antibody to CD68 in the mucous membrane of the colon of patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis during examination microscopy, by counting the absolute number of $CD68^+$-cells and determining their brightness coefficient.

Comparative analysis of the obtained results revealed an increased number of macrophages in the colonic mucosa of patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis, with the greatest increase observed in acute uncomplicated diverticulitis. The brightness coefficient decreased in patients with symptomatic uncomplicated diverticular disease and, more markedly, in acute uncomplicated diverticulitis, indicating increased expression of the studied monoclonal antibody by macrophages. In these patients, macrophages acquired elongated and irregular shapes and increased in size. The observed decrease in the brightness coefficient of $CD68^+$-cells, together with changes in cell shape and enlargement, indicates activation of the morphofunctional state of macrophages in diverticular disease.

Subsequently, the results obtained in the groups allowed us to construct a scatter plot with a trend line, which is shown in Figure 5. A linear approximation model was constructed for the results in groups 1-3.

The trend line (regression) equation, as shown in Figure 5, was as follows: $y = -0.0037 \times x + 0.7015$, where «y» is the brightness coefficient and «x» is the absolute number of macrophages. As shown in the figure, with increasing absolute macrophage number, the brightness coefficient decreased; the coefficient of determination (R^2) was 0.9962. The coefficient of determination ($0 \le R^2 \le 1$), as is well known, characterizes the degree of agreement between experimental data and data obtained from the approximation model. The coefficient of determination equals the proportion of observed outcomes explained by the model equation. In our study, this corresponded to 99.62% of all observations.

The increase in the number of macrophages and the activation of their morphofunctional state in the mucous membrane of the colon in patients with symptomatic uncomplicated diverticular disease and, especially, acute uncomplicated diverticulitis, in our opinion, has not only physiological but also pathological significance. Macrophages in the colon wall in diverticular disease not only perform a protective function, contributing to the restoration of damaged structures, but also induce inflammation, causing the onset and progression of sclerosis. Sclerotic changes in the colon wall lead to degenerative changes and atrophy of muscle fibers in the lamina propria and muscularis. The qualitative and quantitative changes detected in the connective tissue cause the development of colonic diverticula [15, 16]. Sclerosis of the vascular walls also occurs, leading to impaired trophic support of all layers of the colonic wall, which may underlie the development of diverticular disease [17].

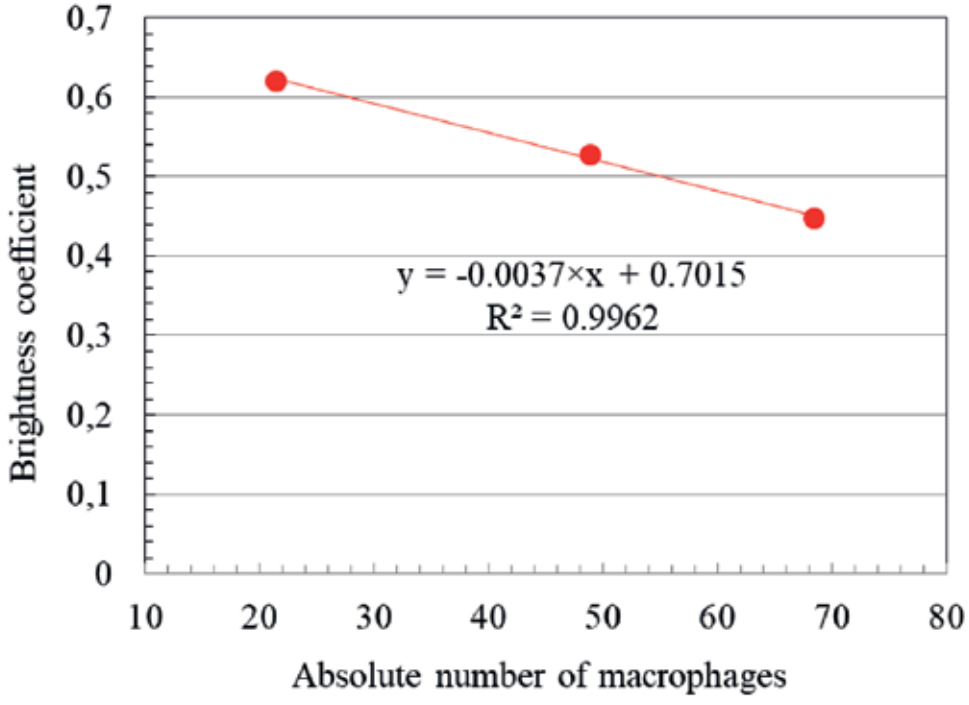

Fig. 5. Graph showing the dispersion of the brightness coefficient and the absolute number of macrophages with a trend line.

Source: Own materials

Macrophage dysfunction and imbalance between their phenotypes cause colonic motility disorders, increased intraintestinal pressure, muscle fiber divergence, and diverticulum formation [4]. Numerous studies have shown that impaired colon motility is the basis for the formation of diverticula [18, 19].

CONCLUSIONS

The mucous membrane of the colon in patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis is characterized by a higher content of macrophages, mainly in the lamina propria, which are characterized by increased morphofunctional activity. The latter manifests itself in increased expression of the monoclonal antibody to CD68 by macrophages, an increase in the size of $CD68^+$-cells, and their acquisition of an elongated or irregular shape. The quantitative and qualitative changes in macrophages in the colonic mucosa identified by the authors are more pronounced in patients with acute uncomplicated diverticulitis compared to patients with symptomatic uncomplicated diverticular disease.

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CONFLICT OF INTERESTS
The Authors declare no conflict of interest

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REVIEW ARTICLE

Special education for individuals with severe mental disorders: Definitions, discourses and contexts at the interface of pedagogy and psychiatry

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ABSTRACT

Aim: The aim of this paper is to define and discuss a new and emerging sub-discipline of special education, namely special education for individuals with mental disorders.

Materials and Methods: This paper is based on a narrative review and theoretical analysis of selected Polish and international literature from the fields of special education, psychiatry, and social sciences. The analysis included monographs and peer-reviewed articles addressing adult education, intellectual disability, autism spectrum disorders, and mental disorders, with particular emphasis on schizophrenia and affective disorders. A critical and comparative analysis of theoretical concepts and paradigmatic changes, especially the development of community-based models of care, was conducted.

Mental disorders constitute an important cause of disability in adulthood and create a new area of interest for special education. The development of community psychiatry and community mental health centers opens opportunities for special educators to work with adults with mental disorders as community therapists, social skills trainers, or recovery assistants. However, the establishment of this professional role requires legal regulations and interdisciplinary cooperation.

Conclusions: Special education pedagogy for people with mental disorders constitutes a new and essential subdiscipline of special education. The expansion of community psychiatry creates structural opportunities for the development of professional roles within this field. Whether community therapists evolve into formally recognized special educators for persons with mental disorders will depend on future legislative solutions, the engagement of universities in developing specialized training programs, and close cooperation between educators and psychiatrists.

KEYWORDS: special education, mental disorders, community psychiatry

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INTRODUCTION

Contemporary special education is predominantly focused on childhood and adolescence [1], with its theoretical foundations heavily relying on the pedagogy of persons with intellectual disabilities [2]. Considerably less attention is devoted to educational work with adults presenting various forms of disability [3, 4]. However, no type of disability that begins in childhood or adolescence ceases upon reaching adulthood; therefore, the focus of special education should also encompass the adult individual who has completed the period of compulsory education. While special education in adulthood may be referred to as special andragogy, and education for older adults as special geragogy, there is a significant gap regarding adults with mental health conditions that are not neurodevelopmental in origin [1, 5, 6, 7]. Principles of holistic pedagogy and social pedagogy suggest that education should be a lifelong process, yet this is rarely applied to psychiatric contexts [8, 9].

AIM

The aim of this paper is to define and discuss a new and emerging sub-discipline of special education: special education for individuals with severe mental disorders, more widely known as severe mental illnesses (SMI). This topic is of particular importance at present, as the dominant paradigm of community-based care in contemporary psychiatry necessitates a redefinition of the roles of the physician, special educator, psychologist, psychotherapist, occupational therapist, and social worker within the community care team [10]. All these professionals collaborate as equal members

Table 1. Mapping of relevant diagnostic categories in ICD-11 and DSM-5

Category	ICD-11 Classification	DSM-5 Classification	Typical Onset
Neurodevelopmental (Excluded from SMI scope)	Mental, behavioral or neurodevelopmental disorders (specifically: Disorders of intellectual development, ASD)	Neurodevelopmental disorders (ID, ASD)	Early Childhood
Psychotic (Focus of this article)	Schizophrenia or other primary psychotic disorders	Schizophrenia spectrum and other psychotic disorders	Early Adulthood
Mood / Affective (Focus of this article)	Mood disorders (Bipolar or related disorders, depressive disorders)	Bipolar and related disorders; Depressive disorders	Adulthood/ Adolescence

Table created by the authors based on conducted research and information from cited articles [16, 17]

of a therapeutic team, providing comprehensive support for individuals with mental disorders [11]. The ultimate goal is to achieve true inclusion, thereby preventing the social exclusion often experienced by this group [12].

MATERIALS AND METHODS

This paper is based on a narrative review and theoretical analysis of selected Polish and international literature from the fields of special education, psychiatry, and social sciences. The analysis included monographs and peer-reviewed articles addressing adult education, intellectual disability, autism spectrum disorders, and mental disorders, with particular emphasis on schizophrenia and affective disorders. A critical and comparative analysis of theoretical concepts and paradigmatic changes, especially the development of community-based models of care, was conducted.

Mental disorders constitute an important cause of disability in adulthood and create a new area of interest for special education. The development of community psychiatry and community mental health centers opens opportunities for special educators to work with adults with mental disorders as community therapists, social skills trainers, or recovery assistants. However, the establishment of this professional role requires legal regulations and interdisciplinary cooperation.

To ensure conceptual clarity and clinical precision, this article explicitly limits its scope to psychotic disorders (especially schizophrenia spectrum disorders) and mood disorders (bipolar and depressive disorders). We differentiate these conditions from neurodevelopmental disorders (such as intellectual disability – ID, and autism spectrum disorder – ASD), which already possess well-established pedagogical methodologies [13, 14]

REVIEW AND DISCUSSION

DIAGNOSTIC CONTEXT: MENTAL DISORDERS *VS* NEURODEVELOPMENTAL DISORDERS

The establishment of the identity of this sub-discipline necessitates the utilization of precise medical terminology in accordance with international standards. In historical pedagogical discourse, various forms of "mental deviation" were often conflated, leading to methodological confusion [15]. However, the prevailing nosology, specifically the International Classification of Diseases 11th revision (ICD-11) and Diagnostic and Statistical Manual of Mental Disorders (DSM-5), demands a clear distinction [16, 17].

According to the ICD-11, the overarching category is "Mental, behavioral or neurodevelopmental disorders" [16]. Within this framework, it is imperative to differentiate between the following:

- Neurodevelopmental disorders (e.g., ID, ASD) manifest typically during the developmental period. The pedagogy of these groups, including legal frameworks for autism and methodologies for ASD, is distinct from the needs of SMI [14, 18].
- Severe mental disorders (SMI), including but not limited to schizophrenia and bipolar disorder, have been observed to manifest predominantly during early adulthood [4].
- Multiple disabilities, where conditions co-occur, requiring complex pedagogical approaches [19]

The distinctions between these categories are illustrated in Table 1, with the aim of preventing the conceptual mixing of these distinct categories.

THE BIOPSYCHOSOCIAL MODEL AND FUNCTIONAL DISABILITY

Historically, disability has been viewed through a reductionist lens, which has been criticized as excessive medicalization [20]. However, in accordance with the World Health Organization's International Classification of Functioning, Disability and Health (ICF), disability is not an inherent attribute of the person, but rather the outcome of the interaction between a health condition and contextual factors (environmental and personal) [21]. Within the paradigm of SMI, disability is not conceptualized as a "failure of the mind"; rather, it is understood as the consequence of barriers to participation and activity limitations [21]. Comprehensive understanding of the subjective experience of disability has been identified as being of crucial importance [22]. In contrast to individuals with congenital ID, those with SMI frequently undergo a disruption in their biological trajectories, often resulting in the loss of previously established social roles, employment opportunities, and interpersonal relationships due to the onset of illness [4]. Consequently, the pedagogical objective transcends mere habilitation, encompassing the principles of rehabilitation and the restoration of social functionality [23].

SEVERE MENTAL DISORDERS AS A CHALLENGE FOR SPECIAL EDUCATION

Psychotic and affective disorders typically emerge during early adulthood [24]. For instance, schizophrenia affects approximately 1% of the adult population, and mood disorders affect a significant percentage of adults [25]. Historically, individuals with such conditions were confined to "total institutions" or relegated to social welfare homes, where educational activity was minimal [11, 26]. Whilst these conditions are frequently chronic, contemporary psychiatry disavows the deterministic perspective of them as unavoidably "lifelong" deteriorations [27]. Instead, it emphasizes the paradigm of recovery, defined as a process through which individuals can live satisfying, hopeful, and contributing lives even with the limitations caused by the illness [28].

Despite the prevalence of SMI being comparable to that of ID, the literature on special education for this group is scarce [4]. Existing paradigms of special education, which are often derived from work with profound intellectual disabilities, are frequently inadequate for the SMI population [29]. This discrepancy can be attributed to the fact that individuals diagnosed with SMI generally engage with the formal education system prior to the manifestation of their condition. Consequently, no distinct sub-discipline of special education has emerged for them, and ther is a lack of legislative frameworks for pedagogical support in adulthood [30].

THE IDENTITY OF SPECIAL EDUCATION PEDAGOGY FOR PERSONS WITH MENTAL DISORDERS

The transition in psychiatry from institutional to community-based care mirrors the processes of normalization and emancipatory paradigms observed in the field of special education [31]. Community psychiatry is predicated on the premise that treatment is most efficacious within the individual's living environment. Nevertheless, the effective delivery of community care necessitates a multidisciplinary approach, which must also encompass the family context, which is frequently impacted by the behaviour of a relative [10, 32].

At present, educational and rehabilitative tasks in community settings are dispersed among psychologists, occupational therapists and social workers. There is an evident need for a specialized professional: a Special Educator for Mental Health. The competencies of this role would serve to bridge the gap between medical treatment and social inclusion. The position would integrate the following:

- The provision in training in social skills.
- The provision of support with a focus on recovery [10].
- Psychoeducation and community therapy [3].

A professional of this kind would assist adults with SMI in "regaining the authorship of their own lives" [33], adapting the humanistic paradigms of special education to the specific needs of the psychiatric population. This perspective is consistent with the notion that special education must perpetually pursue the "escaping subject" of its inquiry, demonstrating adaptability to emerging social realities [23].

CONCLUSIONS

The field of special education pedagogy for individuals with SMI has emerged as a novel and pivotal subdiscipline. The basis of this study is twofold: firstly, it is grounded in modern medical classifications (ICD-11/DSM-5), and secondly, it is anchored in the biopsychosocial model of disability. The development of community psychiatry has created structural opportunities for this profession, emphasizing the complementarity between psychiatry and special pedagogy. It is imperative that future legislative solutions and academic programmes acknowledge the distinctive educational and rehabilitative requirements of adults with SMI, shifting the discourse beyond the emphasis on childhood neurodevelopmental disorders.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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REVIEW ARTICLE

Effectiveness and safety of antiestrogens in the treatment of functional hypogonadism in men with obesity

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ABSTRACT

Functional hypogonadism in men is a syndrome characterized by low testosterone levels and clinical signs of hypogonadism without organic disease of the hypothalamus-pituitary-gonadal axis. It is most often caused by obesity, diabetes type 2, exogenous steroid abuse, excessive physical exercise, opioid use, co-occurring diseases, and is most prevalent among middle-aged and older men as so-called late-onset hypogonadism. It seems that the most important epidemiological factor causing functional hypogonadism is excess adipose tissue. Obesity is currently one of the most important public health problems and its prevalence is higher in men than in women. Complications of obesity include not only cardiovascular diseases or type 2 diabetes mellitus, but also lead to decreased testosterone levels, reduced libido and erectile dysfunctions, and impaired fertility in men. These disorders in men with obesity are associated with decreased gonadotropins and testosterone secretion due to hyperinsulinemia, hyperleptinemia, chronic inflammation and oxidative stress. Currently, it seems that an important complication of obesity is the deterioration of semen parameters (sperm concentration, motility, and morphology) and reduced fertility. Theoretically, obesity-related functional hypogonadism in men is reversible, so the basis of initial treatment is primarily lifestyle modification and effective treatment of chronic diseases but primarily reducing body fat mass (physical exercise and low-caloric diet). Unfortunately, lifestyle changes are often insufficient to significantly increase testosterone levels and resolve the clinical symptoms of hypogonadism. Because in men seeking fertility conventional testosterone replacement therapy is contraindicated, alternative pharmacological methods, such antiestrogens (selective estrogen receptor modulators and aromatase inhibitors) are often used.

KEYWORDS: men, obesity, functional hypogonadism, aromatase inhibitors, selective estrogen receptor modulators

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INTRODUCTION

Functional male hypogonadism (FH) is a clinical syndrome caused by a dysfunction of the hypothalamic-pituitary-gonadal (HPG) axis, which leads to decreased testosterone (T) secretion and impaired spermatogenesis [1]. FH is recognized in patients without apparent organic disease of the HPG axis, and in young and middle-aged men is observed in such conditions as overweight and obesity, type 2 diabetes, exogenous steroid abuse, excessive physical exercise, prolongated stress, and others chronic comorbidities [2]. For several years also the so-called late-onset hypogonadism (LOH) occurring in older men and caused by the ageing process is classified as FH. However, it seems that excess adipose tissue and obesity in men are the main causes of FH, which is why the term Male Obesity Secondary Hypogonadism (MOSH) is often used [3]. The prevalence of FH varies from 2.1% to 12.3% [4] and although it is a common disease and treatment recommendations have been developed, it still raises much controversy, particularly regarding therapeutic management.

The data obtained by the National Institute of Public Health in Poland reported in 2020, that overweight was observed in 64% and obesity in 12% men [5], so they are an increasingly common health problem in men and can cause endocrine disorders, and are a risk factor for associated comorbidities, like type 2 diabetes, atherosclerosis and cancer.

FH is a potentially reversible syndrome, therefore lifestyle modification and effective treatment of chronic diseases [6]. However, it seems that a significant reduction in the amount of adipose tissue (physical exercise and low-caloric diet), is the most important guarantee of resolving the symptoms of FH and increasing testosterone (T) levels. It may also have a beneficial effect on their fertility.

Unfortunately, lifestyle changes are often insufficient to significantly increase T levels and resolve the clinical symptoms of FH. Recently, much attention has been paid to the importance ofglucagon-like peptide-1 (GLP-1) analogues in the treatment of FH, and they effectively reduce fat mass, but their effect on fertility remains unclear. Because in men seeking fertility conventional testosterone replacement therapy is strictly contraindicated, alternative pharmacological methods, such selective estrogen receptor modulators (SERMs) and aromatase inhibitors (AIs) are often used. SERMs and AIs might be especially beneficial for young and middle-aged men desiring fertility. The aim

of this review is to update information about SERMs and AIs in the treatment of male hypogonadism.

AIM

The aim of this review is to update information about SERMs and AIs in the treatment of men with obesity and FH.

MATERIALS AND METHODS

A systematic research was performed using the electronic database PubMed in January 2025. Search terms covered all the research on the intended population (men with functional hypogonadism and obesity or hypogonadism and impaired fertility) combined with the intervention (aromatase inhibitors and selective estrogen receptor modulators). On purpose, search terms for outcomes were not used, to search all possible results. We included studies published in the last 20 years (2004 to 2024) and applied the following filters: English publication, human study, and only male subjects. The initial search yielded a total of 34 clinical trials, and 7 a systematic review and meta-analyses. The results were screened using the following criteria: (1) hormonal assessment and preferably evaluation of symptoms of hypogonadism during treatment of hypogonadal men with obesity; and (2) influence of SERMs and AI of sperm parameters in men with impaired fertility and/or functional hypogonadism. Further screening of the retrieved titles were excluded 23 of clinical trial for the following reasons: very small group (11), group heterogeneity (8), and inconclusive diagnosis of functional hypogonadism or obesity (4). After verification, 11 clinical trials and 7 systematic reviews and meta-analyses were qualified for final analysis and discussion.

REVIEW AND DISUSSION

PATHOGENESIS OF FUNCTIONAL HYPOGONADISM IN MEN WITH OBESITY

FH is defined as the low serum T concentrations occurring in the absence of intrinsic structural HPT axis pathology and specific pathologic conditions suppressing the HPT axis in middle-aged or older men" [1, 6]. The European Male Aging Study revealed that T levels decline with age, comorbidities (e.g., obesity) may further reduce T concentration [4]. FH is potentially reversible, if the underlying causes are removed or treated, and the most important cause of FH is obesity (MOSH), but in practice the term FH is a complex concept and also includes other causes of T deficiency such as advancing age, stress, excessive sport, and comorbidities.

It was shown, that men with a body mass index (BMI) of 35-40 kg/m2 can have up to 50% less free and total T when compared to age matched men BMI <25 kg/m2 [6], and an 8.7-fold increase in risk of hypogonadism in men with a BMI >30 kg/m2 has been demonstrated [7].

In men with obesity, excess adipose tissue negatively affects the central and peripheral HPG axis, leading to hypogonadotropic hypogonadism. This unfavorable phenomenon is caused by excess estradiol (E) produced in adipose tissue. In men with BMI >30 kg/m2, the increased fat mass leads to enhanced aromatase activity, resulting in elevated E levels, which reduce pulsatile GnRH secretion by hypothalamus and in consequence, reduction of luteinizing hormone (LH) secretion in pituitary. Finally, T secretion from tests is decreased. In the other hand, low T levels may directly upregulate adipogenesis and increase subcutaneous, ectopic, and visceral fat [8]. But increased aromatization of T to E in fat tissue seems to be the main but not the only mechanism underlying causes of FH in obesity, because it has been observed, that reduced T is accompanied by decreased E levels in obese and diabetic men.

The next potential mechanism leading to FH in obese men is associated with sex hormone binding globulin (SHBG). E2 stimulates the hepatic synthesis of SHBG, which is important in T action. In the circulation, about 58% of T is bound SHBG, 40% to albumin (40%), and only 1-2% it is free T, which acts on tissue androgen receptors. Therefore, in men with elevated E levels (in obesity), a decrease in free T levels and signs of hypogonadism are often observed. But again, in men with obese this phenomenon is partially compensated by low SHBG levels. These decreased SHBG levels are likely related in part to insulin resistance (hyperinsulinemia) and in part to increased proinflammatory cytokines (IL-6 and TNF-α) in obese men. The cytokines interfere with the HPG axis at the hypothalamus level by impairing GnRH, contributing to a further decrease in T [9].

In obese men with body composition disturbances (multipotential mesenchymal cells of fat tissues are preferentially differentiate to adipocytes instead to myocytes, hyperinsulinemia and insulin resistance are a common phenomenon, which also inhibits hypothalamic kisspeptin-dependent signaling and reduces pulsatile gonadotropin-releasing hormone (GnRH(secretion leading to FH [10]. Also, leptin is the next factor associated with FH risk in obese men, which leptin produced by adipocytes, and acts to regulate energy balance ensuring reduced fat storage. The role of leptin is to stimulate kisspeptin neurons which, in turn, stimulate GnRH secretion, while T inhibits the secretion of leptin in adipocytes and regulates T-leptin-kisspeptin-GnRH feedback [11]. However, in obese men leptin secretion is increased and a central leptin resistance at the hypothalamo-pituitary level is observed. Thus, hyperinsulinism and leptin resistance are important causes of FH in men with obesity.

In summary, it is currently believed that the key element directly regulating GnRH secretion is kisspeptin, which through specific receptors (KISS1R) in the hypothalamus regulates the secretion of T and also spermatogenesis. Leptin resistance, hyperinsulinism and hyperestrogenism cause disorders of the kisspeptin-GnRH axis and, consequently, a decrease in T synthesis in obesity. However, it should be remembered that obesity is a risk factor for FH, but hypogonadism promotes obesity leading to a vicious cycle, and the most effective way to increase T levels is to reduce body fat mass.

However, it should be emphasized, that a single mechanism is almost never responsible for the symptoms of FH in obese men. Usually, a combination of several factors (besides excess body fat) is observed.

Currently, such a common mechanism is the adverse effects of chronic stress, which can also cause FH, because it activates the hypothalamic-pituitary-adrenal axis and, consequently, increases the release of corticotrophin-releasing hormone (CRH), and as a result, increases secretion of adrenocorticotrophic hormone (ACTH) from the pituitary. This mechanism leads to increased secretion of glucocorticoids from the adrenal glands. Because both axes, HPT and HPA, are functionally closely related, elevated cortisol suppress GnRH secretion, and finally LH, follicle-stimulating hormone (FSH) and T levels are decreased [12]. Also, kisspeptin, a key regulator of GnRH release, is sensitive to stress signals, and the suppression of kisspeptin neurons by stress-related factors, not only cortisol but also by inflammatory cytokines, disrupts HPG axis function and leads to FH.

Obesity is often not the only chronic health problem in men. Other conditions such as cardiovascular diseases, type 2 diabetes, kidney, and liver diseases, as well as chronic obstructive pulmonary disease (COPD) as well as sleep apnea syndrome, often co-occur in men with obesity and may cause or exacerbate the symptoms of FH. These conditions are often observed in middle-aged and elderly men, and associated with elevated levels of pro-inflammatory cytokines, leptin and insulin resistance and exacerbate the decline in T levels [1].

One of the significant causes of FH is excessive or irrational physical activity. In obese men, it does not play a significant role, as obese men are usually characterized by a lack of adequate physical activity. However, some men, despite being significantly overweight, attempt to reduce body fat mass through excessively intense (for their abilities) physical activity. This phenomenon may also exacerbate FH in obese men, but the mechanisms underlying this process are not fully understood in men. Probably, as in younger and non-obese men, the relative energy deficiency in sport (RED-S), resulting from restrictive diets and/or intense exercise syndrome may play a role (53). Now the more recent definition - low energy availability (LEA) – is often used, and it was observed, that FH secondary to LEA, with decreased LH, FSH, T and sperm count, may develop after >10 hours a week of exercise or weight loss of >10% in men [13]. The increased risk of FH in men with LEA appears to be related to the suppression of GnRH stimulation via kisspeptin.

As mentioned earlier, T secretion decreases with age with an annual drop of 0,4% in total T and 1,3% in free T, beginning in the fourth decade of life (44). Late-onset hypogonadism (LOH) is another factor that further exacerbates testosterone deficiency in obese men, and is caused by the age-related, gradual deterioration of all components of the HPG axis. With age, we often observe impaired pulsatile release of GnRH and the hypothalamus and pituitary become less responsive to circulating T and E levels, and secretion of LH and FSH in decreased [1, 9-10].

Also, insulin and leptin sensitivity decline with age, which further exacerbates the changes observed in obese men. On the other hand, Leydig cells in the testicles undergo functional and structural changes with age, including a reduction in their number and efficiency.

DIAGNOSIS OF FUNCTIONAL HYPOGONADISM IN MEN WITH OBESITY

FH should be diagnosed only on the basis of the presence of clinical symptoms and signs of T deficiency in combination with consistently low morning serum T concentrations, but FH usually presents a more subtle, non-specific clinical symptoms [6]. Furthermore, FH symptoms often overlap with those arising from co-morbidities and the effects of ageing in older men. It is currently believed that the „classic" symptoms of FH are decreased libido, decreased frequency of morning erections and erectile dysfunction [4].

According of the European Academy of Andrology, the most important is the recognition of obesity, particularly World Health Organization (WHO) class III, as a major and increasingly common cause of low T levels in middle-aged and elderly men, since this is reversible after weight reduction [6]. Therefore, a reduction in adipose tissue mass is necessary and often sufficient to increase T levels.

Clinical diagnosis of FH should be confirmed by fasting morning blood samples obtained on two different days. Hypogonadism is highly unlikely with T levels >12 nmol/L (>350 ng/dL) but more likely in patients with T concentrations <8 nmol/L (<231 ng/dL). In those patients with T levels between 8-12 nmol/L (231-350 ng/dL) free T (fT) or calculating fT measurements can often be helpful, especially in men with conditions that alter SHBG levels [6]. As pointed earlier, obesity is frequently associated with insulin resistance, low SHBG concentration and hence low total T (but fT remains normal). On the other hand, elevated SHBG (liver disease and old age) may lead to high or normal total T, potentially masking the diagnosis of hypogonadism.

FH should be diagnosed after exclusion of organic causes of hypogonadism. In addition to total T, also LH levels should be measured in all patients with suspected FH to differentiate the primary from secondary causes. From the point of view of pathophysiology, most patients with FH have secondary or mixed hypogonadism with low to normal LH [4,6]. But it should be emphasized that if the T concentration is at the lower limit of normal or slightly below normal and the LH concentrations are normal, then in reality LH level is inappropriately low, the feedback mechanism is disturbed and, in such patients, we also can recognize FH. Low T concentration accompanied by elevated LH and FSH is characteristic to classical primary hypogonadism due to tastes failure.

MRI scanning of pituitary should be considered in patients with visual disturbances, headache, hyperprolactinemia, or T levels <6 nmol/L (<175 ng/dL). However, in the majority of patients with acquired and reversible FH, no abnormalities are observed on pituitary MRI.

TREATMENT OF FUNCTIONAL HYPOGONADISM IN MEN WITH OBESITY

Lifestyle changes

As first-line treatment, lifestyle changes, particularly specialized dietary interventions and structured physical

activity, should be preferred in obese men with FH, since weight loss may increase total testosterone levels in a significant percentage of patients. Clinical evidence demonstrates that weight loss – whether obtained by a low-calorie diet, bariatric surgery, or physical activity – improves T levels proportionately to the degree of weight reduction [2.6]

Dietary Interventions

The primary goal of dietary management is the reduction of visceral adipose tissue, which is metabolically active and contains high levels of aromatase enzyme. To reduce suppressing effect of aromatase on testosterone production, a deficit of 300-800 kcal/day is recommended, with a particular focus on:

- low glycemic load – reducing consumption of simple sugars and highly processed carbohydrates is crucial to mitigate hyperinsulinemia. High insulin levels acutely suppress GnRH secretion and decrease the production of SHBG in the liver, leading to lower total T levels.
- high protein intake – maintaining a high protein intake (1.5-2 g/kg of body mass) helps preserve lean muscle mass during the process of weight loss, which is vital for maintaining a healthy basal metabolic rate.
- healthy fats – diets should not be overly restrictive in healthy fats (monosaturated and Omega-3), as cholesterol is the basic structural precursor for steroidogenesis.

Physical activity

Maintaining physical activity is recommended as an addition to the diet. Not only it increases the energy expenditure, but also it directly modulate hormone sensitivity. It is worth distinguishing two types of physical activity:

- resistance training: Strength training, especially using moderate to heavy weights with emphasis on progressive overload in particularly effective in men with FH. It increases the expression of androgen receptors in muscle tissue and stimulates the acute release of growth hormone and testosterone.
- cardiovascular training: Aerobic and anaerobic activities should be implemented, either as High-Intensity Interval Training (HIIT) or as more steady activities such as running or walking. It is worth noting that moderate-intensity training should ideally be performed within the second heart rate zone (Zone 2), typically corresponding to 60-70% of the maximum heart rate (approximately 100-140 BPM for the majority of the adult population). This type of activity has been shown to improve insulin sensitivity, thereby mitigating the inhibitory effects of hyperinsulinemia on the HPG axis. Furthermore, consistent cardiovascular exercise contributes to increasing the basal metabolic rate (BMR) and facilitates a shift in body composition by reducing visceral adiposity, which is primary driver of FH.

However, it is worth noting that overtraining should be avoided. It is essential to balance intensity, because an excessive chronic endurance exercise without adequate recovery can lead to an increase in cortisol, which antagonistically suppresses T production.

Also, withdrawal or modification of drugs (opiates, anabolic steroids, and glucocorticoids) interfering with T production are recommended. For men practicing sports (competitively or recreationally), it is particularly important to avoid or discontinue of anabolic-androgenic steroids (AAS).

ANTIESTROGENS

Antiestrogens (estrogen antagonists or estrogen blockers) prevent E2 from mediating their biological effects in the body. They act by blocking the estrogen receptor (ER) and/or inhibiting or suppressing E2 synthesis. In men two types of antiestrogens are often used: selective estrogen receptor modulators (SERMs) like tamoxifen or clomiphene, and aromatase inhibitors (AIs) like anastrozole or exemestane.

SERMs and AIs have been used for many years in patients with FH and idiopathic infertility (oligoasthenoteratozoospermia) as off-label treatment or so-called "empiric" treatment of these conditions. Although the use of these drugs in men is often criticized due to the insufficient number of RCTs, inadequate outcome data, short duration of the trials as well as small numbers of subjects enrolled, are frequently used and we have extensive experience in their effectiveness and safety of therapy [14].

Selective estrogen receptor modulators

Clomiphene citrate (CC) and tamoxifen stimulate endogenous T production by blocking estradiol-receptors (ERs), thereby reducing negative feedback on the HPG axis. Many studies have reported a significant increase in T levels with these drugs in men with secondary hypogonadism, like in men with FH. The underlying mechanism of action is the stimulation of endogenous T synthesis through elevating LH, FSH levels, thus these drugs stimulate the HPG axis. However, data supporting their efficacy and safety in alleviating hypogonadal symptoms has so far been inconsistent.

In a study performed in 292 men with obese men with FH treated with CC or enclomiphene citrate in dose 25 to 50 mg every day for 1.5-4 months a significant increase in total serum T levels both with CC and enclomiphene citrate were observed [14]. In another study a total of 153 men with FH, CC significantly increased TT, fT, and LH levels, and in 74% of the patients alleviation of hypogonadism symptoms was observed [15]. This study was long-term (up to 8 years) and allowed for the assessment of the safety of the therapy. Only few side effects were reported during the trial and no significant changes in PSA or hematocrit were observed. Also an earlier study evaluating long term safety and efficacy of CC treatment in a total of 400 men with FH for a mean of 25.5 months (range 0 to 84) showed that in men receiving CC for more than 3 years 88% achieved eugonadism, 77% reported improved symptoms and only 8% reported side effects (changes in mood, blurred vision and breast tenderness) with no severe adverse events. So, this data suggests that CC might be a safe and effective option for long-term treatment of male hypogonadism [16].

Also, clomiphene treatment was associated with a significant increase in T levels in patients with normal or minimal increased baseline gonadotropin levels [18]. However, in other study it was observed, that LH in the lower normal range was

predictive for a better T response to CC, an observation similar to an earlier study (68). So, it appears that patients with slightly elevated gonadotropin levels also benefit from CC treatment. This is a remarkably interesting observation, because patients with FH do not always have reduced LH levels. Often, the concentration is normal or even slightly elevated (but usually < 12 IU/l). It is important to remember that LH is secreted 24 hours a day, and a single morning LH measurement is not always predictive of CC treatment effectiveness, and baseline E levels also cannot be used to anticipate treatment response to CC in hypogonadal men [19].

These observations indicate that the qualification of patients with FH for SERMs treatment should be based mainly on morning TT or fT levels and the presence of clinical symptoms of hypogonadism, while LH and E2 levels should not be the decisive indication for treatment.

Data on the effectiveness of SERMs in increasing total testosterone (TT) levels and FH treatment are provided by meta-analyses conducted primarily in men with fertility disorders. In a meta-analysis which included 18 studies for analysis (n = 731) and 15 studies for meta-analysis (n = 566) TT, FSH and LH concentrations were higher during therapy and side effects included headache, gynecomastia, dizziness, visual changes, mood swings and fatigue were observed, however, the severity of these symptoms was mild and decreased after reducing the CC dose [20].

Also a meta-analysis of 14 randomized trials evaluating four treatments of impaired fertility in men (CC, tamoxifen, AIs, and anti-oxidants) and their combinations in 1342 men concluded that CC was the most effective in increasing T levels and sperm concentration, though none of the treatment modalities proved superior to placebo, but the overall quality of included studies was low [21].

The improvement in semen parameters shown in the discussed meta-analyses only indirectly proves the effectiveness of SERMs in the treatment of FH, but the hormonal mechanism of spermatogenesis is based on the action of gonadotropins and a sufficiently high concentration of T in the testicles. Therefore, hormonal changes observed in these analyses should be considered beneficial, especially in obese patients with FH.

Studies comparing testosterone replacement therapy (TRT) and SERMs treatment in patients with FH have also been conducted, and the authors concluded, that both are similarly efficacious for increasing serum TT, and patients on CC and T gels reached comparable T levels and reported improvement of hypogonadism symptoms similar to that of men treated with T injections [22]. However, TT levels are more stable during SERMs treatment and supraphysiological TT levels are not observed, therefore, patients often experience slightly improved well-being.

One of the most common side effects of TRT is polycythemia associated with the direct, anabolic effect of supraphysiological doses of T on hematopoiesis, and regarding safety profile study investigated the prevalence of polycythemia in men treated with CC, the incidence of polycythemia was significantly lower compared to TRT [23], thus SERMs proved to be well-tolerated in long-term treatment.

CC treatment in men with secondary hypogonadism, FH and fertility disorders is generally well-tolerated, with no serious adverse effects reported during and is associated with low risk of polycythemia. Therefore, the use of SRRMs in these patients appears to be an effective and safe alternative to TRT, and in addition, has a beneficial effect on fertility compared to TRT. The safety of SERMs therapy also depends on the drug doses, as excessively high doses of CC can cause supraphysiological TT concentrations, which can secondarily impair spermatogenesis.

However, there is limited data on efficacy and safety of SERMs in older population who potentially could benefit from such treatment, and appropriate long-term randomized, placebo-controlled clinical trials are needed.

Aromatase inhibitors

Aromatase inhibitors - anastrozole and letrozole - inhibit the conversion of T to E and weakening estrogens negative feedback at the pituitary level, hence, increasing endogenous T levels. Their use in men with FH is also off-label, and they have been associated with adverse effects such as reduced bone mineral density. According to the latest recommendations, they are not routinely recommended for patients with FH and fertility disorders. However, they are widely used in both Europe and USA.

In older studies investigating AIs in FH nearly all subjects experienced improvement in their hormonal profile, including an increase in fT concentrations, a decrease in E and improved T/E ratio after a 6-month therapy with Ais [24]. In next study performed in hypogonadal men with decreased fertility, and with BMI >25 kg/m2 anastrozole improved T levels and semen parameters after a 5-month follow up [25]. In study performed in obese men with FH AIs combined with weight loss were effective in improving T levels with no severe adverse events, however, it did not lead to reduction of hypogonadism symptoms [26.

A meta-analysis on efficacy of AIs in male FH found, that AIs significantly improve hormonal profile (TT and fT concentrations increased significantly) as well as semen parameters, and no severe side effects were noticed [27].

A study of a new AI leflutrozole in obesity-associated male FH founded that leflutrozole significantly increased T, LH, FSH, as well as improved semen parameters, and however, reported side effects included raised hematocrit, hypertension, increased PSA, and headaches. Moreover, reduction in bone mineral density was observed in the lumbar region, regardless of dosage [28]. It seems, that also in the case of SERMs side effects depend on the TT concentrations obtained during treatment; the higher the T concentration, the greater the risk of polyglobulia and an increase in prostate-specific antigen (PSA) concentration.

The previously cited meta-analysis showed similar efficacy of AIs and SERMs in improving TT levels in men with FH, but quality of included studies was low, which resulted in significant inconsistency [21].

Also the most current meta-analysis demonstrated the high efficacy of AIs in increasing endogenous TT levels in men with FH associated with obesity and aging after a six-month

treatment but also drop in bone mineral density at the lumbar spine was observed, but not at total hip or femoral neck [29].

The AIs are effective in improving T levels and semen parameters in men with FH. Side effects of AIs are mild and well tolerated, however, the adverse effect on spine bone mineral density remains a concern in obese, ageing males. Optimal estradiol levels (20-40 pg/ml) are essential for achieving and maintaining normal bone density in men. The greatest challenge in treating patients with FH is administering appropriate doses of AIs to achieve TT levels sufficient to reduce the severity of symptoms of hypogonadism. However, E levels should not be too low (<20 pg/mL), as this is associated with reduced bone mineral density and the risk of osteoporosis.

TESTOSTERONE TREATMENT

Testosterone therapy should never be used as the first treatment of choice. TRT is generally contraindicated in men who wish to preserve their fertility, especially if they and their partner are planning a pregnancy within the next 6-12 months. It should be emphasized, that according to the currently applicable definitions and recommendations [2,6], FH is a reversible disease and it is not directly related to the dysfunction of the HPG axis but to external factors influencing its functioning, like obesity and others conditions, therefore it is necessary to try to identify and treat the causes of FH. In many cases, pharmacological treatment is not necessary, unfortunately, in many patients, causal treatment is impossible or ineffective and only in these men can pharmacotherapy be considered.

Only in individual cases where patients cannot or do not want to use off-label medications testosterone therapy may be considered. In patients not seeking fertility, TRT can be used periodically, but the lowest possible doses of T and short-acting preparations (transdermal T preparations) should be used (minimal suppression of GnRH and LH secretion).

GONADOTROPHIN TREATMENT

Gonadotrophin therapy is also not a causal treatment and should be reserved only for men with FH when fertility is desired. Long-term use of human chorionic gonadotropin (hCG) or human menopausal gonadotrophin (hMG) in patients with FH are not recommended [6].

CONCLUSIONS

One of the main causes of FH in men is obesity. It is reversible, and treatment should begin with weight loss, physical exercise, and lifestyle changes. If these measures are ineffective, pharmacotherapy can be initiated, but not sooner than after six months of non-pharmacological interventions. TRT is routinely used, but it is contraindicated in patients wishing to preserve fertility. Furthermore, it does not address the underlying cause and may worsen secondary hypogonadism (inhibition of GnRH and LH secretion).

An alternative is the use of SERMs and AIS, which are effective in increasing TT concentrations and are relatively safe. The most important principle of their use is to select the drug dose so that the resulting TT concentrations are not too high and do not secondary inhibit spermatogenesis. It is also important when using AIS drugs not to reduce E2 concentrations to too low values because this may increase the risk of osteoporosis. Their efficacy in FH needs to be further established in randomized clinical trials, however, considering their relatively good safety profile they remain a reasonable alternative to TRT. The advantage of SERMs and AIS over classic TRT is their beneficial effect on semen parameters and the drugs are used to treat male fertility disorders.

It should be emphasized that the use of SERMs and AIS is an off-label treatment, there are no long-term clinical trials using a placebo and the available data often comes from relatively small groups of patients.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CASE STUDY

Fibromuscular dysplasia as a rare cause of ischaemic stroke – a case report

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ABSTRACT

Fibromuscular dysplasia (FMD) is a rare cause of ischemic stroke. It is an important element of differential diagnosis that should be considered, especially in patients who do not have common cardiovascular risk factors. A 65-year-old man was admitted with transient left arm weakness and drooping of the mouth, lasting about 3 minutes, followed by left-sided paresthesia. A similar episode had occurred a week earlier. On admission, the patient was alert and oriented. Neurological exam showed subtle flattening of the left nasolabial fold and a tendency to a positive ipsilateral Babinski sign, with no other abnormalities. Brain MRI revealed a subacute ischemic lesion in the anterior deep structures and a hyperacute focus at the right temporo-occipital border. CT angiography showed bilateral internal carotid artery changes suggestive of FMD, critical stenosis of the right MCA (M1), and aneurysmal dilation of the left ophthalmic artery origin. Digital subtraction angiography (DSA) confirmed a wide-neck saccular aneurysm of the left internal carotid artery (ICA). Rehabilitation and multidisciplinary consultations were undertaken. The patient was discharged in good condition without significant neurological deficits. Regular blood pressure control, atherosclerotic diet, physical activation and taking medications (antihypertensive drugs, acetylsalicylic acid and statin) regularly were recommended. Due to the diagnosis of a saccular aneurysm of the left ICA, a referral was issued to the neurosurgery department. The described case illustrates the need for a comprehensive and interdisciplinary approach to a patient with stroke in order to determine the etiology of the stroke and implement secondary prevention.

KEYWORDS: fibromuscular dysplasia; stroke; case report

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INTRODUCTION

Fibromuscular dysplasia (FMD) is a rare, idiopathic, segmental disorder of the muscularis arteriosus, showing no signs of inflammation or atherosclerosis, leading to narrowing of medium-sized vessels. Although it can occur at any age, it is diagnosed on average at about 50 years of age [1,2]. It is currently estimated that the disease affects the carotid and intracerebral arteries with a similar frequency to the renal arteries, but data on neurological events and symptoms associated with FMD are still limited. More than half of patients with cerebrovascular FMD have concomitant changes in the renal artery [3]. The exact incidence of the disease is difficult to estimate, but clinically significant disease occurs in about 0.4% of adults (four times more often in women) [4]. Men are more likely to develop vascular complications, such as intermittent claudication, renal infarction, or stroke [5]. Risk factors considered for the development of the disease include exposure to female gender hormones, stress, and cigarette smoking. Although the disease occurs sporadically and in families, only a few genetic mutations predisposing to the development of FMD have been identified to date, including a mutation in the PHACTR1 gene located on chromosome 6 [6]. Genetic tests for the detection of mutations in asymptomatic relatives of patients with fibromuscular dysplasia are not recommended, as their specificity for FMD is limited [7]. Symptoms, if they occur, most often depend on the location of the vessel affected by FMD. Many patients do not report any symptoms. In the case of renal artery involvement, hypertension is a typical symptom [8]. In the case of women, headaches, neck pain, the presence of a murmur over the neck vessels, and tinnitus should raise particular alertness. FMD should be considered in the differential diagnosis, especially in men with renal dissection, renal infarction or arterial aneurysm [9]. Histologically, there are three main types of dysplasia, depending on the layer of the artery wall affected by the disease, i.e. intimate, medial and adventitial type. Due to the fact that only few cases of FMD are examined histologically, a diagnostic classification based on angiography has been proposed [10]. The angiographic classification of FMD includes three types: multifocal type, tubular type and focal type. In the case of multifocal type, which is characterized by multiple vascular narrowings, a "string of pearls" pattern is distinguished [11]. Currently, FMD diagnosis is based on the results of spiral computed tomography of blood vessels (angio-CT) or magnetic resonance angiography (MRA). The diagnostics can be supplemented with an ultrasound examination [12]. Differential diagnosis includes

atherosclerotic aneurysms, Ehlers-Danlos syndrome, Marfan syndrome, neurofibromatosis type 1, or polyarteritis nodosa (PAN) [13]. FMD is treated with antiplatelet drugs when the patient is asymptomatic. In patients who qualify for surgery, percutaneous balloon angioplasty is recommended. Patients with aneurysms should have a stent placed or an open surgical procedure performed [14].

CASE REPORT

Sixty-six-year-old man checked into neurological emergency room of the provincial specialist hospital because of transient weakness of the left upper limb and drooping of the left corner of the mouth, which occurred on the day of admission. After about three minutes, the above symptoms gave way to left-sided paresthesia. A similar episode had occurred a week earlier. The patient had no family history of neurological diseases, no chronic illnesses, denied allergies or other situations that could lead to muscle weakness, denied stimulants. His previous medical history included prostate removal. In the neurological examination conducted on admission, the patient was conscious and in logical contact. A slight shallowing of the left nasolabial fold was noticeable, apart from that there were no deficits in the innervation of the cranial nerves and no speech disorders or deficits in muscle strength in the upper and lower limbs. There was a visible tendency towards a positive Babinski sign on the left side, apart from that - normal sensation and coherence, normal gait, Romberg's test normal. The patient was admitted to the Neurology Department for extended diagnostics.

A blood test package was performed, including: blood count, APTT, PT (INR), plasma albumin concentration, C-reactive protein (CRP), lipid profile, glucose level, creatinine level, urea, thyroid-stimulating hormone (TSH), sodium and potassium concentration in serum. The following abnormalities were found: slightly reduced mean hemoglobin concentration in red blood cells (MCHC) - 31.6 g/dl and a decrease in hemoglobin (HGB) from 15.6 g/dl to 13.9 g/dl in a control test conducted four days later. Abnormalities found in other tests include: slightly elevated fasting glucose level - 108 md/gl and elevated total cholesterol level - 223 mg/dl. No other abnormalities were found.

During hospitalization, magnetic resonance imaging (MRI) of the head was performed in the stroke protocol, using the DWI (Diffusion Weighted Imaging) technique with an ADC (apparent diffusion coefficient) map, T2 Flair (Fluid Attenuated Inversion Recovery), T2*, T1 Flair multiplanar (Fig. 1). The examination revealed a subacute focus of ischemic stroke in the anterior part of the right deep structures, as well as a hyperacute focus of ischemic stroke measuring 17x9 mm on the border of the temporal and occipital lobes of the right cerebellum.

The sulci and fissures of the cerebellum were slightly widened. No other abnormalities were observed. Computed tomography angiography (angio-CT) of the carotid and cerebral arteries was performed using the spiral technique, in 0.6 mm thick layers, after intravenous, dynamic administration of a contrast agent. The examination revealed changes in the walls of both internal carotid arteries (ICA) giving a "string of pearls" image and aneurysmal dilatation of the origin of the left ophthalmic artery. In addition, a critical stenosis focus of 4 mm in length was visualized in the first segment of the right middle cerebral artery (M1 RMCA), as well as small, morphologically insignificant atherosclerotic plaques in the imaged arteries. No other changes were found.

The diagnostics were extended with digital subtraction angiography (DSA), which diagnosed a saccular aneurysm of the C6 segment of the left ICA in the origin of the left ophthalmic artery and changes in the initial segment of the RICA, which corresponded to RICA type III fibromuscular dysplasia. Apart from that, the image of the cerebral vessels was normal. A Doppler-duplex (UDP) examination of carotid arteries was also performed, which revealed small atherosclerotic changes without affecting the hemodynamic flow. The patient also underwent transcranial Doppler ultrasound (TCD) - the examination did not reveal any abnormalities in the flow recording in both middle cerebral arteries (MCA), anterior cerebral arteries (ACA) and posterior cerebral arteries (PCA). During the stay in the ward, an echocardiographic examination of the heart (ECHO) was performed, which revealed concentric hypertrophy of the left ventricular muscle and mild mitral and aortic valve insufficiency, with a normal ejection fraction (EF) of 65%.

During the hospital stay, the patient was consulted by an internist, speech therapist, and psychologist; and the rehabilitation was performed. Arterial hypertension was diagnosed and amlodipine (5 mg per day) and ramipril (5 mg per day) were applied, besides acetylsalicylic acid (150 mg per day) and rosuvastatine (20 mg per day) were introduced into the treatment. The patient was diagnosed with ischemic stroke of the right hemisphere caused by stenosis of the right internal carotid artery in the course of FMD. Additionally, left ventricular hypertrophy, mild mitral and aortic valve insufficiency and atherosclerosis were diagnosed. Due to the diagnosis of a saccular aneurysm of the left internal carotid artery, a referral was issued to the neurosurgery department.

After 8 days of hospitalization, the patient was discharged home in good general and neurological condition. Regular blood pressure control, atherosclerotic diet, physical activation and taking medications regularly were recommended.

DISCUSSION

In the case of previous ischemic stroke, it is important to correctly determine its etiology. It is necessary to identify risk factors and differentiate the patient's clinical picture based on the clinical examination, as well as other diagnostics tests. This allows for the rapid implementation of appropriate therapy and then effective secondary prevention, reducing the risk of subsequent vascular incidents in the future [15].

The described patient had several risk factors for vascular incidents, such as hyperlipidemia, impaired fasting glucose, hypertension and older age, because the risk of stroke doubles with each decade after the age of 55 [16].

Angio-CT of the carotid and cerebral arteries performed using the spiral technique allowed for the determination of

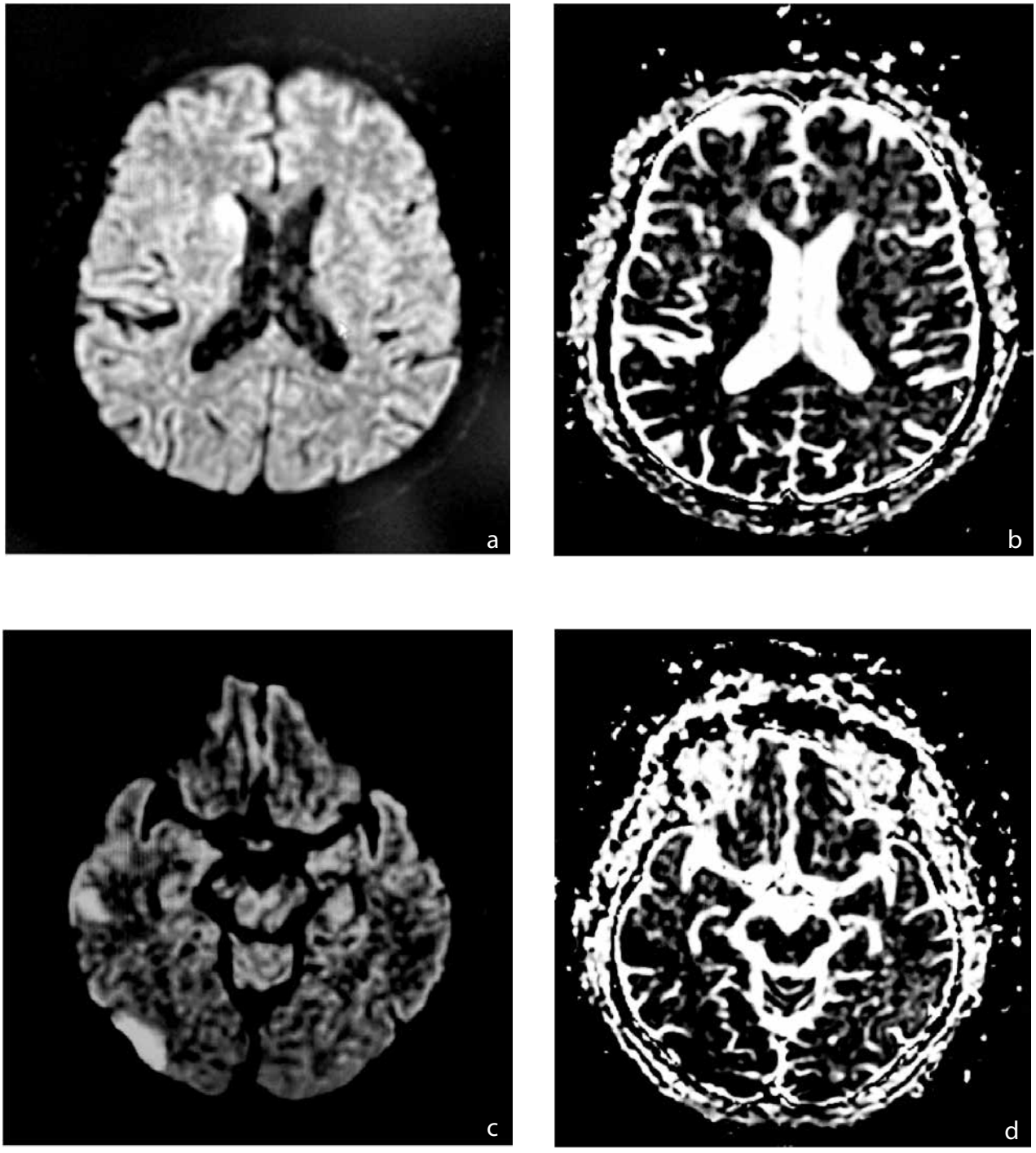

Fig. 1. MRI of the head presenting subacute focus of ischemic stroke in the anterior part of the right deep structures (1a - DWI; 1b - ADC) and hyperacute focus of ischemic stroke on the border of the right temporal and occipital lobes (1c - DWI; 1d - ADC)

Source: Own materials

stenosis of the right internal carotid artery in the course of FMD as the most probable cause of stroke in the patient. Involvement of cerebral vessels in the course of the disease may account for up to 80% of cases. Despite its often asymptomatic course, FMD may manifest itself as carotid artery dissection or intracranial aneurysms, which lead to ischemic strokes or subarachnoid hemorrhages [17].

Information on similar cases of stroke in the course of FMD in the literature is limited. In the described case, the patient had an extracranial form of dysplasia, with significant stenosis located in the right carotid artery. Similar cases are rarely described in the literature [18].

According to the US FMD registry, the incidence of stroke in the course of FMD is about 10%. The vascular stenosis

is caused by fibrous septa, described in angiography as a "string of pearls". They can cause platelet deposition and block the flow, leading to TIA, embolism or ischemic strokes. According to the "2021 Guideline for the Prevention of Stroke in Patients With Stroke and Transient Ischemic Attack: A Guideline From the American Heart Association/American Stroke Association," for secondary prevention, the strong recommendation is antiplatelet therapy, blood pressure control, and lifestyle modification to prevent future ischemic events. Carotid angioplasty may be reasonable in patients with recurrent ischemic stroke when other causes have been excluded, but this is a weak recommendation [19].

CONCLUSIONS

FMD is a rare etiological cause of ischemic stroke. It is an important element of differential diagnosis that should be considered when making a diagnosis, especially in patients who do not have standard cardiovascular risk factors, with accompanying abnormal vascular images, such as „string of pearls" stenoses or intracranial aneurysms. The described case indicates the importance of advanced imaging diagnostics, including spiral angio-CT, as well as classical arteriography (DSA), in identifying FMD and its accompanying vascular complications. They include the occurrence of aneurysms, which may require neurosurgical treatment, even with minimal or transient neurological deficit. FMD in men may be characterized by a more complicated course, and strokes and organ thrombosis pose an increased risk despite a reduced overall incidence in this gender group. This confirms the need for special diagnostic control also in the male population. Early initiation of antiplatelet therapy, statin therapy and blood pressure regulation are in line with current guidelines and are also the basis for effective secondary prevention of ischemic stroke in FMD. This case illustrates the importance of interdisciplinary care in the clinical setting for a neurological patient. The composition of the interdisciplinary team should include neurologists together with interventional radiologists, as well as internists, cardiologists and neurosurgeons. Rapid diagnosis combined with effective treatment together with protection against serious complications resulted from the coordination of these activities. This clinical case report significantly complements the limited information on stroke in fibromuscular dysplasia. It presents real diagnostic and therapeutic difficulties and emphasizes that additional studies are important to optimize management approaches in this condition.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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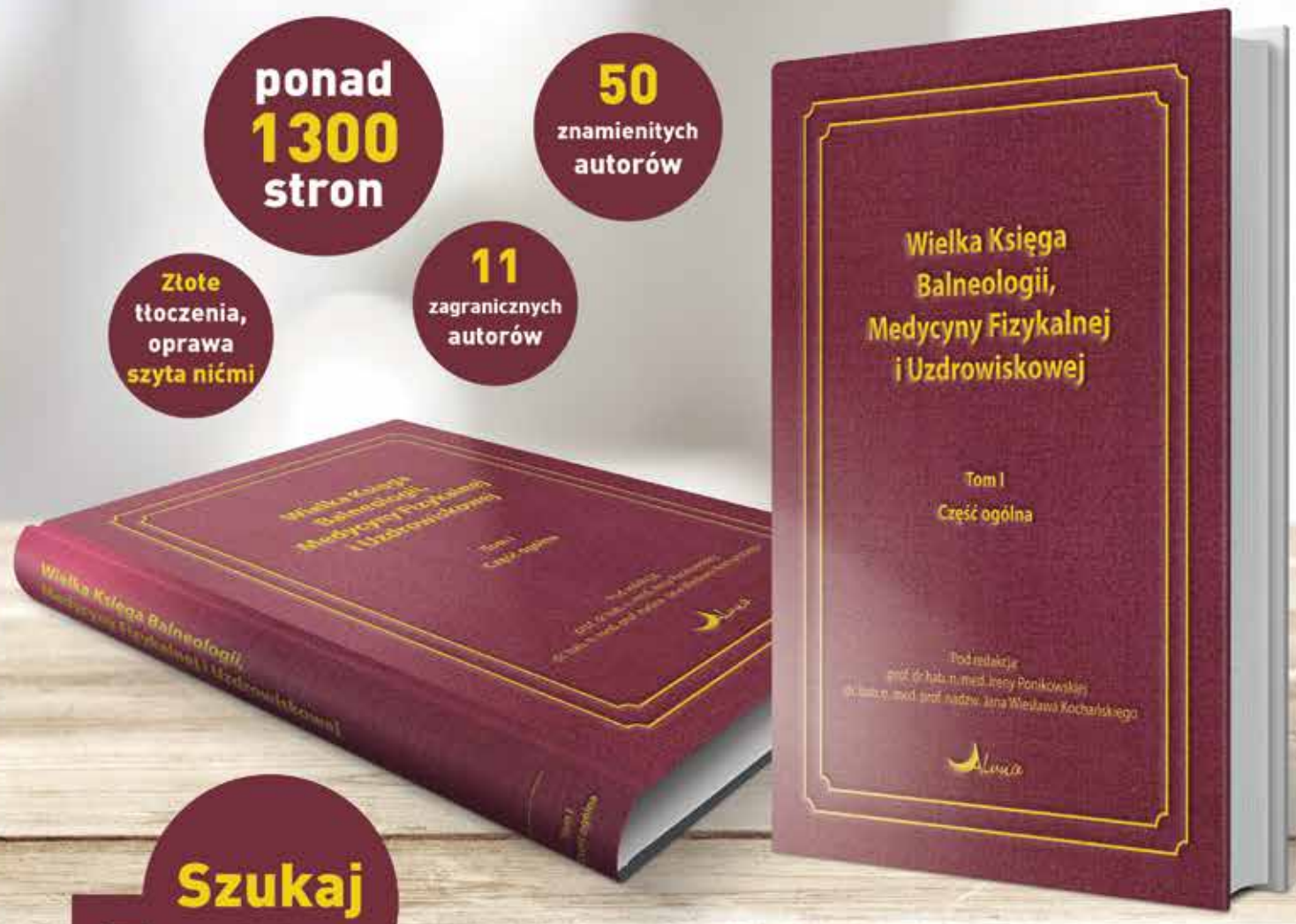

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