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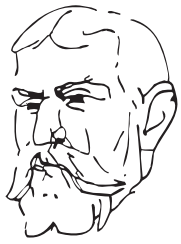

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ORIGINAL ARTICLES

ORIGINAL ARTICLE

SURGICAL TREATMENT OF ACUTE SMALL BOWEL OBSTRUCTION: CLINICAL AND LABORATORY PARAMETERS ASSOCIATED WITH STRANGULATION AND EARLY MORTALITY AFTER SURGERY

DOI: 10.36740/WLek202212101

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ABSTRACT

The aim: The study aimed to evaluate some criteria for preoperative diagnosis of strangulation and significant indicators of the prognosis of short-term outcomes in patients with small bowel obstruction.

Materials and methods: The results of the treatment of 123 patients aged 18–70 years with SBO were evaluated.

Results: All of these patients underwent emergency surgery, and 22 patients (17.9%) have died. It has been shown that four lab parameters (blood leukocytes, lactate, intestinal fatty acid-binding protein, and C-reactive protein levels) and one instrumental (involving the mesentery of the small intestine, free fluid in the abdomen during CT) with 80% probability or more were associated with the strangulation type of SBO (= 0.276, p = 0.000). Three lab indicators (WBC count, serum lactate, and intestinal fatty acid-binding protein levels) and two clinical parameters (abdominal perfusion pressure level and the presence of abdominal sepsis) were associated with early mortality after surgery (= 0.66, p = 0.000) with the same probability. Immediate results of the treatment in these patients depended on the development of intra-abdominal complications after surgery (P = 0.024) and the need for early reoperation (P = 0.006) as well as the development of cardiovascular dysfunction (P = 0.000) and respiratory dysfunction (P = 0.000).

Conclusions: There were confirmed parameters that were significantly associated with strangulation before surgery and short-term in-hospital mortality with an 80% probability or more. This made it possible to develop new mathematical models for the diagnosis of strangulated bowel obstruction and early postoperative mortality with an accuracy of 84.5% and 84.2%, respectively.

KEY WORDS: diagnosis, prognosis, surgical treatment, strangulation, mortality, acute obstruction of the small intestine

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INTRODUCTION

Despite advances in medicine, acute small bowel injury in patients with small bowel obstruction (SBO) remains one of the most difficult problems in emergency abdominal surgery. Most studies have shown that this is a heterogeneous syndrome caused by an unbalanced host response to ischemia/necrosis of the small intestine and infection, which leads to organ dysfunction, and its main manifestations are characteristic both for patients with acute adhesive intestinal obstruction and for patients with acute occlusive or, in rare cases, non-occlusive ischemia In all these cases, ischemia, necrosis, and perforation of the intestinal wall due to vascular compromise in the loop of the small intestine can be observed [1,2]. At least 300,000 surgical procedures are performed annually in the United States [3], and about 40% of cases are associated with small bowel strangulation. Meanwhile, nonviable asphyxia of the small intestine is about 16% of the SBO, which four times increases the risk of death compared to the rates in patients with viable asphyxia [4], and in patients with impaired SBO, the mortality rate is 2–10 times higher than in patients with simple SBO [5]. In addition to the well-known scores of severity [6,7], a lot of laboratory indicators [8, 9] are used for earlier diagnosis and detection of complications associated with hypoxia, ischemia, and necrosis of the small intestine in these patients [10–13]. It should be noted, however, that it is often difficult to objectively predict the severity of a patient's condition based on prior information obtained from initial contact with a patient in the emergency department during the initial treatment phase before and after surgery.

In recent years, several serum markers have been identified that can be detected in SBO. One of them includes factors released by damaged enterocytes, such as intestinal fatty acid-binding protein (I-FABP) as known that enterocytes are rapidly damaged in the early stages in critical patients and its biomarker can be easily detected in both urine and plasma. It opens up promising opportunities for the use of I-FABP as a biomarker for early detection of injury of the small intestine including small bowel strangulation

[14,15]. Accordingly, such an indicator could be considered as an indicator of intoxication syndrome and used together with the definition of markers of pro-inflammatory and hypoxia status (C-reactive protein, serum lactate, etc.) to diagnose and assess the treatment of patients.

THE AIM

The study aimed to evaluate some criteria for preoperative diagnosis of strangulation and significant indicators of the prognosis of short-term outcomes in patients with small bowel obstruction.

MATERIALS AND METHODS

PATIENT CHARACTERISTICS AND RESEARCH METHODS

A two-centre retrospective study was conducted at Kharkiv National Medical University, which involved 123 patients aged 18 to 70 years, hospitalized in the intensive care unit in the immediate postoperative period. The study was conducted from September 1, 2014, to November 30, 2021, with the approval of the University Ethics Committee (protocol No. 3, September 20, 2021).

INCLUSION CRITERIA

The study included men and women who were admitted to the hospital with SBO. Based on some of the clinical data, the inclusion criteria for patients with SBO met one of the following conditions: simple abdominal radiograph or abdominal ultrasound showing certain multiple air-liquid levels in the small intestine, but no evidence of gas in the colon in it; X-ray and/or computed tomography (CT) of the abdomen, indicating SBO; confirmation of SBO during laparotomy or laparoscopy.

EXCLUSION CRITERIA

Patients with an inguinal hernia, mechanical obstruction of the colon, and early postoperative SBO less than 30 days after abdominal surgery; patients with ascites; comorbidity with acute myocardial infarction and stroke; severe acute pancreatitis with small bowel obstruction or necrosis; post-resuscitation disease due to the arrest of effective blood circulation and refractory shock; pregnancy; history of cancer.

When examining patients, the following data were collected: personal data (age, gender, previous operations on the abdominal organs and abdominal trauma); body mass index (BMI); laboratory examination: peripheral blood leukocyte count, platelets, hematocrit, D-dimer, lactate, C-reactive protein; in the dynamics of patient treatment, systolic blood pressure (SBP) was monitored and the following scores were calculated for each patient: Acute Physiology and Chronic Health Evaluation (APACHE) II, Sequential Organ Failure Assessment (SOFA); the level of I-FABP was determined using commercial kits "I-FABP, Human, ELISA kit"; data on the nature of the treatment and complications were collected and the results were assessed: short-term mortality up to 3-7 days and mortality within 30 days after surgery. To determine the level of intra-abdominal hypertension, we used the classification of the World Society of Abdominal Compartment Syndrome (WSACS, 2007) with the calculation of the abdominal perfusion pressure (APP). Patients were screened for IAH (defined as IAP≥12 mmHg) with the Foley-Manometer method (Denmark). The WSACS classification was used to assess the level of IAH with the classification of its according to the developed recommendations**:** I degree IAP registered at IAH 12-15 mm Hg; II degree – at 16-20 mm Hg; III degree – at 21-25 mm; IV degree - > 25 mm Hg.

The CT parameters included: a decrease in the density of the intestinal wall, dilatation of the small intestine; the large wall of the small intestine; involvement of the mesentery in the form of its thickening; free fluid in the abdominal cavity; air/fluid level in the small intestine; and volvulus. For the diagnosis, laboratory parameters were taken into account in two clinical categories: patients with reversible ischemia of the small intestine who underwent emergency laparoscopy or laparotomy, and patients with irreversible ischemia of the small intestine who underwent urgent laparotomy, which required resection of the small intestine, and for the prognosis, laboratory parameters were also taken into account for the prognostic indicators of short-term in-hospital mortality.

All patients were divided into two groups depending on the results of treatment: the first included patients with a positive outcome (survivors, n = 101), the second, those with an unfavourable outcome (non-survivors, n = 22).

Using it in the clinical setting, we took into account the existing WSES recommendations for this category of patients, taking into account local conditions and opportunities [16,17].

STATISTICAL ANALYSIS

Initially, statistical analyses were performed using descriptive statistics. A comparison of data from survivors and non-survivor patients was analyzed using the normality of distributions (Shapiro-Wilk's test) of the selected indicators. Continuous data were presented as mean and standard deviation (M±SD). At a significance level of $p > 0.05$, zero hypotheses (H0) in statistical tests were rejected. Odds ratio (OR) and discriminant analysis of the parameters were used to identify risk factors for the development of strangulation of the small intestine before surgery and an unfavourable outcome of the treatment in the early stages. After studying of odds ratio for all the indicators that were researched, those that had a probability of 80% or more in the preliminary distribution were taken into account for further construction of mathematical models. Statistical analysis was performed using the STATISTICA 13.3 EN software.

RESULTS

CHARACTERISTICS OF THE PATIENTS

A total of 123 patients were recruited, and all of them met the inclusion criteria. The patients' characteristics are shown in Table I.

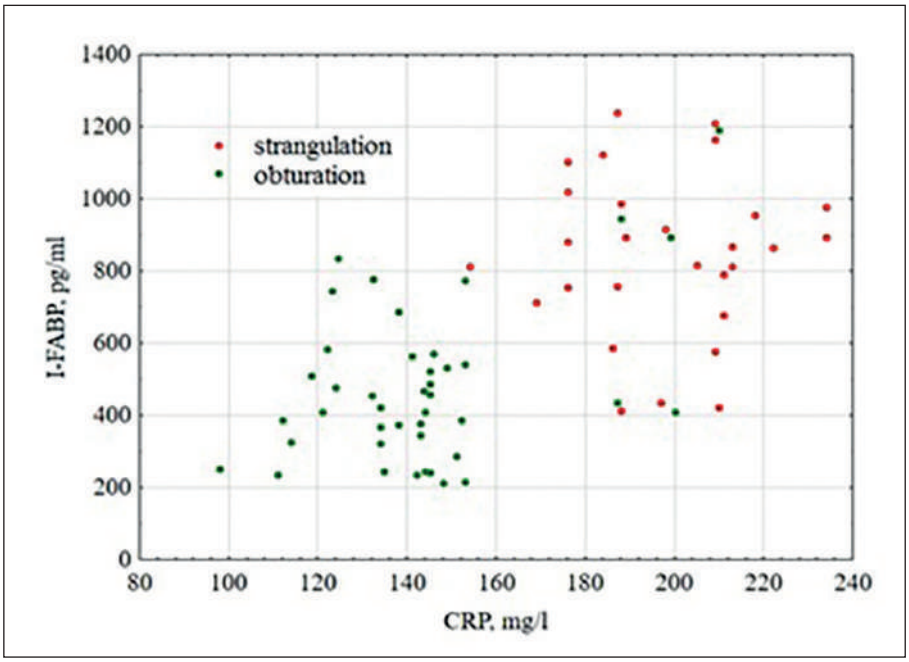

Fig. 1. A scattering graph for CRP and I-FABR indicators in patients with SBO

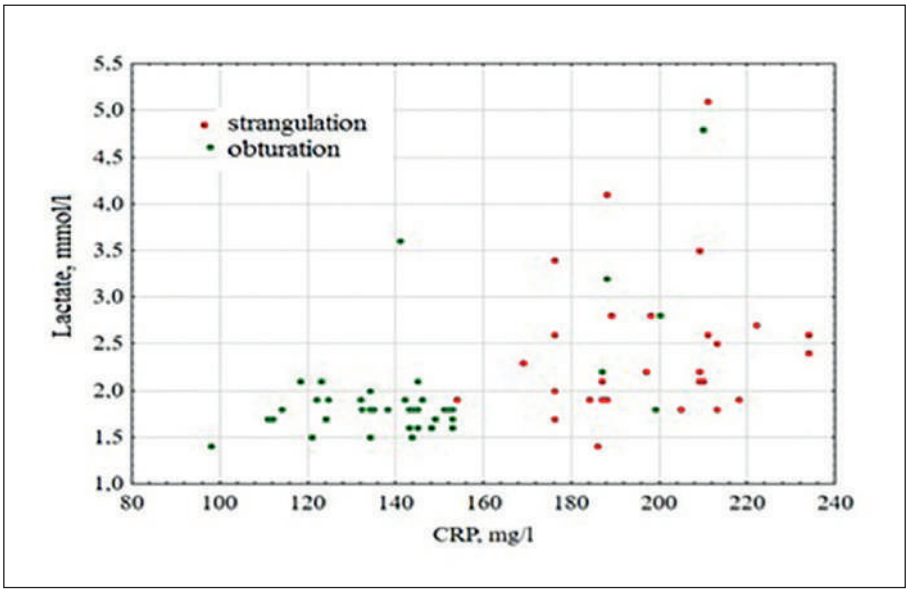

Fig. 2. A scattering graph for CRP and and serum lactate indicators in patients with SBO

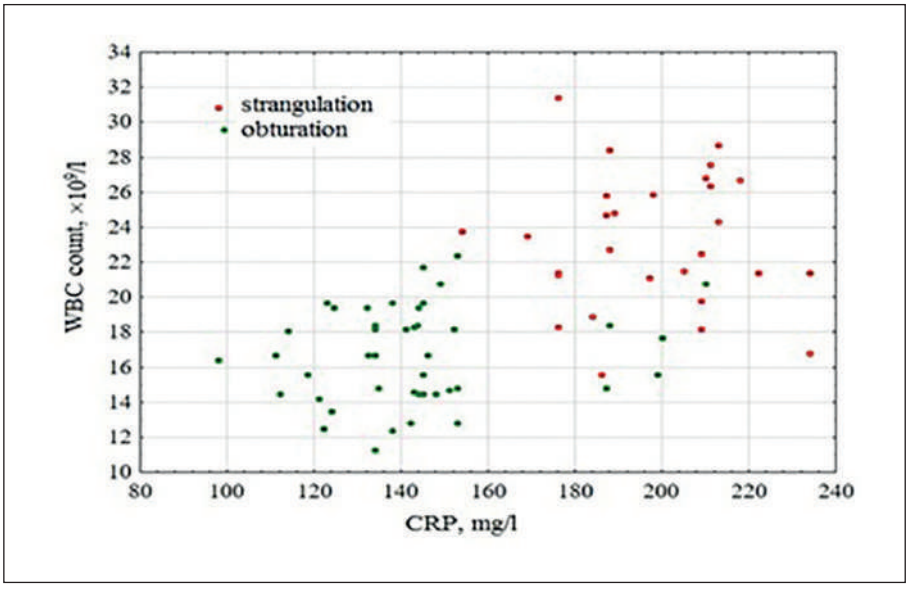

Fig. 3. A scattering graph for CRP and white blood cells indicators in patients with SBO

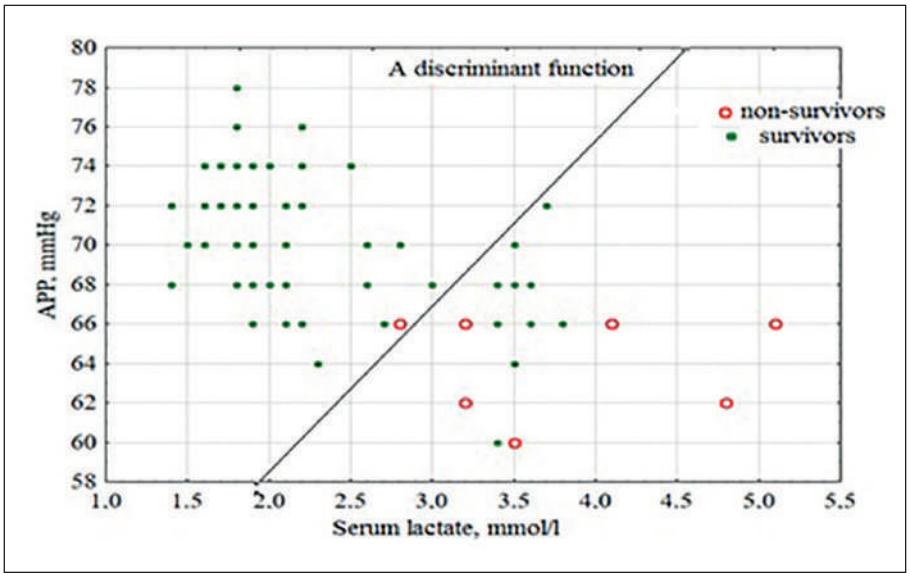

Fig. 4. A scattering graph for serum lactate and APP indicators in patients with SBO and early in-hospital mortality

Table I. Patients' Characteristics and Surgical Data

Indicators	Outcomes		P-value
	Survivors (n = 101)	Non-survivors (n = 22)	
Years	56.43±11.19	51.59±6.23	0.052
Sex *: - men - women	 44 (43.6%) 57 (56.4%)	 13 (59.1%) 9 (40.9%)	0.417
BMI, kg/m2	26.2±5.08	28.14±3.12	0.088
Respiratory rate beats/min	18.88±2.59	25.05±2.61	0.000
Hypotension at diagnosis (SAP < 90 mm Hg), (%) * Fluid resuscitation before surgery, (%) * Vasopressors before surgery, (%) *	 12 (11.9%) 101 (100%) 5 (4.95%)	 15 (68.2%) 22 (100%) 14 (63.6%)	 0.000 0.868 0.000
Surgical approach, (%) *: - laparoscopic - laparotomy - laparoscopy + laparotomy	 8 (13.6%) 87 (76.3%) 6 (10.1%)	 0 20 (83.3%) 2 (16.7%)	0.000
Type of surgery, (%): - adhesiolysis, NIT, drainage - small bowel resection, NIT, anastomosis - small bowel resection, stoma formation	 58 (57.4%) 15 (14.9%) 28 (27.7%)	 16 (72.7%) 1 (4.6%) 5 (22.7%)	0.309
Operation time (min)	146.5±17.65	137.7±18.43	0.038
The amount of intraoperative fluid administration, (mL)	2365.5±311.8	2980.9±286.5	0.000
Intra-abdominal complications, (%) *	26 (25.7%)	15 (68.2%)	0.024
Re-laparoscopy or re-laparotomy, (%) *	4 (4%)	6 (27.3%)	0.006
Cardiovascular dysfunction, (%) *	7 (6.9%)	14 (63.6%)	0.000
Respiratory dysfunction, (%) *	7 (6.9%)	18 (81.8%)	0.000
Length of hospital stay (days),	13.46±5.2	5.5±4.4	0.000

Abbreviations: Values are presented as mean ± SD; P-value - Student's criterion; * Statistically significant difference by criterion χ2; NA - not applicable; BMI - body mass index; NIT - nasal-intestinal tube.

During the study period, all of these patients underwent emergency surgery operations for SBO: the adhesive disease was the main cause in 101 (82.1%) patients with SBO, obstruction of the small intestine by gallstones was in 2 (1.6%), and volvulus was the cause in 20 (16.3%) patients. 14 (11.4%) patients had high SBO. There were 49 (39.8%) patients with irreversible ischemia; generalized peritonitis was present in 24 (19.5%) patients, of which 18 (75%) had abdominal sepsis according

Table II. Laboratory Findings in the types of SBO and Patients' Severity Assessment by Outcomes

Indicators	Before surgery		P - value	Before surgery		P - value
	Patients with a viable small intestine (n = 74)	Irreversible ischemia of the small intestine (n = 49)		Survivors (n = 101)	Non-survivors (n = 22)	
WBC count (×109/l)	14.56±3.92	20.95±4.09	0.000	16.78±4.15	22.74±5.77	0.000
	95% CI: –7.844 to –4.936			95% CI: –8.044 to –3.876		
Haemoglobin (g/L)	154.6±8.09	156.7±4.37	0.099	146.8±9.56	151.4±8.11	0.038
	95% CI: –4.601 to 0.4014			95% CI: –8.943 to –0.2567		
Platelets (×109/l)	218.1±77.72	236.6±48.18	0.140	223.3±98.7	237.5±97.8	0.541
	95% CI: –43.14 to 6.136			95% CI: –60.1 to 31.7		
Hematocrit (%)	58.74±8.6	59.58±9.2	0.448	49.2±5.4	56.6±3.9	0.000
	95% CI: –4.464 to 1.884			95% CI: –9.809 to –4.991		
D-dimer (ng/mL)	253.6±51.51	264.3±52.75	0.266	238.9±63.47	262.3±61.32	0.118
	95% CI: –29.66 to 8.263			95% CI: –52.79 to 5.993		
Lactate (mmol/l)	1.54±0.78	2.32±0.52	0.000	1.81±0.43	2.53±0.76	0.000
	95% CI: –1.031 to –0.5289			95% CI: –0.9543 to –0.4857		
CRP (mg/l)	139.6±31.34	189.6±24.33	0.000	145.8±42.12	204.7±32.21	0.000
	95% CI: –60.49 to –39.51			95% CI: –77.8 –40		
I-FABP, pg/mL	276.2±61.86	881.8±179.3	0.000	486.2±248.7	762.2±270.5	0.000
	95% CI: –624 to –587.2			95% CI: –393.7 to –158.3		
SBP (mm Hg)	128.6±13.23	122.4±21.55	0.055	124.2±14.18	88.4±15.23	0.000
	95% CI: –0.007532 to 12.41			95% CI: 29.1 to 42.5		
APP (mm Hg)	73.31±4.85	72.29±5.61	0.286	72.84±2.92	65.51±3.08	0.000
	95% CI: –0.8633 to 2.903			95% CI: 5.957 to 8.703		
APACHE II score	11.14±3.16	12.54±4.67	0.044	10.76±4.23	14.22±5.56	0.000
	95% CI: –2.762 to –0.03809			95% CI: –7.551 to –3.369		
SOFA score	8.21±1.58	8.96±1.87	0.018	7.34±1.78	9.92±1.72	0.000
	95% CI: –1.37 to –0.1298			95% CI: –3.404 to –1.756		
AGI grade	1.81±0.5	2.07±0.7	0.018	1.73±0.55	2.67±0.19	0.000
	95% CI: –0.4742 to –0.04576			95% CI: –1.176 to –0.7042		

Abbreviations: Values are presented as mean ± SD; P - Student's criterion; CRP – C-reactive protein; SBP - systolic blood pressure; APP – abdominal perfusion pressure; AGI - acute digestive injury; 95% CI - 95% confidence interval for the difference.

to Sepsis-3 criteria. After the operation, 22 patients (17.9%) died: in the early stages (the first 72 hours), 17 patients (77.3%) and the rest (22.7%) up to 30 days of the postoperative period.

LABORATORY RESEARCH

INITIAL CHARACTERISTICS

The results of the distribution of patients by changes in laboratory data and severity depending on the outcome are presented in Table II.

As it follows from the data presented in Table II, the laboratory data of patients with strangulation SBO and non-survivors patients differed significantly from those of patients with obstructive SBO and survivors in many parameters before surgery.

An analysis of the OR and probability of signs in patients with strangulation SBO before surgery showed (Table III) that the correspondence of both of these indicators to the level of 80% and above was shown by the following data before surgery: WBC count (81.2%), lactate (80.1%), CRP (81.7%) and I-FABP (82.4%) levels, and CT data indicating the presence of such signs as mesenteric involvement of the small intestine and free fluid in the abdomen (84.1%).

DISCRIMINANT ANALYSIS OF SEVERAL BIOMARKERS FOR DIFFERENTIAL DIAGNOSIS OF OBTURATION AND STRANGULATION

For two types of SBO (obturation and strangulation) the task was to determine one canonical root (canonical discriminant function), which divides the n-dimensional

Table III. Analysis of the odds ratio and probability of signs for patients with strangulation SBO before surgery

Variables	Odds Ratio (95% CI)	Probability (%)	χ2	P-value
WBC count	4.301 (1.921 – 9.505)	81.2	11.48	0.0007
Haemoglobin	1.136 (0.517 – 3.848)	53.2	0.11	0.074
Hematocrit	2.084 (1.016 – 4.544)	67.7	2.76	0.0965
D-dimer	2.153 (1.036 – 7.424)	68.3	3.05	0.0807
Lactate	4.022 (1.814–8.918)	80.1	10.49	0.0012
CRP	4.451 (2.038–9.943)	81.7	12.01	0.0005
I-FABP	4.673 (2.007 – 10.815)	82.4	11.45	0.0007
SBP	2.182 (1.016–4.543)	68.6	3.20	0.0738
APP	2.054 (0.932 – 9.025)	67.2	2.62	0.1054
APACHE II score	1.841 (0.812–4.524)	64.8	1.29	0.2567
SOFA score	2.143 (0.923–5.115)	68.2	7.33	0.1348
AGI grade	2.543 (1.221–75.517)	71.8	4.89	0.0270
Signs of peritonitis	2.213 (1.012–4.813)	68.9	3.34	0.0674
CT: mesenteric involvement, free fluid in the abdomen	5.283 (2.449–11.811)	84.1	14.71	0.0001

Table IV. Analysis of the odds ratio and probability of early in-hospital mortality for patients with SBO before surgery

Variables	Odds Ratio (95% CI)	Probability (%)	χ2	P-value
WBC count	3.321 (1.521 – 7.505)	76,9	7.19	0.0088
Haemoglobin	1.563 (0.624 – 3.943)	60.9	0.53	0.4665
Hematocrit	1.252 (0.522 – 3.213)	55.6	0.05	0.8280
D-dimer	1.874 (0.842 – 4.535)	65.2	1.37	0.2419
Lactate	4.362 (2.014–9.618)	81.3	11.89	0.0006
CRP	3.531 (1.638–8.0341)	77.9	8.12	0.0044
I-FABP	5.614 (2.124 – 14.732)	83.6	10.66	0.0011
SBP	3.153 (1.316–7.643)	75.9	5.43	0.0198
APP	4.552 (2.031 –10.153)	82	11.20	0.0008
APACHE II score	3.451 (1.612–7.644)	77.5	8.44	0.0037
SOFA score	4.183 (1.923–9.015)	80.7	11.97	0.0005
AGI grade	2.733 (1.218–6.117)	73.2	5.02	0.0250
The presence of abdominal sepsis	4.453 (2.149–9.611)	81.9	13.31	0.0003

Table V. Matrix of the factor structure of indicators for diagnosis of strangulation of the small intestine

Indicators	Canonical correlation
CRP (mg/l)	–0.751
WBC count (×109/l)	–0.619
I-FABP, pg/mL	–0.508
Lactate (mmol/l)	–0.217

space of indicators into two areas corresponding to different types of disease. The selection of indicators for the discriminant function was carried out sequentially after determining the OR of these indicators at the level of 80% and more of its probability. The statistical significance of the obtained discriminant function was estimated based on Wilk's Λ-statistics and was $\Lambda = 0.276$ at $\chi 2 = 86.24$ ($p <= 0.000$). Thus, a discriminant function was obtained that contained four indicators: CRP, WBC, lactate and serum I-FABR. The contribution of each indicator to discrimination and the division of patients by SBO types was judged by the contribution of each indicator that correlated with the discriminant function. As can be seen from the factor structure matrix (Table V), serum lactate played the least role in the discrimination.

The classification of patients according to SBO types was performed using the classification functions 'F obturation' and 'F strangulation'. The patient belonged to the type whose classification function was greater:

F obturation $= -32.89 + 0.29X_1 + 1.55X_2 - 0.72X_3 - 0.0027X_4$;

Table VI. The matrix of a posteriori classification for patients with different types of SBO

The groups of the patients with SBO	Shown the rows: the groups that were observed Shown the columns: the groups that were predicted		
	Percentage of correct observations	Obturation	Strangulation
Obturation	83.7%	36	7
Strangulation	85.7%	4	24
Total	84.5%	40	31

Table VII. Matrix of the factor structure of indicators for prognosis of early in-hospital mortality

Indicators	Canonical correlation
Lactate (mmol/l)	0.925
APP (mmHg)	–0.74

F strangulation = $-63.23 + 0.4\ X_1 + 2.13X_2 - 2.29X_3 + 0.0018X_4$,

Note: X_1 – CRP, mg/l; X_2 – WBC, $\times 10^9$/l; X_3 - lactate serum, mmol/l; X_4 - I-FABR serum, pg/mL.

To simplify the calculations for the two types of SBO, it is possible not to compare the values of F obturation with F strangulation and it is necessary to consider their difference: ΔF = F obturation – F strangulation. In this case, if DF ≥ 0, the patient is classified as a patient with obturation, and if DF < 0 the patient is classified as strangulation type SBO:

ΔF = F obturation – F strangulation = $30.35 - 0.11X_1 - 0.58X_2 + 1.56X_3 - 0.0045X_4$

A clear idea of the quality of discrimination can be obtained from scatter plots projected on the planes of CRP and I-FABR (Fig 1), CRP and serum lactate (Fig 2), CRP and white blood cells (Fig 3).

As shown in table 6, the accuracy of the a posteriori classification was 84.5% in this mathematical model, which for the statistical significance of the discriminant function indicates the adequacy of the constructed model.

DISCRIMINANT ANALYSIS OF SEVERAL BIOMARKERS FOR PREDICTING EARLY MORTALITY

The statistical significance of the obtained discriminant function was estimated based on Wilk's Λ-statistics and was $\Lambda = 0.626$ at $\chi 2 = 20.31$ ($p = 0.000$). The obtained discriminant function contained only two indicators: serum lactate and abdominal perfusion pressure. Therefore, the so-called hyperplane, which divides the n-dimensional space of indicators into two areas and is built using a discriminant function, is transformed into a line on the plane of indicators of serum lactate and APP. It is shown in the scattering graph of these indicators in patients with SBO and early in-hospital mortality (Fig 4). The contribution of each of these indicators to discrimination and patient selection based on treatment outcomes can be judged by the magnitude of the canonical correlation and how the selected indicators correlate with the discriminant function can be seen from the matrix of factor structure (Table VII). It was shown that the contribution to discrimination based on survivors' or non-survivors' status in serum lactate and APP was approximately the same.

The resulting classification functions «**F non-survivors**» and «**F survivors**» were used to predict the outcome of the operation for up to 72 hours. The patient was related to the outcome of treatment after surgery, whose classification function was greater:

F non-survivors = $-308.72 + 24.76X_1 + 8.15X_2$;

F survivors = $-322.42 + 22X_1 + 8.48X_2$,

Note X_1 - Serum lactate, mmol/l, X_2 – APP, mmHg.

When we also considered only two groups (non-survivors - survivors) for simplicity, we can not compare F survivors and F non-survivors, and consider their difference: ΔF = F survivors - F non-survivors. If ΔF ≥ 0, the patient is classified as a survivor; if ΔF < 0 the patient belongs to the group of patients who died.

ΔF = F survivors – F non-survivors = $13.7 - 2.76X_1 + 0.33X_2$.

A clear idea of the quality of discrimination can be obtained from the scatter plots of serum lactate and APP, where the discriminant function is shown in a straight line (Fig 6). The same accuracy of the discriminant model is obtained when using the classification functions 'F non-survivors' and 'F survivors' to predict the outcome of treatment. This is the so-called a posteriori classification when the result is considered unknown and is predicted using the functions «F non-survivors» and «F survivors» (Table VIII). As can be seen from Table 8, the accuracy of the a posteriori classification was 84.2%, which together with the statistical significance of the discriminant function indicates the adequacy of the constructed model.

Table VIII. The matrix of a posteriori classification for the patients with different outcomes of the treatment

The groups of the patients with SBO	Shown the rows: the groups that were observed Shown the columns: the groups that were predicted		
	Percentage of correct observations	Obturation	Strangulation
Non-survivors	85.5%	6	1
Survivors	84.1%	10	54
Total	84.2%	16	55

DISCUSSION

Since SBO is a life-threatening disease, a timely and accurate diagnosis of strangulated obstruction of the small intestine is important as it often leads to ischemia with loss of plasma and the formation of a large number of toxic substances, sequestration of blood in a closed cycle causes an increase in intraluminal pressure, necrosis, and perforation of the intestine [16-17].

Our statistical analysis confirmed the following predictors of strangulation in SBO for laboratory findings before surgery: WBC, serum lactate, CRP, and I-FABP ($P = 0.000$). All these signs were present in 41 (83.7%) of 49 patients with irreversible ischemia of the small intestine before surgery ($P < 0.001$). The statistical significance of the obtained discriminant function was $\Lambda = 0.276$ at $\chi 2 = 86.24$ ($p < 0.000$). A discriminant function was obtained that contained four indicators: CRP, WBC, lactate, and serum I-FABR. Thus, the first mathematical modal was built, and the accuracy of the a posteriori classification was 84.5% in this mathematical model. In addition, the OR also confirmed that the following indicators are important predictors of early mortality: serum lactate, serum I-FABP, level of APP before surgery, and the presence of abdominal sepsis. All these indicators had a probability contribution to mortality of 80% or more. When conducting the discriminatory analysis, only two of these indicators were selected (serum lactate (its canonical correlation was 0.925) and APP (its canonical correlation was −0.74) for the mathematical model of mortality, and the accuracy of the a posteriori classification of this model was 84.2%.

As has been shown early in our studies, some biomarkers, such as lactate, fatty acid-binding protein (I-FABP), and others, can be used as possible markers to determine the severity of patients in urgently patients as had shown early in our studies [18-21]. As is known, the intestine plays a central role in the pathogenesis of MOF in SBO and other urgent surgical diseases, when against the background of a defect of all parts of the immune system is the penetration of bacteria and their toxins through the intestinal mucosa [22,23]. Hypoxia of the intestinal wall, activation of the different processes in the membrane structures of epitheliocytes, as well as suppression of immune reactivity, leads to transient bacteria in the blood and lymphatic system, which then causes increased vascular spasm, and subsequently stable dilatation [24,25]. Intestinal insufficiency, indeed, becomes the «motor» of the pathogenesis of many critical disorders, since there are many risk factors for mortality in SBO including early MOF, the use of vasopressors, mechanical ventilation, surgical stress, etc. As you know, the digestive tract function is very complex, many researchers complex, and to develop various assessment systems to assess its severity in the ICU. The AGI score, proposed by the ESICM working group (2012), which includes abdominal signs and symptoms, IAP scores, and organ function, is considered an important indicator for assessing AT function in ICU patients. This classification is now considered «classical» and accepted by various medical societies. The results obtained in this study showed that for such an indicator as AGI grade the OR was 2.543 with the probability of 71.8% of the development of strangulation SBO before surgery and OR was 2.733 with the probability of 73.2% of the development of the early mortality after surgery. Nonetheless, using such an objective biomarker of enterocyte damage, which is I-FABP, along with the presented indicators can be used to predict strangulation SBO before surgery according to the proposed model with a probability of 84.5%. It should be noted that with almost the same probability (84.2%) it is possible to predict early mortality after surgery using the level of lactate and abdominal perfusion pressure in our proposed mathematical model.

The indicators used for prognosis in this study are not unique and new, but they give us valuable information that must be taken into account when diagnosing and predicting mortality in this category of patients. As it was being, clinicians should take into account the variables that we have analyzed since none of them is specific enough individually, but they are useful in aggregate in the preoperative judgment about the patient [26,27].

CONCLUSIONS

The study confirmed that four lab parameters (blood leukocytes, lactate, intestinal fatty acid-binding protein, and C-reactive protein levels) and one instrumental (involving the mesentery of the small intestine, free fluid in the abdomen during CT) with an 80% probability or more were significantly associated with the strangulation type of SBO ($\Lambda = 0.276$ at $\chi 2 = 86.24$, $p = 0.000$), as well as three lab indicators (WBC count, serum lactate, intestinal fatty acid-binding protein levels) and two clinical parameters (abdominal perfusion pressure level, the presence of abdominal sepsis), was associated with short-term in-hospital mortality after surgery ($\Lambda = 0.626$ at $\chi 2 = 20.31$, $p = 0.000$) with the same probability. Using the proposed two mathematical models for the differential diagnosis of obstruction and strangulation of the small intestine suggests an earlier diagnosis and adequate surgical treatment that will reduce the incidence of irreversible vascular compromise in the intestine and the development of complications after surgery. Given the complexity and ambiguity of the problem, in the future, it is necessary to continue studying the laboratory and potential factors affecting the risk of intestinal strangulation and predict mortality in SBO in different age groups.

ABBREVIATIONS

95% CI - 95% confidence interval
AGI - Acute Gastrointestinal Injury grade
APACHE II - Acute Physiology and Chronic Health Evaluation score
APP - abdominal perfusion pressure
BMI - body mass index
CRP – C-reactive protein
CT - computed tomography

EI - endogenous intoxication
I-FABP - intestinal fatty acid-binding protein
OR - odds ratio
SBO - small bowel obstruction
SBP - systolic blood pressure
SOFA - Sequential Organ Failure Assessment

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The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

MOLECULAR STUDY OF BLASTOCYSTIS HOMINIS ISOLATED FROM DIFFERENT REGIONS OF DIYALA GOVERNORATE

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ABSTRACT

The aim: To detect the infection rate of *Blastocystishominis* in children less than 10 years old with diarrhea in Diyalaby polymerase chain reaction (PCR) method, to determine the subtype of *Blastocystishominis* by sequencing the product of the positive result, and to determine the association between *Blastocystishominis* infection and different factors such as gender, age, the level of mother education and the presence or absence animals in their houses.

Material and Methods: A cross-sectional study was conducted on children with diarrhea at Al-Batool Teaching Hospital in Diyala governorate, during the period from November 2020 to April 2021, a total of 100 children 55 males and 45 females, then, stool samples were collected and examined by conventional polymerase chain reaction.

Results: The rate of infection with the parasite *Blastocystis hominis* was 8%, 8 out of 100. The infection was higher among females 62.5% than to males 37.5%, while the positive result was higher in the age group less than two years 75%, the highest percentage occur with patient whose mothers were incomplete primary and primary education was reached 37.5% and 25%; respectively and the study showed the highest percentage was with those who kept animals at homes was 75%.

Conclusions: According to the genetic analysis of the sequence of eight samples that were positive for *Blastocystis hominis* parasite using the conventional polymerase chain reaction and they were back to the subtypes 3.

KEY WORDS: diarrhea, *Blastocystis hominis*, molecular detection

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INTRODUCTION

Parasitic infection, particularly intestinal parasites, is one of the most frequent infectious diseases worldwide, with developing countries being particularly vulnerable [1]. *Blastocystis* are a common anaerobic protist found in the gastrointestinal tracts of humans and many animals. It belongs to the *Stramenopiles* taxonomic group [2]. Due to the genetic diversity of *Blastocystis sp*., deciphering its taxonomy and developing meaningful terms has proven difficult. By 2013, 17 subtypes had been acknowledged, but *Blastocystis* is currently divided into 22 subtypes (STs) based on polymorphisms of small subunit of rRNA genes (SSU rDNA) consisting of ST1-ST17, ST21, and ST23 to ST26 have been identified in humans and domestic and wild animals worldwide [3-4]. *Blastocystis* are transferred through contaminated food or drink via the fecal–oral route, although there are conditions that encourage transmission, such as poor hygiene and zoonotic contact [5]. Contaminated water is the leading cause of spreading this parasite. *Blastocystis* infections can be transmitted through drinking water, fresh vegetables or fruits, and unwashed hands infected with cysts, because cysts can survive in water for up to 18 days at normal temperatures [6-7]. Vacuolar, granular, amoeboid, and cyst are the four primary morphological types of *Blastocystis* [8]. The main methods of diagnosing *Blastocystis sp.* bydirect examination using the light microscope or by *in vitro* cultivation method [9].Molecular diagnosis by polymerase chain reaction (PCR) detection is an effective tool for detecting and analyzing subtypes of *Blastocystis* spp. from stool specimens, and is increasingly being utilized for the detection of enteric parasites in both people and animals [10], and real-time PCR assay is considered to be a more sensitive method for detecting *Blastocystis* [11]. The prevalence of *Blastocystis* infection varies by nation, depending on the diagnostic methodology employed and the hygienic-sanitary conditions in place [12].

THE AIM

The aim of this study is to detect the infection rate of *Blastocystishominis* in children less than 10 years old with diarrhea in Diyalaby polymerase chain reaction (PCR) method, to determine the subtype of *Blastocystishominis* by sequencing the product of the positive result, and to determine the association between *Blastocystishominis* infection and different factors such as gender, age, the level of mother education and the presence or absence animals in their houses.

MATERIAL AND METHODS

STUDY DESIGN

Cross-sectional study was carried out for patients with diarrhea who presented to the Emergency Department

Table I. Sequence of primers used in this study.

Primer	Sequence	Tm [°C]	GC [%]	Product size
Forward	5′-GGAGGTAGTGACAATAAATC- 3′	47.6	40	486-512 bp
Reverse	5′-TAAGACTACGAGGGTATCTA- 3′	48.3	40	

Table II. The condition of thermal cycling for DNA amplification.

N	Phase	Tm [°C]	Time	Number of cycles
1.	Initial Denaturation	94°C	7 minutes	1 cycle
2.	Denaturation -2	94°C	60 seconds	40 cycles
3.	Annealing	56°C	45 seconds	
4.	Extension-1	72°C	45 seconds	
5.	Extension-2	72°C	7 minutes	1 cycle

Table III. Distribution of Blastocystis hominis in accordance with demographic characteristic.

Variable factors		Positive N(%)	Negative N(%)	Total
Gender	Males	3(37.5%)	52(56.5%)	55
	Females	5(62.5%)	40(43.5%)	45
Age group	0- 1 year	6(75%)	75(81.5%)	81
	2- 3 year	2(25%)	10(10.9%)	12
	4- 5 year	0(0%)	6(6.5%)	6
	6- 7 year	0(0%)	1(1.1%)	1
The presence of animals in house	Present	6(75%)	27(29.3%)	33
	Absent	2(25%)	65(70.7%)	67
Maternal educational level	Incomplete primary edu.	3(37.5%)	22(23.9%)	25
	Primary education	2(25%)	33(35.9%)	35
	Secondary education	2(25%)	21(22.8%)	23
	High education	1(12.5%)	16(17.4%)	17
Total		8(100%)	92(100%)	100

of Pediatrics at Al-Batool Teaching Hospital in Baqubah city, during the period from November 2020 to April 2021.

STOOL SAMPLES COLLECTION AND DNA EXTRACTION

Stool samples were taken from 100 children who were suffering from diarrhea, ranging in age from one to seven years. Disposable gloves and wooden sticks were used to transfer small volumes of diarrhea to Eppendorf tubes, which were then labeled. For storage, these were placed in a deep freeze (-20 to -80°C). The DNA was extracted from stool samples using Quick-DNA™ fecal/soil microbe miniprep kit (Cat no D6010, Zymo research, U.S.A.)

GENE AMPLIFICATION BY CONVENTIONAL PCR

The sequence of primers Bohme et al., 1997; Reverse Stensvold et al., 2007 were used in this study to amplify 486-512 bps fragment of the SSU rRNA gene of *Blastocystis hominis* are listed in table I.

PRIMER PREPARATION

The primers were lyophilized, they dissolved in the nuclease free water to give a final concentration of 100 pmol/µl as stock solution and keep a stock at -20.To prepare 10 pmol/µl concentration as work primer suspended, 10 µl of the stock solution in 90 µl of the nuclease free water to reach a final volume 100 µl, was investigated by IDT (Integrated DNA Technologies company, Canada).

PROTOCOL OF CONVENTIONAL PCR

A conventional PCR was used to amplify the SSU rRNA gene of *Blastocystis hominis*, a (486-512 bps) fragment was amplified by using forward and reverse primers, the PCR reaction mix was performed in (25 µl) total volume, (1.5 µl) template DNA, (1 µl) of each forward and reverse primer, and (16.5 µl) nuclease free water, all of which were added to tube containing 5 µl of TaqPCR Pre Mix according to protocol kit then thermal cycling was listed in table II.

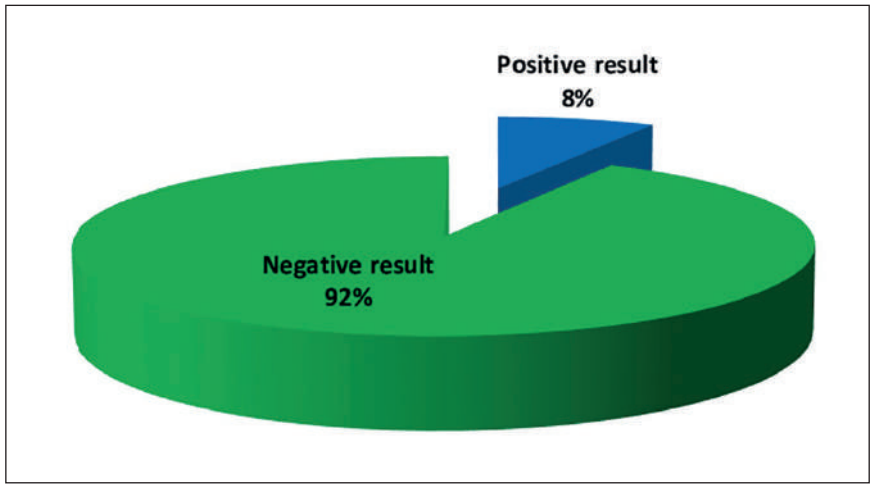

Fig. 1. Rate of Blastocystis hominis infection in accordance with conventional PCR.

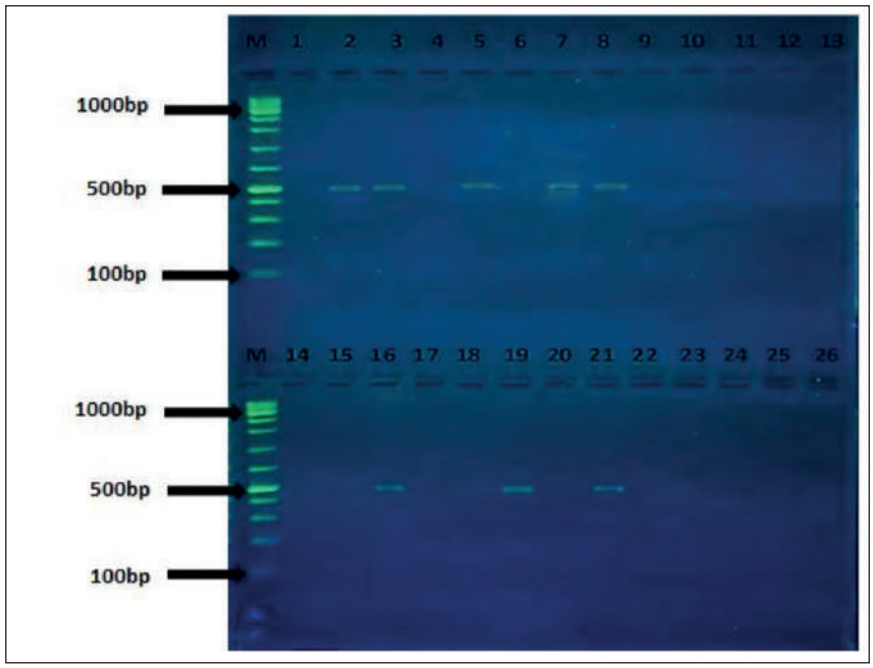

Fig. 2. Gel electrophoresis image show the positive bands for the SSU rRNA gene of Blastocystis hominis, stained with red safe stain on 2% agarose gel, M: ladder marker (100-1000 bp).

SEQUENCING OF PCR PRODUCTS AND DATA ANALYSIS

While successful amplification of the target regions of *Blastocystis hominis* by conventional PCR, (25 µl) of product together with primers, were sent to Macrogen in South Korea for direct sequencing. Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at http://www.ncbi.nlm.nih.gov and BioEdit program.

STATISTICAL ANALYSIS

In the present study, the Statistical Analysis System-(SAS 2012) program was used to detect the influence of difference agents in study parameters, and chi-square test was used to significant compare between percentage 0.05 and 0.01 probability.

RESULTS

There were eight samples which a positive result (8 out of 100) for *Blastocystis hominis* among children with diarrhea when tested by conventional PCR as show in figure 1 after electrophoresis on the agarose gel as show in figure 2.

Results of *Blastocystis hominis* infected children was 8 positive, 5 cases were females and 3 cases were males. According to age group, 6(75%) of the positive result in age group 0-1 year, 2 25% of the positive in age group 2-3 year, but no positive case was noticed with other age groups and the rate of *Blastocystis hominis* according to the presence of animals in house or nearby, the education levels of mothers of the patients were with highest rate with incomplete primary education 37.5%, followed by 2(25%) for each primary education and secondary education, and only 1(12.5%) mother with high education as shown in table III with significant 0.05.

GENETIC ANALYSIS AND PHYLOGENETIC TREE

The *Blastocystis hominis* sub-type 3 small subunit ribosomal RNA gene were registered after correspondence with the National Center for Biotechnology Information, the accession number was assigned, and it became a reference

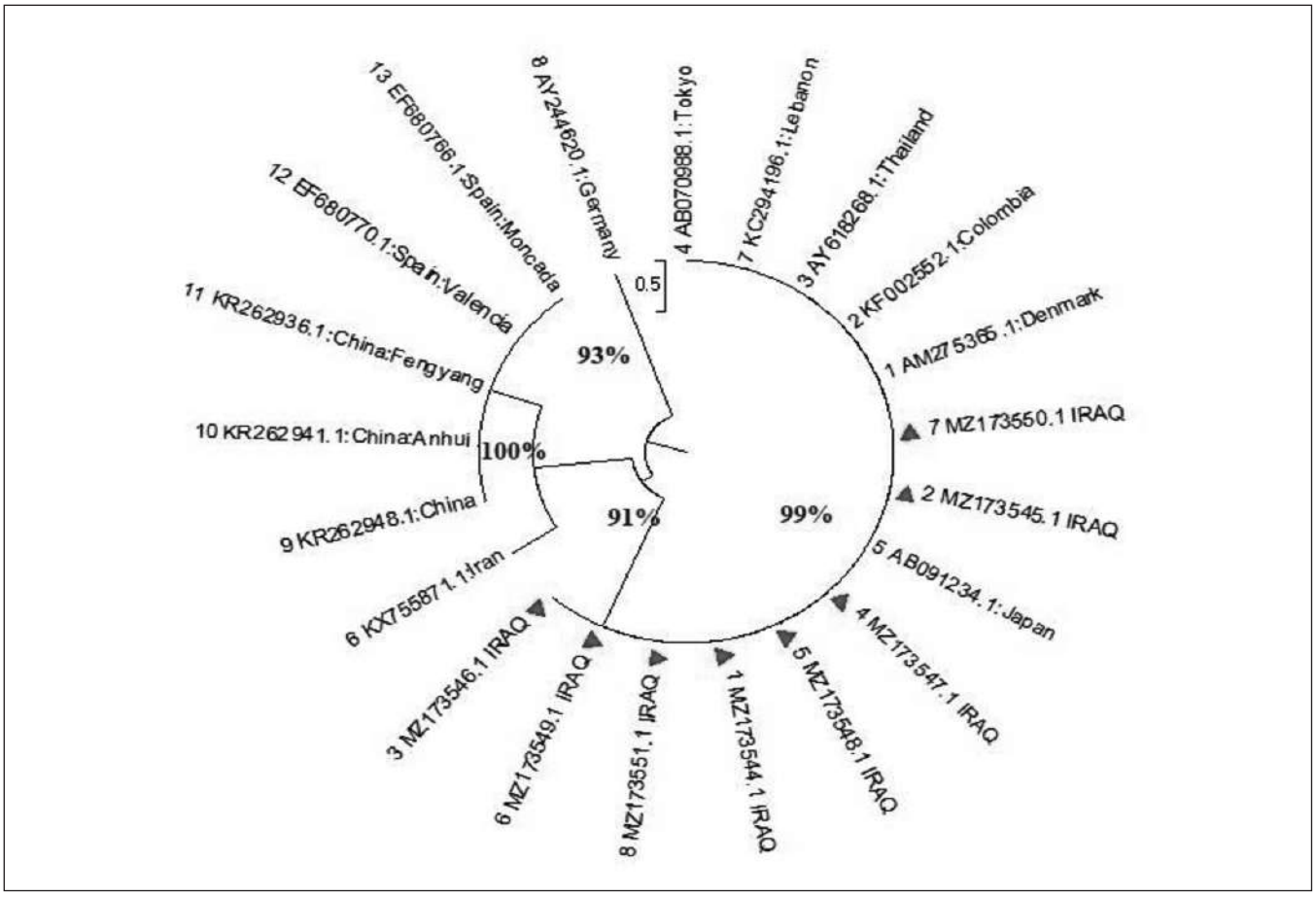

Fig. 3. Neighbor-joining tree Blastocystis hominis subtype 3 small subunit ribosomal RNA gene, involved 21 nucleotide sequences (13 sequences reference from Gene Bank). Current isolates are indicated with red triangle.

for Iraq, the Middle East, and the rest of the world. As more type strains are disclosed, this set will be expanded, and it is accessible for download at ID: MZ173544.1, MZ173545.1, MZ173546.1, MZ173547.1, MZ173548.1, MZ173549.1, MZ173550.1 and MZ173551.1 (Fig. 3).

DISCUSSION

The result of this study was 8% infection rate with *Blastocystis hominis* using PCR, This outcome was compared to the approach infection rate of *Blastocystis hominis* studies around the world like 8.4 in Switzerland [13] and 8.5% in Saudi Arabia [14]. However, the infection rate in this study was higher than the 3.33% seen in Southwestern Iran [15] and 2.9% infection rate in Qatar [16]. Other studies showed higher rates of infection than this study such as 63.7% in Lebanon [17]. This wide variation among studies could be attributed to the time and period of samples collection; number of samples, the age of the study population, the season, the techniques is used to diagnose the parasite. Variations in behaviors, regional variables, temperature changes, and individual immunological and nutritional conditions could all have a role in the disparity in parasite prevalence rates between studies. In the present study, the distribution of *Blastocystis hominis* in this study according to PCR method showed high prevalence in female 5(62.5%) more than males 3(37.5%) this agree with other studies like in Iraq the percentage of females infected was 56.7%, while for males was 43.3% [18]. we suggest that there is no relationship between gender and infection with *Blastocystis hominis*, and it is possible that the infection depends on the individual's immunity. The results of this study according to age conventional PCR result showed 0-1 year's rate 75% and 2-3 years at rate 25%. A study in Iraq showed the rate was higher 20.79% in the children group [19] and in Denmark; Children under the age of three years had the fewest positive instances 6% [20]. The reason for increasing the infection in this age group was due to the fact that most of the samples collected were from the age group under 2 years and this age group considered less knowledgeable about cleanliness and had a desire to play and pick up unclean objects, motivated by exploring things around them. The result of the PCR method where it was 6(75%) for those who raised animals and 2(25%) for those who did not raise animals, This agreed with study conducted in Mexico, which showed consumption of *Blastocystis*-contaminated food or water, as well as exposure to domestic animals infected with this parasite, have all been associated to a higher incidence of *Blastocystis* [21], while according to PCR the rate of incomplete 37.5%, primary education 25%, secondary education 25% and high education 12.5%. This in comparison with study in Iran, demonstrated that the prevalence of *Blastocystis*

hominis declined with higher educational levels, with the highest percentage of positive cases 23.8% in those with an elementary education and the lowest percentage of positive cases in those with a university education 9.1% [22]. Poor education caused a lack of awareness of hygiene and health care matters that caused disease reduction, and continuing ignorance of disease transmission methods, and this was one of the reasons that pose a health risk to society. phylogenetic analysis for *Blastocystis hominis* isolates from stool samples identified eight isolates, according to the findings (ZaMoSh-1 to ZaMoSh-8) (Sequence ID: MZ173544.1, MZ173545.1, MZ173546.1, MZ173547.1, MZ173548.1, MZ173549.1, MZ173550.1 and MZ173551.1). This is local isolates sequenced alignment with 13 references isolates software Mega 6. The eight isolates were discovered to be related to subtype 3 and cluster with *Blastocystis hominis* ST3 isolate 05/RS158.2 from Denmark with identical 99%. *Blastocystis hominis* subtype 3 was the most predominant subtype obtained in human samples of both symptomatic and asymptomatic patient, followed by ST1 in most studies such as in Qatar [16], in Kirkuk province [23], in Saudi Arabia [24], in Iran [25], in Denmark [20]. In the current study was obtained only subtype 3 without others subtype, this agree with a study in Egypt [26].

CONCLUSIONS

The rate of *Blastocystis hominis* was 8% in children with symptom of diarrhea in Diyala province. Most of the infection rate of *Blastocystis hominis* was observed among females and the age group was less than two years. The degree of education of the mother played a significant influence, as children whose moms had just a primary or partial education were more likely to be infected. Most of the infected children had animals in their homes. Eight *Blastocystis hominis* isolates obtained in this study belonged to subtype 3.

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ORIGINAL ARTICLE

FEATURES OF COAGULOPATHY AND SYSTEMIC INFLAMMATION IN PATIENTS AFTER COVID-19 INFECTION

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ABSTRACT

The aim: To determine the peculiarities of laboratory data concerning blood coagulation and systemic inflammation in COVID-19 patients in three months after discharge and recovery. The state of coagulation, anticoagulation, and fibrinolytic systems, as well as their prognostic value having been well studied in hospitalized COVID-19 patients, their state three months after hospitalization, are not yet well understood.

Materials and methods: Methods of randomization, anthropometry, ECG, standard clinical blood testing, immunoenzymometry, immunoanalysis, and primary statistical analysis were used in the study. Anthropometric measurements of patients (n=20), blood samples, blood serum samples, urine samples, and statistical data were the materials of the study.

Results: Indices of coagulation and systemic inflammation in studied patients after COVID-19 were obtained (PTT, s ; PATPT, s; Fibrinogen, g/L; Platelets ×109 /L; PCT, ng/mL; DD, µg/L; CRP, mg/L; IL -6, pg/mL; IL -10, pg/mL; Cortisol (nM/L); CIC (IU/mL); Ig A (g/L).

Conclusions: Summing up the results obtained, it is possible to assert micro- and macro-vascular thromboses to be common in COVID-19 cases; they are associated with poor prognosis for diseased patients and are not completely investigated; the role of thromboses in COVID-19 course and complications are to be studied as well as the strategies of fibrinolytic therapies for such condition are to be justified. The presence of specific rheological and serological changes in patients even three months after surviving COVID-19 needs further study to understand the necessity of anti-thrombolytic drug uptake for a relatively long time.

KEY WORDS: COVID-19, Inflammation, Thrombolytic Therapy, Blood Platelets, Immunoglobulins, Interleukins

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INTRODUCTION

Viral respiratory infections including coronavirus agent of severe acute respiratory syndrome (SARS-CoV), coronavirus agent of Middle East respiratory syndrome (MERS) as well as SARS-CoV-2 pandemic agent initiate coagulopathies, lead to intravascular thrombi and fibrinogen deposits formation [1], this infection consequences being associated with unique pro-thrombotic pathophysiology [2].

In COVID-19 patients, usual blood coagulation anomalies imitate often other systemic coagulopathies associated with severe conditions such as disseminated intravascular coagulation (DIC); however, coagulation anomalies in COVID-19 patients possess some marked differences [3].

Deep vein thromboses (DVT) and lung artery embolism (LAE) are found in 20-30% of COVID-19 patients [2, 3]; in Holland, the cumulative incidence of large vessels thromboses reached 49%, the majority of them being LAE; in Italy the level of such events was 21% (27.6% of them were found in intensive care units and 6.6% in general population); in France the thromboses level was as high as 20.6%. In meta-analysis including 49 investigations having been realized with 18,093 patients, the common LAE incidence reached 17.0% (9.8-33.0%) [4]; in large randomized controlled studies 6-10% of LAE were found in cases of anticoagulants use in prophylactic doses; when anticoagulant compounds have been used in therapeutic doses, the LAE levels were lower (4-8%) [5]; other systemic reviews indicate the LAE incidence to be 28%, its fluctuations reaching 19-24% in cases of clinical diagnostics and 36-46% for routine screening [6]. All the authors agree the blood coagulation system to be activated and disturbed in SARS-CoV-2 infected humans; however, the properties of coagulopathies associated with this condition are different from other well-known blood coagulation disorders [7]. Such disorders include immunomediated thrombotic mechanisms, complement activation, syndrome of macrophages activation, anti-phospholipid antibodies syndrome, hyper-ferritinemia, and regulative disturbance of renin-angiotensin system [1].

J. Connors, J. Levy [8] have summed up the data being associated with the SARS-CoV-2 coagulopathy; they have found such a coagulopathy to possess some specific features including a marked increase of D-dimer (DD) levels as well as of fibrin/fibrinogen degradation products; at the same

time, changes of prothrombin time (PTT), partially activated thromboplastin time (PATPT), and platelets quantity are insufficient; such features are not common for the usual DIC syndrome. The hemostasis damage described for the COVID-19 infection includes the increase of DD and fibrinogen levels [9], changes of platelets quantities and of their activation levels, increase of the von Willebrand factor (VWF) level [10] as well as changes of other coagulation parameters. A constantly increased risk of thromboembolism events being observed even at the beginning of prophylactic anticoagulation therapy testifies the presence of hypo-fibrinolysis process in addition to hyper-coagulation found in SARS-CoV-2-infected patients [11]. All researchers agree the coagulopathy manifestations in these patients do not completely correspond to the DIC-syndrome determination; however, the dynamical monitoring of C-reactive protein (CRP) levels as well as of fibrinogen (FIB) and DD ones and of their ratios in sera (CRP/FIB, FIB/DD) are to optimize the timely disease diagnostics, management, and disease course prognosis [12].

Summing up all the data obtained it is possible to approve that micro- and macro-vascular thromboses are rather common; they are COVID-19-associated and are markers of poor prognosis; these thromboses possess their own etiopathogenetic properties being yet not completely studied and needing further investigations in the field of their role in disease course and prognosis as well as for justification of fibrinolytic therapy approaches in COVID-19 cases.

THE AIM

To determine the peculiarities of laboratory data concerning blood coagulation and systemic inflammation in COVID-19 patients in three months after discharge and recovery.

The state of coagulation, anticoagulation, and fibrinolytic systems as well as their prognostic value having been well studied in hospitalized COVID-19 patients, their state in three months after hospitalization are not yet well understood.

In this regard, in the course of the study it is planned to study the role of thrombosis in the course and complications of COVID-19, as well as to substantiate the strategies of fibrinolytic therapy of this condition, to evaluate the changes in prothrombin time, partially activated thromboplastin time, the number of platelets, fibrinogen, D-dimer and PCT, to identify their relationship relationship with systemic inflammation, i.e. IL-6, IL-10, IgA, CIC, cortisol and CRP levels.

In addition, it is necessary to detect and evaluate rheological and serological changes in patients three months after the transfer of COVID-19.

MATERIALS AND METHODS

During 2020-2021, 20 patients were examined in 3 months after their treatment at the hospital because of the COVID-19, the studies having been realized in the Center of Primary Health Care N3 of the Sviatoshyn region of Kyiv-City. The SARS-CoV-19 was diagnosed according to the recommendations of the World Health Organization [13], clinical guidelines «Clinical Management of COVID-19 Patients» [14], Order of the Ministry of Health Care of Ukraine № 762 (02.04.2020) (the version of the Order of the Ministry of Health Care of Ukraine N 358, 22.02.2022) «Protocol for the provision of medical care for the treatment of coronavirus disease (COVID-19)» [15], Order of the Ministry of Health Care of Ukraine N 771 (20.04.2021) «Protocol for the provision of rehabilitative aid to patients with the diagnosis of coronavirus disease (COVID-19) and convalescents" [16]. The COVID-19 diagnosis in our patients has been earlier confirmed in clinical laboratories by the detection of the SARS-CoV-2 RNA [16].

All the patients received all the necessary information for patients and signed their "Voluntary informed consent for the realization of diagnostics, management, carrying out of operation and anesthesia" (form №003-6/o) and "Voluntary informed consent for the treatment of patient's data" according to the Order of the Ministry of Health Care of Ukraine № 110 (14.02.2012) [17]. The research protocol was prepared according to the Helsinki Declaration. According to the Order "Protocol for the provision of medical care for the treatment of coronavirus disease (COVID-19)" [18] was used after the patients' voluntary informed consent. The inclusion criterion was the following: patients aged 45-65 years after laboratory confirmed COVID-19 infection who have been under the supervision of family doctors during 3 months. The exclusion criteria were the following: patients with comorbidities, aged > 65 years with habits and states able to change their clinical parameters during the natural COVID-19 development.

The patients were divided in 2 groups by a randomization method, each group contained 10 persons. The patients' mean age for groups 1 and 2 were 58.16±1.32 and 60.37±1.03, respectively; their mean height was 171.24±1.23 and 172.50±1.35 cm, respectively. The patients' mean body mass reached 83.24±1.94 kg and 85.00±2.00 kg for groups 1 and 2, respectively, the Quetelet index reaching 28.35±0.54 and 28.42±0.53 kg/m^2, respectively. The group 1 included 50% of males and 50% of females, the group 2 contained 40% of males and 60% of 40% of females. Twenty per cents of patients examined were smoking abusers, 80.0% of them being non-smokers. Among 20 patients having survived COVID-19 infection, 60.0% were persons with higher and 40% – with secondary education. The group 1 contained 10 patients with proved COVID-19 course of moderate severity, the group 2 containing 10 patients with proved COVID-19 infection of severe course. Our investigation was carried out according to the "Protocol for the provision of rehabilitative aid to patients with the diagnosis of coronavirus disease (COVID-19) and convalescents" given in the Order of the Ministry of the Health Care of Ukraine N 771 [16]. The data concerning the disease progress were taken from medical documentation and from detailed interviews with our patients. According

to the Order mentioned, the following up of COVID-19 patients includes the main results of investigations obtained for persons having survived acute respiratory failure in 6-8 weeks after discharge as well as different measures aiming to improve breathing and physical exercises. The persons having survived COVID-19 with present/continuous lung function damage in 6-8 weeks after discharge underwent a complex lung rehabilitation program according to established international standards (Quality Standards for Pulmonary Rehabilitation in Adults, 2014 [19], British Thoracic Society Guidelines on Pulmonary Rehabilitation, 2013 [20]; American Thoracic Society, Assembly on Pulmonary Rehabilitation «Guidance for Re-opening Pulmonary Rehabilitation Programs», 2020 [21]). The program of lung rehabilitation used in our study included the assessment of patients' condition as well as individualized rehabilitation program including also physical exercises, elements of sanitary education, and psycho-correction. We tried to improve both physical and psychological condition of patients with chronic respiratory diseases, to increase their desire to become healthy, and to understand the significance of different risk factors [16].

All the patients were examined by a cardiologist using ECG; common clinical blood analyses were realized (count of blood elements and hematocrit determination) as well as biochemical urine and blood analyses (in order to determine kidney and liver functions as well as levels of serum creatine kinase, lactate dehydrogenase, and glucose, enzymes of myocardium and CRP) [according to the Order of the Ministry of Health Care of Ukraine N 771 (20.04.2021) "Protocol for the provision of rehabilitation aid to patients with the diagnosis of coronavirus disease (COVID-19) and convalescents [16]. We estimated such indices as PTT, PTPT, FIB, and DD values [16]. IL-6 and IL-10 values were measured using a standard of human cytokines 27-Plex. A panel of analyses and a system Bio-Plex 200 (Bio-Rad, Hercules, Ca, USA) were used according to the manufacturer's instructions [22].

The cortisol level determination in post-COVID patients' sera was carried out using solid phase immunoenzyme analysis and test kits "Cortisol-EIA" of the firm "Khema" («Хема»), Ukraine, and a photometer "Stat Fax 303" for registration of immunoenzyme analysis results [22].

The level of circulating immune complexes (CIC), mostly of IgG-binding C1q, was determined by a precipitation approach using a Stat Fax 303 photometer and taking into consideration the referent data for adults (0.025-0.045 IU/mL) [22].

The IgA concentration was determined by a solid immunoanalysis approach using a photometer Stat Fax 303 [22] and immunoenzyme kits "Common IgA – EIA" («Загальний IgA – ІФА») from the "Khema" firm (Ukraine).

The mathematical processing of results obtained was made using mathematical statistics approaches. The statistical description of investigation results was realized by methods of primary statistical analysis [23]. Having determined the arithmetic mean values (M) and arithmetic mean errors (m), we have found the distribution of indicators on normality using the Kolmogorov-Smirnov criterion. We have stated the distribution of the majority of indicators to be different from the normal one on the significance level 0.05. The distribution analysis was carried out for each criterion studied. The Student's t-test was taken for evaluation of scatter of random collections with "normal" distribution. In cases the collections with distributions being different from "normal" ones, the U-test according to the Mann-Whitney method was used. In cases of qualitative signs distribution different from the normal one median and interquartile range were determined (Me (25.0%; 75.0%)).

RESULTS

The PTT as an important blood coagulation index measures the time necessary for blood plasma coagulation. Although a routine PTT determination has been recommended for the evaluation of COVID-19 associated coagulopathy at the beginning of this epidemic, in the majority of COVID-19 patients this parameter is normal or near normal; there are sometimes mentions about elongated PTT in patients with severe disease course [24]. In all our patients as well as in patients of groups 1 and 2 the PTT values were 12.7, 12.6, and 12.8 s (Table), its level being below 11.5 s in 30.0%, 20.0%, and 10.0% of patients examined, respectively; 10% of all patients and 10% of them in each group showed the PTT value above 14.5 s.

Usually the index of PATPT in COVID-19 patients is normal and not associated with severity of this condition course. The PATPT elongation may be an indicator of blood coagulation factor, presence of specific coagulation inhibitors (antibodies to the factor VIII) or a laboratory artefact because of the presence of anti-phospholipid antibodies. In some COVID-19 patients the artefact PATPT elongation was found because of lupus erythematosus or increased heparin resistance because of high levels of fibrinogen or factor VIII [25]. In all our patients and in patients of groups 1 and 2 the PATPT values were 34.4, 34.1, and 34.6 s, respectively (Table).

The main platelets function is their participation in the system of blood coagulation and fibrinolysis processes. In all patients examined in our study as well as in patients of groups 1 and 2 the quantities of platelets were 206, 188 and 224×10^{9}/L, respectively (Table), their quantity below 100×10^{9}/L having been found only in 15.0 and 30.0% of all patients and of patients from the group 1; in 15.0% of all patients as well as in 10.0 and 20.0% of patients from groups 1 and 2 the platelet concentrations were above 300×10^{9}/L.

Fibrinogen is an important factor of blood coagulation system responsible for the final stage of thrombi formation, their stabilization, and stop of bleeding. In the interstitial tissue fibrinogen develops a background for fibroblasts and histiocytes growth; in any tissue being damaged fibrinogen and fibrin concentrations are increased; there they intensify the migration of granulocytes producing different growth factors and realizing necrosis products phagocytosis;

Table I. Indices of coagulation and systemic inflammation in patients after COVID-19

Indices of coagulation and systemic inflammation	Patients after COVID-19		
	All the patients	Group 1	Group 2
PTT, s	12.7 [11.3; 13.1]	12.6 [11.7; 13.1]	12.8 [12.2; 13.7]
PATPT, s	34,4 [29.5; 37.2]	34.1 [29.9; 37.6]	34.6 [29.0; 38.3]
Fibrinogen, g/L	4.1 [3.1; 4.63]	4.0 [3.03; 4.68]	4.2 [3.8; 5.28]
Platelets ×109 /L	206 [159; 235]	188 [152; 218]	224 [176; 277]
PCT, ng/mL	0.10 [0.05; 0.27]	0.12 [0.04; 0.26]	0.08 [0.04; 0.12]
DD, µg/L	271 [123; 282]	220 [135; 378]	321 [228; 560]
CRP, mg/L	6.5 [6.2; 6.7]	5.3 [5.0; 5.6]	7.7 [7.5; 7.95]
IL -6, pg/mL	4.3 [2.4; 5.8]	4.5 [2.2; 11.8]	4.1 [2.6;10.3]
IL -10, pg/mL	3.0 [0.9; 3.9]	2.5 [0.9; 3.8]	3.4 [1.9; 4.9]
Cortisol (nM/L)	209.3 [193.33; 233.05]	195.6 [191.38; 212.88]	223.0 [196.3; 269.9]
CIC (IU/mL)	0.037 [0.025; 0.047]	0.045 [0.033; 0.054]	0.028 [0.022; 0.036]
Ig A (g/L)	1.6 [1.3; 1.95]	1.8 [1.4; 2.2]	1.4 [1.1; 1.8]

these processes are especially important for damaged tissue regeneration. Products of both fibrinogen and fibrin degradation possess anti-coagulant activity and are able to inhibit the process of fibrin formation. Fibrinogen is a valuable hemostasis index. A soluble fibrinogen precursor belongs to acute phase proteins, its concentration becoming higher in cases of inflammation processes of different etiologies. The fibrinogen level becomes quickly increased in cases of acute inflammation or tissue damage, its concentration being able to become 10 times higher. Fibrin takes part in adhesion processes as well as in trans-endothelial migration of monocytes and neutrophils; it stimulates the chemokines secretion by macrophages in extra-vascular space helping the strengthening of the immune response during the inflammation process. In all our patients and in patients of groups 1 and 2 the fibrinogen levels were 4.1, 4.0, and 4.2 g/L, respectively (Table I), this level being below 2 g/L only in 5% of all patients and in 10% of group 1 patients; the level above 4 g/L was found in 55.0% of all patients as well as in 50.0 and 60.0% of patients belonging to groups 1 and 2, respectively.

Our results are similar to data obtained in numerous investigations and confirm the opinion such a combination of anomalies to testify the presence of coagulopathy similar DIC, being, however, phenotypically different from DIC associated with other inflammation syndromes, such as hemophagocytic lymphohistiocytosis (HLH) accompanied by marked hypofibrinogenemia, and sepsis with significant thrombosis [26]; no significant deviations concerning fibrinogen level and quantity of platelets were found in patients with cases of severe and critically severe COVID-19. Besides, DIC-imitating coagulopathy in cases of COVID-19 does not meet the evident DIC criteria confirmed by the International Society of Thrombosis and Hemostasis (ISTH) [27].

DIC-syndrome is accompanied by the development of systemic or common vascular thrombosis leading to non-adequate blood delivery to different organs [28]. DIC-syndrome is a rather common, but fatal consequence of cytokine storm and a significant death precursor in cases of this pathology; however, it is not the main driving force for coagulopathy and critical illness seen in COVID-19 cases. Our results obtained for coagulation parameters suggest the coagulopathy to be associated with deep inflammation and to mediate the SARS-CoV-19-accompanying thrombotic complications.

COVID-19-associated coagulopathy is characterized by almost normal platelets level and PTT in the majority of patients, as well as by evenly high levels of DD and fibrinogen [24]. As a contrast, the DIC-syndrome seen rarely only in patients with severe COVID-19 cases is characterized by lowered platelets quantity, increased DD levels, PTT elongation and decreased fibrinogen level; it is associated with poor prognosis in COVID-19 patients [29]. Recent studies comparing the properties of coagulation in cases of severe COVID-19 due to pneumonia and cases of pneumonia due

to other factors show he quantity of platelets to be higher in SARS-CoV-2 infected persons, the relative levels of DD and PTT elongation being, however similar [30].

DD is the main fragment of the fibrinogen degradation used as a biomarker of coagulation and fibrinolysis [31]. During the SARS-CoV-2 pandemic the DD was widely investigated. Now it is known DD levels to be increased in the majority of patients hospitalized because of COVID-19. The DD levels reach the highest levels in 5 days after hospitalization and are significantly higher in critically-ill patients [32]. The extended study of the DD levels in 2,377 COVID-19 patients in hospitals has revealed an association between the initial and peak DD level – a situation associated with thromboses, acute kidney damage, and mortality due to all causes [33]. Another review demonstrates the DD levels may be used for identification of COVID-19 patients needing CT and lung angiography for diagnostics of lung artery thromboembolism, the boundary DD levels may reach ≥1000 μg/L [34]. In all our patients investigated and in patients of groups 1 and 2 the DD levels were 271, 220, and 312 μg/L, respectively (Table), its level above 300 μ/L having been found in 40.0% of patients; at the same time, 20% of all the patients examined in our clinic as well 20% of them belonging to groups 1 and 2 showed DD levels above 500 μg/L.

PTC is a precursor of the hormone calcitonine being synthesized by thyroid C-cells. In some pathological conditions PCT may by produced by other tissues and organs including liver, kidneys, muscles, and fat tissue. We are talking about the cascade of extra-thyroid PCT synthesis being switched on as a response on the aggression of some microorganisms or toxins. The rapid increase of this biomarker level is seen in cases of systemic inflammation answer due to bacterial or fungal invasion as well as in cases of protozoan infections. The PCT levels are usually not changed if a human is infected only by any viral agent. In all our patients and in the patients of groups 1 and 2 the PCT levels were 0.10, 0.12, and 0.08 ng/mL, respectively (Table I).

Cytokines are known to be able to disturb the normal human hemostasis in some pathological conditions; they are incredibly among the main factors of thrombotic SARS-CoV-2 potential, contributing to disbalance in pro-thrombotic and anti-coagulating pathways including the loss of action of tissue factor pathway inhibitor (TFPI), decrease of the regulation of thrombomodulin expression in endothelial cells, and decrease of anti-thrombin (AT) III level in the blood serum. Such cytokines changes are associated with coagulopathy in cases of sepsis and lead to the process known as immunothrombosis. Different thrombotic triggers produced during this event including also cytokines, activated thrombocytes, extracellular traps of complement and neutrophils play a certain role in the inflammation process. The analysis having been carried by M. Ranucci shows 16 patients with COVID-19 accompanied by the acute respiratory distress-syndrome (ARDS) to have the IL-6 levels correlating with thrombosis and hyper-coagulation markers [34].

The IL-6 is produced by different cell types – by macrophages, T- and B-lymphocytes, fibroblasts, by endothelial, epidermal, and microglial cells as well as by chondrocytes and osteocytes. It promotes the production of acute phase proteins and corticotropin, induces fever development, terminal B-cell differentiation, and antibody production; in cooperation with other cytokines it accompanies the stem cells proliferation and differentiation as well as the activation of CD4+-, CD8+-, and T-lymphocytes. It is a pro-inflammation cytokine.

The cytokine IL-6 is a pleiotropic inflammation mediator and the central stimulus of the acute phase response. This cytokine plays an important role in the pathological response to inflammation leading to the severe COVID-19, high serum levels of the IL-6 being a marker of more severe consequences in hospitalized COVID-19 patients. The IL-6 is a biomarker of the severe COVID-19 infection as well as an important etiopathogenetic factor. Although IL-6 is a such marker, it cannot be used for differentiation of COVID-19 and other possible factors of severe disease. Meta-analysis of 19 investigations (1,245 patients) shows the increased IL-6 levels in critically ill COVID-19 patients to be, however, significantly lower comparing to its levels in cases of sepsis not associated with COVID-19 and in cases of ARDS [35]. The data obtained suggest the necessity of determination of the IL-6 levels as a prognostic marker in cases of severe COVID-19 course, its exact association with the thrombosis development being, however, not found [36].

IL-10 is a suppressive factor produced mostly by Th2. It inhibits the function of Thl, NK cells and monocytes decreasing the production of immunocytokines (gamma-IFN, PNP, IL-1, IL-8). IL-10 increases also the proliferation of B-lymphocytes and tissue basophiles. In such a way, IL-10 belongs to the most important regulatory cytokines determining the results of the immune response; the Th1-regulated cell response is inhibited under the IL-10 influence, the humoral response (Th2) becoming stimulated. IL-10 belongs to anti-inflammatory cytokines. In all our patients examined and in patients of groups 1 and 2 the IL-6 levels were 4.2, 4.4, and 4.0 pg/mL, respectively, the IL-10 levels in the same patients being 3.0, 2.5, and 3.3 pg/mL, respectively (Table I).

R. Bodnar, C. Yates, A. Wells [37] think IL-10 to activate the cell-medicated immune response of the T-helper type. This substance is produced by macrophages, endothelial cells, and fibroblasts; it acts as a chemoattractant for a lot of cells including, in particular, macrophages and T-cells. IL-10 inhibits proliferation, induces endothelial cells apoptosis and prevents the gaining of their mobility.

An important factor is the synergism between IgA and non-specific defense mechanisms, such as complement, lysozyme, cells having been phagocytated and their enzymes; such synergism leads to higher antibacterial defense increasing its total efficacy. In all our investigated patients as well as in persons of groups 1 and 2 грyп the IgA levels were 1.6, 1.8, and 1.4 pg/mL.

One of the most important biological functions of immunoglobulins is antigen fixation and immune complexes

(IC) formation – a physiological process being constantly realized in the organism and directed on the support of its inner environment. The IC formation is one of normal immune response components. The most important IC ability is complement system activation determining the IC role in the inflammation development and regulation of functional immune system regulation. In all patients and persons from groups 1 and 2 the levels of CIC were 0.037, 0.045 and 0.028 IU/mL, the cortisol levels being 209.3, 195.6, and 223.0 nM/L, respectively.

CRP is a known marker of acute inflammation in a lot of diseases. It is produced by the liver as a response to inflammation-accompanying cytokines, especially to IL-6. The levels of both CRP and IL-6 in COVID-19 patients become higher and correlate positively with disease severity and mortality [36]. Meta-analysis of 20 studies including 4,843 COVID-19 patients found four times higher risk of complications and death for patients with increased CRP level [38]. A large analysis having been realized in the USA using data for 2,782 COVID-19 patients shows > 97% of these persons to have increased CRP levels at the moment of hospitalization; high initial CRP levels are associated with thromboses, acute kidney damage, and mortality due to different factors [11]. The data mentioned show a positive correlation between CRP levels and COVID-19 severity; contrary to IL-6, the CRP levels are a prognostic factor for thrombosis risk [39].

The CRP induces the increase of expression and activity of the tissue factor (TF) as well as the decrease of expression of tissue factor pathway inhibition (TFPI); it causes activation of inflammation and coagulation, disturbance of endogenic fibrinolytic ability as well as stimulation or increase of platelets adhesive activity and sensitivity. Epidemiological studies show the increased CRP concentrations to be associated with venous thromboembolism risk (VTR) [40]. In all patients examined in our study as well as in patients of groups 1 and 2 the CRP levels were 6.5, 5.3, and 7.7 mg/L (Table), its level above 10 mg/mL having been shown in 50.0, 40.0, and 60.0% of all the persons examined and patients of groups 1 and 2; at the same time the CRP level was above 30.0 mg/mL in 20.0% patients in each group.

DISCUSSION

At the moment, besides the treatment of the leading disease cause, the International Society of Thrombosis and Hemostasis (ISTH) recommends to treat thrombotic COVID-19 complications using prophylactic systematic introduction of low molecular weight heparin (LMWH) to all the hospitalized patients [27]. LMWH is a better choice than non-fractioned heparin, taking into consideration its use once per day and low risk of heparin-induced thrombocytopenia. Renewed scientific recommendations as well as standardized recommendations include the use of moderate doses of LMWH (enoxaparin, 40-60 mg daily); the drug dose is to be titrated especially for patients with obesity, severe thrombocytopenia or damaged renal function. Besides, because of the absence of specific data concerning COVID-19, the ISTH recommends to continue the thromboprophylaxis during 2-6 weeks using LMWH; the American Society of Hematology (ASH) and the American College of Cardiology [27] confirm these conclusions. The ASH proposes also an approach approved by the USA Food and Drug Administration (US FDA), i.e. the use of certain drugs (enoxaparin, dalteparin, tinzaparin) or peroral anticoagulants (rivaroxaban, betrixaban) after patient's stay in hospital, taking into consideration the low bleeding risk. However, in cases of significant BTE risk (advanced age, immediate resuscitation after hospitalization, malignant tumors, BTE in anamnesis, thrombophilia, severe immobility and increased DD level the patient management protocol is different. American schemas of prophylaxis after discharge include rivaroxaban (10 mg daily) during at least 31 days or betrixaban – 160 mg daily for the first day and the uptake of this drug during at least 35 days (80 mg daily) [21, 27].

It should be also taken into consideration disturbances of coagulation, anticoagulation, and thrombolytic blood systems to be often kept in former COVID-19 patients during three months after disease. In persons with severe COVID-19 course the mean PTT value was 12.8 s, its level being below 11.5 s and above 14.5 s in 10.0% of patients, respectively; the mean PATPT value in these patients reaches 34.6 s; their quantity of platelets was found to be 224×10^9/L, the values above 300×10^9/L having been seen in 20.0% of patients examined. The mean FAB content was 4.2 g/L, the values above 4 g/L having been found in 60.0% of persons examined; the mean DD level was 312 μg/L, this value being above 300 μg/L in 40.0% of patients and exceeding 500 μg/L in 20.0% of patients. The mean PCT level in these patients was 0.08 ng/mL.

Even in persons with COVID-19 course of moderate severity the mean PTT value was 12.6 s, its level being below 11.5 s in 20.0% of patients and above 14.5 s in 10.0% of patients. The mean PATPT value was 34.1 s and the platelets content reached 188×10^9/L; the platelets concentration above 300×10^9/L was seen in 10.0% of patients examined. The mean FAB content was 4.0 g/L, its level above 4 g/L was seen in 50.0% of patients. The DD level was 220 μg/L, its value being above 300 μg/L in 40.0% of patients examined and even above 500 μg/L in 20.0% of patients. The mean PCT level in these persons was 0.12 ng/mL.

As it has been underlined above cytokines disturb normal human hemostasis and belong to the main factors of SARS-CoV-2 thrombotic potential contributing to the disbalance in thrombotic and inner anti-coagulating processes. In persons with severe COVID-19 course the mean levels of IL-6, Il-10, IgA, CIC, and cortisol were the following: 4,0 pg/mL, 3,3 pg/mL, 1,4 pg/mL, 0.028 IU/mL, and 223.0nM/L, respectively. The mean CRP value was 7.7 mg/mL, its level being above 10 mg/mL in 60.0%, of patients and above 30 mg/mL in 20.0% of them. In patients having earlier had even moderate COVID-19 severity the mean IL-6 and IL-10 levels were 4.4 pg/mL and 2.5 pg/mL, respectively; the mean IgA level was 1.8 pg/mL; the mean levels of CIC, cortisol, and CRP were the following: 0.045 IU/mL, 195.6 nM/L, and 5.3 mg/mL, respectively. The CRP values above

10 mg/L were found in 40.0% of patients and above 30 mg/mL – in 20.0% of them.

The presence of certain rheological and serological changes in patients even in three months after survived COVID-19 needs further study in order to understand the necessity of anti-thrombolytic drugs uptake during a rather long time.

CONCLUSIONS

1. Summing up the results obtained it is possible to assert micro- and macro-vascular thromboses to be common in COVID-19 cases; they are associated with poor prognosis for diseased patients and are not completely investigated; the role of thromboses in COVID-19 course and complications are to be studied as well as the strategies of fibrinolytic therapies for such condition are to be justified.
2. In three months after the past COVID-19, the persons with severe disease course kept continuously unfavorable changes of PTT (12.8 s), PATPT (34.6 s), quantity of platelets (224×10^9 /L), FIB (4.2 g/L), DD (312 μg/L), and PCT (0,08 ng/mL); they are associated with systemic inflammation, i.e. with the levels of IL-6, IL-10, IgA, CIC, cortisol, and CRP reaching 4.0 pg/mL, 3.3 pg/mL, 1.4 pg/mL, 0.028 IU/mL, 223.0nM/L, and 7.7 mg/L, respectively.
3. In three months after the past COVID-19, in patients with the moderate disease course we have registered disturbances of PTT (12.6 s), PATPT (34.1 s), quantity of platelets (188×10^9 L), FIB (4.0 g/L), DD (220 μg/L), PCT (0.12 ng/mL); such indices were associated with systemic inflammation, the levels of IL-6, IL-10, IgA, CIC, cortisol, and CRP being 4.4 pg/mL, 2.5 pg/mL, 1.8 pg/mL, 0.045 IU/mL, 195.6nM/L, 5.3 mg/L.
4. The presence of certain rheological and serological changes in patients even in three months after survived COVID-19 needs further study in order to understand the necessity of anti-thrombolytic drugs uptake during a rather long time.

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ORIGINAL ARTICLE

EVALUATION OF SEROLOGICAL SCREENING AND PCR-AMPLIFICATION OF HEPATITIS B VIRUS DNA AMONG IRAQI BLOOD DONORS

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ABSTRACT

The aim: Infection with the hepatitis B virus (HBV) caused by blood transfusion is a big problem throughout the world. The aim of study is to determine the faster and more accurate methods for detection of hepatitis B infections by serological screening and PCR- amplification. **Material and Methods:** A total of 140528 donors were tested for HBsAg and total anti-HBc from January to October 2021 in Iraq's National Blood Transfusion Center; however, only 100 samples with HBsAg (-) and anti-HBc (+) were collected and tested for HBV DNA using quantitative real-time PCR.

Results: From 2015 to 2021, the percentage of HBsAg positive donors was 0.33 percent in 2015, 0.32 percent in 2016, 0.30 percent in 2017, 0.28 percent in 2018, 0.23 percent in 2019, 0.22 percent in 2020, and 0.27 percent in 2021. Between January and October of 2021, the overall anti-HBc rate among the (140528) donors was 4.42 percent. According to our findings, only 7% of blood samples from NBTC donors with HBsAg (-) anti-HBc (+) were positive for HBV DNA. The results showed no significant change in HBs Ag (+) and total anti-HBc rates among blood donors between 2015 and 2021.

Conclusions: HBV infection could be transmitted from a blood donor with OBI. PCR (RT PCR) is substantially more sensitive and effective. Despite this the use of an anti-HBc test for blood donors could be seen as a second choice to control HBV from spreading during blood transfusions.

KEY WORDS: hepatitis B, nucleic acid test, hepatitis B, PCR

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INTRODUCTION

The hepatitis B virus is highly infectious because it may readily be passed from one person to another via blood transfusion. The presence of blood-borne diseases in silent donors' blood cells is the most common way for infectious agents to be transmitted during blood transfusion [1]. Hepatitis B virus (HBV) infection is a major global health issue. Around 2 billion people are infected. Globally, almost 350 million people are chronically infected with HBV. The prevention of infection transmission by blood transfusion is a difficult topic to solve globally. Despite screening for HBV utilizing HBs Ag using highly sensitive and precise methods, new cases of hepatitis B virus infections arise [2]. To avoid post-transfusion hepatitis, it is suggested that every donated blood be tested for HBV before transfusion. Hepatitis B surface antigen (HBsAg) screening of donated blood was adopted in the 1970s and it significantly reduced post-transfusion hepatitis. Despite the screening methods, it has been discovered that HBV infection can occur even when HBsAg is not detected, a condition known as "occult HBV infection (OBI)" This phenomena is becoming more widely recognized in a variety of therapeutic settings all around the world [3]. Chronic HBV infection can be divided into three phases, each of which can be distinguished serologically. The first is chronic Hepatitis B, which is defined as a six-month or longer period of detectable HBsAg in the serum. The second is an inactive HBV carrier, in which HBsAg is detected in the serum but no HBeAg is present. The third is (OBI), which is an uncommon clinical condition [4]. OBI is simply the presence of circulating HBV DNA in individuals who are serologically negative for HBsAg. If an OBI is antibody positive (anti-HBc alone or in combination with anti-HBS), it is known as a seropositivity OBI [5, 6]. OBI was found in 39.1% of HBsAg-/HBcAb+ patients. OBI was found in the blood of 3.9 percent of Iraqi blood donors in Diyala and 14 percent of Iraqi blood donors with positive HBc Abs results in Basra. OBI is more common in neighboring and foreign countries.0.25 Percent was reported in Saudi Arabia and zero in Turkey, Iran, and Greece. Many developed countries now require NAT testing for all transfused blood units because that OBI has been confirmed by numerous NAT (nucleic acid testing) tests on donors [7, 8]. HBV had a moderate endemicity in Iraqi populations, with a prevalence of 3%, while another study found a prevalence of 1.6%. It was more common in men over 40 and more prevalent in Iraqi

males than females. PCR testing and patients with strong clinical profiles, including low-risk patients who are correctly administered, proved that the current generation test has excellent sensitivity and specificity 99.8%. Anti-HBc is often used in place of NAT testing in resource-constrained situations because it is less expensive, but is still preferred for blood screening due to its sensitivity. These examples, despite their lower sensitivity, are nonetheless significant. Screening for HBsAg in donated blood has been done at the National Blood Transfusion Center in Baghdad since 1973. The Food and Drug Administration (FDA) now recommends and requires that all blood and plasma donations, injectable plasma derivatives should also pass a serologic screening for hepatitis B surface antigen (HBsAg) and hepatitis C virus [9-15]. All donors were tested for HBsAg and total anti-HBcAb by using of an enzyme linked immunoassay (ELISA) although HBeAg was not included in the screening. The NBTC has developed fresh preparations and arrangements to use a more precise architectural technique for donor screening. In 2022, NAT will be employed for the first time to screen for HBV-DNA, HCV-RNA, and HIV RNA.

THE AIM

The purpose of study is to determine the faster and more accurate methods for detection of hepatitis B infections by serological screening and PCR-amplification.

MATERIALS AND METHODS

SAMPLING

Each sample's serum was extracted by centrifugation and divided into two aliquots of 1.5 ml tubes, which were then stored in a deep freeze (-70°C) until testing.

TESTING IN A LABORATORY

Hepatitis B virus surface antigen (HBs Ag) detection
The ELISA technique was used to detect hepatitis B virus surface antigen (HBsAg) (Imbian ELISA kit, Novosibirsk, Russia).

Anti-Hepatitis B Core Antibody (HBc) detection

Total antibodies were determined using the anti-HBc II kit (Abbott, Wiesbaden, Germany), which employs a chemiluminescent micro particle immunoassay (CMIA) performed on the ARCHITECT i2000SR Immunoassay system platform (Abbott Diagnostics, the USA).

Real-time PCR was used to detect hepatitis B virus DNA
The detection of HBV DNA comprised three steps:

- DNA extraction with the help of (Zrviral DNA Kit, the USA)
- DNA purification
- A quantitative real-time PCR method can detect hepatitis B virus DNA in human plasma or serum (Sakash kit, Italy).

A total of one hundred volunteers with HBsAg-/anti-HBc+ were studied, and blood samples were collected from each of them. Blood donors who accepted to participate in the study and had HBsAg (-)/anti-HBc+ were provided written informed consent.

STATISTICAL ANALYSIS

The Statistical Analysis System – SAS (2012), program was used to detect the effect of various elements on research percentage. The Chi-square test was used in this investigation to determine a significant comparison between percentages.

RESULTS

The total number of screened blood donors was 1117691. For the period between 2015 and 2020 were found 3204 (28%) for HBsAg (+), while for the period from January to October 2021 found 392 (27%) for HBsAg (+), with no significant differences (P>0.38) when compared to the years between 2015 and 2021 (Table I).

When comparing the rates of anti-HBc (+) among blood donors in NBTC at the years (2015, 2016, 2020) and during the period of our research work (from January to October 2021) which was 6231 (4.42%) from 140528 blood donors, and the result showed that there is no significant difference between these rates (Table II).

HBV DNA was found in 7% of the samples tested in this study by using of quantitative real-time PCR (qRT-PCR), which was chosen from 100 samples with HBsAg (-) and anti-HBc (+) (Table III).

DISCUSSION

When the hepatitis B virus infects the liver, it can lead to hepatitis B, which can be fatal (HBV). It's a major public health issue around the world. It can lead to a long-term infection and raises the possibility of cirrhosis and liver cancer as a result [16-19]. HBsAg immunoassays have difficulty detecting early acute and occult HBV infections. 100 samples with HBsAg (–) and Anti-HBc (+) HBV-DNA yielded OBI samples in this study, which is consistent with previous research. Between 2015 and 2020, HBsAg was found in 3204 (or 0.28%) of the 1117691 blood donor samples tested (Table I). According to El-Zayadi et al. screening blood donors for HBsAg can reduce the risk of transfusion-transmitted HBV infection [18]. Blood transfusion is a major source of OBI infection, particularly when blood donors are not properly vetted [20-24]. HBV DNA has been demonstrated to be more sensitive than serological HBsAg, when using nucleic acid technology (NAT) or HBA DNA. As a result, NAT is more sensitive than an HBsAg assay in detecting HBV-DNA and preventing HBV or OBI transmission by blood transfusion [25, 26]. A blood screening technique, integrating HBsAg and anti-HBc detection with an HBV NAT test, enables for the detection of window-period infection in several developed

Table I. The rates of HBs Ag (+) among the total numbers of blood donors in NBTC for years from 2015 to 2021.

Years	The total numbers of donor samples	The rates of HBsAg(+)	Chi-Square (χ^2)	P-value
2015	185171	619 (0.33%)	0.974	0.382 (NS)
2016	188404	603 (0.32%)		
2017	196535	604 (0.30%)		
2018	178966	506 (0.28%)		
2019	190773	476 (0.23%)		
2020	177842	396 (0.22%)		
Total	1117691	3204 (28%)		
2021 (From January to October)	140528	392 (0.27%)		

NS: Non-Significant at $P > 0.05$

Table II. The total anti-HBc, founded in blood donors for years 2015, 2016, 2020 and 2021.

Years	The total number of donor samples	The rates of anti-HBc(+)	Chi-Square (χ^2)	P-value
2015	185171	7952(4.3%)	1.159	0.237 (NS)
2016	188404	7875(4.1%)		
2017	196535	N/A		
2018	178966	N/A		
2019	190773	N/A		
2020	177842	4907(2.75%)		
2021 (from January to October)	140528	6231(4.42%)		

N/A: None available; NS: Non-Significant at $P > 0.05$

Table III. Techniques for detecting HBV serologically and molecularly in blood donors.

Test	Techniques Assay	Samples Tested	Positive Samples
HBs Ag	ELISA	140528	392 (0.27%)
Total anti-HBc	Chemiluminescent Micro particle Immunoassay (CMIA)	140528	6231 (4.42%)
Hepatitis B virus DNA	Quantitative real time PCR	100	7%

nations. Following the introduction of the hepatitis B core antibody in 2014 to routine examinations, it appears, that the infection rate with viral hepatitis is based on statistics from NBTC following the above-mentioned results [27]. Clinical tests, such as those that detect viral hepatitis antigens and antibodies and the type of infection, have been recommended in the past, but recent studies have found a variety of new and ambiguous results for these tests, including cases of hepatitis OBI, defined as the presence of low-level HBV DNA in serum with serological markers of previous infection OBI (source: CDC). More than 20% of patients had no detectable antibody titer, leaving only the presence of HBV DNA as a marker of infection [28-30]. According to the results of this study, the prevalence of Hepatitis B infection among blood donors was 0.27 percent positive for HBsAg and 4.42 percent positive for Anti-HBc, respectively (Table III). This finding is consistent with the findings of a study conducted in Iraqi Kurdistan, which found the prevalence of HBV infection among HD patients to be 3.2% [31, 32]. And more similar to the results of a study conducted in 2015 in Karbala, Iraq, to detect infection using HBsAg in an ELISA assay 0.6% [33] they were discovered in the current investigation during our effort from January to October 2021. Although the percentage of HBV-DNA positive samples (7/100) diagnosed as OBI by serological and molecular assay among blood donors is lower than in a research conducted in 2000, it has been reported that 20% of OBI cases are seronegative for all HBV markers [34]. Covalently closed circular DNA (Ccc DNA) of the virus persists as an episome in the nucleus of infected cells during chronic infections [35]. In some countries, OBI is more prevalent than in others. According to HBV DNA detection methods, sample size and whether HBV DNA is detected using nested or real-time methods, this ranges from one to the other. OBI's prevalence ranges from 1 percent to 87% over the world. Several low-endemicity sites for HBV have reported cases of OBI [2, 3]. Only 0.016 percent of the population in South Korea tested positive for HBV DNA, compared to India's 10.22 percent and Turkey's 0.015 percent of the population. Mutations in the S region of the HBV genome have been associated to a decreased expression of HBV surface proteins. Because of

this, mutations in the preS1/preS2 promoters are prevalent in OBI patients, resulting in the loss of HBsAg testing [30, 31]. Immunoassays may be unable to detect the presence of HBsAg because of factors such as low analytical sensitivity, anti-HBs, HBsAg mutations that reduce HBsAg production and/or secretion, and HBV mutations that reduce HBsAg production and/or secretion to levels below current HBsAg assay detection limits. It has recently been found that HBV-infected blood donors may have viral levels below the detection threshold, according to a recent study [34, 35].

CONCLUSIONS

Since OBI testing is urgent, the best option is real-time polymerase chain reaction (PCR), or NAT which is regarded the current gold standard for detecting OBI because of its high sensitivity for detecting viral DNA in blood. Because anti-HBc is detectable during asymptomatic infections and throughout life, blood donors with OBI who do not have detectable HBsAg but have anti-HBc positive and HBV DNA are a possible source of HBV infection. PCR (RT PCR) is more accurate and sensitive than the serological HBsAg test, but the use of anti-HBc tests for blood donors can be a second method to decrease on HBV transmission through blood transfusion.

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ORIGINAL ARTICLE

BONE MINERAL DENSITY AND PROBABILITY OF OSTEOPOROTIC FRACTURES IN WOMEN WITH TYPE II DIABETES MELLITUS

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ABSTRACT

The aim: To assess bone mineral density and 10-year probability of major osteoporotic and hip fractures using the Ukrainian FRAX® version for postmenopausal women with Type II diabetes mellitus and to determine the need for OP treatment according to the algorithm FRAX and BMD.

Materials and methods: 690 females aged 50-89 years (mean age 67.0±7.7 years) were divided into two groups: Group I (n=345) was made of mostly healthy women, Group II (n=345) – patients with Type II diabetes mellitus. Bone mineral density was measured using dual-energy X-ray absorptiometry, 10-year probability of major osteoporotic and hip fractures was calculated using the Ukrainian FRAX® model.

Results: Bone mineral density and 10-year risk of major osteoporotic and hip fractures did not differ depending on the Type II diabetes mellitus presence, however the frequencies of low-energy, vertebral and all previous fractures were higher in Group II. 19% of women with diabetes mellitus and 38% of healthy ones required antiosteoporotic treatment according to dual-energy X-ray absorptiometry and only 8% and 2%, respectively, according to the FRAX. These indices became higher after recalculation of FRAX taking into account bone mineral density, however they were lower in patients with diabetes mellitus compared to the corresponding rate in the Group I (FRAX (high risk) + bone mineral density: 26% and 41%; $\chi 2 = 18.2$; $p < 0.001$).

Conclusions: The use of FRAX in combination with bone mineral density resulted in an increased necessity for antiosteoporotic treatment, indicating the urgency of using both indices for osteoporotic fractures prediction in patients with Type II diabetes mellitus.

KEY WORDS: osteoporosis, FRAX, hip fractures, dual-energy X-ray absorptiometry, major osteoporotic fractures

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INTRODUCTION

Diabetes mellitus (DM) is one of the most common and severe chronic diseases associated with an increase of disability and mortality rates among both young and older subjects. The Global Burden of Disease study (2017) [1] presented the DM epidemiological data over 28 years, showing that the number of Type 2 diabetes mellitus (T2DM) cases in the world increased by 103% from 1990 to 2017, with an increase in the age-standardized incidence rate from 234 to 285 per 100,000 people, which is more pronounced for T2DM compared to the corresponding Type 1 DM (T1DM) (estimated annual percentage changes were 0.89 (95% Confidence Interval (CI): 0.80-0.97) and 0.34 (95% CI: 0.30-0.39), respectively).

Both types of DM are characterized by a range of complications, including retinopathy, nephropathy, neuropathy, cardiovascular events, and an increased risk of osteoporotic fractures (OFs) [2-4]. The recent studies confirm the importance of a reduced bone mineral density (BMD) for the development of OFs in patients with T1DM [5, 6], while the mechanisms of an increased risk of fractures in patients with T2DM, although widely studied, are not fully understood [5, 7, 8].

Due to the higher BMD values of patients with T2DM, dual-energy X-ray absorptiometry (DXA), which is considered the "gold standard" of osteoporosis diagnostics, may reduce its capacity to diagnose osteoporosis in a timely manner. Another modern risk calculation tool, developed to evaluate the OFs risk of patients, the FRAX considers only T1DM among the risk factors for secondary osteoporosis. Despite the available data on the increased risk of OFs in patients with T2DM [2-4], is important to assess BMD and FRAX in this category of patients. In recent years, there have been some studies [9, 10] underestimating the risk of OFs in patients with T2DM, and some researchers suggest factoring T2DM, as well as T1DM [11] in the FRAX algorithm.

Ukraine has lately developed its own version of the FRAX algorithm with cut-off values for initiating antiosteoporotic treatment or additional BMD measurement in women [12]; however, the study of patients with T2DM has not been conducted before, this fact underlying the study.

THE AIM

The aim of the study is to assess BMD and 10-year probability of major osteoporotic fractures (MOFs) and hip

fractures (HFs) using the Ukrainian FRAX version for postmenopausal women with T2DM and determine the need for osteoporosis treatment according to the algorithm FRAX and BMD.

MATERIALS AND METHODS

In a cross-sectional study, we have examined 690 postmenopausal women aged 50-89 years (mean age (M±SD): 67.0±7.7 years) at the State Institution «D. F. Chebotarev Institute of Gerontology of the NAMS of Ukraine». The study was approved by the Ethics Committee of the Institute (Protocol №5 of 17.05.2017) and was conducted from December 2017 to December 2019. The research was performed in accordance with the Declaration of Helsinki, with every participant signing informed consents.

All the examined females were divided into two groups: Group I (n=345) was made of mostly healthy individuals without any pathology or condition with a potentially negative effect on the BMD and the risk of OFs, and Group II (n=345) was made of patients with T2DM. Among the all women, 19.3% were aged 50-59, 43.8% – 60-69 years, 31.6% – 70-79 years, and 5.4% females aged 80-89 years.

The main anthropometric indices (height, body weight) were measured using routine methods. The body mass index (BMI) was calculated according to the generally accepted formula. Depending on the BMI, the females were considered to have a normal body mass (BMI=18.5-24.9 kg/m^2), overweight (BMI=25.0-29.9 kg/m^2), or obesity (class I: BMI=30.0-34.9 kg/m^2, class II: BMI=35.0-39.9 kg/m^2 and class III: BMI=40 kg/m^2 and more).

The ten-year probability of major MOFs and HFs was calculated without and with BMD, using the Ukrainian FRAX model published on the official FRAX website (https://www.sheffield.ac.uk/FRAX). Ten-year probability of MOFs and HFs calculates automatically taking into accout the data of gender, age, body weight, and height, as well as information about clinical risk factors for fractures (Yes or No), such as previous fractures, hip fractures in parents, current smoking, alcohol intake, glucocorticoid use, presence of rheumatoid arthritis, and risk factors for secondary osteoporosis (hypogonadism or premature menopause 45 years, T1DM, osteogenesis imperfecta in adults, untreated long-standing hyperthyroidism, chronic malnutrition, or malabsorption and chronic liver disease), are entered on the questionnaire's website. Also, the BMD of the femoral neck can be added to the calculation, but it is possible to determine the risk of fractures without this indicator, i.e. without the use of DXA instrumental bone examinations. The 10-year probability of fractures is determined by an algorithm developed based on population studies in different countries. The Ukrainian version is formed from research conducted in Ukraine.

The need for an antiosteoporotic treatment was determined according to the latest International Society for Clinical Densitometry (ISCD) recommendations [13], being based on BMD (≤-2.5 SD) and age-dependent intervention thresholds of the Ukrainian FRAX version [12]. According to the above-mentioned criteria, we have collected a group of women who necessitated the additional examination (BMD measurement) or antiosteoporotic treatment without assessment of DXA values. For the analysis, they were divided in the following manner: 1). women who do not require treatment or additional examination (FRAX-MOFs values below the lower assessment threshold); 2) females who necessitated the treatment regardless of BMD measurement (FRAX-MOFs values above the upper assessment threshold); 3) ones who after the 10-year probability of MOFs was calculated should be referred to an additional DXA examination (indices between the lower and upper assessment threshold).

Measurements of BMD were performed using the two DXA devices: PRODIGY (GEHC Lunar, Madison, WI, USA) and DISCOVERY (Wi, Hologic, Inc. USA) with an automatic T-score calculation by Densitometer Software (criterion of the osteoporosis diagnosis for the postmenopausal women). The evaluation was performed at the level of the femoral neck, total hip and lumbar spine (L_1-L_4).

The statistical analysis was carried out by means of «Statistica 13.0» software. The sample was checked for normality of distribution by Kolmogorov-Smirnov test. Quantitative data were presented as mean and standard deviations (M±SD) with 95 % Confidence interval (CI) (under the rule of normal data distribution), as well as the median and lower and upper quartiles (Me [25Q–75Q]) under conditions of discrepancy. In order to compare the two independent samples, we used Student's t-test for independent samples or the Mann-Whitney U Test, while estimating the differences (%) of two independent samples χ^2 was used. The strength of relationships between quantitative variables was assessed using Pearson's correlation test. The differences in indices were considered significant provided $p<0.05$.

RESULTS

The subjects did not differ significantly in terms of their age and height, although the indices of body weight and body mass index (BMI) were significantly higher in the group of females with T2DM ($p<0.001$ for both ones, Table I). Analysis of women's menstrual function did not reveal significant differences in the age of menarche, although the age of menopause was significantly lower in the patients with T2DM ($p<0.001$), and the duration of the postmenopausal period, respectively, longer ($p<0.001$) compared with the Group I (Table I).

Depending on the presence of T2DM, analysis of women's DXA results did not reveal significant differences in BMD of the hip (respectively, for women of Groups I and II: 0.73±0.16 and 0.73±0.15 g/cm^2; t=0.56, p=0.58). While T values were significantly higher in women with T2DM both at the level of the lumbar spine (respectively, -0.48±1.65 and -1.29±1.84 SD; t=6.09, $p<0.001$) and femoral neck (-1.37±1.19 and -1.70±1.20 SD; t=3.59, $p<0.001$).

Patients with T2DM and low-energy fractures had significantly lower BMD values compared with patients

Table I. Clinical characteristics of the examined patients.

Parameters / Groups	Almost healthy women n=345		Women with T2DM n=345		t	p
	M±SD	95% CI	M±SD	95% CI		
Age, years	66.9±8.3	65.3-67.1	67.5±7.1	66.7-68.2	1.9	0.07
Height, m	1.61±0.1	1.60-1.62	1.60±0.1	1.59-1.61	1.4	0.17
Weight, kg	73.5±14.6	71.9-74.9	83.2±15.3	81.4-84.7	8.5	<0.001
Body mass index, kg/m^2	28.2±5.3	27.6-28.7	31.7±5.6	31.1-32.3	8.5	<0.001
Age at menarche, years	13.7±1.6	13.5-13.9	13.5±1.6	13.3-13.7	1.6	0.10
Age at menopause, years	50.6±3.1	50.2-50.9	49.0±5.0	48.3-49.5	5.1	<0.001
Duration of postmenopausal period, years	15.8±8.4	14.7-16.6	18.7±8.7	17.1-19.1	4.4	<0.001

Note: the data are presented as the mean and standard deviations (M±SD) with a 95 % Confidence interval (CI); t – differences in indices were assessed using Student's t-test.

Table II. Proportion of postmenopausal women with a low BMD depending on the presence of T2DM.

Parameters / Groups	I	II	χ^2	P
Osteoporosis (L_1-L_4), n (%)	84 (24.3)	30 (8.7)	30.64	<0.001
Low BMD (L_1-L_4), n (%)	225(65.2)	148 (42.9)	33.32	<0.001
Osteopenia (L_1-L_4), n (%)	141 (40.9)	118 (34.2)	3.27	0.07
Osteoporosis (hip), n (%)	93 (27.0)	49 (14.2)	17.17	<0.001
Osteopenia (hip), n (%)	167 (48.4)	192 (55.7)	4.04	<0.05
Low BMD (hip), n (%)	260 (75.4)	241 (69.9)	2.63	0.11
Osteoporosis (L1-L4 and / or hip), n (%)	130 (38)	64 (19)	31.24	<0.001

Table III. Risk factors for OFs in postmenopausal women depending on the presence of T2DM.

Parameters / Groups	I	II	χ^2	p
History of fractures, any age, n (%)	152 (44.1)	187 (54.2)	7.10	0.01
History of low-energy fractures, n (%)	130 (37.7)	167 (48.4)	8.09	0.005
Low-energy major osteoporotic fractures #, n (%)	104 (30.1)	134 (38.8)	5.77	0.02
Vertebral fractures, n (%)	0	53 (15.4)	57.41	<0.001
A history of parental hip fractures, n (%)	0	30 (8.7)	31.36	<0.001
Smoking, n (%)	9 (2.6)	12 (3.5)	0.44	0.51
Alcohol consumption (3 or more units per day), n (%)	0	1 (0.3)	1.00	0.32
Secondary osteoporosis, n (%)	0	48 (13.9)	51.59	<0.001
Rheumatoid arthritis, n (%)	0	23 (6.7)	23.79	<0.001
Glucocorticoid use, n (%)	0	0	-	-
Early menopause, n (%)	3 (0.9)	44 (12.8)	38.38	<0.001

Notes: BMI – body mass index; # – hip, shoulder, forearm and clinical spine fractures.

with T2DM without fractures (0.66±0.11 and 0.77±0.15 g/cm^2, respectively; t=7.5, p<0.001), as well as T-score at the level of both lumbar spine (t=4.1, p<0.001) and total hip (t=7.6, p<0.001) against the background of no significant differences in BMI (t=1.4, p=0.2).

The number of patients who necessitated the treatment according to BMD of the lumbar spine and hip was also higher in the Groups I (Table II). In total, 19% of females with T2DM and 38% of women in the Groups I required treatment according to results of DXA scan.

Estimation of 10-year probability of MOFs and HFs according to the Ukrainian version of FRAX did not reveal any significant differences depending on the T2DM presence (Fig. 1). However, the 10-year risk of HFs taking into account the BMD (Z=2.59; p<0.01) was significantly lower than in the Groups I, but the analysis of risk factors for osteoporosis and its complications revealed some specific features (Table III). Thus, women with T2DM had significantly more often both a history of any fracture (p=0.01) and low-energy fractures (p=0.005). In patients with T2DM, MOFs index (p=0.02) was higher and vertebral fractures (p<0.001) were detected significantly more often. Additionally, analysis of the FRAX-included factors detected a significantly higher percentage of parental HFs, underlying factors of secondary osteoporosis,

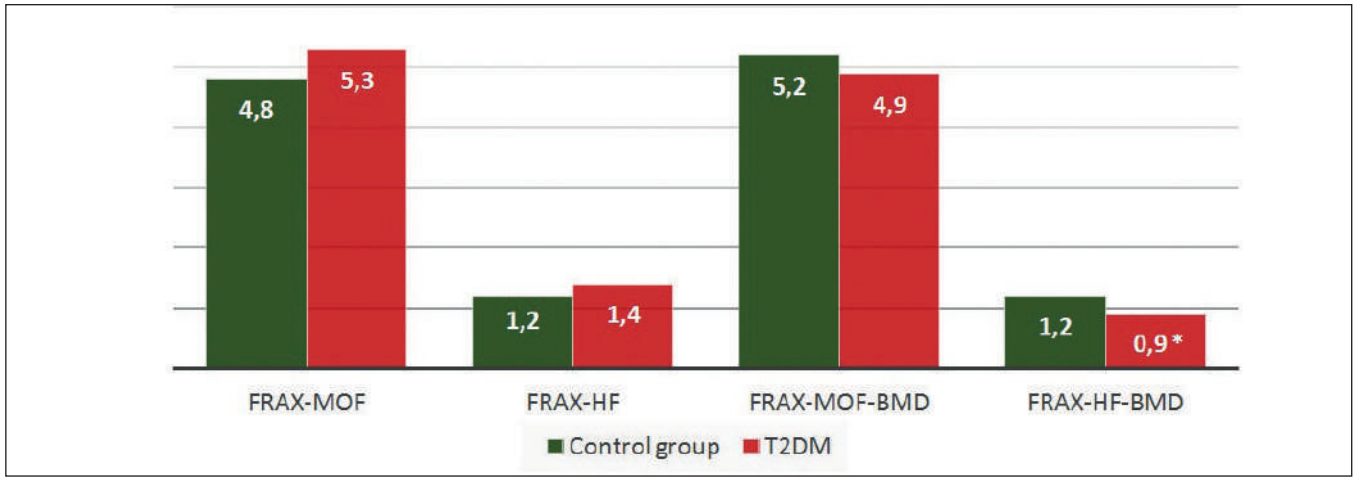

Fig. 1. 10-year probability of MOFs and HFs according to FRAX in women depending on the presence of T2DM.
Note: the data are presented as the median, the differences between groups (*) assessed using the Mann-Whitney U test; FRAX-MOF – 10-year probability of MOF without BMD; FRAX-HF – 10-year probability of HF without BMD; FRAX-MOF-BMD – 10-year probability of MOF with BMD considered; FRAX-HF-BMD – 10-year probability of HF with BMD considered.

most common being early menopause, and rheumatoid arthritis ($p<0.001$ for both indices) and no differences in the rate of smoking and alcohol intake (Table II).

Assessment of the need for antiosteoporotic treatment according to the FRAX without taking into account the BMD revealed a significantly higher proportion of women that required antiosteoporotic treatment among patients with T2DM compared with those in the Groups I (8.4 and 1.7%, respectively). While significant differences in the proportion of subjects who do not require additional examination or treatment were observed in Groups I and II (36.5 and 40.3%, respectively), subjects who require additional DXA examination with BMD considered were numbered 61.7 and 51.3%, respectively.

The analysis of anthropometric data did not reveal significant differences in the proportions of overweight females (Groups I and II: 36.8 and 31%, $\chi^2=2.59$; $p=0.11$). However, among patients with T2DM there was a significantly higher number of women with obesity class I (31.3 and 23.5%; $\chi^2=5.31$; $p=0.02$), class II (18.6 and 7.8%; $\chi^2 = 17.33$; $p<0.001$) and class III (8.7 and 2.3%; $\chi^2=13.48$; $p<0.001$). The relationship between hip BMD and BMI was weak, but significant in both groups (Groups I and II, respectively: $r=0.38$; $p<0.001$; and $r=0.27$; $p<0.001$).

Analysis of the combined use of DXA (T=-2.5 SD) and the high probability of MOFs detection by FRAX (above the upper assessment threshold, according to the Ukrainian guidelines), the proportion of individuals necessitating antiosteoporotic treatment was significantly lower in the group of T2DM compared to the corresponding rate in the Groups I (23 and 39%; $\chi^2=18.32$; $p<0.001$). Similar differences were found after the recalculation of FRAX taking into account the BMD level (FRAX-BMD: 26 and 41%; $\chi^2=18.20$; $p<0.001$).

DISCUSSION

Diabetes mellitus and systemic osteoporosis are among the most common non-communicable diseases, whose incidence has been progressively increasing in the recent years [1]. Both diseases lead to a decreased life expectancy, an increased rate of disability and deteriorated quality of patient's life. Furthermore, along with other serious complications (retinopathy, nephropathy, neuropathy, cardiovascular events, etc.), systemic osteoporosis and its grave complications – low energy fractures, are frequent outcomes of T2DM, which aggravates the course of the underlying disease; so early detection of risk groups and timely antiosteoporotic treatment is extremely important.

The DXA and FRAX, up-to-date methods of osteoporosis and its complications risk assessment are the most evidence-based in terms of probability of osteoporotic fracture evaluation, but their informative value in terms of data collected from patients with T2DM is insufficiently studied; the issue underlying our study.

Our study revealed a significantly higher overall percentage of fractures, and specifically, the low-energy OFs and vertebral fractures in patients with T2DM compared with the control group; this fact is confirmed by the results of most [3, 4], though not all meta-analyses [14] of the negative effect of T2DM on fracture risk. The meta-analysis by Moayeri A. et al. [3], comprising 30 studies, found an increased overall risk of fractures (relative risk – RR=1.05, 95% CI: 1.04-1.06), which was exacerbated with age, duration of disease and insulin therapy; with a significant positive relationship between T2DM and hip and vertebral fractures, but not with forearm or shoulder fractures. Another meta-analysis [4] of 8 studies showed an increased risk of vertebral fractures in patients with T2DM (RR=2.03; 95% CI: 1.60-2.59; $p<0.0001$). The risk was higher in both men (2.70; 95% CI: 1.34-5.43; $p=0.005$) and women (1.93; (95% CI: 1.18-3.13; $p=0.008$)), and did not differ in both prospective (1.81; (95% CI: 1.19-2.75; $p = 0.006$)) and retrospective studies (2.23 (95% CI: 1.60-3.10; $p<0.0001$)).

Moreover, the present study confirms the results of our previous study [15] on the relationship between BMI and BMD in women with metabolic syndrome that often accompanies

T2DM. Although this relationship is weak, the effect of overweight on BMD may be one of the explanations for the higher densitometry indexes in women with T2DM.

Recently, the use of FRAX in the assessment of fracture probability in patients with T2DM is of interest to the researchers [9-11, 16-18]. Thus, in a study by Giangregorio L. M. et al. [10], the authors explored whether T2DM is a risk factor for MOFs and HFs independently of FRAX. The study was conducted with participation of 3518 men and women with T2DM, and 36085 people in the control group aged 50 years and older. To the same extent as in our study, the authors did not reveal any differences in the 10-year probability of MOFs and HFs depending on the presence of T2DM, but the latter was a reliable predictor of subsequent MOFs (hazard ratio – [HR]=1.61, 95% CI: 1.42-1.83). The study took into account age, gender, drug use and other factors included in the FRAX algorithm, namely BMD. The presence of T2DM was also associated with an increased risk of HFs ($p < 0.001$). The authors concluded that FRAX underestimated the risk of MOFs and HF in the individuals with T2DM (adjusted for competing mortality), and T2DM increases the risk of fractures independently of FRAX, and should be the pre-requisite for listing this disease among the fracture risk factors with a further adaptation of FRAX.

Our study confirms the results of other studies [17], which also showed a decrease of BMD in patients with T2DM and a history of fractures compared to subjects without fractures, as well as a higher T-score (DXA) at the level of lumbar spine and hip in subjects with T2DM compared to the corresponding control indices.

We did not reveal any significant differences in the 10-year probability of MOFs and HFs depending on the T2DM presence, except for the lower 10-year risk of HFs with BMD in subjects with T2DM. Higher BMD in patients with T2DM and lower FRAX parameters can underestimate the risk of developing OFs in patients with T2DM which complicates timely and effective fracture risk assessment and requires special attention.

In recent studies, the authors are attempting to factor T2DM in the calculation of FRAX. Thus, a study by Hu L. et al. [11] explored the clinical value of FRAX in predicting the risk of fractures in 1047 Chinese patients with T2DM. While calculating the FRAX, they substituted T2DM for the rheumatoid arthritis (RA) in the algorithm. The FRAX-HFs sensitivity, specificity, and Youden's index in the RA-adjusted patients with T2DM were 0.48, 0.96, and 0.44, respectively; while the corresponding FRAX-MOFs values were 0.008, 1.00 and 0.008. Correction of antiosteoporotic treatment recommendations based on BMD and RA-adjusted FRAX in patients with T2DM showed an acceptable consistency for HFs (κ=0.49), but not for MOFs (κ=0.01). When patients were divided into two groups based on the results of HFs risk assessment, considering the DXA and FRAX (adjusted for RA) data, namely the same treatment tactics according to both DXA and FRAX, or opposite tactics by the above-mentioned methods, the healthcare providers obtained significantly higher values of BMI, hip BMD and the number of men making up the first group ($p<0,001$) in the absence of significant differences in HbA1c. The authors concluded that the RA-adjusted FRAX may be useful in predicting HF risk in males with T2DM and accurate fracture risk predictor for males with T2DM, for individuals with high BMI or BMD values.

Another retrospective study [18] aimed to explore how taking into account glycosylated hemoglobin (HbA1c) indices can improve the prognostic value of FRAX in 6355 50-year-old (and over) patients with T2DM. The authors found that BMD of T2DM patients was significantly higher compared to control, while the FRAX values were higher in the control group. A significant negative correlation was found between HbA1c and FRAX: for each additional percentage increase in HbA1c, the probability of FRAX-MOFs decreases by 0.9 points, and for FRAX-HF – by 1.4 points, and the introduction of a correction factor for HbA1c in patients with T2DM, resulted in obtaining the FRAX values which were equivalent to those of the control.

Recent publications [19] on the necessity of factoring other, clinically significant factors in the FRAX algorithm, namely T2DM, appear more and more often. Nowadays, FRAX does not take into account either the dose-dependent effects of drugs that adversely affect the rate of bone loss or time effects. Due to the accumulated data on the negative factors impacting the bone tissue, some researchers suggest including such indices and diseases as glucocorticoid dose, frequency of falls, BMD of the lumbar spine and trabecular bone score (TBS), the length of hip axis, chronic kidney disease and T2DM [19]. However, it is obvious that listing the above-mentioned risk factors instead of those previously existing in the algorithm may lead to a distorted interpretation of risk indices and requires detailed study. It is believed that in connection with the active development of artificial intelligence, the new algorithms for assessing the risk of fractures will be invented, and thy will include numerous factors of negative impact on bone tissue.

Among the limitations of this study, we observe the inclusion of only female subjects, recruitment of but one research center in Ukraine, which may not be fully representative of all women with T2DM. In addition, the design which we used does not allow us to estimate the prognostic value of DXA and FRAX in the assessment of fracture risk for this category of women; the task which requires prospective studies.

CONCLUSIONS

BMD in patients with T2DM did not differ significantly from healthy subjects, however, T values at the lumbar spine and femoral neck were significantly higher in women with T2DM and their need for antiosteoporotic treatment taking into according to DXA results was significantly lower.

The 10-year probability of MOF and HF according to FRAX did not differ significantly in patients with T2DM, except for the 10-year probability of HFs, taking into account the BMD, which was significantly lower compared to the control group. The use of FRAX with consideration of hip BMD resulted in an increased necessity for antiosteoporotic treatment (26 and 41%, respectively), however, the latter was lower in patients with T2DM compared to healthy postmenopausal females,

indicating the necessity of using both methods (DXA and FRAX) for OFs prediction.

Our data confirm that the risk of OFs in patients with T2DM can be underestimated which complicates timely and effective initiation of antiosteoporotic treatment and requires special attention.

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ORIGINAL ARTICLE

EXPLORING THE INFLUENCE OF NEUTROPHIL-LYMPHOCYTE RATIO ON OUTCOME PREDICTION OF SEVERELY-ILL PATIENTS WITH COVID-19

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ABSTRACT

The aim: The study conducted to have a better understanding on the role of neutrophil-lymphocyte ratio in the determination of the prognosis of COVID-19 and to assist in predicting disease severity.

Material and Methods: A total of 96 patients within age group 18-80 years who were verified positive for the COVID-19 by PCR, and admitted to (Al-Sader Medical City) in Al-Najaf City between (July to October 2020) were enrolled in a cohort retrospective study, Neutrophil to lymphocyte ratio was calculated via taking the absolute neutrophil count divided by the absolute lymphocyte count. Other parameters like (renal function tests, D-dimer, C-reactive protein, serum ferritin) also has been studied in relation to outcome of patients with COVID-19.

Results: The Neutrophil-lymphocyte ratio was significantly associated with low oxygen saturation and poor outcome. A significant difference was found between two clusters in CRP, serum ferritin, and D-dimer level. In addition, age and obstructive airway disease were important clinical predictors for poor outcome.

Conclusion: The study was a useful prognostic marker linked with poor outcome in patients admitted for COVID-19 pneumonia. Other inflammatory markers, such as ferritin, CRP, and D-dimer were also associated with critical illness and increased mortality from COVID-19 disease.

KEY WORDS: COVID-19, NLR, D-dimer, prognosis

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INTRODUCTION

Coronaviruses are essential animal and human pathogens. Toward the end of 2019, a novel coronavirus was recognized as the origin of a group of pneumonia cases in Wuhan, China. It swiftly spread, causing an epidemic all over China, afterwards a growing amount of cases in further countries all through the world. In February 2020, the WHO labeled the illness COVID-19, which represents coronavirus disease 2019. On 11th of March, 2020, the WHO announced the outbreak of COVID-19 as international pandemic [1]. Laboratory findings: a large spectrum of laboratory markers can support the identification and follow up of COVID-19 case. Considerably low absolute white blood cell count or neutrophil count throughout initial course of illness has been largely established [2]. However, with progression of the disease, both absolute leukocyte as well as neutrophil counts was noticeably greater in the severe to critical patients. This could be as a result of superimposed bacterial infection with severe disease or the likelihood of cytokine storm [3]. Lymphopenia is similarly one of the utmost recognized hematologic abnormalities that serve as a great prognostic tool. Lymphopenia can be utilized as a biomarker to predict outcome. Patients with severe infection may present with severe lymphopenia of fewer than 1.5 x 10^3/μl in nearly eighty percent of cases. It is undoubtedly linked to severity, the deceased as a result had considerably low lymphocyte count than the recovered. Various healthcare centers and professionals used particular parameters to predict outcome for instance neutrophil-to-lymphocyte ratio or platelet-to-lymphocyte ratio [4]. It has been revealed that the ACE2 receptors are expressed on the lymphocytes cell surface, therefore COVID-19 may invade them and eventually causing cell lysis [5]. Moreover, the cytokine release storm is described by significantly rising interleukins levels and tumor necrosis factor (TNF), which may stimulate lymphocytic apoptosis [6]. Extensive activation of cytokines may be as well related to degeneration of lymphocytic organs, involving the spleen and more impairs turnover of lymphocyte [7]. Additional hematologic finding is thrombocytopenia that may be due to various reasons. The etiology for thrombocytopenia in both initial and advanced stages of infection is probably multifaceted, for direct and indirect aspects, which hinder production, activation or consumption of thrombocytes. The likely mechanism of low platelet count includes viral myelosuppression. Others like increased consumption due to excessive thrombosis, reflected excessive coagulation activity marked by significantly higher levels of D-dimer [8]. Those who have severe infection seem to have signs of liver derangement than patients with mild disease. Elevated liver enzymes and bilirubin level has been detected in lots

of intensive care patients [9]. The evolving part of certain biomarkers similar to ferritin, procalcitonin, LDH, IL-6, and CRP has forced intense investigation on their potential place in management [10]. The deceased showed more frequently with raised procalcitonin, LDH, increased IL–6 and high serum ferritin. High CRP and LDH have also been linked to increased chance of ARDS, intensive care support or even death, which might presumably be elucidated by acute systemic inflammatory response triggered by a series of cytokine activation, a state of hemodynamic instability and multi-organ failure, namely, cytokine release syndrome (CRS) [11]. It can be described that the viral direct impact, immunological response and immense release of inflammatory mediators ultimately participate to this life-threatening event [12, 13]. Correlation of unstable coagulation variables with unfortunate outcome has been identified. Those who died from severe infection have shown considerably greater levels of fibrin degradation products and plasma D-dimer, higher PT and PTT paralleled to survivors [14]. Defective coagulation and disseminated intravascular coagulopathy seem to be related to higher mortality. Among these coagulation parameters, elevated D-dimer of greater than 1 ug/L was the most important independent biomarker for poor outcome [15].

THE AIM

The study's objective is to have a better understanding on the role of neutrophil-lymphocyte ratio in the determination of the consequence of COVID-19 and to assist in predicting disease severity.

MATERIALS AND METHODS

A total of 96 patients (male: 44 and female: 52) within age group 18-80 years who verified positive cases for COVID-19 based upon the nucleic genome detected from a nasopharyngeal and oropharyngeal swab using PCR, who were admitted to (Al-Sader Medical City) in Al-Najaf City, Iraq, between July to October 2020, were enrolled in a cohort retrospective study.

EXCLUSION CRITERIA INCLUDE

- Pregnancy
- Patients with a known malignancy

The data of 96 COVID-19 patients were reviewed, after granting permission from Al-Sader Medical City ethics committee, for patients' medical history, laboratory tests, the treatment regime, and outcomes. The neutrophil-lymphocyte ratio was calculated via taking the absolute neutrophil count divided by the absolute lymphocyte count using a complete blood count with differential that taken within 24 hour of admission and correlated with O_2 saturation and outcome of patients with COVID-19. Other data were including CBC, hemoglobin level, platelet count, renal function tests, D-dimer, C-reactive protein, serum ferritin.

STATISTICAL ANALYSIS

The statistics were scrutinized by utilizing SPSS program version 24. Continuous variable presented by mean and standard deviation, these were analyzed by t-test. Categories variables presented as number and percent, these variables underwent chi-square test to study the significance among variables. Via application of the receiver operating curve (ROC) analysis, the ideal cut-off values of the continuous variables were calculated. To exclude the effect of the confounding factors on the outcome (age, gender, chronic diseases), we conducted binary logistic regression analysis. The test was considered significant when P-value ≤ 0.05.

RESULTS

The study enrolled 96 patients with mean age were 58.3 ± 13.8 year. The male patients were constituted 45.8%. The deceased were thirty individuals. The mean of average oxygen saturation at admission for all patients was 78.33 ± 6.2% (Table I). The mortality rate was 31.25%.

There was no significant relation between oxygen saturation and patients' variables, including age, gender, and history of diabetes, hypertension, heart failure, and obstructive airways disease; however, there was significant relation with chronic kidney disease (Table II).

There was a statistically significant association between NLR, CRP, D-dimer and serum ferritin, and oxygen saturation at the time of presentation. However, the platelet count and hemoglobin levels failed to reveal a significant impact (Table III).

In this study level of mortality from COVID-19 infection was significantly more in the old age group and in patients with obstructive airway disease. Even though the mortality ratio was greater in male sufferers and in those with DM and CKD, the figures were statistically insignificant (Table IV).

Regarding the laboratory findings, a statistically significant difference was noted in the mean of neutrophil-lymphocyte ratio between deceased patients and those who recovered. In addition, a significant difference was found between two clusters in CRP, serum ferritin, and D-dimer

Table I. Baseline data of the study population.

Variable	Results No.
Age in year (mean ±SD)	58.3 ± 13.8
Gender	M: 44, F: 52
Average stay in the hospital [days]	11.64 ± 4.6
Total death	30
Diabetes	36
Heart failure	10
Obstructive airways disease	12
Chronic kidney disease	7
Hypertension	60
Average oxygen saturation at admission	78.33 ± 6.2%

Table II. The relationship between clinical background and oxygenation saturation in COVID-19 patient at presentation.

Variable		O_2 saturation		P-value
		≥80% (80-88) (No. 52)	<80% (No. 44)	
Age [years]	20-60 (N: 51)	30	17	0.8
	>60 (N: 45)	22	27	
Gender	M (N: 44)	23	21	0.7
	F (N: 52)	29	23	
DM*	Yes 36	20	16	0.3
	No 60	32	28	
HT**	Yes 60	33	27	0.4
	No 36	19	17	
HF***	Yes 10	3	7	0.7
	No 86	49	37	
OAD****	Yes 12	4	8	0.5
	No 84	48	36	
CKD*****	Yes 7	1	6	0.03
	No 89	51	38	

W *Diabetes mellitus, **Hypertension, ***Heart failure, ****Obstructive airways disease, *****Chronic kidney disease.

Table III. The relationship between hematological variables and oxygenation saturation in COVID-19 patients at presentation.

Parameter	Mean ±SD	Oxygen saturation	No.	p-value
NLR	9.85±3.427	>80	52	0.001
	15.93±3.26	<80	44	
HB [g/dl]	12.1±1.434	>80	52	0.43
	11.89±2.082	<80	44	
Platelet/mm^3	220880.3±108441.4	>80	52	0.61
	236410.1±146497.5	<80	44	
CRP [mg/L]	71.26±22.211	>80	52	0.001
	94.15±19.96	<80	44	
Ferritin [ng/ml]	755.82±450.33	>80	52	0.001
	1336.4±497.491	<80	44	
D-dimer [µg/ml]	2.89±3.13	>80	52	0.002
	6.443±4.855	<80	44	

level. However, there were no statistical differences in hemoglobin level and platelet count (Table V).

After excluding the effect of confounding factors on the outcome (age, gender, hypertension, diabetes, heart failure, chronic kidney diseases and obstructive airway diseases) by conducting a binary logistic regression analysis, NLR and D dimer were independent influencer on the outcomes of COVID-19 disease (Table VI, Fig.1).

DISCUSSION

In this study, the average age of the patients was 58.3 ± 13.8. Male patients were constituted about 45.8% and females were 54.2%. This figure is consistent with a study by Li et al. who revealed that the mean age of the patients was 59 years, with a swing between 15-89 years [16]. Thus, they described that there were no cases in those under the age of 15 years, but they recorded the male infected more than the female [17]. In contrary to that, a study by Burugu et al., who recorded a male incidence of 74% in his patients' sample [18], in addition a study by Elza et al. reported high female than the male with mean age equal to 60 years [19]. Many patients in the study had comorbidities, such as hypertension in 62.5%, diabetes in 37.5%, obstructive airway disease in 21.8%, heart failure 10.4%, and renal impairment in 7.2%. In accordance to study by Ai-Ping Yang whose found the hypertension presented in 66%, diabetes 54%, heart failure 37.%, and kidney dysfunction in 33.3%. Patients with chronic diseases were showed high frequency of severe disease presentation [20]. The mortality rate in this study was 31.25%; which is higher than worldwide mortality, this perhaps related to the selected admission

Table IV. The influence of clinical background on the consequence of patients with COVID-19.

Variables		Survivor	Death	p-value
Age (years)	20-60 (N: 47)	38	9	0.02
	> 60 (N: 49)	28	21	
Gender	M (N: 44)	26	18	0.07
	F (N: 52)	40	12	
DM*	Yes (N: 36)	24	12	0.4
	No (N: 60)	42	18	
HT**	Yes (N: 60)	44	16	0.3
	No (N: 36)	22	14	
HF***	Yes (N: 10)	7	3	0.7
	No (N: 86)	59	27	
OAD****	Yes (N: 12)	5	7	0.04
	No (N: 84)	61	23	
CKD*****	Yes (N: 7)	3	4	0.2
	No (N: 89)	63	26	

*Diabetes mellitus, **Hypertension, ***Heart failure, ****Obstructive airways disease, *****Chronic kidney disease.

Table V. The association between laboratory variables and the outcome in COVID-19 patients.

Laboratory variables	Mean ±SD	Outcome	p-value
NLR	18.01±1.626	Death	0.001
	10.20±2.9	survivor	
HB [g/dl]	12.05±1.97	Death	0.9
	11.99±1.651	survivor	
Platelet/mm^3	207670.2±129829.3	Death	0.2
	237240.2±125358.1	survivor	
CRP [mg/L]	101.2±18.62	Death	0.001
	73.02±20.32	survivor	
Ferritin [ng/ml]	1435.3±489.83	Death	0.002
	834.1±474.16	survivor	
D-dimer [µg/ml]	8.71±5.135	Death	0.003
	2.6188±2.10525	survivor	

Table VI. Regression analysis indicators between levels of NLR and outcomes of COVID-19.

Variables	OR (95% CI)	p-value
Hypertension	1.02(1.07-2.1)	0.2
Diabetes	1.58 (0.65–1.4)	0.3
Heart failure	1.2 (1.1–2.4)	0.1
Obstructive airways disease	1.4 (1.03–1.6)	0.04
Chronic kidney diseases	1.09 (1.2–1.32)	0.8
NLR at admission	1.626 (1.359–1.946)	0.001
CRP at admission	1.45 (0.671–1.24)	0.1
D-dimer at admission	1.32 (1.08–1.390)	0.001

of COVID-19 patients in Al-Sader hospital, where only those with severe infection were admitted.

Moreover, there was significant difference in mean of NLR between the recovered and deceased patients. The mean of NLR in non-survivor patients was 18.01 ± 1.626, which is higher than that for the recovered patients 10.20±2.9; in addition, there were significant difference between two groups in CRP, serum ferritin and D-dimer levels. However, there were no statistical differences in hemoglobin levels and platelet counts. The result illustrates the independent

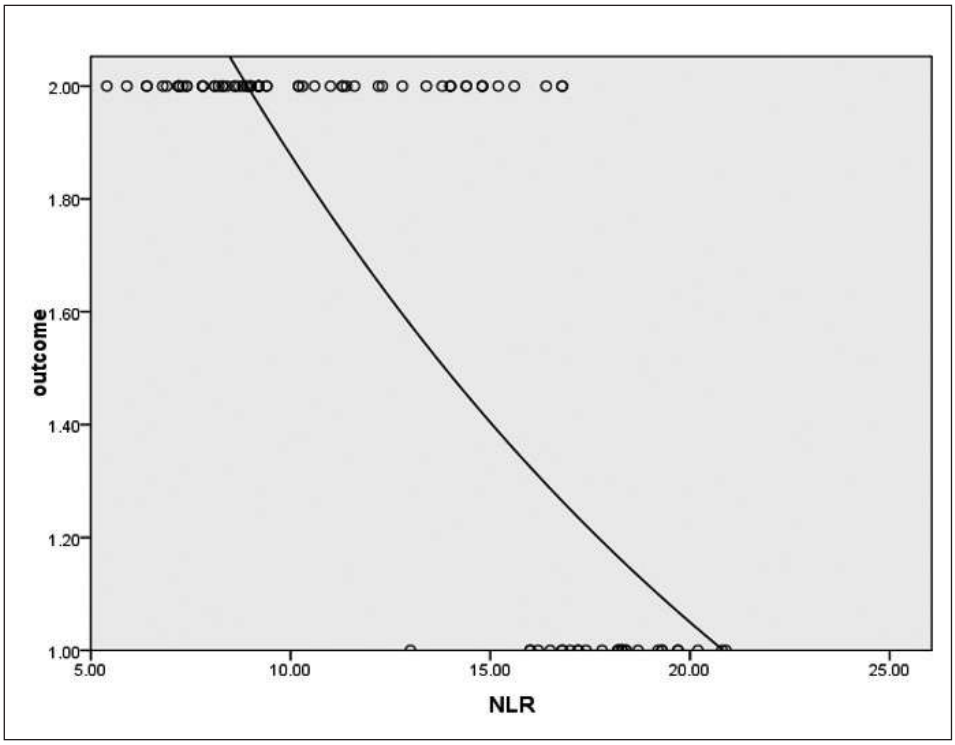

Fig. 1. Logistic regression curve (the value of 1= death, the value of 2 = survivor) between the outcome and NLR.

influences for progression of COVID-19 disease after excluding the effect of gender, age, and co-morbidities. With the aid of multivariate regression analysis, our study reported NLR was independent discriminator for the disease progression as higher levels on admission associated with lower oxygen saturation and a doomed outcome. These results are consistent with other studies that reported the incorporation of raised NLR as a predictive factor that could result in enhanced prognosis [20]. The study results were steady with the aforementioned studies concerning the association of NLR and the outcome of further contagious illnesses [21]. Many reasons may be behind these results. Neutrophils considered a main component of the leukocytes gathering nearly (50-70%) of all leucocytes which act and migrate from the venous circulation to the immune organs and other systems including the lungs [22]. Neutrophils discharge a huge amount of reactive oxygen species (ROS) which stimulate cellular DNA damaging and viral releasing from cells. By way of this action, the virus becomes free and destroyed via antibody-mediated immunity. Furthermore, neutrophils interact with a distinctive cell population and release numerous types of cytokines and growth vascular endothelial growth factors that lead to hyper-inflammation and more damage in the alveolar lining that is unconnected to the viral deleterious effect [20]. In addition, Neutrophil action can be stimulated by numerous virus inflammatory factor, for example IL-6 and IL-8, TNFs and GSF that produced by lymphocyte and endothelial cells [22]. Moreover, immune response against viral infection by the human body specifically relies on lymphocyte [23], on the other hand, systematic inflammation considerably reduces cell immunity, which meaningfully depresses CD4+ T-lymphocyte and upsurge CD8+ suppressor T-lymphocytes [21]. For these reasons inflammation that triggered via virus elevated NLR. This increase in NLR encourages progression of COVID-19 disease. Additionally, the clinical features become severe and escalate the possibilities of ICU admission or invasive ventilation [20]. The NLR is used in many applications in clinical fields and it a very useful indicator, it specifically used in cancer patients, autoimmune disorders, bacterial infections and tuberculosis [25], additionally, further researchers had revealed that NLR was predominant prognostic biomarker of avian H7N9 infection [24]. In the meantime, a new study by Qin et al. described that the NLR was significantly elevated in sufferers with severe COVID-19 illness in a group of hospitalized patients [26]. Meanwhile, the study of Zhang et al., found the neutrophil was much higher in severe cases and patients of poor outcome such as patients hooked on mechanical ventilation or those admitted in an ICU. They were concluded increase neutrophil count might be due to excess inflammation response [27]. In fact, the exact mechanism behind lymphopenia in COVID-19 patients is still unidentifiable. Lymphocytes massive apoptosis is encouraged by pro-inflammatory cytokine, metabolic acidosis-mediated inhibition of lymphocyte, and lymphocytes migration to the target organs, principally the lungs, as well as suppression of hematopoietic precursors [28]. Numerous researchers stated that lower lymphocyte number and lymphocyte percentage in comparison to neutrophils are intensively associated with disease severity, in a same way it can predict a progression to critical illness [29], when recovered, in almost all cases, lymphocyte counts return back to normal levels [30]. Besides, a meta-analysis study that encompasses six studies with more than 800 patients exhibited higher NLR as a worthy prognosticator of clinical severity and patients of poor outcome [31]. The study results show the death increase with advance age, which are in line with

study by Nan Li whose indicated that the fatality was chiefly in older individuals, especially patients at 80 years or older (nearly 15%), and 70-79 years 8.0% [17]. There was a rise in serum ferritin levels in non-survivor 1435.3±489.83 more than patients who survivor 834.1 ± 474.16 in accord with study by Burugu et al., who revealed the amplified levels of serum ferritin were detected in most patients with COVID-19, it was highly raised in deceased patients with COVID-19 when compared to the improved patients [18]. Kai Kapper was concluded serum ferritin could be reflected both predictive and classifying factors which could be pay to the therapeutic decision-making regarding patient of COVID-19 [32]. The study had designated that CRP was significantly raised in deceased patients 101.2 ± 18.62 than those who ultimately recovered 73.02 ± 20.32, which correlates with the degree of inflammation. This comes along with study by Matsumoto that exhibited CRP values in severe COVID-19 infection [33]. One study by Mo P demonstrates that individuals who could not survive from severe COVID-19 pneumonia had nearly tenfold greater CRP ranges than those who ultimately discharged from the hospital [34]. Another finding is that D-dimer was statically different between the recovered and the dead. D-dimer levels were high in the non-survivor group, which are consistent with Hai-Han Yu study [35].

CONCLUSION

The study concluded that elevated NLR at admission was a useful prognostic marker linked with poor outcome in patients admitted for COVID-19 pneumonia. Other inflammatory markers, such as ferritin, CRP, and D-dimer were also associated with critical illness and increased mortality from COVID-19 disease.

LIMITATIONS AND RECOMMENDATIONS

A COVID 19 is a new pandemic disease; studies with large size cohort are needed to further understand the disease characteristics, diagnostic, therapeutic and prognostic parameters. We recommend that our results better to be reinforced in the future by a multicenter study to afford optimal set of patients' data with comprehensive laboratory investigations and to ensure that longer period of follow up.

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Conflict of interest

The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

OCCUPATIONAL SAFETY AND HYGIENE OF HEALTHCARE PROFESSIONALS IN THE CONTEXT OF HOSPITAL ENVIRONMENT SAFETY

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ABSTRACT

The aim: This study was conducted to carry out a comprehensive assessment of the occupational safety and health of medical workers.

Materials and methods: The study involved a questionnaire survey and full-scale physiological and hygienic research using bibliosemantic, questionnaire, hygienic, and statistical methods.

Results: In the course of research it was recorded that the work of surgeons (according to the criteria of the current "Hygienic classification of work") is characterized by high intensity (class 3.2), high probability of exposure to infectious agents (class 3.3, and 4), harmful effects of physical factors (class 3.1), which according to the criteria of the general hygienic assessment of working conditions according to the degree of harmfulness and danger allows being assigned to class 3.3 ("harmful") and 4 ("dangerous"), respectively. Such working conditions provoke an increase in chronic morbidity (industrial and temporary disability), lead to the development of occupational diseases, and can pose a threat to the lives of workers. The need to focus attention on assessing the safety of hospitals in emergencies not only on issues of occupational safety and health but also on the stability and reliability of hospitals in such extreme conditions.

Conclusions: The work process of doctors is characterized by the impact on their body of a complex of unfavorable production factors of psycho-physiological, biological, physical, and chemical nature, among which the leaders are high labor intensity due to intellectual and emotional load, irrational mode of work and rest, and biological factors.

KEY WORDS: medical staff, occupational hygiene, occupational safety, safe hospital environment

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INTRODUCTION

The scientific community around the world is actively demonstrating its interest in the problem of ensuring the safety of the hospital environment. However, at present, most scientific publications are devoted to the study of patient safety, and the problem of occupational safety and health in the medical sector is paid much less attention [1-4].

And this is although, according to the WHO, there are more than 59 million health workers in the world. In particular, in Ukraine (according to the Center for Medical Statistics, Ministry of Health of Ukraine), as of 2021, there were 147,400 doctors of all specialties and 273,526 paramedics. At the same time, health care systems in many countries are leading among other areas of economic activity in terms of losses due to temporary disability, and occupational injuries, and the average lifespan of doctors in the world, according to the WHO, is only 54 years, dentists - 51 years much less than the lifespan of the population as a whole [5-6].

All this indicates the relevance and timeliness of research aimed at studying working conditions in the medical field and developing measures to prevent their negative impact on the health of medical staff [5-8].

THE AIM

The aim was to comprehensively assess the occupational safety and health of health workers.

MATERIALS AND METHODS

The work was performed in two stages, including a questionnaire and full-scale physiological and hygienic research.

At the first stage of the study, a survey of 135 health workers of 10 hospitals in Kyiv, Zhytomyr, and the Zhytomyr region was conducted using a questionnaire created by the authors, which contained 54 questions and covered safety and hygiene aspects of the work of medical staff [9-10].

The second stage of the study performed a hygienic assessment of the conditions and nature of work on the health of health professionals, taking into account the possible impact of hazardous and harmful factors in the working environment, as well as the severity and intensity of the work process according to criteria of "Hygienic classification of labor by indices of harmfulness and danger of factors of the working environment, the weight and intensity of the

labor process", approved by the order of the Ministry of Health of Ukraine № 248 dated 08.04.2014.

The research was conducted in surgical departments based in two multidisciplinary city clinical hospitals in Kyiv. Measurements were performed at permanent workplaces of medical staff (in operating rooms, dressings, manipulation, residencies, nurse posts, and wards).

The analysis was performed based on staffing schedules, schedules of scheduled and emergency shifts, protocols of surgical interventions, protocols of anesthesia, and job descriptions of doctors and according to the timing of surgeons' working hours.

The intensity of work was assessed based on time trials on intellectual workloads, nature of work performed, sensory workloads, size of the object of observation, emotional loads and degree of responsibility, degree of risk to one's own life, and degree of responsibility for other people's safety.

The difficulty of the surgeons' work was assessed by the working posture and inclinations of the body.

Studies of microclimatic conditions (air temperature, ° C; adhered humidity, %; air velocity, m / s; indoor temperature, ° C; THC index) were performed in the warm season with the help of devices: infrared thermometer "Nimbus- 2000 ", ball thermometer" Tensor-41 ", thermoanemometer. Hygienic assessment of microclimate parameters was carried out by the "Sanitary norms of the microclimate of industrial premises" sanitary standard 3.3.6.042-99 and GOST 12.1.005-88 "General sanitary and hygienic requirements for the air of the working area".

The intensity of ultraviolet radiation was measured using a radiometer of energy exposure of illumination of the ultraviolet range (UV meter) in the spectral range from 200 to 400 nm (regions A, B, and C). The results were evaluated by sanitary norms № 4557-88 "Sanitary norms of ultraviolet radiation in industrial premises".

Noise measurements were performed using the Larson-Davis 812 device following GOST 12.1.050-86 "Methods of measuring noise in Hygienic assessment of industrial noise was carried out following sanitary norms 3.3.6.037-99 "Sanitary standards of noise, ultrasound, and infrasound", "Sanitary standards of permissible noise generated by medical devices in the premises of medical institutions" № 3057 -84, "Sanitary standards noise in residential and public buildings and residential areas" № 3077-84.

Industrial lighting was measured and evaluated using a luxmeter Yu-117 by DSTU B B.2.2-6-97 (GOST 24940-96) "Buildings and structures. Methods of measuring illuminance "and DBN B.2.5-28-2006" Natural and artificial lighting ".

RESULTS

In the first stage of the research, we conducted a subjective assessment of working conditions in hospitals in Kyiv, Zhytomyr, and the Zhytomyr region through a questionnaire. The obtained data show that the vast majority of medical workers are affected by increased nervous and emotional stress (89.7% of Kyiv and 74.0% of Zhytomyr doctors). Zhytomyr doctors in 22.9% of cases experience fatigue in the middle of the work shift, 66.7% at the end of the work shift. Among Kyiv doctors, these figures are slightly higher at 28.2% and 79.5%, respectively.

Among harmful chemical occupational factors, Kyiv (71.8%) and Zhytomyr (57.3%) doctors most often mentioned the presence of biocides and medicines - 46.2% and 39.6%, respectively. Interviewed Kyiv doctors noted as a manifestation of the influence of chemical factors: odor - 41.0%, irritation of mucous membranes or skin - 35.9%, and allergic reactions - 18.0%, and their Zhytomyr colleagues had the value of these manifestations at 51, 0%, 45.0%, and 33.3% respectively.

Sufficient lighting in the workplace and sufficient ventilation efficiency were considered by 90.6% and 50.0% of doctors in the Zhytomyr region and only 51.3% and 30.8% of doctors in Kyiv, respectively.

Zhytomyr doctors among the physical factors noted the negative impact of ultraviolet radiation (22.9%), second place - noise (20.8%), and third place (16.7%) - ionizing radiation. Ultraviolet radiation (48.7%) and noise (20.51%) were also in first and second place among their Kyiv colleagues, and electromagnetic radiation was in third place (18.0%).

In addition, health care workers are at risk of injury due to falls, burns, and electric shocks.

Data from our study show that during 2019-2020, almost every 5th Zhytomyr doctor was injured due to a slippery floor (1 to 10 times). Approximately at the same level, the problem of injuries was noted by Kyiv doctors.

Previous research by us [10-11] has shown that the risk of contracting SARS CoV 2 and the risk of dying from COVID-19 in health care workers is many times higher than in the general population (for example, at the end of 2020 the corresponding excess was recorded at 2.7 and 1.4 times). And this dependence, despite the reduced risk of infection and death of medical personnel throughout the pandemic, persists to this day.

It should also be noted that according to the literature, the incidence of SARS, influenza, tonsillitis, pharyngitis, laryngotracheitis, bronchitis, intestinal infections, pustular skin lesions, and herpes infection is higher by an average of 1.5 - 3 times higher than the population in general [12].

Thus, the data of our survey show that the formation of working conditions of CHP staff is influenced by biological, physical, and chemical, factors, and high physical and neuro-emotional stress. In combination, they enhance the effect of each other and cause the functional accumulation of fatigue in health professionals.

At the next stage of the study, we conducted an in-depth assessment of the working conditions of medical staff by conducting full-scale physiological and hygienic studies in individual surgical departments of the two cities of Kyiv. Surgical departments were chosen because they have the widest (compared to other departments) range of harmful and dangerous factors in the production environment.

Assessment of the severity and intensity of work shows that the work of medical staff, and especially surgeons, is characterized by a significant intellectual load, requires a large amount of information and long-term memory, and

Table I. Classes of working conditions of surgeons in terms of labor intensity

Indicators of the intensity of the labor process	Characteristics of the indicator	Class of working conditions
1. Intellectual loads		
1.1. Content of work	Heuristic (creative) activity that requires solving complex problems in the absence of an algorithm; personal guidance in difficult situations.	3.2
1.2. Perception of signals (information) and their evaluation	Perception of signals followed by a comprehensive assessment of interrelated parameters. Comprehensive assessment of all production activities.	3.2
1.3. Distribution of functions according to the degree of complexity of the task	Control and preliminary work on the distribution of tasks to others	3.2
1.4. The nature of the work performed	Work in conditions of shortage of time and information with increased responsibility for the result	3.2
2. Sensory loads		
2.4. Load on the visual analyzer 2.4.1. The size of the object of distinction (at a distance from the eyes of the worker to the object of distinction, not more than 0.5 m), at the duration of concentrated observation (% of shift time)	1.0-0.3 mm more than 50% of the time	3.1
3. Emotional load		
3.1. The degree of responsibility for the result of their activities. Significance of the error	Responsible for the functional quality of the final product, work, and tasks. Wrong decisions can be life-threatening	3.2
3.2. The degree of risk to one's own life	Reliable	3.2
3.3. Degree of responsibility for the safety of others	Is responsible for the security	3.1
4. Operating mode		
4.1. Working day length (hours)	More than 8 hours	3.1
4.2. Variability of work	Irregular variability with work at night	3.2

Table II. Classes of working conditions of surgeons by indicators of difficulty

Indicators of the severity of the labor process	Characteristics of the indicator	Class of working conditions
Working posture	periodic stay in an awkward and/or fixed position from 25% to 50% of the time of change; being in a standing position from 60% to 80% of the time change	3.1
Slopes of the case (forced, more than 300), the number per shift	120	3.1

contains elements of creativity. Doctors are forced to solve complex problems in the absence of an algorithm, personally manage the work in difficult situations; distribute tasks to others and monitor their implementation; work in conditions of shortage of time and information with increased responsibility for the result.

When performing work, surgeons are subjected to loads on the visual analyzer, which consists in distinguishing small objects (1.0-0.3 mm) for a long time (more than 50% of the time): during surgery, bandaging, examination of patients, and registration of medical documentation. The density of signals (light, sound) for one hour of work is up to 75.

Periodically, surgeons monitor the screens of video terminals (up to 2-3 hours per shift), sometimes using optical devices.

The significant emotional burden on doctors is associated with a great responsibility for the possible danger to the life and health of patients, probable risk to their own lives, and responsibility for the safety of others. Daily contact with people of different ages, social statuses (patients and their relatives, managers, colleagues), and different psychological characteristics requires medical professionals constant nervous and emotional stress. Estimation of working conditions of surgeons on indicators of the intensity of labor process is given in table I.

The working day of surgeons lasts 7 hours. 42 min (from 8^{30} to 16^{12}), no lunch break. In addition, each surgeon has several shifts during the month, lasting 7; 16, or 24 years. In this case, after each shift on a working day, the surgeon continues to work during the next working day, thus, the duration of continuous working time increases. There are no regulated breaks during working hours according to the work schedule.

About 30% of doctors work part-time, which increases the total time of contact with harmful factors in the work environment.

Based on the analysis of the protocols of operations, it was found that one surgeon has about 185 hours of surgery per year. One operation lasts, on average, 1 hour 12 min.

The nature of the surgeon's work is associated with elements of difficulty, as it is characterized by periodic, from 25% to 50% of the time of the work shift in an awkward and/or fixed position, as well as being in a forced "standing" position from 60% to 80%.

When performing the main types of their activities, surgeons perform forced tilts of the body at an angle of more than 300 to 120 times per shift.

Often irrational working posture is due to non-compliance of medical equipment with ergonomic requirements.

Estimation of working conditions of surgeons on indicators of difficulty of labor process is given in table II.

The general assessment of the severity of the surgeon's work was performed based on auxiliary indicators (working posture, body tilts), during the main activity - surgical interventions. Taking into account the coefficients of the significance of auxiliary indicators, the sum of points in severity is less than 1, which corresponds to class 2 - acceptable working conditions.

Assessment of the microclimate of industrial premises showed that the air temperature does not meet the optimal parameters. Thus, at some workplaces (in manipulation, operating, and patient wards) the air temperature exceeded the permissible parameters by 1.0-1.3^{0}C and corresponded to the class of working conditions "harmful" - 3.1. At other workplaces, the air temperature was within the permissible values and corresponds to the class of working conditions - 2 "permissible".

Relative humidity in most workplaces is optimal (Class 1 working conditions), except for operating and dressing, where it is acceptable (Class 2 working conditions).

The speed of air movement corresponded to the optimum at all studied workplaces and corresponded to the 1st class of working conditions "optimal".

The temperature of the internal surfaces in some rooms exceeded more than 2 ^{0}C optimal values.

Thus, the work of doctors of surgical departments in terms of microclimate by the criteria of the "Hygienic Classification of Labor…" mainly belongs to the 2nd class of working conditions (permissible). However, in some workplaces (in manipulation, operating, and patient wards) - up to class 3.1 (harmful).

During the sanitary and hygienic study of air exchange, it was found that in all rooms the openings of mechanical ventilation were blocked by artificial screens. This created conditions for impaired ventilation and led to an increase in room temperature.

During the assessment of the intensity of UV radiation, it was found that the medical staff in operating rooms and dressings are affected by UV radiation of the spectral region C (from 0.02 to 11 mW / m2), which should not be at all (class of working conditions 3.1 (harmful 1 degree UV radiation in sections A and B did not exceed the maximum allowable levels.

At all workplaces, except for the wards for patients, noise levels exceed the maximum allowable values from 2 to 8 dBA (class of working conditions 3.1 (harmful 1 degree). than 5 dBA.

Most nurses' offices did not have natural light, as they were located in remote corners of corridors without windows. However, DBN B.2.5-28-2018 regulates the level of natural light for these types of premises. The lighting of workplaces at such posts was provided only by general artificial lighting, the level of which was 93-146 lux, which was significantly lower than the standardized lighting indicators (300 lux).

Levels of artificial lighting in the workplaces of surgeons comply with hygienic regulations for the relevant types of premises.

The work of surgeons by the "Hygienic Classification of Labor…" on the level of illumination in the workplace belongs to the 2nd class (permissible), and nurses on duty - to the class of working conditions "harmful" 3.2.

Among the biological factors that affect surgeons, there are two groups - pathogens of infectious diseases and antibiotics and antibiotic-containing drugs.

According to the data of microbiological passports of surgical and traumatological departments of hospitals in the treatment of patients surgeons are exposed to pathogenic and opportunistic microorganisms: Strep. viridans, b-Strep. hemolytic, Staph. aureus, Staph. epidermidis, Staph. fecal, Klebsiella pneumonia, E. Colli, etc. Surgeons use a large number of antibiotics to treat wounds infected with these microorganisms: erythromycin, tetracycline, gentamicin, lincomycin, rifampicin, ofloxacin, cefoperazone, gentamicin, etc., and which can directly affect the doctor's body.

During the deteriorating epidemic situation of Covid-19 (a particularly dangerous disease) in Kyiv, the study wards of hospitals were reorganized to provide medical care to this category of infectious patients.

According to the "Hygienic classification of work…" working conditions of surgeons under the influence of the above pathogenic microorganisms belong to class 3.3 ("harmful" 3 degrees), and under the influence of pathogens of particularly dangerous infections (SARS-CoV-2 virus) - up to class 4 ("Dangerous").

Working conditions of surgeons in terms of harmfulness and danger of factors of the working environment, severity, and intensity of the labor process by the criteria of "Hygienic classification of labor…" belong to class 3.3 ("harmful" 3 degrees), and working with patients with Covid-19 - up to 4th grade ("dangerous").

DISCUSSION

As mentioned in the introduction, the scientific community's interest in ensuring hospital environment safety is actively growing. Despite this, scientific publications devoted to the patient safety study predominate, and less attention is paid to other safety aspects (safety of healthcare workers) [1-4].

And here it is worth noting that the specific form of a safe environment in hospitals includes close interaction between individual components of the safe environment. Above all, it concerns patient safety with the safety and occupational hygiene of healthcare workers [13-14]. That significantly actualizes the need for a more active introduction into the practice of research aimed at studying medical personnel's safety and occupational hygiene.

At the same time, it is worth noting that conducting field studies to assess the hygienic working conditions of medical workers require additional funding and specialized equipment, which complicates their conduct, especially in emergencies of various natures. The authors emphasize the proposed methodology's prospects for assessing medical personnel's working conditions.

In particular, I would like to draw attention to the consonance of the results of the questionnaire, conducted using the questionnaire developed by the authors of the article, and the data of classical natural physiological and hygienic research, performed by the requirements and criteria of the current "Hygienic Classification of Labor".

The course of field research confirmed the presence and qualitatively and quantitatively assessed the impact of psycho-emotional factors, irrational lighting, high noise, exposure to ultraviolet radiation, and chemical factors on the production environment. Similar results are also presented in the scientific literature [5, 15].

This demonstrates the effectiveness of the method proposed by the authors, as an additional, for assessing the working conditions and safety of medical staff in emergencies of various kinds. An objective quantitative assessment of physical, chemical, biological, and psychophysiological factors can be provided only as a result of appropriate laboratory and instrumental studies.

However, in the absence of opportunities for full-fledged physiological and hygienic research, the proposed method can be effectively applied and allow for rapid assessment of the situation and influence the most important risk factors. In addition, this technique makes it possible to conduct a preliminary assessment of working conditions during the planning of physiological and hygienic research, to optimize them.

CONCLUSIONS

Conducted sociological (through a questionnaire) and physiological and hygienic studies to study the working conditions of medical staff allowed to draw the following conclusions:

1. The labor process of doctors is characterized by the impact on their body of a complex of adverse production factors of psycho-physiological, biological, physical, and chemical nature, among which the leaders are high labor intensity due to intellectual and emotional stress, irrational work and rest, and biological factors.
2. The work of surgeons is characterized by high intensity (class 3.2), high probability of exposure to infectious agents (class 3.3 and 4), and harmful effects of physical factors (class 3.1), which according to the criteria of general hygienic assessment of working conditions according to the degree of harm and danger refer to class 3.3 ("harmful") and 4 ("dangerous"), respectively.
3. The working conditions of surgeons are characterized by such levels of harmful factors of the production environment and labor process of class 3.3 ("harmful"), which, in addition to increasing chronic morbidity (industrial and temporary disability), lead to occupational diseases. And dangerous working conditions of the 4th class - pose a threat to life, and a high risk of acute occupational injuries, including severe forms.
4. The effectiveness of the proposed method of assessing the working conditions of medical staff through questionnaires, which can be used in emergencies of various kinds (in the absence of classical research) and in the planning of physiological and hygienic research (to optimize their conduct).
5. As hospitals are currently operating in a dual emergency of medical, biological, and military origin, further research, in addition to occupational safety and health, should focus on the sustainability and reliability of hospitals in such extreme conditions.

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ORIGINAL ARTICLE

PRETREATMENT WITH ERYTHROPOIETIN ALLEVIATES THE RENAL DAMAGE INDUCED BY ISCHEMIA REPERFUSION VIA REPRESSION OF INFLAMMATORY RESPONSE

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ABSTRACT

The aim: This study aimed to examine the anti-inflammatory, and antiapoptotic effects of erythropoietin against kidney injury inducted by ischemia reperfusion in experimental model.

Material and Methods: 20 male Sprague Dawley rats were randomly divided into 4 equal groups: sham (subject to median laparotomy only), control (subject to 30 minutes ischemia and 2hours reperfusion), vehicle (injected by distilled water and subjected to the same procedure of ischemia reperfusion), erythropoietin group (as in vehicle group but the rats pretreated with 1000 U/kg of erythropoietin). The left kidney and blood specimen were collected. The blood utilized to assess serum creatinine. While kidneys utilized to assessed MCP-1, TLR2, and caspase-3 in addition to histopathological evaluation.

Results: Control and vehicle samples showed that a significant elevation in serum creatinine, TLR2, caspase-3, and MCP-1 as compared with sham group. The histological evaluation showed a significant rise in kidney injury scores. Kidneys and blood samples of erythropoietin pretreated rats established histopathological and functional improvement as evidenced via reduced kidney injury scores in addition to the reduction in serum creatinine, as well as there were a significant diminished in caspase-3, MCP-1, and TLR2 levels when compared with control and vehicle groups.

Conclusion: Erythropoietin has renoprotective effect against ischemia and reperfusion, which achieved by decrease the inflammatory response as well as antiapoptotic effect

KEY WORDS: erythropoietin, ischemia reperfusion injury, apoptosis, TLR2, caspase-3, MCP-1

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INTRODUCTION

Ischemia reperfusion injury (IRI) describes a restriction of blood flow to any organ followed by restoration of blood supply and reoxygenation. The inescapable injuries may happen subsequent to infarction, sepsis, and the transplantation of organs. These phenomena worsen the damage of tissue via initiating an inflammatory cascade such as cytokines, chemokines, activation of leukocytes, in addition to reactive oxygen species. Renal IRI participate in pathological condition termed as acute kidney injury (AKI) which is clinical syndrome with rapid dysfunction of kidney and high rates of mortality [1]. Inflammation is the main contributor of the pathogenesis of ischemic AKI, though, effective therapies that improve the outcomes of AKI via reduction of inflammation are yet limited [2] Innate immune response is nowadays being recognized as paramount in initiating acute inflammatory responses to give early defenses to nonspecific injury, like ischemia reperfusion damage before, during and next organ transplantation. Toll like receptors (TLRs) have been recognized to amplify innate immune defense mechanism in addition to play a main role in initiating and modulation adaptive immune response, T helper cells differentiation, in addition to immune tolerance. Liberation of inflammatory cytokines and chemokines from immune as well as nonimmune cells induce after TLRs stimulation by endogenous and exogenous ligands [3]. TLR2 is identified to have a main role in initiating inflammatory response and ischemic damage progression in the kidney. TLR2 is expressed intensely in Bowman capsule epithelial cells and glomerular endothelial cells [4]. Through IRI induced AKI, TLR2 is upregulated and stimulated in epithelial cells of renal tubules that activates intracellular signaling pathways. Afterward upregulation of mediators like interleukin 1beta (IL-1β), interleukin 6 (IL-6), monocyte chemoattractant protein 1(MCP-1), and tumor necrosis factor alpha (TNF-α) which induce inflammatory responses, resulting in acute tubulointerstitial damage [5]. MCP-1 is a chemotactic factor belongs to cytokines super family that may facilitate the infiltration of mononuclear macrophage into kidney tissue and induction of Ca^{+2} increment in macrophages/monocytes. This Ca^{+2} increment lead to respiratory burst, release of lysosome, in addition to production of oxygen

derived free radicals to cause renal injury [6]. Apoptosis is one of the mechanisms of kidney cell death through renal IRI. The investigation of apoptosis is performed via caspase, which is a group of cysteine proteases. Numerous of caspase have been implicated in apoptotic cell through kidney IR like caspase-3 and caspase-9 [7]. Cell apoptosis is mostly initiated by 2 distinct signaling pathways (extrinsic and intrinsic pathways), extrinsic (death receptor dependent) pathway which is triggered by binding the cell surface death receptor with their ligands, intrinsic (mitochondrial dependent) pathway which is mediated by damage the outer membrane of the mitochondria. This damage causes liberation of the mitochondrial proteins like cytochrome c from the mitochondria into the cytosol. Then caspase-9 is activated that in turn stimulates the apoptotic caspase activation like caspase-3. Caspase-3 is the main executive caspase in both cell apoptosis pathways. Caspase-3 cleavage lead to its activation therefore facilitating its proapoptotic effects [8]. Erythropoietin is a glycoprotein that is control the production of erythroid progenitor cells. It is formed by the kidney to induce the erythroid precursor's cells in response to hypoxia in order to increase the mature red blood cells within the circulation [9-10]. Erythropoietin has numerous of bio-protective effects like antioxidant, antiapoptotic, pleiotropic, and anti-inflammatory effects. The bio-molecular impacts of erythropoietin are mediated via binding its cell-surface receptor, and the existence of the functional erythropoietin receptor in the mesangial and the tubular epithelial cells indicates the potential role of erythropoietin in the kidney [11]. Previous studies have been shown that erythropoietin administration diminished the circulation proinflammatory cytokines, conserved the integrity of the microvascular endothelial cells, as well as diminished the lipid peroxidation associated with oxidative stress [9].

THE AIM

This study aimed to examine the anti-inflammatory, and antiapoptotic effects of erythropoietin against kidney injury inducted by ischemia reperfusion in experimental model.

MATERIAL AND METHODS

SITE OF THE STUDY AND ANIMAL ETHICAL CONSIDERATION

This research was done in University of Kufa, Faculty of Medicine, Department of Pharmacology and Therapeutic. The research was accepted by Center Committee of Bioethics and its branch in the Faculty. All the steps were performed regarding to the committee approvals.

ANIMAL GROUPING

Adult male Sprague Dawley rats' weight about 230-290 g were obtained from Center of the Control and Pharmaceutical-research/Ministry of health. The animals were housed with identical temperature, humidity, and light-dark cycles for 10 days before starting the procedures. In this research, the animals were randomly separated into four isolated groups (five in everyone):

1. Sham group: the rats were undergoing to anesthesia and median laparotomy for 150 minutes but without clamping.
2. Control group: the rats were undergoing to anesthesia and median laparotomy followed by half hour ischemia and 2 hours reperfusion.
3. Vehicle group: all rats intraperitoneally injected with distilled water as vehicle 30 minutes before surgery.
4. Erythropoietin group: the rats were injected intraperitoneally with 1000 U/kg body weight erythropoietin half hour before surgery [12].

The rats in vehicle and erythropoietin groups undergo to the same ischemia reperfusion procedure as in control group.

INDUCTION OF RENAL-IRI MODEL

The animals were anaesthetized by using a mixture consisting from ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg) injected intraperitoneally, placed the rats on heating plate to reserve their body temperature near 37°C. Abdominal area hair was removed and swabbing by antiseptic then making midline incision. Bilateral renal pedicles were clamped for 30 minutes with non damaged vasculo-clamps, infusion 1ml of warm 0.9% saline on the peritoneal to maintain well hydration. Once the ending of ischemic period, the vasculo-clamps were removed, after that the wound was sutured and covered with sterilized gauze damped with 0.9% saline to prohibit the dehydration. After 2 hours of reperfusion, the suture was opened and withdraws 3 ml of blood from the heart and then used to measure serum creatinine, afterward bilateral nephrectomy which cleaned from the blood by precooled phosphate buffer saline. Lastly, the rat was sacrificed via heart puncture. Cut the left kidney sagittal into two halves, kept the first one in the deep freeze for assessment of bio-molecule. Insertion the other half in 10% formalin followed by embedded within paraffin for histological analysis and immunohistochemistry [13-14].

FUNCTIONAL ASSESSMENT OF THE KIDNEY

The withdrawal blood was placed in serum-separating (gel) tube (free of anticoagulant) and left it for half hour at laboratory temperature followed by centrifugation for 15 minutes at 4000 rpm in order to obtained serum to measure the concentration of serum creatinine during 3 hours via commercially available assay kits.

ASSESSMENT MCP-1

The small fragments of frozen kidneys was washed by cooled phosphate buffer saline followed by weighted and homogenization firstly via mortar and pestle by mixing with 0.1M of cooled phosphate buffer saline (pH=7.4)

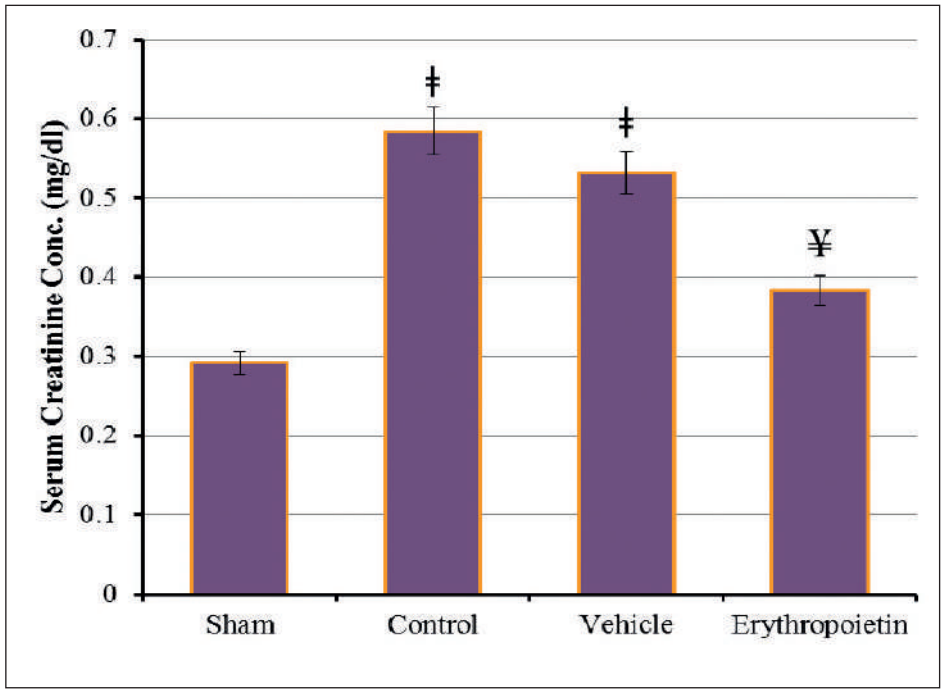

Fig. 1. Means of serum creatinine concentration in different groups.
ǂ significant with sham group
¥ significant with control and vehicle groups

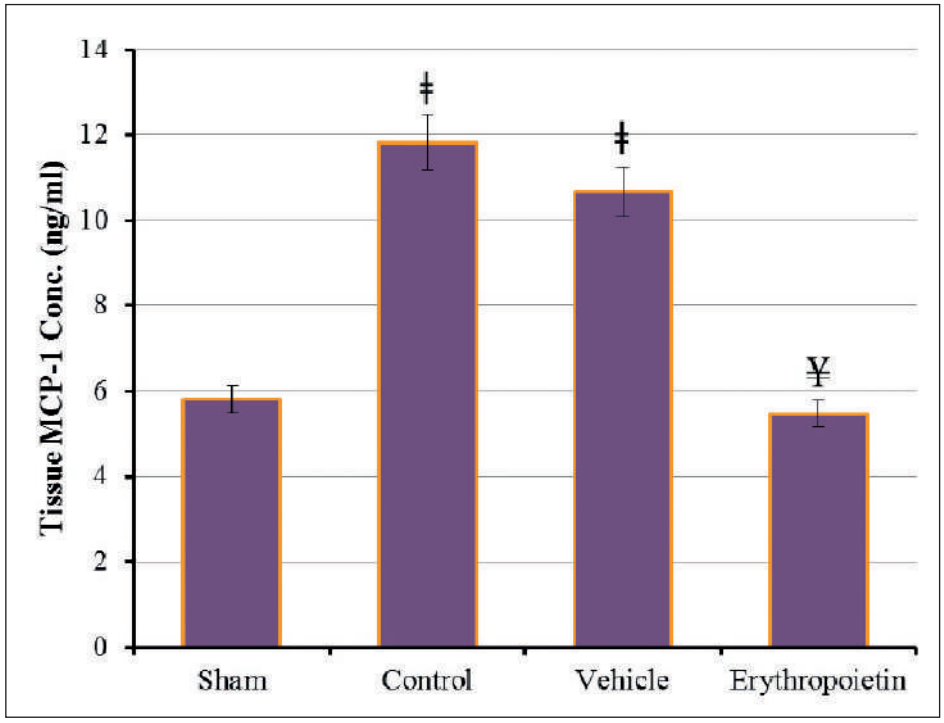

Fig. 2. Means of tissue MCP-1concentration in different groups.

consist of 1% protease inhibitor cocktail in addition to 1% triton-100X in ratio 1:10 (W/V). For additional homogenization, the homogenate suspension was sonicated by an ultrasonic cell disrupter, finally centrifugated the suspension according to manufacture procedure, then, the supernatant was used to determine MCP-1 levels via ELISA kits [14-15].

HISTOPATHOLOGICAL ANALYSIS

Dehydration half of left kidney then embedded in paraffin. 5µm thickness sections were cut via the rotary microtome. After that, the tissue sections were fixed on slides, stained by hematoxylin eosin dye, then covered the slides in order to examine by microscope. Two experienced pathologists did evaluation of kidney tissue injury in a sightless way that was grouping the sections in scale design for evaluation the kidney damage such as swelling and desquamation of the epithelial cells, formation of the eosinophilic cast, necrosis of tubular, brush border loss, and inflammation. The scoring model was utilized composed of 5 scores which are: score of zero for a normal renal tissue, score of one for a renal injury area fewer than 25%, score of two for a renal injury area 25-50%, score of three for a renal injury

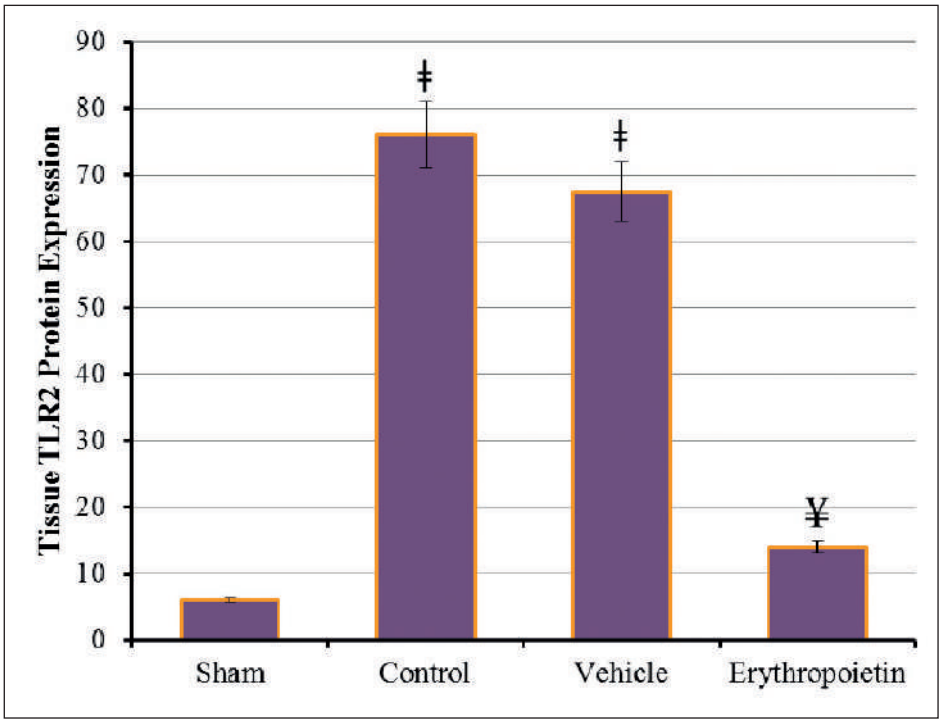

Fig. 3. Means of tissue TLR2 protein expression in different groups.
ǂ significant with sham group
¥ significant with control and vehicle groups

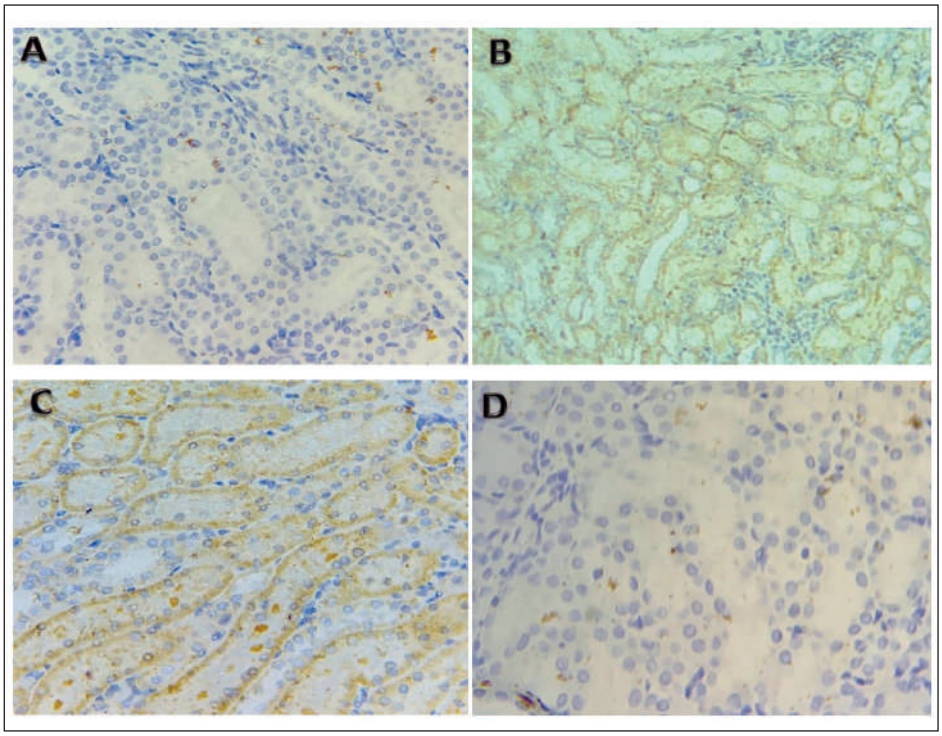

Fig. 4. Immunohistochemical staining of kidneys sections by TLR2 polyclonal antibody; A: sham shows renal tubules negative stain IHC (x400). B & C: control and vehicle show renal tubules positive cytoplasmic stain IHC (x100 and x400). D: erythropoietin shows renal tubules negative stain IHC (x400).

area 50-75%, score of four for a renal injury area greater than 75% [16].

IMMUNOHISTOCHEMISTRY

Immunohistochemistry performed to assessment of TLR2 and caspase-3 in renal tissue. Staining of 5 μm of paraffin embedded sections via using immunostaining procedure. Briefly, subjection the slices to deparaffinized, rehydration, exposing to retrieval buffer in order to repairing the antigen, and endogenous peroxidase activity inhibition via 3% H2O2, incubation of the sections with TLR2 polyclonal antibody (Bioassay Technology Laboratory) or caspase-3 monoclonal antibody (Bioassay Technology Laboratory) at 4°C overnight. Sections were later washed and incubated with conjugated secondary antibody for 1 hour, then washed and horseradish peroxidase was subjected for 30 minutes. After that the slices were incubated for eight minutes with fresh 3,3'-diaminobenzidine. Lastly, tissue slices stained by hematoxylin. The stained slide was observed under the clinical microscope. TLR2 and caspase-3 protein expression were calculated via H-score method (0-300) that calculated via multiplying the intensity by the percentage of the colored zone. The

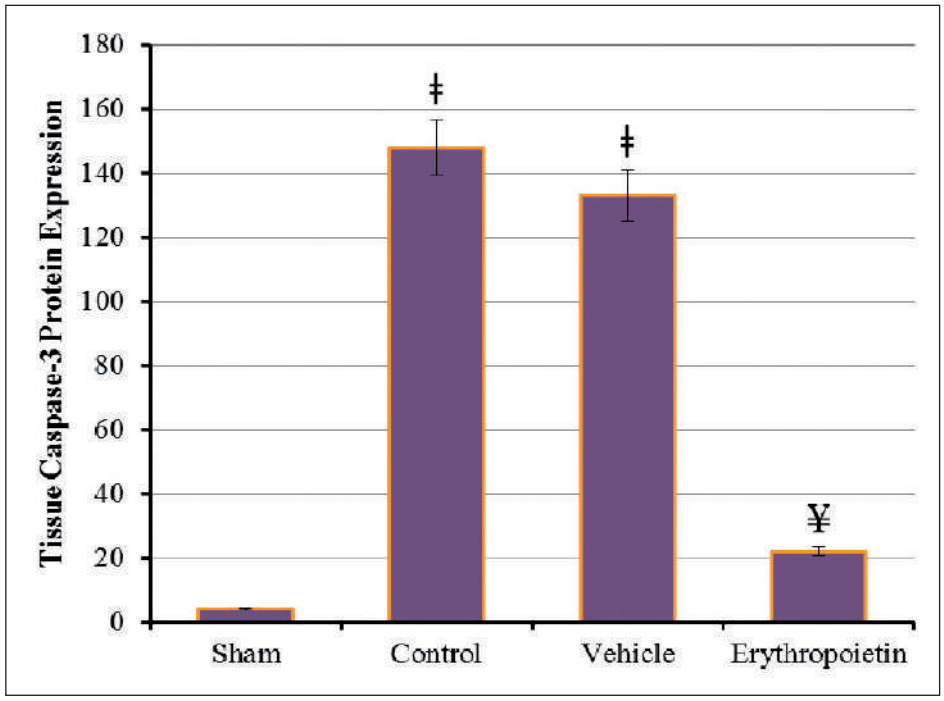

Fig. 5. Means of tissue caspase-3 protein expression in different groups.
‡ significant with sham group
¥ significant with control and vehicle groups

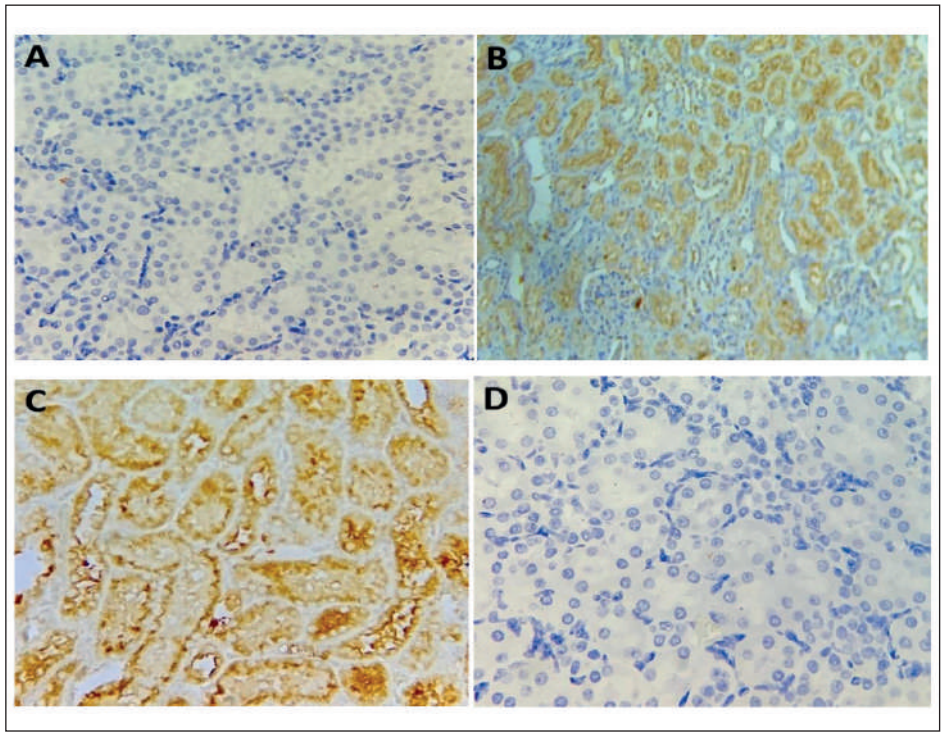

Fig. 6. Immunohistochemical staining of kidneys sections by caspase-3 monoclonal antibody; A: sham shows renal tubules negative stain IHC (x400). B & C: control and vehicle show renal tubules positive cytoplasmic stain IHC (x100 and x400). D: erythropoietin shows renal tubules negative stain IHC (x400)

intensity of the staining area was scored as 0 for negative-stain, 1 for weak-stain, 2 for moderate-stain, and 3 for strong-stain. The percentage of stained zone was graded 0-100% [17].

STATISTICAL ANALYSIS

The values were presented as mean ± standard error mean, the variable were subjected to the normality and homogeneity tests in order to select the appropriate statistical test for analysis, using one way analysis of variance (ANOVA) test, when the values of these tests >0.05, otherwise using Krukal-Wallis as well as Mann-Whitney U test to assess the difference among groups. The statistical analysis was done via utilizing SPSS 21. If the P-value of the result was less than 0.05, it considered statistically significant.

RESULTS

ERYTHROPOIETIN IMPROVED THE RENAL FUNCTION

Following ischemia-reperfusion, serum creatinine was significantly raised in ischemic and vehicle-ischemic groups versus sham group. Erythropoietin pretreatment significantly reduced level of serum creatinine comparing with control group (Fig. 1).

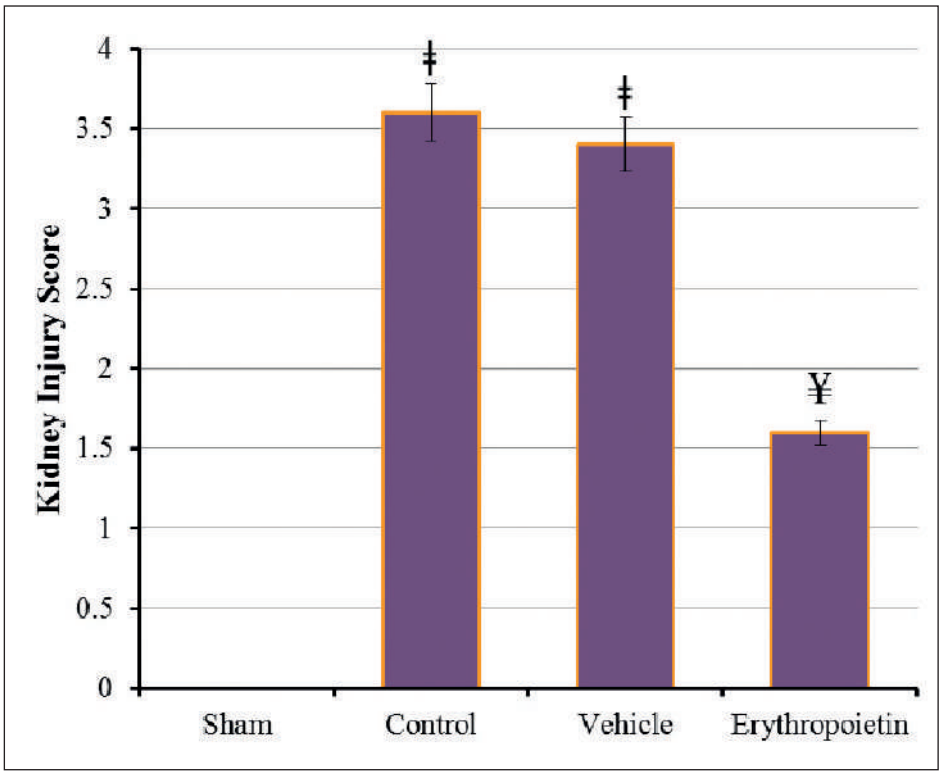

Fig. 7. Means of kidney injury score in different groups.
‡ significant with sham group
¥ significant with control and vehicle groups

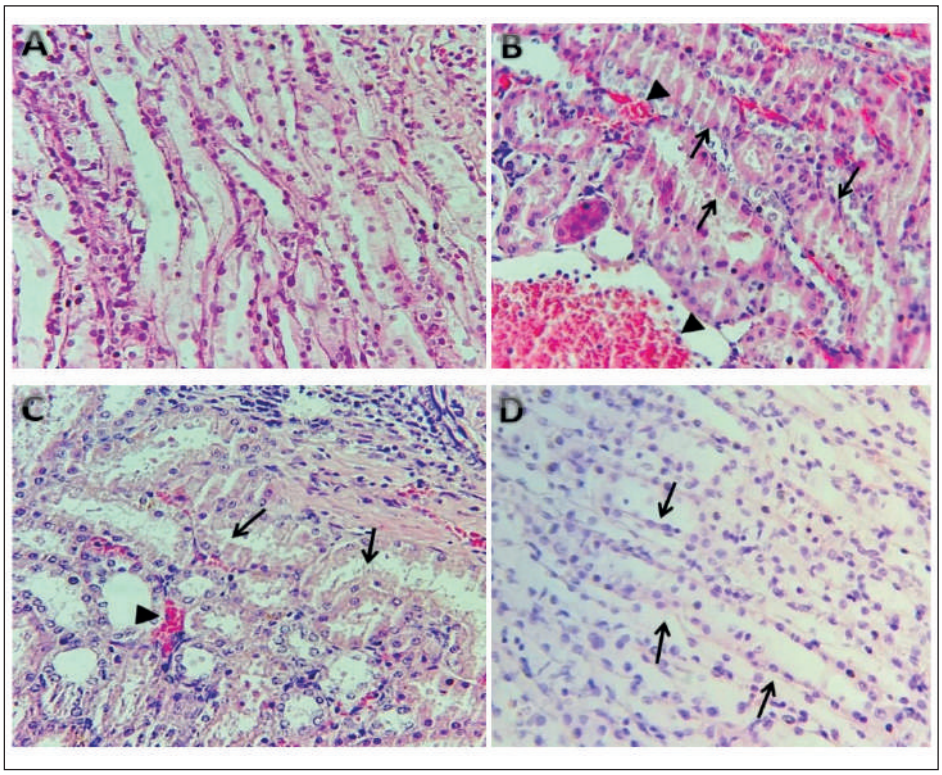

Fig. 8. Kidneys sections stained by H&E; A: sham shows normal renal tubules. B & C: control and vehicle show cellular swelling, increase cytoplasmic eosinophilia and loss of brush border (arrows), and hemorrhage (arrow head). D: erythropoietin shows normal renal tubules. X400

ERYTHROPOIETIN ALLEVIATED MCP-1 IN RENAL TISSUE

Kidney homogenate of control and vehicle rats exhibited a significant elevation in proinflammatory mediator (MCP-1) comparing with sham group. However, MCP-1 level was significantly reduced in rats pretreated with erythropoietin comparing with ischemic non-treated rats (Fig. 2).

ERYTHROPOIETIN DOWNREGULATE THE TLR2 EXPRESSION

Ischemia for 30 minutes and 2 hours reperfusion significantly raised the protein expression of TLR2 in control and vehicle rats comparing with sham rats. Furthermore, erythropoietin exhibited a significant downregulation in TLR2 protein expression as compared with control group (Fig. 3-4).

ERYTHROPOIETIN DIMINISHED CASPASE-3 EXPRESSION

The expression of caspase-3 was measured via immunohistochemical assessment. The result confirmed that the caspase-3 protein expression was significantly augmented in ischemic and vehicle-ischemic rats related to sham rats, while, erythropoietin pretreated group significantly reduced the caspase-3 protein expression comparing with sham group (Fig. 5-6).

ERYTHROPOIETIN IMPROVED KIDNEY INJURY

Histological investigation showed no renal damage in sham group, while there is a raised in injury percent of tubules in ischemic and vehicle-ischemic animals comparing with sham animals ($p< 0.05$). Erythropoietin pretreated group showed a statistically significant decrease in renal tissue injury scores in contrast with control and vehicle animals (Fig. 7-8).

DISCUSSION

Renal IRI is a complex process; the kidney is submitted to morphological as well as functional injury through the ischemic period and undergoes additional insult during reperfusion [9]. In spite of, the recent advances in the treatment of renal I/R injury by surgical, medical, and pharmacological modes, the later remains the main problem that needs additional examination on the probable underlying mechanisms and that for discovery of a novel line of therapy [7].

EFFECT OF IRI AND ERYTHROPOIETIN ON RENAL FUNCTION

Serum creatinine level measurement used to evaluate the renal dysfunction that was associated with IR as several studies reported. This study observed that 30 minutes ischemia and 2 hours reperfusion induced a significant rise in serum creatinine. This in agree with Ding et al. [18] study showed that a significant rise in serum creatinine in 1 hour after IRI and the peak levels was reached after 24 hours of reperfusion as compared with sham group. Najah et al. [19] found that 30 minutes renal ischemia and 2 hours reperfusion significantly elevate the Serum creatinine when compare with sham group. A single intraperitoneal dose of erythropoietin was given 30 minutes before onset of ischemia lead to a significant reduction in serum creatinine as compared with ischemic and vehicle-ischemic groups. This in agreement with Qin et al. [20] found a significant reduced in Serum creatinine in erythropoietin pretreated group compared with control group. Yazihan et al. [21] study indicates that at the end of 45 minutes renal vascular nontraumatic clamping and 4 hours of reperfusion a significantly decreased of serum creatinine in erythropoietin pretreated comparing with non-treated group.

EFFECT OF IR AND ERYTHROPOIETIN ON PROINFLAMMATORY MEDIATOR (MCP-1)

The effect of 30 minutes of bilateral renal ischemia and 120 minutes reperfusion significantly increased a proinflammatory mediator (MCP-1) comparing with non-ischemic group. This in agreement with Thu-Alfeqar et al. [22] showed that a significant increase in MCP-1 level in rats that subjected to 30 minutes ischemia and 2 hours reperfusion as compared with sham group. Peng et al. [23] indicated that MCP-1 level significantly increased after 30minutes reperfusion. This study indicated that a significant reduce in the concentration of MCP-1 in erythropoietin treated group comparing with control and vehicle groups. Ha Nee Jang et al. [24] showed that MCP-1level was significantly decreased in erythropoietin treated ischemic mice comparing with non-treated group. Kwak et al. [25] indicated that erythropoietin administration significantly reduced the expression of MCP-1 in renal ischemia reperfusion group.

EFFECT OF IRI AND ERYTHROPOIETIN ON TLR2

This study observed that there is a significant elevation in TLR2 expression in control and vehicle rats comparing with sham rats. This agree with Najah et al. [26] study which showed that TLR2 expression significantly increased in 30 minutes ischemia and 2 hours reperfusion groups as compared with sham group in rat model. Tan et al. [5] rat model study found that the protein expression of TLR2 was significantly increased in ischemic reperfusion group comparing with sham group. The present study found that erythropoietin administration significantly reduced TLR2 ($p<0.05$) in comparison to control and vehicle groups. Liu et al. [27] confirmed erythropoietin anti-inflammatory effect by significant reduced TLR2 expression in hepatic ischemia reperfusion injury in rat model.

EFFECT OF IRI AND ERYTHROPOIETIN ON APOPTOSIS

The current study demonstrated that there is a significant rise in caspase-3 expression in control group besides vehicle group comparing with non ischemic group. Ding et al. [18] study showed that ischemia reperfusion significantly elevated caspase-3 expression comparing with sham group Alghamdi et al. [7]. The immunohistochemical examination of caspase-3 showed a significant rise in caspase-3 expression ischemic group as compared with sham group. The current study showed that there is a significant reduction in caspase-3 in the erythropoietin pretreated rats kidney that subjected to ischemia and reperfusion as compared with control and vehicle groups. Yazihan et al. [21] study showed that the pretreatment with erythropoietin was significantly reducing the caspase-3 level in renal ischemia reperfusion groups. Zhang et al. [28] study evaluated the renoprotective effect of erythropoietin administration in rat model. The data confirmed the antiapoptotic effect of erythropoietin by significant reduction the renal expression of caspase-3 as compared with ischemic non-treated group.

EFFECT OF ERYTHROPOIETIN ON RENAL PARENCHYMAL

In the current study, histopathological evaluation showed that a significant rise in renal damage scores in groups of control and vehicle when comparing with sham. IRI

lead to tubular changes which were shown by shedding of brush border, tubular dilatation and necrosis as well as Neutrophils infiltration and cast formation. Kidneys tissues that were obtained from erythropoietin pretreated rats confirmed a significant less kidney damage. This in agreement with Hussein et al. [29] showed that erythropoietin pretreated rats which exposed to ischemia reperfusion significantly attenuated in renal damage scores comparing with control group. Ahmadias et al. [30] confirmed that erythropoietin treatment reduced the histological changes related to the renal ischemia reperfusion injury.

CONCLUSION

Erythropoietin has renoprotective effect against ischemia and reperfusion, which achieved by decrease the inflammatory response as well as antiapoptotic effect

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Conflict of interest

The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

LABOUR ANALGESIA AND THE RISK OF POSTPARTUM DEPRESSION

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ABSTRACT

The aim: To find association between postpostpartum depression incidence and mode of labour analgesia.

Materials and methods: This is a prospective observational study conducted at the Kyiv City Maternity Hospital №5 (from March 2020 to May 2021). Using google-form and face-to-face interviews, 321 women completed Childbirth Experience Questionnaire on the 2- 3-rd day in the postpartum period. After the first survey, only 35% of women agreed to screen for postpartum depression (PPD) by Edinburgh Postnatal Depression Scale. Univariate logistic regression method was used to assess the risk relation between PPD and factors.

Results: Women who used nitrous oxide (50:50) and non-pharmacological methods of labour analgesia were associated with the decreased risk of PPD (p = 0,044), OR = 2.83 (95% CI 1,03–7,79), compared to women with patient-control epidural analgesia. On the other hand, there are factors which do not have impact on the risk of depressive symptoms, such as age (p = 0,266); parity (p = 0,713); mode of delivery (p=0,959); pain intensity (p=0,931).

Conclusions: Our findings confirmed the association between nitrous oxide and the alternative methods of labour analgesia usage and decreased risk of development PPD.

KEY WORDS: labour analgesia, postpartum depression, childbirth satisfaction

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INTRODUCTION

Women's childbirth satisfaction is a multicomponent event. A negative childbirth experience affects emotional well-being [1-6]. Postpartum depression (PPD) or "baby blue" is a common mental health disorder after childbirth, with over 300 000 women suffering from it [1]. Additionally, over 75% of women in low and middle-income countries had symptoms of depression [2], more than 50% of these cases are undiagnosed and untreated [3].

The essential predictors of PPD are mood disorders, anxiety during pregnancy/delivery, history of psychiatric illness [4], marital relationship [7]. Although severe labour pain has a direct correlation with depression. Therefore, effective management of labour pain is a great possibility for the prevention of PPD. Two meta-analyses supported the negative relationship between labour epidural and PPD [8]. It was proven, that appropriate usage of neuraxial analgesia was associated with a decreased risk of PPD in 14%, compared with no epidural is 34.5% of PPD at 6-8 weeks [9]. Other methods of pain relief in labour, such as, nitrous oxide doesn't have an impact on Edinburg Postpartum depression scale (EPDS) scores, also increase women's childbirth satisfaction [10,11].

According to some studies, alternative methods of labour analgesia don't have complications or side effects, and successfully reduce negative psychological symptoms in labour. Musicotherapy [12-20], aromatherapy [15], acupressure [14], water emersion [17], breathing and relaxation techniques [18], labour massage [16] are non-invasive and non-expensive methods that effectively prevent postpartum depression symptoms and increase women's childbirth satisfaction.

Women who experience PPD experience such symptoms: depressed mood, anhedonia, sleep disturbance, suicidal ideation and hallucination (inside voice tells to hurt child) [18]. Those symptoms influence child development. It can lead to low mother-child connection, breastfeeding problems and an increased risk of psychological disorders during infancy [19].

Therefore, it is important to prevent PPD for a high level of infant mental health and maternal outcomes.

In our study, primary hypothesis was that woman who had non-pharmacological methods of labour analgesia and nitrous oxide (50:50) would have lower risk of PPD.

THE AIM

The aim was to find association between postpartum depression and mode of labour analgesia.

MATERIALS AND METHODS

The design was a prospective observational study conducted at the Kyiv City Maternity Hospital №5. Data collection was in the period from March 2020 to May 2021.

INCLUSION CRITERIA

Mothers over 18 years of age, intended vaginal delivery at 37-41 gestation age; 1st or 2nd parturitions; childbirth with the birth of the alive fetus;

EXCLUSION CRITERIA

Mothers over 45 years of age; inability to obtain the informed consent of the patient or his legal representative; allergy to bupivacaine; patients with contraindication for epidural analgesia – infection at the site of needle insertion, thrombocytopenia (platelet count < 50 x lOP/litre) or clinical coagulopathy.

The study protocol was approved by the Bogomolets National Medical University Ethics Committee №170.

We identified 605 women to join the research. On the 2-nd and 3-rd days in the postpartum period, 321 women completed a Childbirth Experience Questionnaire (CEQ). All participants have signed informed consent. It was validated in Sweden on the population of nulliparous pregnant women to assess the level of women's satisfaction with childbirth. CEQ is a multidimensional scale that is divided into 4 parts: own capacity, professional support, perceived safety, participation [20]. There are 22 statements, the response format is a 4-point Likert Scale.

On the 6 -24 weeks in the postpartum period, only 35% of women (out of 321) agreed to complete the electronic form of the EPDS (table I). EPDS was developed to detect women with the risk of postpartum depression. It has good sensitivity (79%) and specificity (85%). EPDS cut off value of 9 points is positive diagnostic criteria, which means an ''increased" risk of postpartum depression.

Univariate logistic regression method was used to assess the risk association between PPD and possible factors. Twelve factors have been selected: age, parity, mode of delivery, methods of analgesia: patient-control epidural analgesia (PCEA), nitrous oxide (50:50) and alternative methods of labour analgesia in ''home" environment and hospital birth without pharmacological analgesia, pain intensity (VAS scores) and 4-dimensions of CEQ.

Alternative methods of labour analgesia in the "home" environment mean hospital deliveries, where the light is dim, quiet and warm; music therapy is used at will; massage/acupressure; constant support in the birth of a doula or partner/relatives; aromatherapy; bath/shower; fitball with a Swedish wall; breathing and relaxation techniques; great choice of positions for childbirth (lying on your side, sitting on a chair for childbirth, squatting, knee-elbow, mattress); within 3-4 minutes to stationary conditions.

Also, we used mean and standard deviation in 4 parts of the overall score of the CEQ and EPDS. Data is presented in Table II.

The database was created in Excel. Statistical data analysis was made in Statistical software EZR v. 1.54.

Table I. Characteristics of the study population, n - 114

Parity	1-st	93 (81,6%)
	2-nd	21 (18,4 %)
Age (18-45 years)	<35	55 (48,2%)
	≥35	59 (51,8%)
Mode of delivery	Vaginal	106 (93%)
	Operative*	8 (7%)
Pain relief for vaginal delivery	Patient-control epidural analgesia (PCEA)	72 (63,2%)
	Nitrous oxide (50:50) and alternative methods of labour analgesia in "home" environment	28 (24,6%)
	Hospital birth without pharmacological analgesia	14 (12,3%)
Visual analogue scale (VAS)	VAS <7 points	20 (17,5%)
	VAS ≥7 points	94 (82,5%)
The suture in perineal injuries	Yes	84 (73,7%)
	No	30 (26,3%)

*Operative includes vacuum extraction and caesarean section.

Table II. Mean and standard deviation in 4 dimensions of the overall score of the CEQ and EPDS.

Surveys	Dimensions	Mean, $\overline{X}$±SD
Childbirth Experience Questionnaire	Own capacity	3,16+0,4
	Professional support	3,02+0,41
	Perceived safety	2,6+0,34
	Participation	3,4+0,65
Edinburgh postnatal depression scale		8,11+4,3

Table III. Coefficients of univariate logistic regressions of the risk PPD prognosis.

Factor variable		Coefficient, b±m	P	OR (95% CI)
Age (18-45 years)	<35		Reference	
	≥35	–0,55±0,49	0,266	–
Parity	1-st		Reference	
	2-nd	–0,14±0,38	0,713	–
Mode of delivery	Vaginal		Reference	
	Operative	0,04±0,73	0,959	–
Suture in perineal injuries	No		Reference	
	Yes	0,60±0,43	0,167	–
VAS scores	<7 points		Reference	
	³7 points	–0,04±0,49	0,931	–
	PCEA	0,28±0,85	0,742	–
	Alternative methods of labour analgesia in ''home'' environment and nitrous oxide usage	-1,04±0,52	0,044	2,83 (1,03–7,79)
	Hospital birth without pharmacological analgesia	0,28±0,59	0,633	–
Childbirth Experience Questionnaire	Own capacity	0,32±0,48	0,496	–
	Professional support	0,00±0,46	0,988	–
	Perceived safety	–0,56±0,56	0,311	–
	Participation	0,18±0,29	0,550	–

RESULTS

In our study, we divided women into two groups where EPDS scores are variable. In the first group, if the EPDS score ≥ 9 points, it means an ''increased'' risk of postpartum depression (Y = 1, n=58). In the second group, if the EPDS score<9 points, we considered it as ''normal'' mental health (Y=0, n=56 patients). We evaluated 13 variables in univariate analysis.

The univariate statistical analysis revealed a significant relationship ($p < 0.05$) between PPD and analgesia methods. Thus, women who used alternative methods of labour analgesia and nitrous oxide (50:50) were associated with decreased risk of PPD (p = 0,044), OR = 2.83 (95% CI 1,03–7,79), compared to women with patient-control epidural analgesia. However, hospital birth without pharmacological analgesia (p=0,633)) don't have an impact on the risk of depressive symptoms.

There was no difference between the PPD and age (p = 0,266), parity (p = 0,713), mode of delivery (p=0,959), pain intensity (p=0,931).

The factor of women's satisfaction of childbirth in each part of CEQ (own capacity (p=0,496), professional support (p= 0,988), perceived safety (p= 0,311), participation (p= 0,550) was not associated with the risk of PPD. Coefficients of univariate analysis of the risk of PPD in the postpartum period are shown in Table III.

DISCUSSION

The literature indicates ambiguous data about factors of risk development PPP and women's experience of childbirth and labour analgesia. PPD has an impact on short- and long-term complications for the woman and infant [20-25]. Thus, the prevalence of depressive symptoms is estimated at 20% (EPDS ≥10 points) [24]. Nevertheless, the relationship between PPD and epidural analgesia is less examined because of heterogeneity in the methods, designs of research etc. Five reviews have shown that women who received epidural have a significantly decreased risk of PPD [26-30]. The other two articles represent that labour epidural analgesia don't have any association with PPD. The same data we found in our study (table 3) [31,32]. Otherwise, three studies demonstrate the incidence of severe labour pain that is associated with the risk of PPD [8]. In view of the adequate analgesia for the mother, neuraxial anaesthesia is mainly used for delivery. Also, we found research about parturients with a poor level of childbirth satisfaction and receiving epidural analgesia during labour [23]. Furthermore, women's satisfaction is an independent factor that improves child and mother outcomes: physical and psychological. Evidence from a review about women's experience of pharmacological and alternative labour analgesia has shown varied results, including psychological maternal outcomes. Women who used pharmacological pain relief with good pain alleviation felt guilt and fear about using medications since they planned natural delivery. Many women with non-pharmacological labour analgesia reported an increased level of self-esteem and the presence of a feeling of labour control [22]. Low self-esteem is a known predictor of depressive symptoms [21]. A key finding of our review is that alternative methods of labour analgesia

were associated with decreased risk of PPD. Interestingly, there are different variations of presented factors that could influence the risk of development of PPD.

CONCLUSIONS

Our findings confirmed the association between nitrous oxide and the alternative methods of labour analgesia usage and decreased risk of development PPD.

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ORIGINAL ARTICLE

EFFICACY OF COMBINATION OF TIOTROPIUM/OLODATEROL IN PATIENTS WITH COPD IN REAL CLINICAL PRACTICE

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ABSTRACT

The aim: Show the efficacy of the Tiotropium / olodaterol combination in real clinical practice.

Materials and methods: 100 patients with the diagnosis of COPD were included onto the study during the period of 2019-2020, an average age was 64.09±1.94 years, 66 were men (66 %) and 34 were women (34 %). There were 68 % of smokers with the average smoking experience of 24.44±4.84 pack-years. Average COPD duration was 9.35±2.42 years. There were 3 visits in the study – visit 1 (baseline), visit 2 (4-6 weeks) visit 3 (1 year). Source documentation was assessed at visit 1 and visit 3 for amount of exacerbations, antibiotic, glucocorticosteroid, methylxanthines use; mMRC and CAT were assessed at all visits.

Results: Combined therapy with tiotropium/olodaterol improves clinical course of COPD, which is characterized by the significant decreased of the amount of exacerbations (2.63±0.29 to 1.63±0.21) and hospital admissions (1.2±0.2 to 0.37±0.11). Improvement of symptoms and amount of exacerbation leads to much less use of antibiotics and glucocorticosteroids. A part of patients that used antibiotics decreased from 86±6.9 % to 67±9.3 %, amount of antibiotic courses from 1.37±0.17 to 0.88±0.15, duration of treatment with antibiotics from 10.85±1.53 to 6.12±1.17 days. Part of the patients that used glucocorticosteroids decreased from 50±9.9 % to 30±9.1 %, duration of treatment with antibiotics reduced from 3.97±1.06 to 1.86±0.91 days. There also was a tendency towards a lesser used of methylxanthines. Combined therapy with tiotropium/olodaterol significantly decreased symptoms of COPD according to the mMRC (2.3±0.14 to 1.87±0.15) and CAT (23.28±1.71 to 15.77±1.58).

Conclusions: Tiotropium/olodaterol combination showed its efficacy in real clinical practice. There was significant reduction in amount of exacerbation and antibiotic, glucocorticosteroid use during the study, which was also accompanied by the reduction is symptoms.

KEY WORDS: COPD, exacerbations, antibiotics, glucocorticosteroids

Wiad Lek. 2022;75(12):2953-2957

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a disease, that is characterized by the progressive course, usually has many symptoms such as productive cough, dyspnea. Presence of COPD increases risk of different co-morbidities, where cardiovascular diseases are the most prevalent. They occur 5 times more often in this group of patients. COPD is also characterized by the great amount of infectious complications, which lead to the great use of antibiotics and promote antibiotic resistance [1]

Around 251 billion people suffer from COPD around the globe according to the data from *Global Burden of Disease study*. COPD is responsible for more and more deaths each year in the world. COPD was a leading cause of death in 3,17 billions patients in 2015 in the world, which was 5 % in the general structure of mortality [2].

The COPD, despite having "pulmonary disease" in its name, is considered systemic disease. A large variety of different intrinsic and extrinsic factors, that might affect the risk of development and course of COPD, can also cause great difference in the population of patients. The main mechanism in the development of COPD is an impact of the extrinsic factors, with tobacco smoke being the most likely risk factor, which might cause much more severe course of COPD with the increase of the smoking experience [3,4]. Different studies showed a correlation between polymorphism of different genes and development, clinical course and treatment efficacy in COPD [5].

Recent studies demonstrated, that reversibility of the bronchial obstruction is not an absolute marker for the differentiatial between different obstructive pulmonary diseases. Some studies showed, that almost 20 % of COPD patients demonstrated positive reversibility after 400 mcg salbutamol, also 5 % of healthy population demonstrated positive reversibility. Patients with response to salbutamol also demonstrated different response to treatment with bronchodillative drugs [6].

So the aim of our study was to assess the impact of the treatment with combination of tiotropium/olodaterol on the clinical course of COPD in real clinical practice.

THE AIM

The aim was to show the efficacy of the Tiotropium / olodaterol combination in real clinical practice.

MATERIALS AND METHODS

Investigation was carried out at the department of propredeutics of internal medicine of Vinnytsia National Pirogov Memorial Medical University and Commercial Non-Profit Enterprise "Vinnytsia City Clinical Hospital № 1". Approval of Local Ethic Committee was obtained prior to any study procedures. Written informed consent was obtained from the patients prior to any study related procedures.

100 patients with the diagnosis of COPD were included onto the study during the period of 2019-2020, an average age was 64.09±1.94 years, 66 were men (66 %) and 34 were women (34 %).

There were 68 % of smokers with the average smoking experience of 24.44±4.84 pack-years. Average COPD duration was 9.35±2.42 years.

Among the 100 patients, there were also patients with confirmed covid-19 disease (or questionable test). Patients received treatment according to the current protocol [7,8]. Some of these patients also took part in our other studies at the same time [9-13].

There were following exclusion criteria: any clinically significant disease, change in laboratory analysis or other information of medical history, that might influence patient's safety during participation in the study, inability of the patient to follow study procedures, abuse of alcohol or drugs in the medical history or at the examination.

Source documentation was assessed at the first visit for all patients, which included the total amount of exacerbations in the previous year, a need in antibiotics, glucocorticoids and xanthines with the amount of separate courses and total treatment duration over the 12 months. mMRC and CAT questionnaires were filled out by the patient. Physical examination, vital signs and anthropometric data was collected in all patients at visit 1, spirometry was performed according to ERS/ATC guidelines [14] with the reversibility test.

All patients were switched to long-acting muscarinic antagonists (LAMA) and long-acting beta-agonists (LABA) according to the GOLD 2020.

At visit 2 in 5±1 weeks aphysical examination, vital signs and spirometry were assessed.

At visit 3 in 1 year ±1 week source documentation of all patients was assessed, which included the total amount of exacerbations in the previous year, a need in antibiotics, glucocorticoids and xanthines with the amount of separate courses and total treatment duration over the 12 months. mMRC and CAT questionnaires were filled out by the patient. Physical examination, vital signs and spirometry were performed. In the further statistical analysis we compared an efficacy of tiotropium/olodaterol in all included patients without separation for the groups.

Statistical analysis was performed using SPSS (Version 26.0 for Windows; USA). Descriptive statystics was determined for each interval parameter and is represented by the mean value ± standard deviation (SD). We used Kolmogorov Smirnov test for the assessment of data distribution in the sample. Chi-square method was used to compare nominal values. Mann-Whitney U-test and T-test were used for the comparison of two independent samples in abnormal and abnormal data distribution correspondingly. Wilcoxon test was used to compare two dependant samples with abnormal data distribution, and t-test for paired samples were used to compare data in two dependent samples with normal data distribution.

RESULTS

There were no group A COPD in the studied population, COPD group B was observed in 19 patients (19 %), COPD group C in 42 (42 %) patients, COPD group D in 39 (39 %) patients.

Average amount of exacerbation in a year prior to the inclusion into the study was 2.63±0.29, such that required hospital admission – 1.2±0.2, such that were treated in outpatient settings – 1.43±0.22. Average amount of exacerbation in a year after the inclusion was 1.63±0.21, such that required hospital admission – 0.37±0.11, such that were treated in outpatient settings – 1.27±0.19. There was statistically significant decrease in the amount of exacerbation ($p < 0,05$), including such with hospital admissions ($p < 0,05$). There was no difference in the amount of exacerbations treated in the outpatient settings ($p > 0,05$) (Fig. 1.).

Treatment with antibiotics received 86±4.9 % in 1 year prior to the participation in the study. The part of the patient, that required antibiotics in a year decreased to 67±5.3 % ($p < 0.05$). There also was a decrease in the part of the patient that required treatment with glucocorticosteroids (GCS) from 50±6.9 % to 30±6.1 % of patients ($p < 0.05$). But there was no statistically significant difference in the part of the patients that required methylxanthines: 38±5.6 % and 31±5.2 % of patients correspondingly ($p > 0.05$) (Fig. 2.).

An average amount of antibiotic courses in a year prior to the study was 1.37±0.17, and amount of antibiotic courses during the participation in the study decreased to 0.88±0.15 ($p<0.05$). An average amount of GCS courses in a year prior to the study was 0.94±0.18, which decreased to 0.72±0.19, but the difference was not statistically significant ($p > 0.05$). There also was no statistically significant difference in the amount of methyxanthines courses, which decreased from 0.79±0.16 to 0.64±0.19 ($p > 0.05$) (Fig. 3.).

An average duration of treatment with antibiotics was 10.85±1.53 days, in a year prior to the study participation, which decreased to 6.12±1.17 days during the study ($p < 0.05$). An average duration of treatment with GCS during the study decreased from 3.97±1.06 to 1.86±0,91 days ($p < 0.05$). There was a tendency towards the decreased of the methyxanthines use, but the difference was not statistically significant, the values reduced from 3.39±1.13 to 1.92±0.85 days ($p > 0.05$) (Fig. 4.).

The baseline values of mMRC questionnaire was 2.3±0.14, which decreased to 2.18±0.13 in 4-6 weeks of the study and to 1.87±0.15 in 1 year of the study. There was a statistically significant difference between visit 3 and visits 1 / 2 ($p < 0.05$). There was no statistically significant difference between the values at visit 1 and visit 2 ($p > 0.05$) (Fig. 5.).

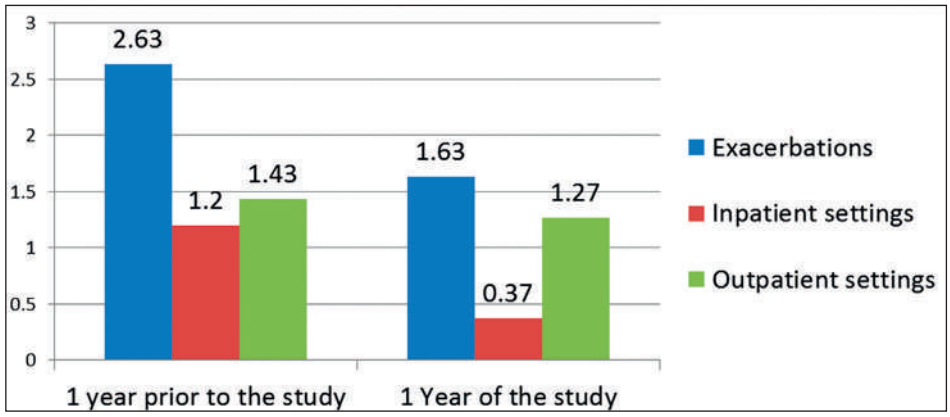

Fig. 1. Amount of exacerbation in patients with COPD 1 year prior to the participation in the study and 1 year of participation.
Source: Author's own elaboration and illustration

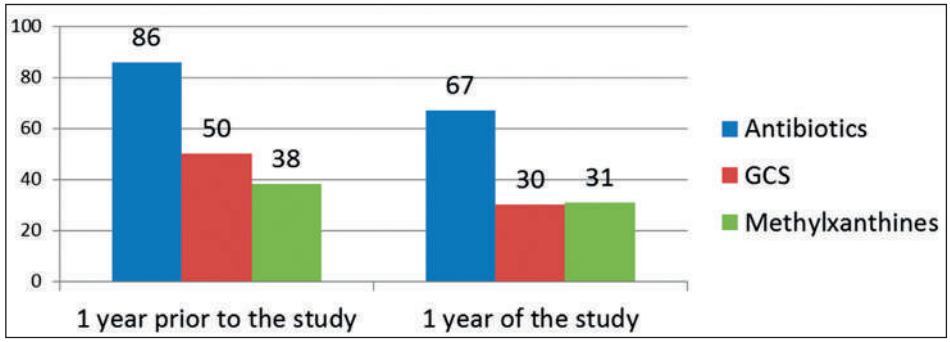

Fig. 2. Percentage of patients that required treatment with antibiotics, glucocorticossteroids and methylxanthines prior and during the study.
Source: Author's own elaboration and illustration

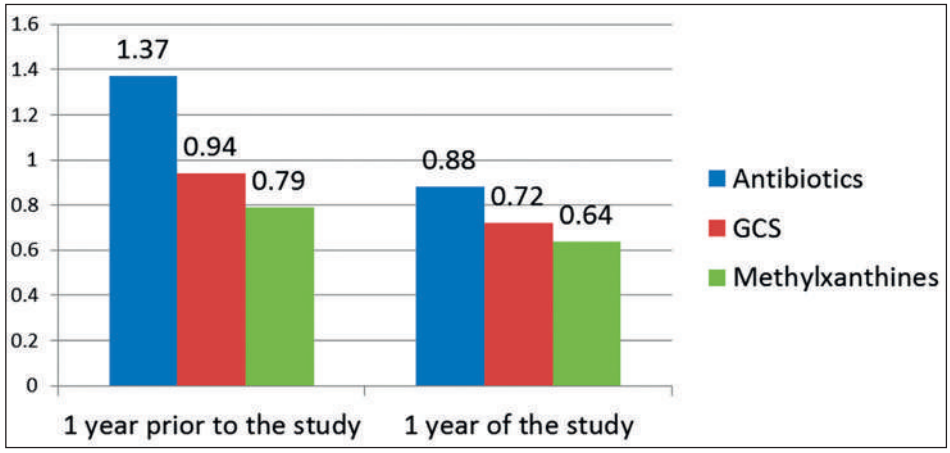

Fig. 3. Average amount of courses of antibiotics, glucocrticosteroids and methyxanthines 1 year prior and during the study.
Source: Author's own elaboration and illustration

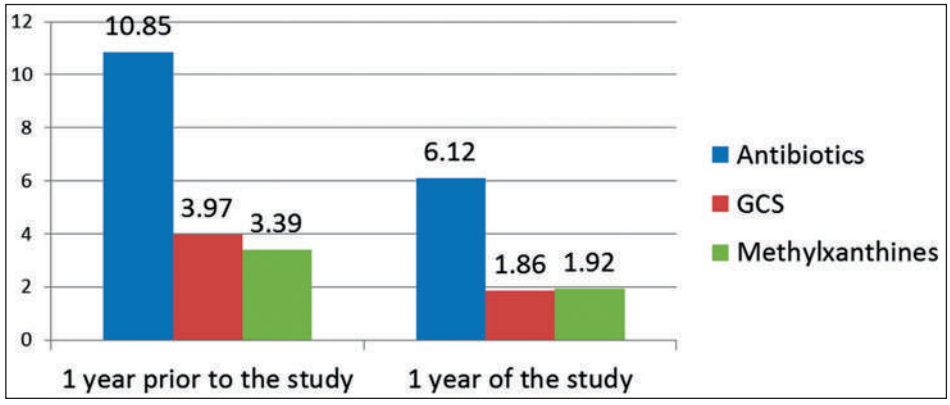

Fig. 4. An average duration of treatment with antibiotics, GCS, methylxanthins before and during the study in days.
Source: Author's own elaboration and illustration

The baseline values of CAT was 23.28±1.71, which decreased to 18.55±1.6 in 4-6 weeks and to 15.77±1.58 in 1 year. There was a statistically significant improvement in CAT values on visit 2 and visit 3 when compared to visit 1 ($p < 0.05$). There was no statistically significant difference between CAT values at visit 2 and visit 3 ($p > 0.05$) (Fig. 6.).

DISCUSSION

In our study we demonstrated a significant improvement of clinical course of COPD in patients on COPD, which is characterized by the significant decreased of the amount of exacerbations (2.63±0.29 to 1.63±0.21) and hospital admissions (1.2±0.2 to 0.37±0.11). Our data corresponds with

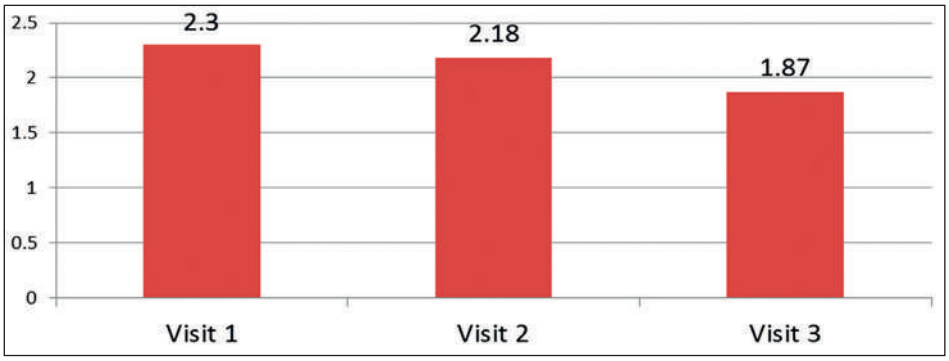

Fig. 5. mMRC values during the participation in the study.
Source: Author's own elaboration and illustration

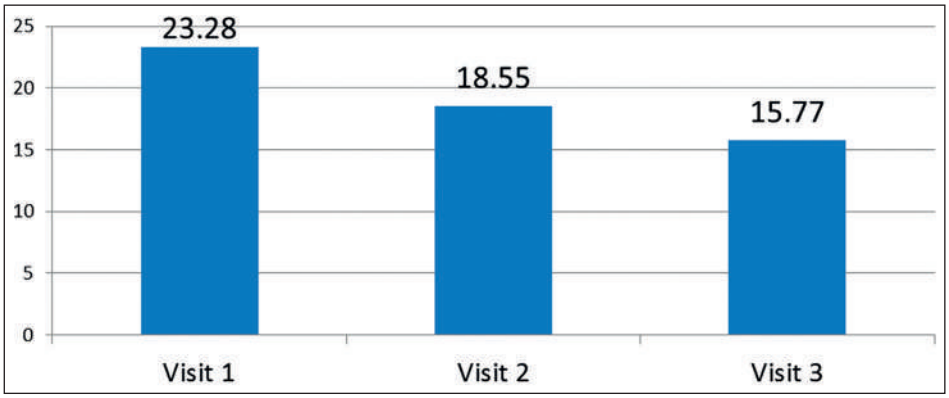

Fig. 6. CAT values during the participation in the study.
Source: Author's own elaboration and illustration

the data from different clinical trials. In post-hoc analysis of 3 major clinical trials – TORNADO-1, TORNADO-2 and DYNAGITO there was a significant difference in the amount of moderate and severe exacerbations when tiotropium/olodaterol was compared to tiotropium alone [15]. In our study we demonstrated more pronounced effect on the amount of exacerbations, which can be explained by the different population, as in the real clinical practice patients were using different type of inhalers. In our study we demonstrated a significant reduction in the amount of antibiotics and glucocorticosteroids use in patients with COPD. There were studies, that compared LABA/LAMA vs triple therapy, which demonstrated lower expenses and lower amount of pneumonias in patients on double vs triple therapy, that can be explained by the absence of ICS in the treatment scheme [16]. As in our study we have a great amount of patients on ICS/LABA treatment prior to the inclusion, such difference in the use of antibiotics. An improvement of symptoms on combination of tiotropium/olodaterol was demonstrated in multiple studies, which also corresponds to the data obtained in our study [17]. Greater improvement can be explained by the great variety in studied population, when compared to the population in clinical trials.

Tiotropium/olodaterol demonstrated greater efficacy in real clinical practice, when compared to the data from different clinical trials, which is easily explained by the different types of inhalers used by the patients in the real world practice. Tiotropium/olodaterol effectively reduced the amount of exacerbations, symptoms (measured by mMRC and CAT), use of antibiotics and glucocorticoids.

CONCLUSIONS

Tiotropium/olodaterol combination showed its efficacy in real clinical practice. There was significant reduction in amount of exacerbation and antibiotic, glucocorticosteroid use during the study, which was also accompanied by the reduction is symptoms.

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ORIGINAL ARTICLE

MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL SIGNS OF PLACENTAL DISORDERS OF WOMEN AFTER SYPHILITIC INFECTION

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ABSTRACT

The aim: To define morphological and immunohistochemical signs of placental disorders of women after syphilitic infection.

Materials and methods: The prospective study of 60 pregnant women with history of syphilitic infection (main group) and 57 pregnant patients without syphilis (control group) was conducted. The morphological and immunohistochemical study of the afterbirth was performed.

Results: In the placentas of women of the main group the following phenomena were found out: circulatory disorders in the form of hemorrhages into the intervillous space and the stroma of villi; accumulation of fibrinoid around villi with dystrophically altered stroma, compensatory-adaptive reactions resulted in hyperplasia of terminal villi and vessels in them, which provoked narrowing of the intervillous space and disruption of blood supply in it. Pathogenic immune complexes containing Ig G, M and C3 of the complement fraction were located in the central part of the placenta – 45.00% of cases, 16.67% – in the regional, 8.33% – in both parts. Immune complexes with Ig M content occurred in 38.33% of cases. The content of pathogenic immune complexes was the most concentrated in the placentas of women with latent forms and secondary recurrent syphilis – 60.00% of cases.

Conclusions: changes in morphohistological and immunohistochemical examination of the placenta of this group of women confirmed the detrimental effect of syphilitic infection in the anamnesis on the structure of placenta during the next pregnancies.

KEY WORDS: syphilis, pathological immune complexes, complement, immunoglobulins

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INTRODUCTION

The problem of sexually transmitted diseases, including syphilis, is quite relevant nowadays. According to the World Health Organization (WHO), the number of new cases of sexually transmitted infections (STIs) is over 376 million per year [1-4]. According to the report by the European Center for Disease Prevention and Control (ECDC, Sweden), the number of syphilis cases in Europe has increased by 70% since 2010. The infection has reached the highest registered level – 33,189 confirmed cases in 28 countries on the European continent. Between 2010 and 2017, the number of sexually transmitted infections more than doubled in five countries, including the United Kingdom [2, 4, 5]. According to experts, the rapid increase in the incidence of syphilis is explained by the presence of many sexual partners with the practice of unprotected sexual intercourse, early sexual intercourse, reduced fear of HIV infection, sex in condition of intoxication (chemosex). The results of social and medical research show a vivid tendency to increasing sexual activity of people, especially young people, around the world. According to the analysis of sexual behaviour of adolescents in Ukraine, it has been revealed that in 16.9% of cases young men and women start having sex before the age of 15. It is the early start of sexual life that boosts the spread of this pathology [4, 6-9].

Today in Ukraine one can observe an unfavorable epidemic situation with syphilis. More than three thousand cases of syphilis are registered annually, a large number of which are recorded in adolescents under 17 years [6, 7, 9-13]. It should be taken into consideration that at the end of the last century there was an increase in the number of syphilis patients among adolescent girls, up to 10% of the total number of cases. It is alarming that a very high percentage of women who have suffered and are suffering from syphilis plan to realize their maternal instinct in the future [14, 15].

Syphilis is a chronic infection in which the human body develops a specific inflammatory process, accompanied by increased synthesis of antitreponemal antibodies, first classes of immunoglobulins (Ig) M and A, then Ig G, as well as antilipid antibodies (reagins), by the formation of circulating immune complexes (CIC), which acquire pathogenicity in combination with the C3 fraction of complement [16-18]. With an increase in the number of CICs in the blood and activation of the complement system, the cascade of formation of immunocompetent cells is launched, accompanied by the release of inflammatory mediators, release of lysosomal proteases, activation of kinin and coagulation system. The most important factor in CIC deposition is the increase in vascular permeability in structures characterized by increased blood pressure and turbulent blood flow [16, 18]. The placenta, which has an extensive microvas-

cular system and a good blood supply, is more prone to CIC deposition. Increased formation of complement-bound CICs, the fixation of which in placental tissue provokes violation of its immune homeostasis and histostructure, contributes to the development of dysfunction of the fetoplacental complex [19]. Disorders of microcirculation in the placenta cause abrupt changes in the latter with the development of atrophic necrosis and subsequent fibrosis, which results in dysfunction in the system "mother-placenta-fetus" [19-21]. It should be taken into consideration that specific treatment of syphilis eliminates CIC in only 50% of patients [15].

One of the significant complications in pregnant women with syphilis is placental dysfunction [21, 22, 23], the mechanism of which has not been completely studied. The placenta, as a temporary organ, carries out an integral relationship between the mother and the fetus, performing transport, trophic, endocrine, metabolic and immune functions necessary for the implementation of the fetal development program and its growth. The complicated course of the gestational period leads to pathological changes in the placenta, which are revealed during histomorphological examination after childbirth. Data on morphological and histochemical changes in the placentas of the specified category of women in labor are few and contradictory. Determination of CIC in the placentas of women who had syphilis in comparison with morphological changes will help to supplement the understanding of the pathogenetic mechanisms of the development of placental insufficiency during pregnancy. So it is the question this work is devoted to.

THE AIM

The aim of the study was to define the morphological and immunohistochemical features of placental disorders in women with history of syphilis.

RESEARCH HYPOTHESIS

Placental disorders due to previous syphilis are reflected in differences in immunohistochemical and morphological parameters of the placenta in women with and without previous pathology; there should be a connection between the forms of transferred syphilis and indicators of pathomorphological changes in placentas.

MATERIALS AND METHODS

117 pregnant women were examined to study the morphological and immunohistochemical features of placental barrier structures and humoral parameters. The main group consisted of 60 pregnant women with history of syphilitic infection, the control group included 57 healthy pregnant women who gave a birth to babies through the natural birth canal. The exclusion criteria were severe somatic and autoimmune diseases, rhesus factor isosensitization or AB0 system isosensitization. For each patient, the pregnancy questionnaire developed by us was filled in, it reflected the data of anamnesis, clinical observation and additional methods of examination. In terms of age, parity, disease severity, the groups were representative and selected according to the copy-pair principle.

METHODS OF HISTOLOGICAL AND HISTOCHEMICAL EXAMINATION

Characterization of the afterbirth was given using the algorithm of macroscopic and microscopic descriptions. The following elements were taken into consideration: placental mass and its area, correlation between placental area and fetal mass, intervillous space, intervillous maternal fibrinoid, stroma and vascular bed of all the villi, intravillous and fetal fibrinoid, pathological changes - functional areas, pseudopharyngeal areas. Histological examination was performed after staining with hematoxylin and eosin according to standard methods, picrofuxin according to Van Giezon, which was used to determine collagen fibers.

Visualization of primary antibodies in immunohistochemical examination of the placenta was performed using the highly sensitive polymer detection system DAKO Advance (DAKO, Denmark) according to the manufacturer's protocol. Drug microscopy and morphometric studies were performed on the Olympus AX 70 Provis microscope (Olympus, Japan) using the Analysis 3.2 Pro image analysis program (SoftImaging, Germany) according to the software manufacturer's recommendations. In each case, 30 fields of view were studied. To calculate the average number of positive cells, the area of each field of view was determined, positively stained cells (brown) were counted, and then the average number of cells per unit area was calculated. To calculate the specific volume of Ig G- and Ig M-positive cells, the study areas ("field of interest") were chosen, then the colour sensitivity threshold was set to select positively stained cells, then the area of the "field of interest" and positively colored areas (brown) were revealed using the "Color Selection" tool, then the specific volume (percentage) of positively colored objects was calculated.

STATISTICAL ANALYSIS

Statistical processing of materials was carried out using variational statistical methods using the standard package of MS Office 365 Excel Windows'10'Home application programs. For quantitative characteristics, after calculating the descriptive statistical parameters within each sample, a check was made on the nature of the distribution. In the case of a normal distribution of similar characteristics in the samples, the Student's test was used to compare them. In the absence of a normal distribution in bound sets of quantitative traits (and also with rank characteristics), the comparison was made using non-parametric Fisher criteria and Mann-Whitney U test. Mismatches between comparable values were considered statistically significant at a significance level of $p < 0,05$.

To confirm the main hypothesis of the study, the probability of the following statistical hypotheses was evaluated:

H0: the distribution of the general set of values of the random variable corresponds to the law of normal distribution - it was checked using the Pearson's Xi-square test.

H0: Spearman's correlation coefficient, which reflects the presence of a relationship between forms of syphilis (not transferred, seroresistance, primary, secondary recur-

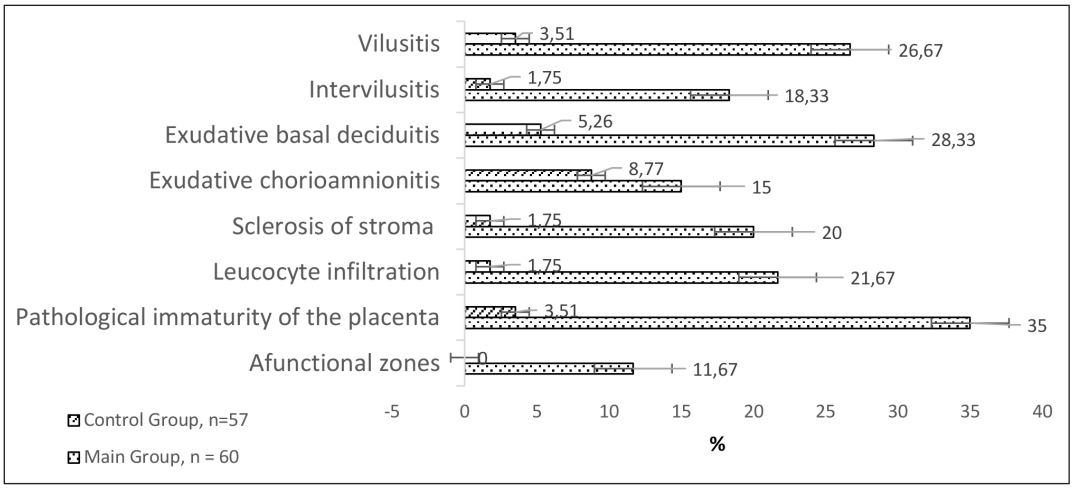

Fig. 1. Characteristics of pathomorphological changes of the placenta in the examined women (%)

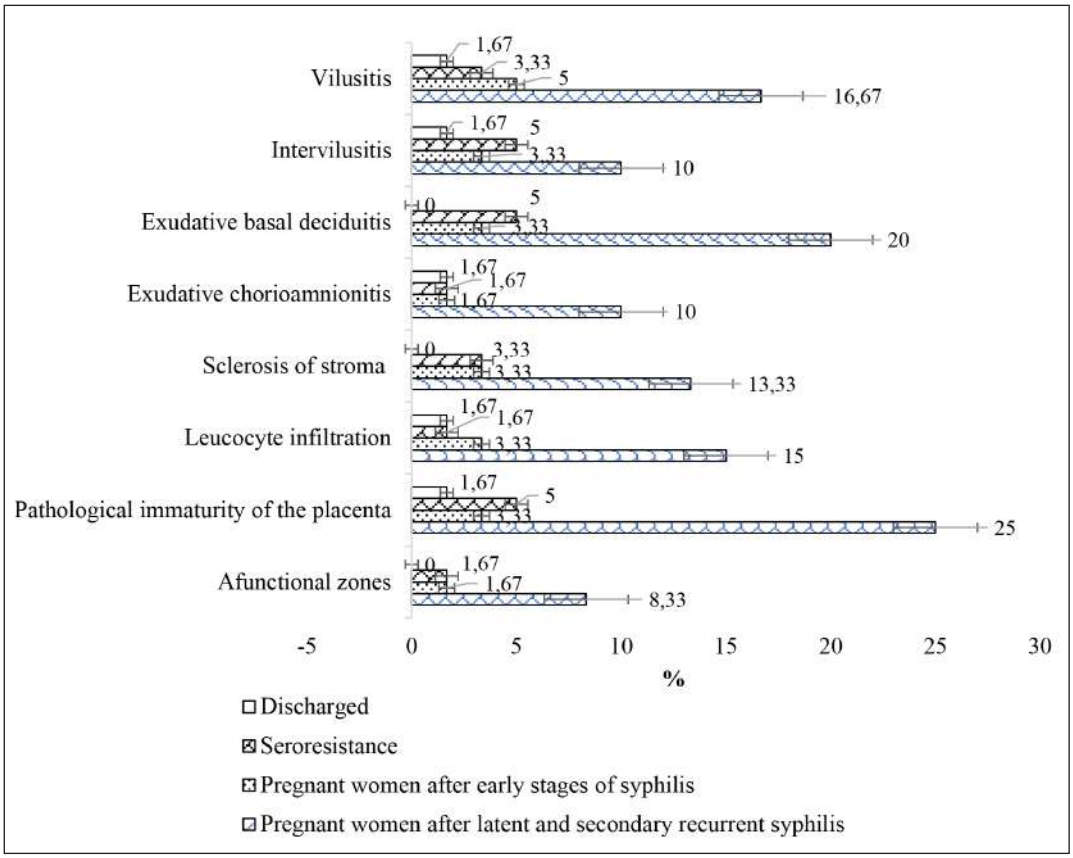

Fig. 2. Distribution of pathomorphological changes in placentas depending on the forms of syphilis in women of the main group (%)

rent and latent forms of syphilis) and morphological and immunohistochemical indicators of the placenta, has no statistically significant differences from 0 – using Student's criteria or Manna-Whitney

H0: the proportions of pregnant women with disorders of the macroscopic parameters of the placenta (for each type of violation) do not differ in the groups of pregnant women who suffered and did not suffer from syphilis – using the Pearson test.

RESULTS

After the study, the data on the forms of syphilis that affected women in the study group in the period 2009-2017 were obtained (Table I).

Macroscopically, the shape of the placenta was round and oval with central or paracentral attachment of the umbilical cord in the majority of cases. Violation of blastocyst implantation in the endometrium in the form of marginal umbilical cord attachment occurred in 7 cases (11.67%) of the main group, which may be explained by the presence of chronic endometritis according to some authors [20-21]. This indicator in the control group was 1.75% or 1 case ($p < 0.05$) (Table II). The surface of the placenta facing the fetus – the chorionic plate – was grey. The length of the umbilical cord did not differ significantly from that in the control group, it was whitish in color with smooth shiny surface, in 5 cases it had spiral form. In 2 cases there were false nodes formed by strongly tortuous arteries, or accumulation of gelatinous substance. The uterine surface of the placenta had lobular structure. The amniotic membranes were represented

Table I. Distribution of women according to the forms of the transferred syphilis

The form of syphilitic infection	Main Group, n = 60	
	Abs.	%
Primary seropositive	6	10,00
Secondary recent	4	6,66
Secondary recurrent	12	20,00
Latent early	25	41,66
Seroresistance	8	13,33
Discharged	5	8,33

Table II. Macroscopic signs of inflammatory lesions of placenta

Type of Pathology	Main Group, n = 60		Control Group, n=57	
	abs.	%	abs.	%
Marginal attachment	7	11,67*	1	1,75
Placenta marginata and placenta circumvallata	8	13,33*	1	1,75
Hypoplasia of the placenta	10	16,67*	0	0,00

Note. * – probable difference in the relevant indicators between groups which are compared

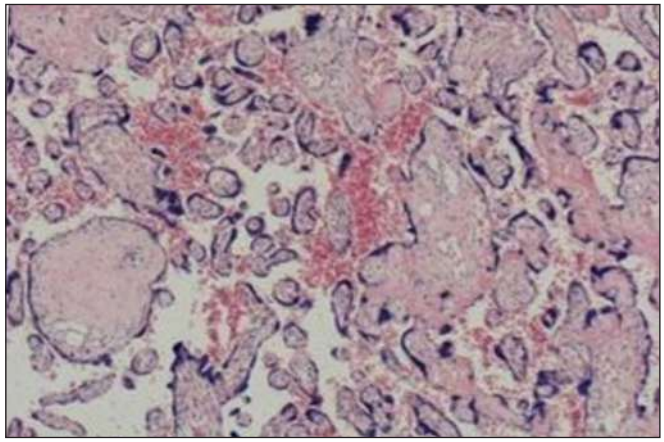

Fig. 3. Fibrinoid deposition, focal sclerosis of the villous stroma with a sharp narrowing of the vascular lumen, focal endothelial proliferation, hypervascularization of the terminal villi. Uneven blood supply to the villi. Hemotoxylin-eosin staining, × 30, edge.

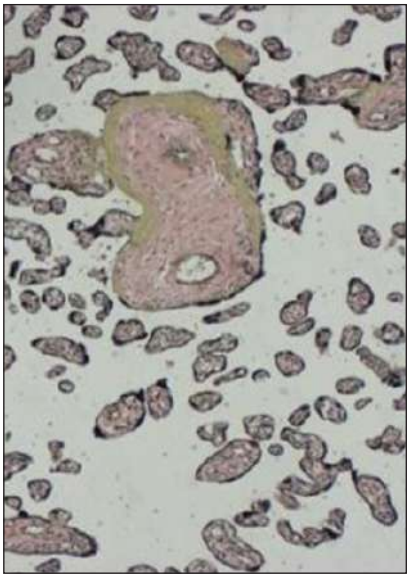

Fig. 4. The growth of collagen fibers in the stroma of the intermediate and terminal villi with compression of blood vessels and narrowing of their lumen.
Staining according to Van Giezon, × 60, center.

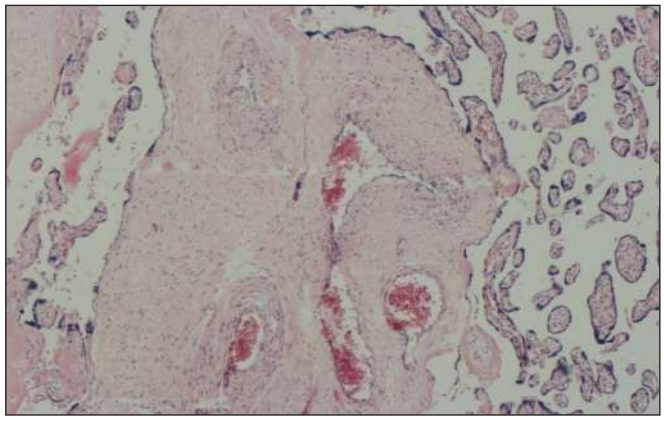

Fig. 5. Sclerosis of the stroma of the stem, intermediate villi, obliteration and thickening of the vessel walls, the phenomenon of endothelial cell proliferation. In the terminal villi - hypervascularization, vasodilation, uneven narrowing of the intervillous space.
Hematoxylin-eosin staining. × 75, center.

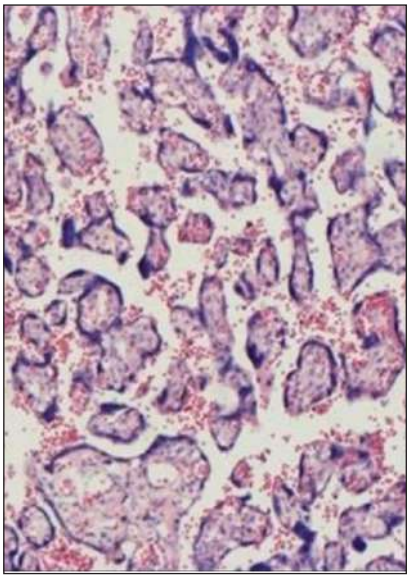

Fig. 6. In the terminal villi - hypervascularization, vasodilation, moderate narrowing of the intervillous space.
Hemotoxylin-eosin staining. × 60, center.

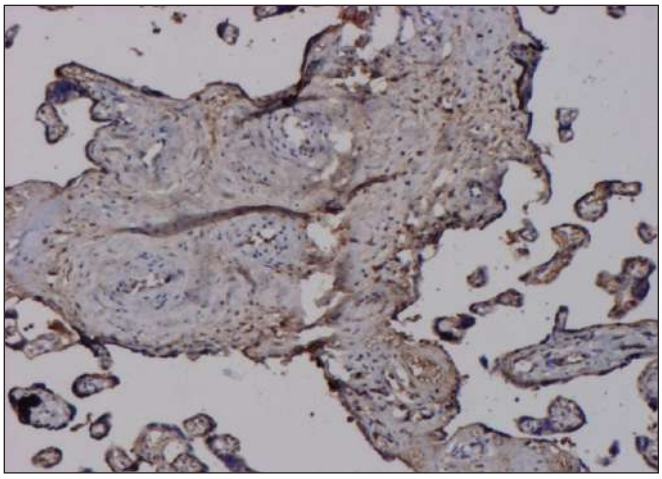

Fig. 7. Granular and single linear C3 deposits in cytotrophoblasts of villi (up to +++), single granular C3 deposits in stroma and on vessel walls (up to ++). C3 expression (produced by DAKO), immunohistochemical staining, imaging system: Poly Vue HRP / DAB Detection System.

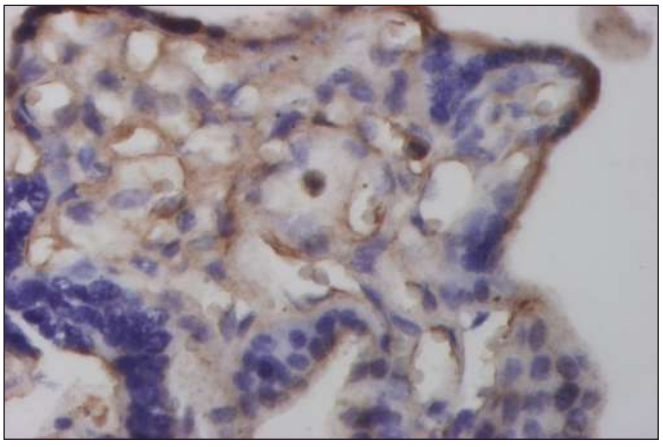

Fig. 8. Linear and focal granular IgM deposits in the cytoplasm of cytotrophoblasts of villi (from ++ to +++), in vascular endothelium focal granular IgM deposits (up to ++). Expression of Ig M-code (produced by DAKO), immunohistochemical staining, visualization system: Poly Vue HRP / DAB Detection System.

by a translucent sac extending from the edge of the placenta, the thickness of the membranes did not exceed 2 mm, macroscopically they were homogeneous, gelatinous, greyish-white. Placental hypoplasia occurred in 10 cases among patients of the main group (16.67%), in the control group such cases were not observed ($p < 0.05$). Organometric parameters of the placenta – weight, its area, the correlation between placenta and fetal weight, were significantly higher in the control group than in the main group ($p < 0,05$). In the main group, the weight of the placenta was 0.48 ± 0.02 g, while in the control group this number was 0.54 ± 0.01 g ($p < 0.05$). When measuring the area of the placenta, the following results were obtained: in the control group, the area of the placenta was 406.79 ± 8.72 cm2, while in the main group, this number was much lower – 298.54 ± 7.24 cm2 ($p < 0.05$). During the study of the placenta, the main attention was paid to histological indicators in the groups after observation, namely, the activity and severity of alternative, infiltrative processes in the placenta – the growth of relative volumes of fibrinoid, petrification, dystrophically altered villi and damaged deciduocytes, delay in their growth rates and differentiation, weak development of compensatory-adaptive reactions, widespread inflammatory infiltration in the structural components of the studied organ. In the placentas of the main group inflammatory changes were common and manifested with increased frequency in the chorionic plate (48.23 ± 8.31%), in the villous part of the placenta (55.00 ± 7.34%), in the basal plate (25.54 ± 9.64%), as well as in the fetal and maternal bloodstream (19.52 ± 10.11%).

Chorionic plate pathology was represented by obliterating vasculopathy, stroma edema, amniocyte degeneration, lymphohistiocytic and leukocyte infiltration of the subchorionic intervillous space, focal diffuse villousitis (Fig. 1). In the basal plate of the placenta, focal deciduitis was accompanied by inflammation of the mouth of the spiral arteries, thrombosis and fibrinoid degeneration of the stroma. In particular, there were foci of sclerosis of the villi stroma with sharp narrowing of their diameter, focal proliferation of vascular endothelium with hypervascularization of the terminal villi. In infiltrates, leukocytes predominated over lymphocytes and plasma cells. In the intervillous space leukocytes were grouped and manifested in clusters. The most pronounced changes were found in women of the main group who suffered from secondary recurrent and latent syphilis. This primarily applies to such pathomorphological changes as vilusitis, exudative basal deciduitis, lymphocytic infiltration, as well as pathological immaturity of the placenta (Fig. 2). Correlation analysis to identify plausible relationships between placental changes in placentas and the form of syphilis in the main group of women showed that women with seroresistance and history of early syphilis had a weak positive connection between the presence of these forms and:

- pathological immaturity of the placenta (respectively $r = 0.18$ and $r = 0.14$, $p < 0.05$),
- exudative basal deciduitis (respectively $r = 0.17$ and $r = 0.15$, $p < 0.05$),
- intervilusitis (respectively $r = 0.18$ and $r = 0.15$, $p < 0.05$).

Probable correlation coefficients, which indicated a medium or even strong connection between the form of the disease and pathomorphological changes, were revealed only in the group of women with latent and secondary recurrent syphilis. Thus, this form of the disease probably contributed to:

- pathological immaturity of the placenta ($r = 0.62$, $p < 0.05$),
- exudative basal deciduitis ($r = 0.57$, $p < 0.05$),
- vilusitis and intervilusitis (respectively $r = 0.42$ and $r = 0.33$, $p < 0.05$),
- lymphocytic leukocyte infiltration ($r = 0.42$, $p < 0.05$),
- sclerosis of the villous stroma ($r = 0.37$, $p < 0.05$),
- exudative chorioamnionitis ($r = 0.30$, $p < 0.05$).

In the placenta of women with syphilis the research revealed circulatory disorders in the form of hemorrhages in the intervillous space, stroma of villi, accumulation of fibrinoid occurred around the villi with dystrophically altered stroma, compensatory-adaptive reactions resulted in hyperplasia of the terminal villi and vessels in them, that caused narrowing of the intervillous space and disruption of blood supply in it (Fig. 3). Diffuse hypervascularization of intermediate and

terminal villi, growth of collagen fibers in their stroma, which led to vascular compression and tissue hypoxia (Fig. 4-6). The vessels of the chorionic plate sometimes contained blood clots. All the indicators of placental pathology in the control group were significantly lower in comparison with the main group. The degree of maturity of the chorionic villi corresponded to the gestational term. Insignificant masses of intervillous fibrinoid were contained, zones of fibrinoid degeneration of epithelium of villi were not revealed.

Fixed immune complexes containing Ig G, M and C3 complement fractions were found in the placentas of women with history of syphilis. Pathological immune complexes were revealed on the basement membrane of chorionic villi and in the vascular endothelium, and in the amniotic membranes – on the basement membrane of the amnion, chorion and decidual tissue (Fig. 7-8). Most often pathogenic IRs were located in the central part of the placenta – 45.00% (27 cases), respectively – 16.67% (10 cases) regional, in 5 cases (8.33%) PICs were found out in both parts. In 30.00% of cases PICs were not revealed at all. Light emission not less than (++) points was taken into account. IR in our observations included Ig of two classes G and M, with the predominance of the latter – 23 cases (38.33%), which was capable of tissue damage. The content of pathogenic immune complexes was most concentrated in women who had latent forms and secondary recurrent syphilis – 36 cases (60.00%). The comparison with the results of immunohistochemical studies of the placenta of women in the control group showed the presence of Ig G in 49.12% (28 cases) associated with trophoblast, which, according to some authors, "indicates the viability of the latter". No pathological immune complexes were detected.

DISCUSSION

An important component of the study of the system «mother - placenta - fetus» is the morphological study of the placenta with quantitative assessment of pathological processes and verification of the degree of placental dysfunction as one of the reserve mechanisms of influence on perinatal mortality indicators [24]. Syphilis, as a chronic infection, the process promotes the formation of CICs, which, when combined with the C3 complement fraction, acquire its pathogenicity [15]. The placenta, with an existing branched microvascular system, has a greater tendency to deposit CIC, the fixation of which in the placenta tissue leads to the formation of a local inflammatory reaction, which subsequently leads to a violation of its morphostructure, and as a result to the development of dysfunction of the fetoplacental complex [16-18]. The detected changes in the placentas of women with a history of syphilis in most cases are accompanied by lymphohistiocytic and leukocyte infiltration, focal deciduit, inflammation of the spiral arteries, thrombosis and fibrinoid degeneration of the stroma, the presence of which is a sign of the inflammatory process, which is confirmed by the data of literary sources [17, 24]. Certain pathomorphological changes are observed in the litter tissues of women whose pregnancy occurred after a syphilitic infection, which differ from the morphological pattern of involutive-adaptive litter changes during physiological pregnancy and are of a non-specific nature. Violation of blood circulation in the vessels of the microcirculation due to sclerosis of the stroma of the villi with a sharp narrowing of their diameter, focal proliferation of the endothelium of the vessels with hypervascularization of the terminal villi, growth of collagen fibers in their stroma leads to compression of the vessels. These signs are a manifestation of chronic hypoxia of placenta tissues [24]. The formation of diffuse hypervascularization of intermediate and terminal villi and vascular hyperplasia is a compensatory reaction that indicates an adequate response to long-term oxygen starvation. A high and increased level of expression of pathogenic immune complexes in the placentas of women after late forms of syphilis (latent and secondary recurrent) has a damaging effect and corresponds to the severity of sclerotic and thrombotic changes in the tissues of the litter, which explains the presence of certain morphological signs. Such a morphological structure shows to the manifestations of angiopathy, which is assigned a leading place in the pathogenesis of the development of placental insufficiency [20, 24].

CONCLUSIONS

Thus, we can conclude that:

1. Morphological examination of the placentas of women after syphilis revealed non-specific pathological changes that are characteristic of the manifestations of compensatory placental insufficiency. Changes in the placentas of women in the control group were involutive and adaptive in nature.
2. A probable correlation of the histological structure of placentas in the form of pathological immaturity, villusitis and intervilusitis, lympho-leukocyte infiltration in the group of women with latent and secondary recurrent syphilis compared with in the main group was determined.
3. The content of pathogenic immune complexes was most concentrated in women who suffered from latent forms and secondary recurrent syphilis, which determines the pattern of pathological changes revealed during histological examination.
4. The pathogenetic mechanisms of the development of placental insufficiency as a result of transferred syphilis have been established, namely: a decrease in the diffusion and perfusion volume of the placenta, its hypovascularization due to a decrease in the number of normally vascularized villi, the number of capillaries in them and obliteration of the vascular circle of the villi, obliterating arteritis.
5. On the basis of the obtained results, it is possible to recommend determination of indicators of the state of the fetoplacental complex from the 28th week of gestation as a comprehensive additional examination and dynamic control of pregnant women with history of syphilitic infection.

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The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

THE NEUROPROTECTIVE EFFECT OF TOCILIZUMAB IN BRAIN ISCHEMIA REPERFUSION INJURY

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ABSTRACT

The aim: This research was conducted to assess the possible neuroprotective effect of Tocilizumab in brain ischemic reperfusion injury in rats.

Material and Methods: 24 adult Sprague-Dawley rats were divided into four groups randomly. The sham group was given anesthesia at the same time as the other groups and was in the same condition as the other groups. Control group: 1 h of ischemia followed by 4 h of reperfusion. The vehicle group was the same as the control, but they were given the vehicle intraperitoneally (1 ml/kg of 0.9 % NaCl) for 7 days before the ischemia. The treatment group as the control group, but they were given tocilizumab (8 mg/kg) intraperitoneally for 7 days before ischemia.

Results: control group, inducing ischemia/reperfusion increased infarction size considerably ($p<0.001$), when comparison to the control and vehicle groups, tocilizumab at dose (8 mg/kg) showed a significantly ($p<0.001$) smaller infraction area.

Conclusions: In a cerebral ischemia/reperfusion, a reduction in infarction area in injected with Tocilizumab medication was considered neuroprotective for cerebral ischemia/reperfusion.

KEY WORDS: Tocilizumab, cerebral ischemia/reperfusion

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INTRODUCTION

A stroke is a clinically recognized phenomenon of rapidly growing symptoms or signs of localized loss of brain function caused by no apparent cause other than a vascular source [1], yet brain function loss can be universal at times (applied to patients in deep coma and to those with subarachnoid bleeding). Symptoms might last for more than 24 hours and result in death. Sacco et al., 2013, in the United States, a neurologic events as a result of altered cerebral circulation are the third greatest cause of death. High blood pressure, a family history of stroke and diabetes mellitus are all risk factors for stroke. Cerebrovascular accident (CVA) has three subtypes: ischaemia, infarction, and hemorrhage [2]. Ischemia and infarction are the result of atherosclerotic thrombus & embolus formation. Tocilizumab is an immunosuppressive medication used to treat rheumatoid arthritis (RA) and systemic juvenile idiopathic arthritis (SJIA), a significant form of arthritis that affects kids. Tocilizumab is a humanized monoclonal anti-interleukin-6 receptor antibody [5]. Inflammation seems to be a key player in the pathophysiology of ischemic stroke and other types of ischemic brain damage. Clinically, systemic inflammatory events increase patients' vulnerability to stroke and their subsequent prognosis; stroke patients with systemic inflammation have lower clinical results [3]. In animal studies, focal cerebral ischemia causes a time-dependent recruitment and activation of inflammatory cells such as neutrophils, T cells, and monocytes/macrophages, and preventing the inflammatory response reduces ischemic size and enhances neurological deficiencies [4].

THE AIM

This research was conducted to assess the possible neuroprotective effect of tocilizumab in brain ischemic reperfusion injury in rats.

MATERIAL AND METHODS

ANIMALS

A total of twenty-four adult Sprague-Dawley rats weighing a total of twenty-four pounds were used (250-350 g). They were purchased from Kufa College of Science's animal house. The animals were kept in the identical conditions in the Kufa College of Science's animal house, at a constant temperature of (25±1°C) and a room humidity of (60–65%), as well as alternating 12-hour light/12-hour dark cycles and free access to water and chow diet. Lastly, the rats were divided into four groups at random, each with six experimental rats [6].

GROUP-1: SHAM GROUP

The bilateral common carotid artery occlusion (BCCAO) was not included in this group, which was subjected to the similar surgical method (anesthesia and time).

GROUP-2: CONTROL GROUP

This group was given anesthesia and BCCAO for one hour and four hour, respectively, for reperfusion.

Table I. Measurements of cerebral infarction size in the sham group, control group, vehicle group, and tocilizumab group obtained by TTC,*vs. sham, # vs. c-vehicle

Parameter	Group	No.	Mean±SEM	P-value
Percentage of infraction	Sham	6	0.00±0.00	
	Control	6	31.967±0.731	<0.001*
	C-vehicle	6	32.667±0.682	<0.001*
	Treatment	6	17.247±0.454	<0.001#

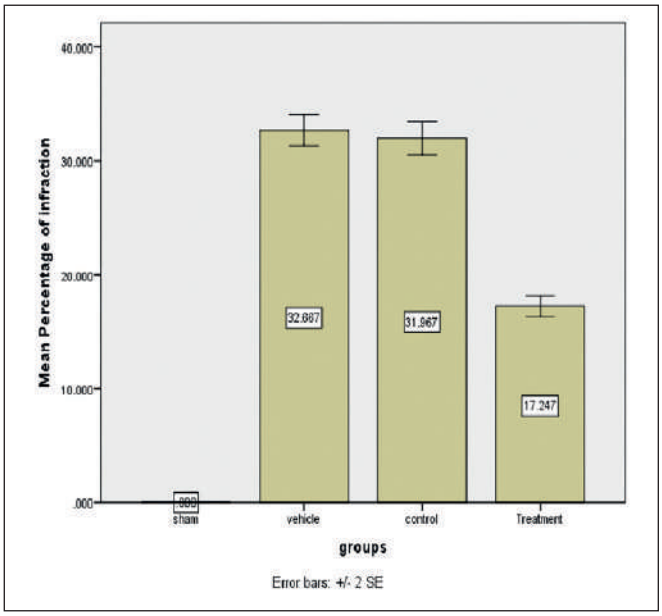

Fig. 1. Measurements of cerebral infarction size [%] in the sham group, control group, vehicle group and tocilizumab group obtained by TTC staining, n=6, *P<0.001 vs. sham, #P < 0.001 vs. c- vehicle groups.

GROUP-3: CONTROL-VEHICLE GROUP (C-VEHICLE)

Rats were given vehicle (0.9% NaCl) for 7 days before ischemia, and then underwent the same anesthesia and surgical technique with bilateral common carotid artery occlusion (BCCAO) for 1 hour and later reperfusion for 4 hours.

GROUP-4: TOCILIZUMAB TREATED GROUP

Rats were given tocilizumab (8 mg/kg/day intraperitoneally) for 7 days prior to the experiment, then anesthesia and surgery with bilateral common carotid artery occlusion (BCCAO) for 1 hour, followed by reperfusion for 4 hours.

PREPARATION OF DRUG AND ADMINISTRATION

Tocilizumab was usually available to be given intraperitoneally to male rats for 7 days prior the ischemic/reperfusion procedure by dose (8 mg/kg/day).

INDUCTION OF GLOBAL BRAIN ISCHEMIA

Bilateral carotid artery blockage resulted in global ischemia [7] and under a light bulb, the animals were kept at a temperature of around 37°C. During this time, ketamine and xylazine (80 mg/kg and 8 mg/kg, respectively) were administered intraperitoneally (IP) to induce complete anesthesia [8]. After the animals were positioned on their backs in a supine position, a small median incision in the neck was made with fine surgical tools, and both carotid arteries beneath the trachea were carefully detached from the vagal nerves. Then, using a vascular clamp, bilateral occlusion was achieved, with both clamped for1 hours ('this stage accounts for ischemia'). The clamp was then withdrawn for four hours to facilitate blood supply or "reperfusion".

PREPARATION OF SAMPLES

ISOLATION OF THE BRAIN

The brain was isolated immediately after decapitation, rinsed in cold 0.9% saline solution, and stored on ice until processing. Then it was separated into (pieces), one of which was utilized for TTC staining with 2, 3, 5-triphenyltetrazolium chloride (TTC). A coronal segment of the brain was obtained by making a brain incision.

TTC STAINING MEASUREMENT OF INFARCTION AREA

According to Li et al. [9] it is possible to measure the infracted brain area, using 2, 3, 5-triphenyl tetrazolium chloride to stain tissues. The brain portion was promptly stained with TTC stain once the ischemia/reperfusion induction was completed. The size of the infarct was determined by using the volume approach and examined by using imaging software (Digimizer). A segment of the brain was cleaned with dissection medium and frozen (-20°C for up to 20 minutes) to make the sectioning process easier. TTC powder was dissolved in PBS at 37°C (2 percent W/V) to prepare a TTC solution, which entailed the production of homogenous coronal slices with a 5 μm thickness [10]. TTC solution was used right away after prepared and kept out of direct sunlight. The brain parts were placed in a relatively small plastic container with TTC solution, which was shielded from light with aluminum foil. Pieces were treated with TTC for 30 minutes at 37°C before being transferred to 10 percent formalin. The use of formalin to keep biological samples is well known [10]. After fixing the brain pieces, image analysis software was used to image and analyze them (Digimizer). Treatment groups were examined to controls after infarcted regions were measured.

RESULTS

ASSESSMENT OF CEREBRAL INFARCT SIZE

The sham group had a normal appearance of the brain tissues with no death lesion, whereas the control and vehicle groups

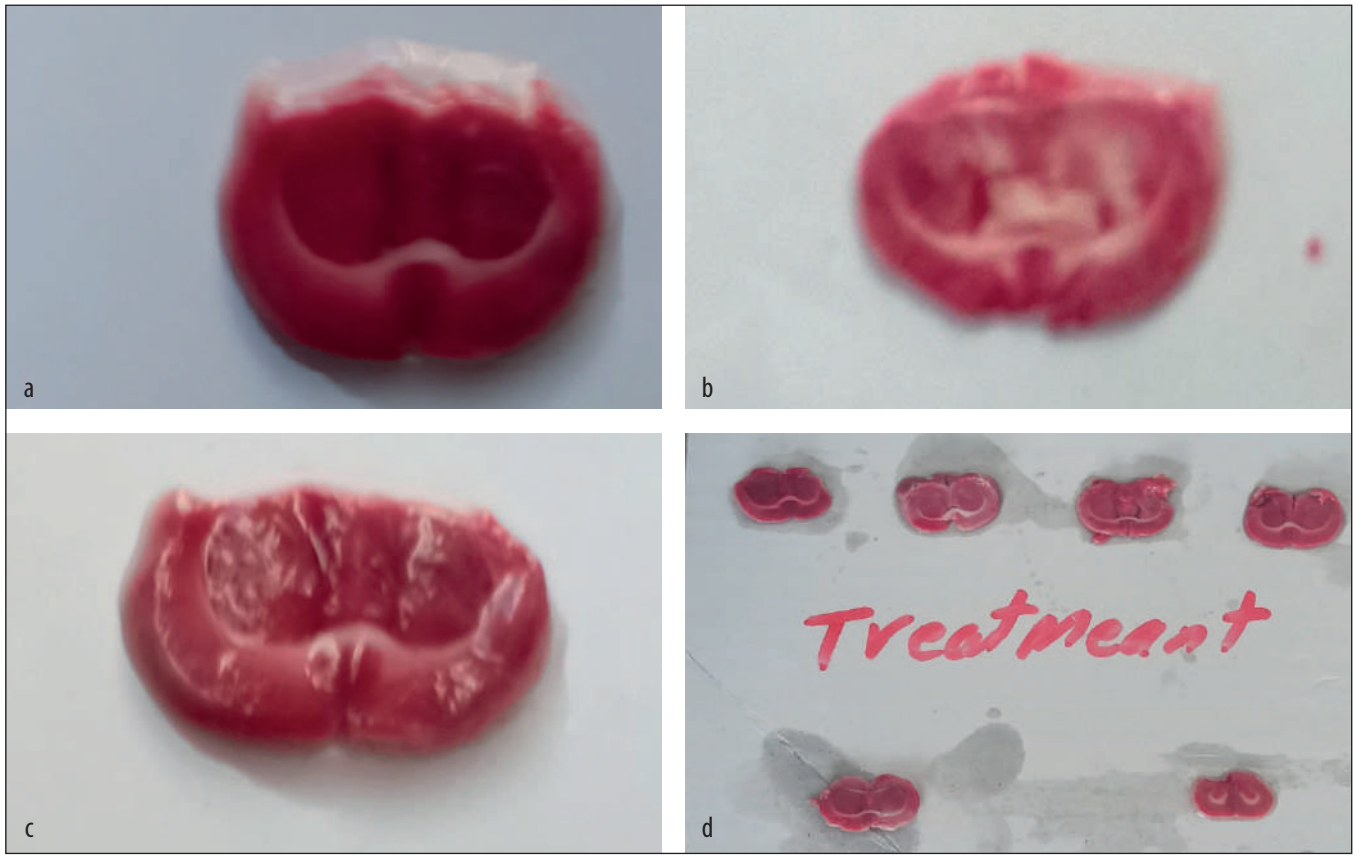

Fig. 2. Cross section of rat Brain from the sham group (A), control group (B), vehicle group (C), and actemra group stained with TTC (D). Normal and slightly damaged brain areas appear red. Those areas subjected to Infarction and cell death

had significantly larger infarction sizes ($p < 0.001$) when compared to the sham group, with infarction sizes of about 31.967±0.731 and 32.667±0.682 in the control and vehicle groups, respectively. There was no discernible difference between the control and vehicle groups. When compared to the control group, tocilizumab treatment resulted in a considerably smaller infarction size ($p<0.001$) (Table I, Fig. 1).

Rat brain sections stained with TTC are shown in figure 2, theses sections have been showed the normal area stained red while infarcted or dead tissue that not stained remain white. Hence, brain section in the sham group looked normal with no infarction or necrosis while rat brains in the control and the vehicle group show widely distributed infarction areas (as seen by white color). On the other hand, in tocilizumab group sections from rat brains have shown significantly lower size of infraction area when compared to the control and vehicle ones.

DISCUSSION

THE EFFECT OF CEREBRAL ISCHEMIA/ REPERFUSION (I/R) ON THE INFARCTION SIZE

One of the factors used to assess the severity of brain damage is the extent of infarcted brain tissue. When compared to the sham group, BACO caused a considerably larger infarction area in the control group. These results are in line with those obtained by [11]. They discovered a link between fraction size and I/R brain damage. These findings are also congruent with those [12-14].

THE EFFECT OF TOCILIZUMAB ON I/R-INDUCED INFARCTION SIZE

When compared to the control and vehicle groups, pre-administered tocilizumab resulted in considerably smaller infarction sizes in rat brains. A similar set of observations was presented by Wang et al. [6]. TUNEL assay was used to investigate tocilizumab's activity in a cerebral infarction rat model, revealing its effects on neuronal cell death in the hippocampus and cortex. The outcomes showed that MCAO operation raised the number of apoptotic neuronal cells in the hippocampus considerably ($P<0.001$), but tocilizumab-treated rats had a lower number of apoptotic neuronal cells than the Operation group ($P<0.05$). Similar findings were found in the cortex, with significant differences ($P<0.01$) between the operation and sham or tocilizumab groups. These findings indicated that TCZ (tocilizumab) may have an anti-apoptotic effect on cerebral infarction rat neuronal cells. In a rabbit subarachnoid bleeding model, tocilizumab decreased delayed cerebral vasospasm, neuronal cell death, and micro clot formation,

and could be a possible treatment to avoid delayed cerebral vasospasm and delayed cerebral ischemia in subarachnoid bleeding patients, according to the study mentioned by Croci et al. [15].

CONCLUSION

In a male rat model of cerebral ischemia / reperfusion, a reduction in infarction area in the treated group with tocilizumab medication is considered neuroprotective for cerebral ischemia / reperfusion.

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Ethical approval:

The current study has been conducted according to the national research guidelines for the care and use of laboratory animals. All protocols have been approved by the High Committee for Review and Approval of Research Proposals of the Faculty of
Medicine, University of Kufa (Ref. 3208, date: 14/02/2021).
Availability of data and materials:
The datasets used and/or analyzed for the present study are accessible from the corresponding author on reasonable request.

Conflict of interest:

The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

RISK OF PLACENTA-ASSOCIATED COMPLICATIONS AT PREECLAMPSIA IN PREGNANT WOMEN WITH THROMBOPHILIA

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ABSTRACT

The aim: To study the distribution and influence of coagulation factor gene polymorphisms, endothelial dysfunction, blood pressure regulator on the development of obstetric and perinatal complications in women with preeclampsia (PE).

Materials and methods: The prospective cohort study included 46 women with PE and maternal or fetal complications and 87 pregnant women with PE, without complications. Genetic polymorphisms of coagulation factors and fibrinolysis (1691 G→A FVL, 20210 G→A prothrombin, 675 5G/4G PAI-1, 455 G→A fibrinogen β), endothelial dysfunction (192 Q→R PON-1, 677 C→T MTHFR) and blood pressure regulator (235 M→T angiotensinogen II) were studied with the help of allele-specific polymerase chain reaction

Results: Markers of predisposition to the development of obstetric and perinatal complications in pregnant women with PE are the following genotypes: 1691 GA by V Leiden factor gene – increases the risk in 2.9 times (95% CI 1.94-4.33), 20210 GA by prothrombin gene – in 2.36 times (95% CI 1.54-3.6), 20210 AA by prothrombin gene – in 3.12 times (95% CI 2.4-4.0). Pathological polymorphisms in the genes of angiotensinogen II 235 M→T, PAI-1 5G/4G, fibrinogen β 455 G→A, paraoxonase-1 192 Q→R do not significantly affect the development of complications during preeclampsia.

Conclusions: The development of PE against the background of the existence of acquired and hereditary types of thrombophilia is associated with a more severe course, early-onset and the development of life-threatening complications for a mother and fetus.

KEY WORDS: MTHFR, factor V Leiden, prothrombin, abruptio placentae, fetal growth retardation, plasminogen activator inhibitor 1

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INTRODUCTION

Preeclampsia (PE) occurs in 5-8% of pregnancies and is the leading cause of maternal and perinatal morbidity and mortality [1-3]. The main threat is preeclampsia-related complications during and after pregnancy. Complications include: abruptio placentae (AP), premature birth, bleeding, eclampsia, fetal growth retardation (FGR), fetal distress and intrauterine fetal death (IFD). Non-obstetric complications include heart failure, pulmonary edema, cardiomyopathy, cerebrovascular disorders, retinal detachment, and an increased risk of cardiovascular disease in future [3,4].

Basic studies have shown that PE is associated with systemic inflammatory response, endothelial dysfunction, oxidative stress, imbalance of angiogenic and antiangiogenic factors, metabolic disorders [5,6]. The starting point of these pathological processes is considered to be inadequate trophoblast invasion [6]. It is now clear that PE, along with premature birth, fetal growth retardation, intrauterine fetal death and other conditions of pregnancy is a multifactorial disease. All of the above conditions are called "major obstetric syndromes" [7,8] that are associated with insufficiently deep placentation that may be associated with varying degrees of reduced remodeling and obstructive damage to the spiral arteries in the joint area or in the myometrium. The introduction of the term "major obstetric syndromes" was intended to explain the failure of works on the prediction and prevention of obstetric diseases, drawing the attention of researchers and clinicians to the etiological heterogeneity of conditions that share common pathogenetic pathways [7]. Common for conditions combined into "major obstetric syndromes" are: etiological heterogeneity, long preclinical phase, fetal disease, ineffective symptomatic treatment, clinical implications have adaptive characteristics, genetic factors and environmental factors play a role in the occurrence.

In recent years, significant progress has been made in the screening and prevention of PE [9,10], but the expected success in the treatment of PE has not been achieved. This is probably due to the fact that PE is the ultimate clinical implication of disorders of different origins. Indeed, the nature of the disease is different if it has developed before 34 weeks or at almost full term pregnancy. Not every case of PE is accompanied by complications, i.e. there is also noted relatively favourable course of the disease. Recently, there has been emerged the evidence of two types of PE: early and late. Early preeclampsia (EOPE) is accompanied by placental dysfunction, increased markers of endothelial dysfunction, early onset, severe course, development of complications and more often ends in induced premature

birth [1]. Maternal mortality is 12 times higher with the development of PE up to 28 weeks of pregnancy, and the period of 34 weeks is most often considered critical [11,12], as it correlates with abnormal placentation in the early stages. Late one (LOPE) is mostly associated with "maternal contribution": metabolic syndrome, hypertension, rarely accompanied by FGR and has a relatively favourable course [7].

The "combination" of EOPE and LOPE in study and development of clinical strategies may complicate the further study of the pathophysiology of this syndrome and the achievement of significant clinical results. Studies on the association of preeclampsia with thrombophilia are conflicting. Clinical heterogeneity of the disease may be one of the explanations [11-13]. A preliminary hypothesis in our study is that the development of maternal and fetal complications in PE or their absence are determined by various pathogenetic mechanisms, namely the existence of pathological genes of polymorphisms (hereditary thrombophilia, mutations in genes of blood pressure regulators and endothelial dysfunction) that are additional factors that cause, maintain and enhance reduced placental perfusion.

THE AIM

To study the distribution and influence of coagulation factor gene polymorphisms, endothelial dysfunction, blood pressure regulator on the development of obstetric and perinatal complications in women with PE.

MATERIALS AND METHODS

The study was conducted at Dnipro State Medical University, Dnipro, Ukraine, in 2018-2020. A prospective cohort study covered 133 women in the second half of pregnancy. The criterion for inclusion into the study is the presence of PE in accordance with the recommendations of the ISSHP [14].

The main cohort (M) consisted of 46 women with PE and maternal or fetal complications. As complications there were considered premature detachment of the normally located placenta (4 women – 8.7%), eclampsia (1 – 2.17%), HELLP syndrome (1 – 2.17%), FGR (23 – 50.0%), IFD (6 – 13.04%), fetal distress during pregnancy (21 patients – 45.65%). The comparator group (C) was formed by 87 pregnant women with PE, without complications. The study did not include pregnant women with a physiological course of pregnancy, since the purpose of the study was to determine the influence of coagulation factor gene polymorphisms, endothelial dysfunction, blood pressure regulator on the development of obstetric and perinatal complications in preeclampsia.

Genetic polymorphisms of coagulation factors and fibrinolysis (1691 G→A FVL, 20210 G→A prothrombin, 675 5G/4G PAI-1, 455 G→A fibrinogen β), endothelial dysfunction (192 Q→R PON-1, 677 C→T MTHFR), blood pressure regulator (235 M→T angiotensinogen II) were studied with the help of allele-specific polymerase chain reaction, followed by detection by electrophoresis in 3% agarose gel. A set of reagents "SNP-Express" (Litech SPF, Russian Federation) was used. DNA from leukocytes of blood, which was isolated using the reagent "DNA-express blood" (Litech SPF, Russian Federation) was used for analysis.

Statistical processing of the study results was performed using licensed computer programs Microsoft Excel 2010 and Graph Pad Prism 5 using methods of parametric and nonparametric statistics. The normality of the distribution of quantitative traits was assessed using Shapiro-Wilk and Kolmogorov-Smirnov criteria, analysis of variance, odd t-test, Mann-Whitney test, $\chi 2$ test with conjugation of conjugation tables and Yates correction, Fisher's exact test were used. Spearman and Pearson correlation coefficients (r) were used to assess the relationship between the indicators. To assess the relationship between impact and outcome, relative risk (RR) and odds ratio (OR) assessments were performed at 95% confidence interval (CI).The difference between the values was considered significant by $p<0.05$ [2,8].

The management of the study was conducted in full compliance with the ethical principles contained in the "Human Rights Declaration" adopted in Helsinki, which follows the Good Practice Rules in the Clinical Study and Legal Regulations and with the approval of the Ethics Committee of the Dnipro State Medical University.

RESULTS

The average age of women and the distribution by age categories between groups almost did not differ: in M group – 26.98 ± 0.9 years, in C group – 28.98 ± 0.65 ($p>0.05$).

Analysis of reproductive function showed that the number of women with a history of childbirth and the average number of births per woman in the group with obstetric complications was less than in the group without complications. Accordingly, the number of first-borns in the M group was significantly higher 35 (76.09%) than in the comparator group 44 (50.57%) ($p <0.05$, OR = 3.1; 95% CI 1.4-6.9, RR = 1.5, 95% CI 1.16-1.96). PE equally often complicated obstetric history in the study groups.

The mean period of PE onset in M group (29.02 ± 0.55 weeks) was 1.19 times shorter than in C group (34.45 ± 0.25 weeks) ($p<0.001$). The duration of PE in the group with complications (4.48 ± 0.47 weeks) is 1.7 times longer than in C group (2.63 ± 0.18 weeks, $p<0.001$). It was found that the onset of preeclampsia at 28 weeks of gestation or earlier increases the relative risk of complications by 34.04 times ($p<0.001$, RR = 34.04, 95% CI 4.69-247.1), and the duration of PE for more than 5 weeks increases the relative risk of complications by 3.57 times (95% CI 1.73-7.38).

The mean gestational age at the time of delivery in M group (33.50 ± 0.57) was 3.44 weeks less than in the comparator group (36.94 ± 0.27, $p<0.001$). This is due to the severity of preeclampsia and the development of complications that required preterm birth. In the main cohort, 7 (15.22%) women had mild preeclampsia; this is

Table I. Frequency of genotypes and alleles in pregnant women from study groups, n (%)

Study group	Genotype		
ANG 235 M→T	MM	MT	TT
M (n=46)	10 (21.74)	22 (47.83)	14 (30.43)
C (n=87)	31 (35.63)	34 (39.08)	22 (25.29)
prothrombin 20210 G→A	GG	GA	AA
M (n=46)	31 (67.39)*	10 (21.74)*	5 (10.87)*
C (n=87)	83 (95.4)	4 (4.6)	0 (0.0)
FVL 1691 G→A	GG	GA	AA
M (n=46)	26 (56.52)*	19 (41.3)*	1 (2.17)
C (n=87)	80 (91.95)	7 (8.05)	0 (0.0)
PAI-1 5G/4G	5G/5G	5G/4G	4G/4G
M (n=46)	7 (15.22)	30 (65.22)	9 (19.57)
C (n=87)	22 (25.29)	44 (50.57)	21 (24.14)
fibrinogen β 455 G→A	GG	GA	AA
M (n=46)	16 (34.78)	26 (56.52)	4 (8.7)
C (n=87)	39 (44.83)	37 (42.53)	11 (12.64)
MTHFR 677 C→T	CC	CT	TT
M (n=46)	21 (45.65)*	17 (36.96)	8 (17.39)
C (n=87)	60 (68.97)	19 (21.84)	8 (9.2)
PON-1 192 Q→R	QQ	QR	RR
M (n=46)	28 (60.87)	13 (28.26)	5 (10.87)
C (n=87)	41 (47.13)	31 (35.63)	15 (17.24)

Note: * – the statistical significance of differences of indicator relative to the P group ($p<0.05$), the $\chi 2$ test and Fisher's exact test are used.

less than in the comparator group (57 – 65.52%, $p <0.001$; OR = 0.09; 95% CI 0.04–0.25). In the group with complications, severe preeclampsia had 39 (84.78%) women, which is more than in C group – 30 (34.48%) ($p <0.001$; OR = 10.59; 95% CI 4.23- 26.5, RR = 2.46, 95% CI 1.79–3.37). Edema of varying severity was noted in most women of both groups: 41 (89.13%) persons of M group, 69 (79.31%) of C group ($p> 0.05$).

Analysis of the results of tests of genes that regulate the hemostasis system, "endothelial system" and genes that regulate blood pressure, revealed a high frequency of pathological polymorphisms in patients with preeclampsia, regardless of complications: 46 (100%) women of M group and 84 (96.5) %) of C group (Table I). The results of testing for the presence of polymorphisms in the gene ANG II 235 M→T, PAI-1 5G/4G, fibrinogen β 455 G→A, PON-1 192 Q→R did not reveal significant changes between the studied groups (Table I).

DISCUSSION

Considering the comparative analysis of frequencies of genotypes and alleles of the FVL 1691 G → A gene (Table I), the frequency of normal homozygotes GG was reduced 1.62 times in the M group compared to the C group ($p <0.001$, OR = 0, 11, 95% CI 0.04-0.3, RR = 0.33, 95% CI 0.22-0.49), and an increase in the number of heterozygotes GA in the M group 5.13 times compared with the C group <0.001, OR = 8.04, 95% CI 3.05-21.22). In carriers of the GA FVL genotype the risk of complications during PE is increased by 2.9 times (95% CI 1.94-4.33). High prevalence of Factor V Leiden 1691G/A variation in preeclamptic patients was determined in a study of Ahmed, N. A. et al [15].

Analyzing the frequency of prothrombin gene genotypes (20210 G→A), it is found that mutations are unique to the group with preeclampsia and complications. In M group, the frequency of heterozygous forms exceeded 4.73 times the rate of C group ($p <0.05$, OR = 5.7; 95% CI 1.7-19.6; RR = 2.36, 95% CI 1, 54-3.6). The frequency of normal homozygotes of GG in M group is 1.42 times less than in C group ($p <0.001$, OR = 0.1, 95% CI 0.03-0.32; RR = 0.34, 95% CI 0, 24-0.5). The number of mutant homozygotes 20210 AA of the prothrombin gene is significantly higher in the M group than in C group ($p <0.05$, OR = 23.19, 95% CI 1.25-429.4), and the risk of complications during PE in carriers of this genotype is increased by 3.12 times (95% CI 2.4-4.0). Our data confirm previous studies [12,15,16].

Analysis of the frequencies of alleles and genotypes MTHFR 677 C→T revealed a decrease in the frequency of the normal genotype of CC in the M group. Its frequency is reduced by 1.5 times compared with C group ($p <0.05$, OR = 0.38, 95% CI 0.18-0.79; RR = 0.54, 95% CI 0.34-0.

86). The number of heterozygotes 677 CT MTHFR, pathological homozygotes 677 TT between study groups did not differ significantly. Thus, pregnant women with *wild-type CC MTHFR 677* had a protection effect against PE complications, that is consistent with Yang, Y. L. et al [17].

Allelic polymorphisms of ANG II 235 M→T, PAI-1 5G/4G, fibrinogen β 455 G→A, paraoxonase-1 192 Q→R were equally common regardless of the presence of complications. That is, the presence of the most pathogenic polymorphisms and their combined action play a role in the occurrence of complications in PE. This is confirmed by correlations between mutations in the prothrombin genes 20210 G→A, FVL, MTHFR 677 C→T and the development of obstetric and perinatal complications in PE r = 0.387, r = 0.421 and r = 0.225, respectively ($p < 0.05$). In a study [16] significant differences in MTHFR A1298C, C677T and FVL polymorphisms between EOPE and LOPE were proved. The synergic effect of MTHFR variants could increase PE and EOPE risk.

It has been suggested that the presence of several pathological genes or polymorphisms is important for the occurrence of complications in pregnant women with preeclampsia, and their pathological effect is summed up. Three or more combined polymorphisms were mostly (2.78 times) determined in the M group (54.3%) than in the C group without complications - 19.5% ($p < 0.001$, OR = 2.58, 95 % CI 1.64-4.05). The adverse effect of pathological polymorphisms on the development of perinatal complications is explained by disorders in the hemostasis system: increased aggregation, adhesion of platelets, coagulation properties of blood, which leads to impaired microcirculation in the utero-placental system.

It has been found that the development of complications in PE is accompanied by ($p < 0.05$): early (1.19 times) onset of the disease (29.02 ± 0.55 vs. 34.45 ± 0.25 weeks), increased (1.7 times) duration of hypertension (4.48 ± 0.47 weeks vs. 2.63 ± 0.18 weeks), less (3.44 weeks) gestational age at the time of delivery (33.50 ± 0.57 vs. 36, 94 ± 0.27 weeks), lower weight of newborns – 1.79 times (1647 ± 103.2 g vs. 2951 ± 71.68 g) and newborns body length – 1.18 times (42.5 ± 0.85 cm vs. 50, 25 ± 0.41 cm), lower Apgar scale score at 1st minute – 1.28 times (5.1 ± 0.32 vs. 5.53 ± 0.24 points), and at 5th minute – 1.25 times (6.07 ± 0.36 against 7.64 ± 0.06 points). Previous report by Lisonkiva S et al [18], showed that early- but not late-onset preeclampsia conferred a high risk of perinatal death/severe neonatal morbidity 16.4 (95% CI, 14.5-18.6) in early-onset and 2.0 (95% CI, 1.8-2.3) in late-onset preeclampsia.

Summarising the above, it should be assumed that there are two types of hypertensive disorders during pregnancy: the first is a severe PE that begins early and is accompanied by life-threatening complications for mother and child, and the second is a mild PE, in late pregnancy and without concomitant complications. Various etiological and pathophysiological changes lie in the development of these types of hypertensive disorders, which differ in time and course. Our opinion is consistent with Lisonkova S. [18], Simcox L. E. et al.[13].

CONCLUSIONS

The development of PE against the background of hereditary types of thrombophilia, namely polymorphism 1691 GA by factor V Leiden, 20210 GA by prothrombin gene, multigenic forms of thrombophilia is associated with more severe course, early onset and development of life-threatening complications for mother, fetus and newborn.

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ORIGINAL ARTICLE

ANTIHYPOXIC ACTIVITY OF 2,6-DIMETHYLPYRIDINE-N-OXIDE

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ABSTRACT

The aim: To study the antihypoxic activity of 2,6-dimethylpyridine-N-oxide in mice using the various experimental models of acute hypoxia under orally or intraperitoneally administration.

Materials and methods: The studies were performed on male CD-1 (SPF) mice. The antihypoxic activity of 2,6-dimethylpyridine-N-oxide was studied in three experimental models of acute hypoxia - hypercapnic hypoxia or hypoxia in a closed space, hemic hypoxia and histotoxic hypoxia at orally administration at doses 0.07; 7.1 and 71 mg/kg (respectively 1/20000, 1/200 and 1/20 of LD50) and at intraperitoneally administration at doses 7.1 and 71 mg/kg in comparison with reference drug Armadin.

Results: It is established, that 2,6-dimethylpyridine-N-oxide shows a antihypoxic activity in the all experimental models of acute hypoxia (hypoxia in a closed space, hemic hypoxia and histotoxic hypoxia). Its antihypoxic activity in acute hemic hypoxia and in acute hypoxia in a closed space was significantly higher than of reference drug Armadin, but during acute histotoxic hypoxia did not differ from Armadin. Also at intraperitoneal administration of 2,6-dimethylpyridine-N-oxide demonstrates less pronounced antihypoxic activity than at oral administration in all experimental models of acute hypoxia, but the coefficient efficiency is higher than in the reference drug Armadin.

Conclusions: 2,6-dimethylpyridine-N-oxide may be recommended for further detailed experimental studies as a perspective antihypoxant.

KEY WORDS: 2.6-dimethylpyridine-N-oxide, antihypoxic activity, hypercapnic hypoxia, hemic hypoxia, histotoxic hypoxia

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INTRODUCTION

Hypoxia is known as one of the most common conditions occurring in various chronic diseases of the lungs and cardiovascular and nervous systems. This condition is also known to occur as a result of various traumas, infectious diseases, as well as intense mental and physical exertion further accompanied by chronic fatigue. [1-3]. Hypoxia develops under insufficient supply of tissues with oxygen. Inhibition of the energy synthesis under oxygen deficiency leads to the energy metabolism disorder, to multisystem and multiorgan functional and metabolic disorders, and in the severe cases - to the fatal outcome. [1, 2]. Hypercapnic hypoxia, or acute hypoxia in a closed space (HCS) is very dangerous and transient type of hypoxia. HCS occurs in the extreme situations: at the accidents on coal mines, during underwater works, on submarines, aircrafts, in the sealed units with artificial oxygen supply, closed-type facilities (storage, underground shelters, etc.) [4, 5].

The pathologies at acute and chronic intoxications of chemical genesis occur due to hypoxia. The most common hypoxants among chemical compounds are pesticides of the various chemical groups (organophosphorus compounds, nitro- and chlorine derivatives of phenol, dipyridyl derivatives), tetrachloromethane, paraaminopropiophenone, cyanides, benzene nitro derivatives, sodium nitrite and others [6, 7]. The organophosphorus pesticides, except cholinergic effects, cause an oxidative stress, lead to multiple organ and system dysfunctions, including a hypoxia. They cause the ultrastructural, biochemical, metabolic and mitochondrial damages for liver, as evidenced by the changes in the liver biomarker enzymes [8]. Organophosphorus pesticides poisoning is accompanied often by the changes in blood system, including anemia, hypoxic conditions, impaired hemoglobin synthesis, hemoglobin oxidation processes, resulting in the formation of the toxic methemoglobin [9, 10].

The mechanism of the dipyridyl herbicides effect, paraquat in particular, is due to high prooxidant activity, disruption of the lipid matrix of the cell membranes and mitochondria, energy deficiency, hypoxia, which leads to the destructive changes in the pneumocytes and other cells [11, 12]. The effect of carbon tetrachloride, paraaminopropiophenone, cyanides, sodium nitrite, is characterized by decreasing hemoglobin concentration, erythrocyte count, and anemia. The main biological effect of sodium nitrite and paraaminopropiophenone on human health is the oxidation of Hb to metHb, which is unable to transport oxygen to the tissues [13, 14]. Nitroderivatives of phenol and benzene are also metHB formers. Methemoglobinemia results in hemic hypoxia affecting parenchymal organs and nervous system, hemolytic anemia developing [15].

Presently, combination drugs composed of 2 to 5 active substances are preferred when implementing a variety of measures to protect agricultural plants from pests and

diseases. This approach can cause potentiation of acute toxicity in biological systems of various levels under certain conditions. In addition to various pesticides, a human body may absorb other chemical substances which are delivered via air, drinking water and food consumption. The combination of these factors may cause various pathologies including hypoxia.

Therefore, it is urgently important to study the mechanisms of combined effects of various xenobiotics as well as develop prophylactic and preventative measures against acute intoxication.

In Ukraine, plant growth regulators (PGRs) based on the N-pyridine derivatives (Ivin and Poteitin) are widely used in agriculture. Mechanism of combined biological action is studied insufficiently. It has been shown [16] that PGRs Ivin and Poteitin reduce the acute toxicity and severity of the clinical symptoms of intoxication of some pesticides at their joint entering the laboratory animals. It is found [17-23] that 2,6-dimethylpyridine-N-oxide stabilizes the membranes of mitochondria of the hepatocytes, reduces the intensity of lipid peroxidation in the liver tissues, has an antioxidant, hepatoprotective and antimutagenic effect, intensifies the protein-synthetic processes, increases the content of DNA, RNA and mitotic index of the rat hepatocytes, which reduces a pesticide intoxication.

Antioxidant activity and membrane stabilizing effect of 2,6-dimethylpyridine-N-oxide just as of various modern antihypoxic medicines significantly facilitate the adaptation to hypoxia [24-26]. Based on this fact the antihypoxic activity of 2,6-dimethylpyridine-N-oxide can be prognosticated too.

Based on these conclusions, the research of pharmacological properties of 2,6-dimethylpyridine-N-oxide will potentially facilitate the interpretation of its combined action with various pesticides. It will also help develop medication based on its formula that will increase survival rates when acute hypoxia is experienced.

THE AIM

The aim of the research was to study the antihypoxic activity of 2,6-dimethylpyridine-N-oxide in mice using the various experimental models of an acute hypoxia under orally or intraperitoneally administration.

MATERIALS AND METHODS

We used the 2,6-dimethylpyridine-N-oxide, 99.9%, synthesized at the V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry, National Academy of Sciences of Ukraine as an antihypoxant.

The antihypoxic drug Armadin (analogue of Mexidol) - 2-ethyl-6-methyl-3-hydroxypyridine succinate produced by NVF «Microchem» (Ukraine) was used As a reference substance.

The studies were performed on male CD-1 (SPF) mice, which acquired from the SPF nursery of small laboratory animals of the «L. I. Medved Research Center for Preventive Toxicology, Food and Chemical Safety, Ministry of Health, Ukraine (State Enterprise)» with a certificate of quality of animal health. The animals were quarantined for 14 days in the vivarium. It is determined [28] that tolerance to hypoxia mostly depends on the body weight of the animals, and significant differences in weight of mice lead to the incorrect results. To avoid the false-positive results there were used mice with the same body weight in all studied groups (22-27 g). The study was conducted following the principles of bioethics and the requirements of humane treatment of animals (European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, 1986).

The antihypoxic activity of 2,6-dimethylpyridine-N-oxide and reference substance was studied in three experimental models of hypoxia - an acute hypoxia in a closed space (HCS), an acute hemic hypoxia (HeH) and an acute histotoxic hypoxia (HiH), in accordance with the Guidelines "Experimental study of the new adaptogens", City of Kyiv, 2009 [29].

For simulation of a "closed space" hypoxia, experimental mice were put in isolated hermetic chambers (volume 250 cm^3). Acute hemic hypoxia was simulated by intraperitoneal injection of an aqueous solution of sodium nitrite at a dose of 300 mg/kg. Acute histotoxic hypoxia was simulated by intraperitoneal injection of an aqueous solution of sodium nitroprusside at a dose of 20 mg/kg. The clinical symptoms of intoxication and time of death of the animals were registered. The animals receiving the hypoxants served as a positive control.

2,6-dimethylpyridine-N-oxide was orally administrated for its antihypoxic activity studying to the relevant groups of mice using an atraumatic probe one time an hour before the simulation of a hypoxia in the non-toxic and subtoxic doses - 0.07; 7.1 and 71 mg/kg (respectively 1/20000, 1/200 and 1/20 of LD_{50}), or intraperitoneally in the doses of 7.1 and 71 mg/kg. In such doses antihypoxic effect of 2,6-dimethylpyridine-N-oxide at orally administration was observed. The reference drug Armadin was administered to mice at an effective dose of 100 mg/kg for both orally and intraperitoneally. The animals of all experimental groups were administered the solution orally (or intraperitoneally) in the same allowable volume. There were 8 animals in each study group.

All data obtained experimentally were processed statistically using the computer program Prizm 6. The results were determined as mean ± standard error (x ± SE). Differences between the groups were determined using the nonparametric Chi square test, where the differences were considered probable at $P < 0.05$.

The criterion for evaluating the efficacy of 2,6-dimethylpyridine-N-oxide was the comparison of the data concerning studied substance in control and reference groups of animals. The effectiveness of the drugs was determined by the values of a Lifetime of animals (T), the Coefficient of Antihypoxic Protection (C_{pr}), and the Relative Indicator of Lifetime Increase of the animals (RI):

; , where $T_{(e)}$ is the mean lifetime (T) of animals in the

experimental group, and $T_{(c)}$ is the mean lifetime (T) of animals in the control or reference groups.

RESULTS

The results of 2,6-dimethylpyridine N-oxide and the reference drug Armadin antihypoxic activity analyzing at oral and intraperitoneal administration in mice in the simulation of acute HCS are set out in Fig 1 and Table I.

As can be seen in Fig 1, N-oxide 2,6-dimethylpyridine at prior (preventive) oral administration in the simulation of acute HCS statistically significant increased a lifetime of mice in all studied doses to comparison with control group. As Table I shows, the Relative Indicator of Lifetime Increase of the animals (RI) in a dose of 0.07 mg/kg is 23.66%, 7.1 mg/kg - 23.98% and 71 mg/kg - 57.81%. The coefficient of antihypoxic protection (C_{pr}) of 2,6-dimethylpyridine-N-oxide, depending on the dose, ranged in 1.24-1.58. Under the preventive effect of the reference drug Armadin in a dose of 100 mg/kg, a lifetime of mice increased, the RI was 19.62%, the C_{pr} was 1.20, which indicates less pronounced antihypoxic effect than 2,6-dimethylpyridine-N-oxide (Figure 1 and Table I).

2,6-dimethylpyridine-N-oxide at preventive intraperitoneal administration to mice in the simulation of acute HCS (Figure 1 and Table I) statistically significant increased the life expectancy of mice in a dose of 7.1 mg/kg by 38.62% and 71 mg/kg - by 49.57%, the C_{pr} was 1.39 and 1.5, respectively. The reference drug Armadin at a dose of 100 mg/kg increased a lifetime of mice by 40.01%, the C_{pr} was 1.4. Therefore, the reference drug Armadin at intraperitoneal administration, compared to oral administration, is slightly more effective. Antihypoxic activity of 2,6-dimethylpyridine-N-oxide at intraperitoneal administration at a dose of 7.1 mg/kg increases and remains at almost the same level in a dose of 71 mg/kg compared with oral administration. More pronounced antihypoxic efficacy of the studied substances at intraperitoneal administration than at oral in acute HCS that is compatible with the literature data [30].

Thus, 2,6-dimethylpyridine-N-oxide in low doses (0.07 and 7.1 mg/kg) slightly exceeds the antihypoxic activity of the reference drug Armadin and in high doses (71 mg/kg) significantly exceeds its antihypoxic activity, especially at oral administration.

The results of the studies of antihypoxic activity of 2,6-dimethylpyridine-N-oxide and the reference drug Armadin at oral and intraperitoneal administration to mice in the simulation of an acute hemic hypoxia are given in Figure 2 and Table II.

As can be seen from Figure 2 and Table II, 2,6-dimethylpyridine-N-oxide at preventive oral administration with simulation of acute HeH increases a lifetime of mice at a dose of 0.07 mg/kg by 34.62%, 7.1 mg/kg - by 82.05% and 71 mg/kg - by 66.67% at comparison with control group. The statistically significant changes in this indicator were observed in two higher doses. The C_{pr} of 2,6-dimethylpyridine-N-oxide, depending on the dose, ranged from 1.35 to 1.82. Under the preventive effect of the reference drug Armadin in a dose of 100 mg/kg, a lifetime of mice increased to 21.38%, the C_{pr} was 1.21. The data obtained indicate that an antihypoxic activity of reference drug Armadin is manifested less than 2,6-dimethylpyridine-N-oxide in all doses studied.

2,6-dimethylpyridine-N-oxide at the simulation of acute hemic hypoxia (Figure 2 and Table II) was preventively intraperitoneally administered to mice at a dose of 7.1 mg/kg that resulted in slightly life expectancy increasing (7.35%) and at a dose of 71 mg/kg it was 28.77%, that statistically significant. The C_{pr} is 1.07 and 1.29, respectively. Respectively reference drug Armadin at a dose of 100 mg/kg increases a lifetime of mice by 21.95%, and the C_{pr} was 1.22.

As can be seen from the given data, the reference drug Armadin at oral and intraperitoneal administration demonstrates the same antihypoxic effect. An antihypoxic activity of 2,6-dimethylpyridine-N-oxide intraperitoneally is significantly lower at both doses than at oral administration, but its antihypoxic effect at a dose of 71 mg/kg was higher than in the reference agent.

The results of the studies of antihypoxic activity of both 2,6-dimethylpyridine-N-oxide and Armadin administrating orally and intraperitoneally in simulation of an acute histotoxic hypoxia are shown in Figure 3 and Table III.

As can be seen from Figure 3 and Table III, 2,6-dimethylpyridine-N-oxide in preventively oral administration at simulation of acute HiH statistically significant increased a lifetime of mice in all applied doses to comparison with control group, in a dose of 0.07 mg/kg the RI was 11.04%, 7.1 mg/kg - 20.84% and 71 mg/kg - 22.47%. The C_{pr} of 2,6-dimethylpyridine-N-oxide depending on the dose, ranged from 1.11 to 1.22. Under the preventive effect of reference drug Armadin at a dose of 100 mg/kg, a lifetime of mice was increasing, the RI was 26.00%, the C_{pr} was 1.26, but these changes were not reliable. The data obtained indicate almost the same level of antihypoxic activity both of Armadin and 2,6-dimethylpyridine-N-oxide at acute HiH.

The research of antihypoxic activity of 2,6-dimethylpyridine-N-oxide and reference drug Armadin in preventive intraperitoneal administration in mice at acute HiH simulation (Figure 3 and Table III) revealed that 2,6-dimethylpyridine-N-oxide slightly increased a lifetime of mice at a dose of 7.1 mg/kg (RI - 14.42%) and at a dose of 71 mg/kg (RI - 28.65%), the C_{pr} was 1.14 and 1.29, respectively. Armadin at a dose of 100 mg/kg slightly increased a lifetime of mice, the RI was 13.03%, and the C_{pr} was 1.13. At the action of a high dose of 2,6-dimethylpyridine-N-oxide and reference drug the obtained changes were statistically significant to comparison with the control group.

As can be seen from the above data, the reference drug Armadin when administered orally and intraperitoneally shows a slight antihypoxic effect. Antihypoxic activity of 2,6-dimethylpyridine-N-oxide when administered intraperitoneally, compared with an oral administration, at a dose of 7.1 mg/kg was less pronounced, and at a dose of 71 mg/kg was higher, but the degree of protective effect was much higher than under the influence of reference drug Armadin.

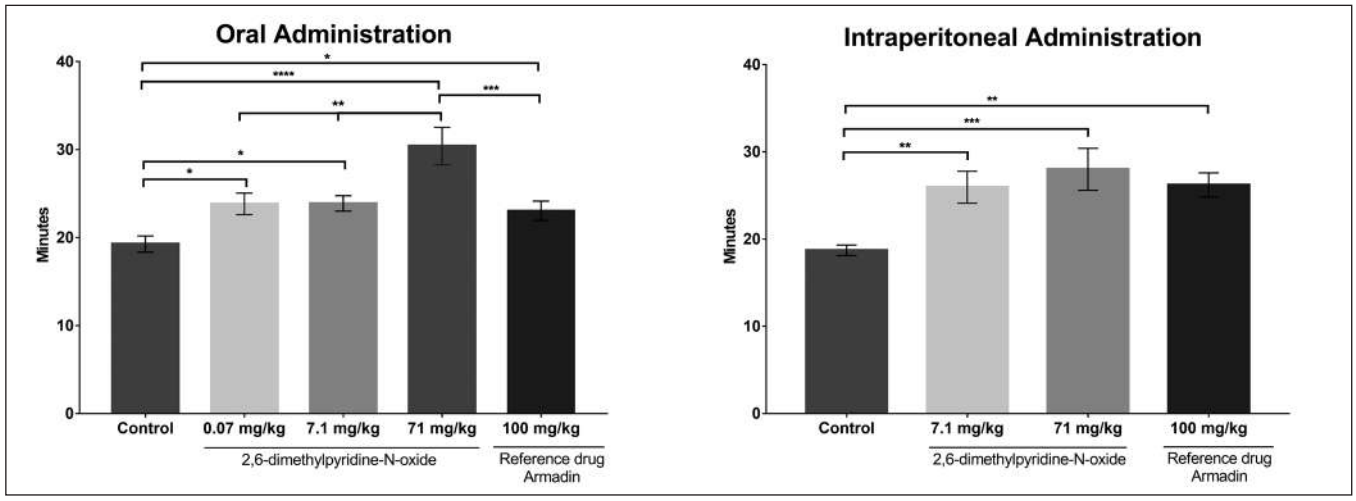

Fig. 1. The average Lifetime of animals (minute) at acute hypercapnic hypoxia or "closed space" hypoxia (HCS)
Note: * - P <0.05, ** - P <0.02, *** - P<0.002, **** - P <0.0001 - statistically significant differences between groups according to the Chi square test.

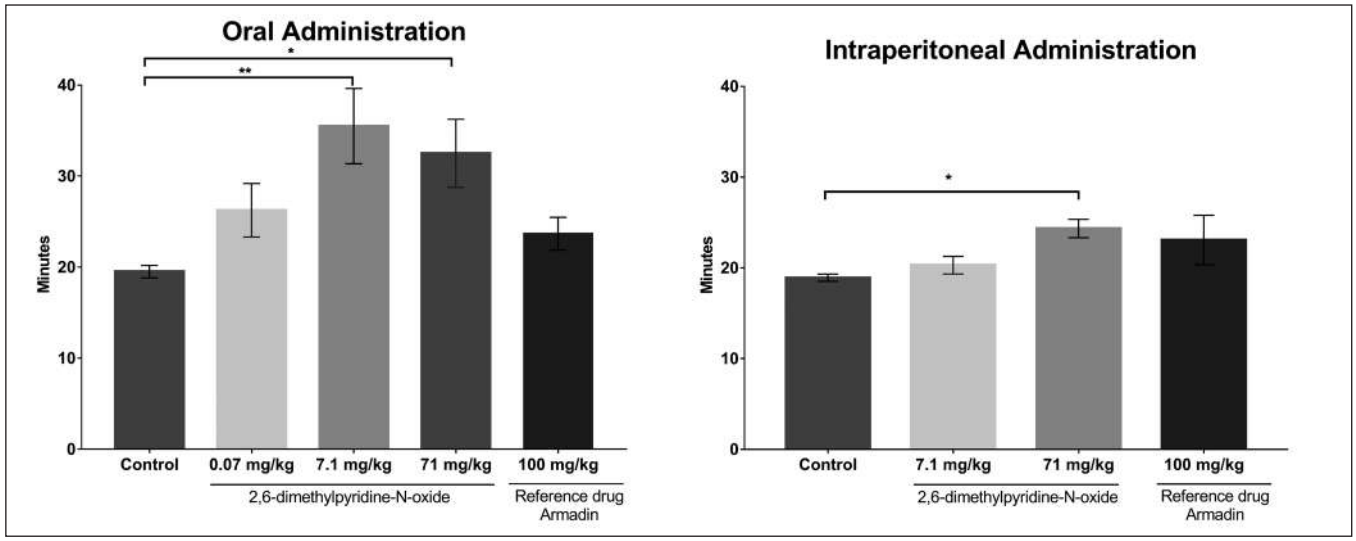

Fig. 2. The average Lifetime of animals (minute) at acute hemic hypoxia (HeH)
Note: * - P <0.05, ** - P <0.02, *** - P<0.002, **** - P <0.0001 - statistically significant differences between groups according to the Chi square test.

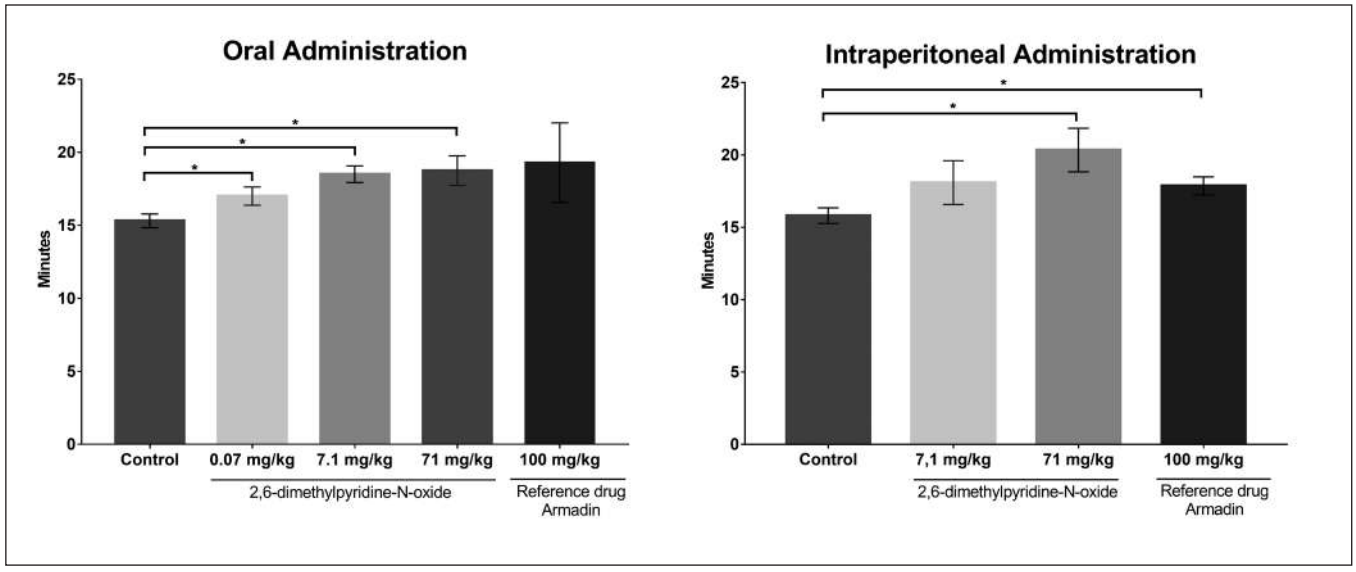

Fig. 3. The average Lifetime of animals (minute) at acute histotoxic hypoxia (HiH)
Note: * - P <0.05, ** - P <0.02, *** - P<0.002, **** - P <0.0001 - statistically significant differences between groups according to the Chi square test.

Table I. Relative Indicator of Lifetime Increase (RI) and Coefficient of antihypoxic protection (Cpr) of animals at acute hypercapnic hypoxia or "closed space" hypoxia (HCS)

Animal groups (n=8)	Dose, mg/kg	Oral Administration		Intraperitoneal Administration	
		RI, %	C_{pr}, relative unit	RI, %	C_{pr}, relative unit
2,6-dimethyl pyridine N-oxide	0.07	23,66	1,24	NO	NO
	7.1	23,98	1,24	38,62	1,39
	71.0	57,81	1,58	49,57	1,50
Armadin	100	19,62	1,20	40,01	1,40

Notes: n – the number of animals in the group, NO – not studied

Table II. Relative Indicator of Lifetime Increase (RI) and Coefficient of antihypoxic protection (Cpr) of animals at acute hemic hypoxia (HeH)

Animal groups (n=8)	Dose, mg/kg	Oral Administration		Intraperitoneal Administration	
		RI, %	C_{pr}, relative unit	RI, %	C_{pr}, relative unit
2,6-dimethyl pyridine N-oxide	0.07	34,62	1,35	NO	NO
	7.1	82,05	1,82	7,35	1,07
	71.0	66,67	1,67	28,77	1,29
Armadin	100	21,38	1,21	21,95	1,22

Notes: n – the number of animals in the group, NO – not studied

Table III. Relative Indicator of Lifetime Increase (RI) and Coefficient of antihypoxic protection (Cpr) of animals at acute histotoxic hypoxia (HiH)

Animal groups (n=8)	Dose, mg/kg	Oral Administration		Intraperitoneal Administration	
		RI, %	C_{pr}, relative unit	RI, %	C_{pr}, relative unit
2,6-dimethyl pyridine N-oxide	0.07	11,04	1,11	NO	NO
	7.1	20,84	1,21	14,42	1,14
	71.0	22,47	1,22	28,65	1,29
Armadin (reference drug)	100	26,00	1,26	13,03	1,13

Notes: n – the number of animals in the group, NO – not studied

DISCUSSION

The study of the antihypoxic activity of 2,6-dimethylpyridine-N-oxide in mice using the various experimental models of an acute hypoxia showed that with preventive oral administration an antihypoxic activity of 2,6-dimethylpyridine-N-oxide was quite high at the simulation of acute HeH and acute HCS, and a little less one - at acute HiH. The antihypoxic protection coefficient of 2,6-dimethylpyridine-N-oxide depending on dose at acute HeH ranged from 1.35 to 1.82; at acute HCS was in range 1.24-1.58, and at acute HiH was in range 1.11-1.22. Despite the fact that the level of an antihypoxic activity of 2,6-dimethylpyridine-N-oxide was the lowest at acute HiH, it was almost the same that occurred with reference drug Armadin. The coefficient of antihypoxic protection with Armadin preventive effect in all three cases of hypoxia was 1.20-1.26 with the most effect at acute HiH simulation.

Therefore antihypoxic effect of 2,6-dimethylpyridine-N-oxide at oral administration is significantly higher at HCS and HeH simulation and is almost the same at HiH simulation compared to the reference drug Armadin.

Contrary to oral administration, 2,6-dimethylpyridine-N-oxide under intraperitoneal administration at acute HiH increased the lifetime of mice at almost the same level as at acute HeH, but less than at acute HCS simulation. The coefficient of antihypoxic protection of 2,6-dimethylpyridine-N-oxide at the maximum dose at acute HeH and acute HiH was the same - 1.29, at acute HCS the coefficient of antihypoxic protection was 1.50.

The reference drug Armadin at intraperitoneal administration contrary to oral under the prophylactic implementing has the highest efficiency at HCS simulation (coefficient of antihypoxic protection – 1.40) and the lowest – at HiH simulation (coefficient of antihypoxic protection – 1.13).

Literature review concerning antihypoxic effect of commonly known medicines demonstrates the highest effect in effective dose of 100 mg/kg at intraperitoneal administration of hypoxen, etomerzol, natrium oxybutyratis, nooglutilum, mexidol at HCS simulation and the lowest one at HiH simulation. At HiH simulation these medicines mostly have medium efficacy [4, 30-32, 36, 37]. The same regularity regarding antihypoxic activity has been revealed in new substances such as metalcomplexes of alkenilimidazole and vinylimidazole derivatives [30, 32].

So both 2,6-dimethylpyridine-N-oxide and the reference drug Armadin at intraperitoneal administration in HeH and HiH simulation have less antihypoxic effect than in HCS simulation that corresponds to literary review.

Varying antihypoxic effect of 2,6-dimethylpyridine-N-oxide depending on hypoxia type can be explained by character and mechanisms of hypoxic effect of substances. Several types of hypoxia are defined considering clinical and metabolic manifestation: hypoxic hypoxia (hypoxemia) – caused by lack of oxygen in arterial blood due to insufficient oxygen saturation of hemoglobin; hemic (anemic) hypoxia – which is caused by oxidization of Hb into metHb, low ability of hemoglobin to bind and transport oxygen to tissues (intoxication by nitrates and carbon oxide); circulatory hypoxia – caused by lack of oxygen in tissues at sufficient volume of oxygen in arterial blood; tissue hypoxia (histotoxic hypoxia) – caused by law ability of affected cells to fully utilize oxygen from blood (cyanide intoxication) and redox processes disorders in tissues occurence; hypobaric hypoxia – caused by reduced partial oxygen pressure in inhaled air [33-35].

The implementation of the effects of hypoxia can occur in at least two ways [1, 11, 33]. The first one is direct affection of cell's bioenergy with further disturbance of its function and the second one is indirect – when stress activity in the neurohumoral chain launches a cascade of non-specific functional and metabolic reactions, causes disorder of blood supply and oxygen delivery to cells.

Therefore depending on pathological process at different types of hypoxia, ability of drug to stabilize cell energy metabolism, function of mitochondrial membranes, hepato-biliary and antioxidant systems the antihypoxic effect of drugs-antihypoxants can be manifested in different ways.

Higher level of 2,6-dimethylpyridine-N-oxide antihypoxic effect occured at intraperitoneal administration contrary to oral administration can be explained by toxicokinetic differences of this substance and peculiarities of biological effect of the specified ways of entering the body.

As it is pointed out by Kurbanov A. et al [4], "the reserve of medicines with antihypoxic effect is limited, quantity and efficacy of treating doses is rather small, and some of them have the effect only at selected type of hypoxia". The therapeutical effect of antihypoxic medicines mentioned above is within 210 [32, 36-38]. Antihypoxic effect of 2,6-dimethylpyridine-N-oxide manifests in dose from 0,07 mg/kg till 71 mg/kg, the index of therapeutical efficacy is over 1000. This fact indicates the advantage of 2,6-dimethylpyridine-N-oxide over other antihypoxants. So it can be provided for further extensive researches of antihypoxic effect and potential mechanism of action.

Considering that the mitochondria are the target for hypoxia [39, 40], one of the further tasks within studying the antihypoxic effect of 2,6-dimethylpyridine-N-oxide is the research of potential oxygen homeostasis regulation at system and cell levels, energy metabolism in cells.

CONCLUSIONS

1. 2,6-dimethylpyridine-N-oxide when administered orally in mice CD-1 (males) in dose range of 0.07 to 71 mg/kg shows a high antihypoxic activity in the models of acute hypoxia with a hypercapnia, acute hemic hypoxia and acute histotoxic hypoxia, increasing life expectancy in experimental animals. Its antihypoxic activity in acute hemic hypoxia and in acute hypoxia in a closed space was significantly higher than of reference drug Armadin, but during acute histotoxic hypoxia did not differ from reference drug.
2. 2,6-dimethylpyridine-N-oxide at intraperitoneal administration to mice CD-1 (males) demonstrates less pronounced antihypoxic activity than at oral administration in all experimental models of acute hypoxia, but the coefficient efficiency is higher than in the reference drug Armadin.
3. 2,6-dimethylpyridine-N-oxide may be recommended for further detailed experimental studies as a perspective antihypoxant.

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ORIGINAL ARTICLE

EVALUATION OF HUMAN ABO BLOOD GROUPS AND BLOOD COMPONENTS AMONG IRAQI PATIENTS INFECTED WITH CORONAVIRUS DISEASE 2019 (COVID-19)

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ABSTRACT

The aim: To evaluate the differences in blood groups, gender and type of Rh factor, as well as the levels of hemoglobin, white blood cells and platelets among patients infected with COVID-19.

Material and methods: A cross-sectional study was performed on 202 patients diagnosed with sever COVID-19 infection who were admitted to the Al-Shefaa center in Al-Hakeem hospital in Al-Najef city.Haematological investigations involved the types of blood groups, Rh factors, haemoglobin (Hb), white blood cells (WBCs), and platelets. In addition, the demographic features including age, gender and presence of any prescribed medications before or at the time of the study were also included.

Results: This study indicated that COVID–19 infected patients with type A blood group are at higher risk of hospitalization than other blood groups, and the majority of these patients were Rh positive. Additionally, WBCs counts indicated that the majority of patients had increased risk of getting infections which demonstrated lower WBC counts than normal. Platelet and Hb levels were normal for the majority of patients.

Conclusions: The findings of this study may help in the diagnosis of the pandemic infection with COVID-19, and prediction of the incidence of some complications caused by COVID-19. Further researches are warranted to confirm our findings.

KEY WORDS: COVID-19, blood groups, Rh, WBCs, haemoglobin, platelets

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INTRODUCTION

COVID-19 is a global pandemic infectious disease. It is an RNA virus in the family Coronaviridae of the order Nidovirales. The spike proteins on the surface of the virus give the name corona for this virus, which have a crown-like appearance under microscopy [1]. The new coronavirus was described in Wuhan, China at the end of 2019, and spread quickly to more than 180 countries [2]. World Health Organization (WHO) previously named the virus as novel coronavirus 2019 (2019-nCoV), while then it was named by the international committee of the Coronavirus Study Group (CSG) as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the disease caused by this virus was called by the WHO as coronavirus disease 2019 (COVID-19) [3]. The viral RNA captures the host cell's machinery to initiate the viral genome replication and polypeptides chain synthesis and form the replication-transcription complex (RCT). This complex is needed to synthesize the sub-genomic RNAs as well as structural proteins (envelope and nucleocapsid). The replication of virus is increased in the late phases of infection, and the integrity of the epithelial-endothelial barrier is compromised. The virus also invades the capillary endothelial cells of the lung, stimulating the inflammatory response and starting the influx of monocytes as well as neutrophils to the infection site [4-5]. Pulmonary edema filling the alveolar spaces with hyaline membrane formation, which is compatible with early-phase acute respiratory distress syndrome (ARDS). Collectively, endothelial barrier disruption, dysfunctional alveolar-capillary oxygen transmission, and impaired oxygen diffusion capacity are characteristic features of COVID-19 [5]. Several observations suggested that ABO blood group types may affect the severity of the infection. Indeed, it was found that COVID-19 patients having blood group type 0 showed less susceptibility than other blood group types [6]. Studies demonstrated that COVID-19 patients with higher risk showed higher proportion of Type A blood group, versus lower proportion of type 0 [7]. The same findings were demonstrated in individuals infected with SARS-CoV-2 pneumonia [8]. Additionally, another study found that the proportion of infected patients with blood group type A was significantly higher than healthy controls, whereas the proportion of infected patients with blood group type 0 was significantly lower in comparison to control group.

Moreover, patients with type A blood group showed higher frequencies of underlying comorbidities [9-10]. Other study demonstrated a higher proportion of type AB blood group in infected patients versus lower proportion of type 0 blood group patients [11]. However, other study reported that there was no correlation between blood groups A and 0 in COVID-19 patients, whereas patients with blood groups type B and AB possessed a higher risk [8]. One potential reason for these differential results is that such studies did not account for various confounders (e.g. age), as well as evaluating comorbidities [10-12]. Anti-A and anti-B antibodies have been suggested to interfere with the interaction of the virus with the human cells. In a study performed on data from about ~1900 patients with COVID-19, patients with anti-A antibodies were importantly less represented in patients with COVID-19 versus individuals do not have anti-A antibodies. Additionally, anti-A antibodies in people having type 0 blood group were shown to exert more protection than anti-A antibodies in blood group type B individuals. High levels of IgG anti-A and B antibodies, circulating in the plasma of patients having blood group type 0, might explain the reason behind this protection [11]. It was shown that there was no correlation between blood groups type A or B with the overall mortality rate. The mortality in patients with COVID-19 has been shown to be low in regions where the prevalence of blood group type 0 is high. Studies further demonstrated the correlation between the Rhesus blood group (e.g. Rh (D) type) and infection with COVID-19. Individuals with Rh (D)-positive have been suggested to show positive results when tested for SARS-CoV-2 [13]. Furthermore, a significant association between Rh (D) blood group, blood group type B, and SARS-CoV-2 has been reported [9]. It has been suggested that several mechanisms could demonstrate the relationship between ABO blood group types and COVID-19 infection. The antigens of type A and/or B blood groups, expressed on the surface of the viral envelope, act as binding sites for the anti-A and/or anti-B antibodies. This antigen-antibody binding could help in the prevention of infection of the target cells [14]. Consequently, this may help describing the differences in susceptibility for COVID-19 infection. In susceptible patients with type 0 blood group, the anti-A antibody can bind with the A antigen on the envelope of a virus inhaled from an infected patient having blood group type A or AB [15]. The later viral proliferation in host patients with type 0 blood group produces a new virus having the ability to express the H antigen on its envelope. Based on the above, the aim of this study is to evaluate the differences in blood groups, gender and type of Rh factor, as well as the levels of hemoglobin, white blood cells and platelets among patients infected with COVID-19.

THE AIM

This study aim is to evaluate the differences in blood groups, gender and type of Rh factor, as well as the levels of hemoglobin, white blood cells and platelets among patients infected with COVID-19.

MATERIALS AND METHODS

A Cross-sectional study was performed on 202 patients, 150 males and 52 females, diagnosed with COVID-19. Enrolled patients were aged between 35-70 years and they were admitted in Al-Shefaa center at Al-Hakeem hospital in Al-Najef city. The study was conducted from September 2020 to February 2021. Data were collected about the hematological laboratory investigations for the subjects enrolled in this study. These hematological laboratory investigations involved the types of blood groups and Rh factors, as well as complete blood counts to evaluate the levels of hemoglobin, white blood cells, and platelet. In addition, the demographic features including age, gender and presence of any prescribed medication before or at the time of the study were also considered. The verbal consents were obtained from the patients and the data collected anonymously then analysed and shown in the results section.

RESULTS

TYPES OF BLOOD GROUP IN PATIENTS INFECTED WITH COVID-19

The data of the study were collected for the people infected with COVID-19 (N=202) then analysed and listed in table I. The results showed that 51 participants (25.2%) were of B blood group, 27 (13.4%) possess AB blood group. The major part of participants 68(33.7%) have an A blood group, and the remaining 56 patients (27.7%) were shown to have an 0 blood group as appeared in table I.

LEVEL OF HEMOGLOBIN IN PATIENTS INFECTED WITH COVID-19

The level of Hb was calculated in the infected patients and results were shown in figure 1. The majority of participants (49.6%) were shown to have normal Hb level, 10.8% of patients possessed high Hb level, while 39.6% of participants had low Hb level.

LEVEL OF WHITE BLOOD CELLS IN PATIENTS INFECTED WITH COVID-19

The data obtained from the infected participant were illustrated in figure 2. It is shown that white blood cells

Table I. Frequency and percentage of the type of blood group in patients infected withCOVID-19.

Blood group	Frequency (f)	Percentage [%]
A	68	33.7
AB	27	13.4
B	51	25.2
O	56	27.7
Total	202	100.0%

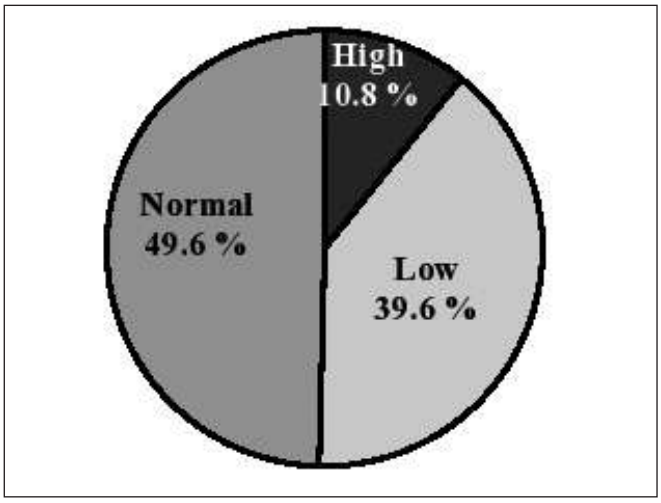

Fig. 1. Level of hemoglobin in patients infected with COVID-19.

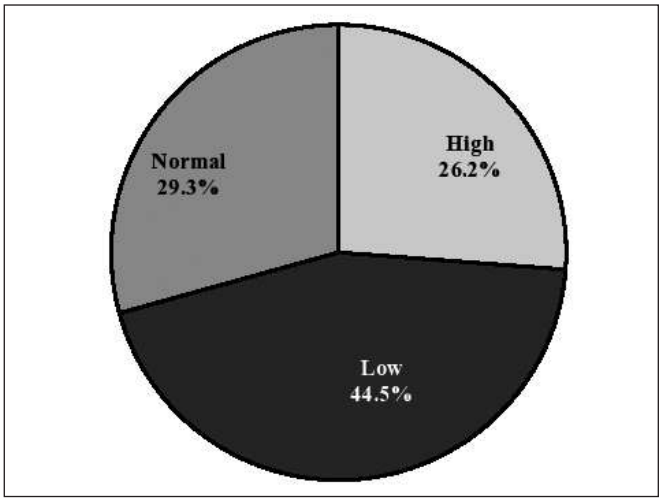

Fig. 2. Level of white blood cells in patients infected with COVID-19.

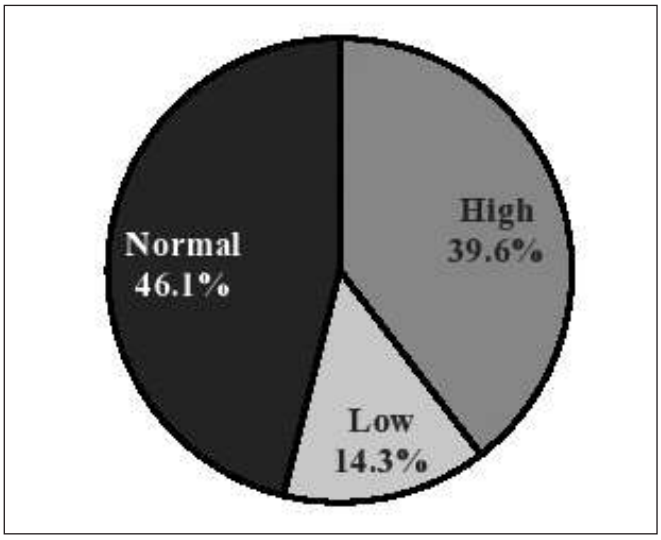

Fig. 3. Level of platelets in patients infected with COVID-19.

level was higher than normal in 26.2% of patients, and it was normal in 29.3% of patients, however, 44.5% of patients showed lower white blood cells level in comparison to normal.

Table II. Types of Rh factor in patients infected with COVID-19.

Type of Rh factor	Frequency	Percentage [%]
Rh negative	34	16.8
Rh positive	168	83.2

Table III. Type of gender in patients infected with COVID-19.

Gender	Frequency	Percentage [%]
Female	150	74
Male	52	26

LEVEL OF PLATELETS IN PATIENTS INFECTED WITH COVID-19

The majority of participants – 46.1% showed normal platelets level, while 39.6% demonstrated higher platelets level than normal. Additionally, 14.3% of patients revealed lower platelets level in comparison with normal see figure 3.

TYPE OF RH FACTOR IN PATIENTS INFECTED WITH COVID-19

As shown in table II, it is noticed that 168 (83.2%) patients had Rh positive, whereas 34 (16.8%) patients had Rh negative.

TYPE OF GENDER IN PATIENTS INFECTED WITH COVID-19

Obtained data demonstrated that 150 (74%) patients were females, while 52 (26%) patients were males, as shown in table III.

DISCUSSION

COVID-19 is a global pandemic infectious disease. The aim of our study is to evaluate frequencies and/or percentages of different blood groups, in addition to assess hemoglobin, white blood cells, platelets, and type of Rh factor among patients with COVID-19. The outcomes of this study may be useful in the diagnosis of this pandemic infection and prediction of the incidence of some complications caused by COVID-19 in the future. As shown in Table I, our cross-sectional study showed that among the 202 patients diagnosed with COVID-19 disease, 68 (33.7%) patients possessed A blood group, 51 (25.2%) patients had B blood group, 27 (13.4%) patients had AB blood group, and 56 (27.7%) patients were shown to have 0 blood group. In a similar study performed by Didar et al., the authors showed that of the 823 patients participated in the study, 351 (42.6%) patients had A blood group, 153 (18.6%) patients had B blood group, 69 (8.4%) patients had AB blood group, and 250 (30.4%) patients had 0 group [16]. In another study reported by Jiao Zhao et al., it was demonstrated that for the 285 patients with COVID-19 from Shenzhen, the type of blood groups A, B, AB and 0 were 28.77%, 29.12%, 13.68% and 28.42%, respectively. Furthermore, AB0 blood groups displayed differential association risks for the infection

with COVID-19. Significantly, blood group type A was associated with an increased risk in patients infected with COVID-19, whereas blood group type 0 was associated with a decreased risk. In the SARS-CoV-1 epidemic, it was shown that the anti-A antibodies in individuals with blood group type 0 prevented the invasion of the S protein into the patient's tissues [17]. In addition, it has been claimed that less severe infection was reported in individuals with blood group type 0 in the SARS-CoV-2 pandemic, and only 69 (8.4%) patients had blood group type AB [16]. Figure 1, showed that the majority of participants, 100 (49.5%), had normal Hb level. Comparable results were reported by other studies which also found normal Hb levels in patients infected with COVID-19, with the exception of adolescents with pneumonia [18-19]. However, other studies showed low level of Hb in 1,336 patients infected with COVID-19, half of them suffered from anemia [20]. Progressive decrease in Hb suggests an unpleasant clinical progression which recommends the need to maintain normal Hb levels. Moreover, these findings also suggest the importance of assessing Hb level, and using effective therapeutic options such as blood transfusion, hematin and/or erythropoietin hormone administration to prevent disease progression. Anemia doesn't predispose severity, rather it's a consequence of clinical course of the disease, taking into account the iatrogenic component due to multiple blood testing and the elevated risk of bleeding due to antithrombotic therapy. The results illustrated in figure 2, showed that 29.3% of participants had normal WBCs level, and 26.2% had high WBC level, whereas 44.5% of participants possessed lower WBC level than normal. These findings were in parallel with another study which was conducted on 137 patients admitted to the respiratory departments in nine tertiary hospitals in Hubei province from December 30, 2019 to January 24, 2020. Of these patients, 80% of them had normal WBC level. Further study reported that 33% of patients had normal white blood cell counts [21]. The determination of platelets level, as shown in figure 3, demonstrated that majority of patients (81.6%) had normal platelets level, while 39.6% of them possessed high platelet level, and only 14.3% of patients had low platelets level. Liu and his research group have demonstrated similar findings in a study performed on 383 patients infected with COVID-19 in the Central Hospital of Wuhan in China. Most admitted patients were found to have normal platelets level, and few had thrombocytopenia [22]. However, other studies revealed a decreased number of platelets in individuals with severe COVID-19 infection. For example, a study was done on 1476 patients found that 20.7% of them suffered from thrombocytopenia [23]. The mechanism by which SARS-CoV-2 produces thrombocytopenia is not clear, even so several important demonstrations were mentioned [24]. Additionally, lung autopsies of patients infected with COVID-19 showed bilateral diffused alveolar damage in addition to cellular fibromyxoid exudates. Interstitial mononuclear inflammatory infiltrates as dominated by lymphocytes were also reported. These abnormal histopathological examinations indicate the presence of immunopathological damage in lung tissues [25]. Platelets activation as well as aggregation in the lungs may be precipitated as a consequence of tissue damage. Consequently, thrombi formation may occur at the damaged sites which may lead to the consumption of platelets and megakaryocytic cells [22]. The determination of Rh factor, as shown in table II, illustrate that most participants, 83.2% were significantly Rh positive, whereas only 16.8% had negative Rh factor. These results were in parallel with a previous study performed on 823 patients in Adana City Education and Research Hospital in Turkey. The later study concluded that 89.9% of COVID-19 infected patients were Rh positive, and10.9% were Rh negative [26]. However, the Rh positive people naturally are more than Rh negative people. Finally, the determination of the gender of patients involved in this study found that 74% of infected people were females, while 26% of them were males. On the other hand, studies reported that the prevalence of COVID-19 infection is not significantly different between males and females, but male gender patients were found to have more severe symptoms with higher mortality rate than females [27].

CONCLUSIONS

The findings of this study may help in the diagnosis of the pandemic infection with COVID-19, and prediction of the incidence of some complications caused by COVID-19. Further researches are warranted to confirm our findings.

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Conflict of interest

The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

DOSE DEPENDENCE OF SUBCHRONIC INFLUENCING OF ACETAMIPRID ON THE ORGANISM OF RATS FROM DATA OF MORPHOLOGICAL RESEARCHES

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ABSTRACT

The aim: To determine the dose dependence of the subchronic effect of acetamiprid on the body of rats based on the data of morphological studies of internal organs.

Materials and methods: The experiment was performed on Wistar Han rats, which were orally administered acetamiprid in doses of 6, 12 and 60 mg/kg for 13 weeks. During the experiment, clinical studies were carried out, the general condition of the animals, body weight were assessed. After necropsy, the absolute and relative weight of internal organs was determined, and morphological studies of the brain, liver, kidneys, and spleen were performed with using an Olympus BX 54 light microscope and an Olympus C-5050 ZOOM camera with software Olympus DP-Soft. The research results were subjected to statistical processing using the Microsoft Excel 2010 computer program package.

Results: The most pronounced manifestations of the toxic effect of acetamiprid were observed at a dose of 60 mg/kg, which indicated its hepatotoxic and nephrotoxic effects, as well as neurotoxic effects with signs of irreversible neurocyte damage.

Conclusions: Morphological studies showed a dose-dependent nature and degree of expressiveness of the toxic effect of acetamiprid. According to the totality and nature of the changes revealed in the conditions of the conducted subchronic experiment on rats, no observed adverse effect level (NOAEL) was determined at the level of 12 mg/kg, no observed effect level (NOEL) – 6 mg/kg.

KEY WORDS: acetamiprid, subchronic toxicity, rats, morphology, dose dependence

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INTRODUCTION

Acetamiprid is an insecticide from the class of pyridylmethylamine neonicotinoids, which exhibits contact and intestinal action against a wide range of pests of agricultural crops and is basic in the integrated system of plant protection. The peculiarity of acetamiprid is high biological efficiency in relation to harmful insects, relative safety for honey bees, systemic and translaminar action in plants, low rates of consumption and multiplicity of treatments.

According to EU Commission Regulation 2018/113 of 24 January 2018, acetamiprid is included in the list of active substances allowed for use in plant protection products. Preparations based on it, often in combination with other insecticides, are also used in Ukraine. Their state registration is preceded by a thorough toxicological assessment and the development of hygienic regulations for safe use [1, 2].

Neonicotinoid pesticides have been known since the 1990s and have become the most widespread class of insecticides in the world [3]. According to the mechanism of action, neonicotinoids are partial or full agonists of neuronal nicotinic acetylcholine receptors in insects and weak agonists of mammalian receptors. Unlike organophosphorus compounds, they disrupt the conduction of nerve impulses due to the formation of acetylcholine competitors, which are not destroyed by acetylcholinesterase.

The risks of using neonicotinoids are related to their toxicity to humans and non-target organisms, their ever-increasing use, high mobility, and a relatively long half-life in water and soil [4, 5].

Acetamiprid is characterized by neurotoxic effects. Evidence of neurotoxicity was obtained in experiments on rats and mice at various doses and exposure periods (tremor, behavior change, dilation of the pupils, cold to the touch, decreased adhesion of the forelimbs), including in studies of neurotoxic effects on the development of offspring [6–10].

According to the results of the toxicological assessment carried out by experts of European Food Safety Authority (EFSA) in 2016, the reference values for acetamiprid - acceptable daily intake (ADI), acceptable operator exposure level (AOEL) were revised downwards from 0.07 mg/kg to 0.025 mg/kg, using the no observed adverse effect level (NOAEL) – 2.5 mg/kg (developmental neurotoxicity) and uncertainty factor (UF) of 100. In the group of male and female rats of the F1 generation at the maximum of the tested doses (45 mg/kg), early postnatal mortality, a decrease in body weight in the period after feeding, a decrease in the auditory response to sound without of neuropathology or changes in the morphological picture of the brain (suppression of the acoustic startle response to 53%) [7].

In Ukraine, the ADI of acetamiprid for humans has been established at the value of 0.01 mg/kg and is based on the inactive level in a chronic experiment for male rats (NOAEL – 7.1 mg/kg), and UF – 500, which guarantees safety its use by man.

In the scientific literature, there is information about the effect of acetamiprid on the liver, kidneys, spleen of rats and mice after oral administration. It should be noted that the expressiveness of morphological changes depends on the duration of exposure and the administered dose of the drug. At the same time, a violation of the hemodynamics of organs (expansion of lumens of veins, capillaries, their blood filling, hemorrhages) is important in the pathogenesis of morphofunctional changes of internal organs and is determined along with dystrophic-destructive and inflammatory processes, the intensity of which is included in the dependence "dose-time-effect" dependence (10 ; 20; 40 mg/kg – 6 weeks; 18.03; 42.72 mg/kg – 5 weeks) [11, 12].

Acetamiprid at a dose of 5 mg/kg for 61 days caused moderate anemia and atrophy of the lymph nodes of the spleen, which indicates a decrease in the functional state of the organ [13].

Characteristic properties of the toxicokinetics of acetamiprid are its rapid and intensive absorption with the highest concentration in the adrenal glands, thyroid gland, liver and kidneys without accumulation potential [7].

The above allows us to conclude that the effect of acetamiprid on the morphofunctional state of organs and systems is an important medical and biological problem. This outlines the relevance and necessity of conducting in-depth morphological studies in order to determine the nature and degree of expressiveness of the toxic effect on the body depending on the administered doses.

THE AIM

To determine the dose dependence of the subchronic effect of acetamiprid on the body of rats based on the data of morphological studies of internal organs.

MATERIALS AND METHODS

Experimental studies were conducted in compliance with the basic principles of bioethical treatment of animals and in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986). Research materials were reviewed and approved by the Commission on Bioethics of the State University "IMP NAMED Y.I. KUNDIEVA NAMN" (protocol dated January 23, 2019, No. 1).

The experiment was carried out in accordance with the recommendations of OECD 408 (OECD Guideline for the Testing of Chemicals Repeated Dose 90-day Oral Toxicity Study in Rodents) on the study of subchronic toxicity.

The test substance - generic acetamiprid, purity min. 99%, wich met international standards [7]. For bioethical reasons, taking into account the lack of sexual sensitivity to the action of the substance, the experiment was conducted on animals of the same sex - female Wistar Han rats. The study used 40 animals with an initial weight of 160-210 g, which were kept in standard vivarium conditions with an average duration of the light period of 11-12 hours, an air temperature of 21-24 °C and a relative humidity of at least 45%, on a standard diet (pelleted feed). Animals were divided into 4 groups, 1 – control, 3 – experimental groups, 10 animals in each group.

Acetamiprid was administered on an empty stomach intragastrically with the help of a metal probe 5 days a week for 13 weeks, in doses of 6, 12, 60 mg/kg of body weight. For administration, aqueous solutions of the substance were prepared with the addition of emulsifier OP-10 – 0.1; 0.2 and 1%, respectively, for doses of 6, 12 and 60 mg/kg. Doses were adjusted every 7 days after determining the body weight of the animals. The doses were chosen according to the existing information on the toxicological evaluation of the original acetamiprid [7].

In the course of the experiment, the animals were examined every day in order to detect any deviations related to the action of the substance. The animals' behavior, feed and water consumption, skin and coat condition, and mucous membranes were assessed. Motor activity, tremors, gait/posture disturbances, the presence of a reaction to care of the animal, the presence of clonic or tonic movements, stereotypic movements (excessive animal grooming, repetitive circling) or chimeric behavior (self-mutilation) were also recorded [14].

The body weight of the animals was determined before the start of the experiment and at regular intervals – every 7 days during the experiment. At the end of the experiment, the animals were euthanized by exposure to 60-70% carbon dioxide (CO2) using the AE0904 unit. In the terminal phase of withdrawal of animals from the experiment, the absolute and relative weight of internal organs was determined.

The object of histological studies was the brain, liver, kidneys, spleen, from which pieces of 1×0.5×0.5 cm size were cut and fixed in a neutral formalin solution for 72 hours. After fixation, they were washed in water, dehydrated in a series of ethanols (80%, 96%, 96%) and embedded in paraffin. Paraffin sections with a thickness of 5-7 μm were prepared using a Thermo NM 325 microtome, mounted on glass slides (3-5 sections). Sections of the brain, liver, kidneys, spleen were stained with hematoxylin and eosin, and additionally the brain with toluidine blue according to the standard protocol [15]. Histological preparations were studied using an Olympus BX 54 light microscope. Detected changes were documented using an Olympus C-5050 ZOOM camera with Olympus DP-Soft software.

The results of the experimental studies were subjected to statistical processing using the Microsoft Excel 2010 computer program package. The arithmetic mean (M), the standard error of the arithmetic mean (m), the Student's criterion «t» and the probable difference of the obtained results were calculated ($p < 0.05$) [16].

Table I. Morphometric indicators of body weight and internal organs of rats after subchronic influencing of acetamiprid in different doses, (M±m)

Experimental group	Body weight, g	Absolute (g) and relative (%) weight of internal organs							
		Liver		Kidneys		Spleen		Brain	
		abs.	rel.	abs.	rel.	abs.	rel.	abs.	rel.
Control, dose 0,0 mg/kg (n=10)	290.0±3.0	9 .86± 0,51	3.40± 0.17	1.78± 0.04	0.62± 0.02	0.74± 0.05	0.26± 0.02	1.80± 0.04	0.63± 0.02
Acetamiprid, dose 6,0 mg/kg (n=10)	285.4±2.8	9.21± 0.35	3.23± 0.11	1.68± 0.04	0.59± 0.01	0.96± 0.07	0.24± 0.02	1.87± 0.04	0.66± 0.01
Acetamiprid, dose 12,0 mg/kg (n=10)	282.2±2.6	9.31± 0.32	3.31± 0.13	1.80± 0.05	0.64± 0.02	0.67± 0.05	0.24± 0.02	1.79± 0.08	0.64± 0.03
Acetamiprid, dose 60 mg/kg (n=9)	236.8±8.2*	9.44± 0.33	3.30± 0.40	1.70± 0.04	0.65± 0.02	0.50± 0.03*	0.19± 0.01*	1.67± 0.04*	0.64± 0.03

Note: * – differences in control group are statistically probable ($p < 0.05$)

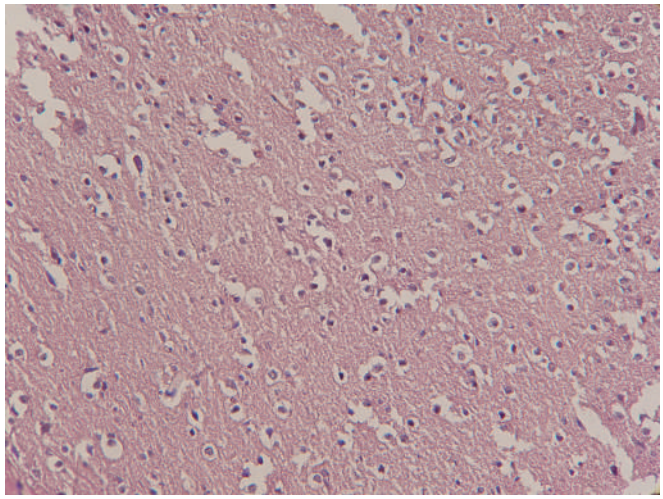

Fig. 1. Histological changes of rat brain neurocytes during oral subchronic administration of acetamiprid at a dose of 60 mg/kg. Pericellular edema in various parts of the white matter of the brain. Hematoxylin and eosin.

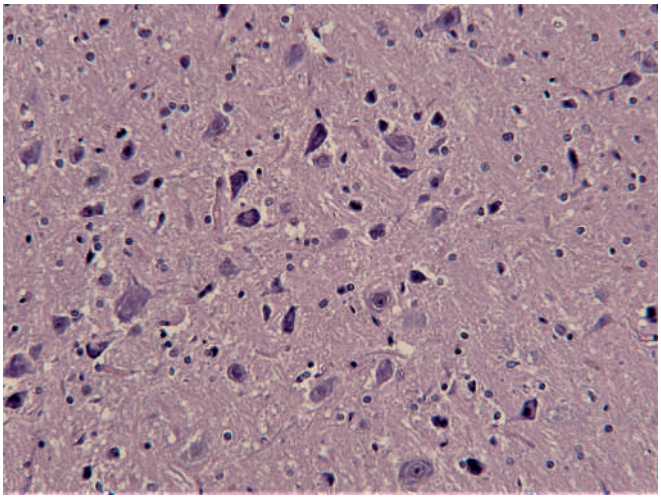

Fig. 2. Histological changes of rat brain neurocytes during oral subchronic administration of acetamiprid at a dose of 60 mg/kg. Hyperchromia of neurons, reduction of their size, chromatolysis and vacuolation of basophilic substance. A small number of neuroglial cells. Hematoxylin and eosin.

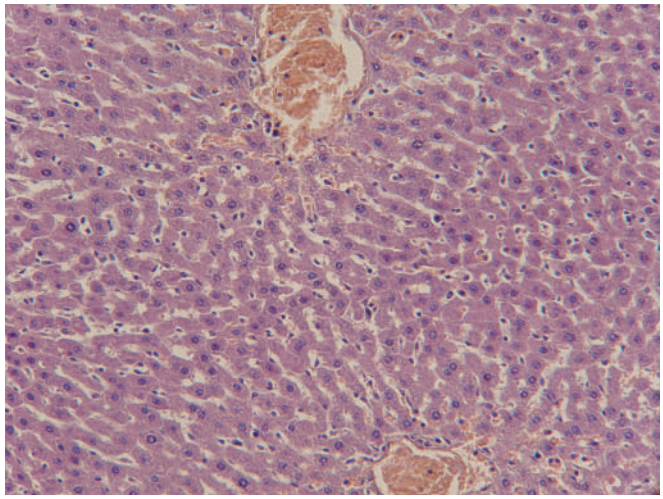

Fig. 3. Histological changes in the liver of rats during oral subchronic administration of acetamiprid at a dose of 60 mg/kg. Expansion of the lumens of sinusoidal capillaries, dystrophic changes of hepatocytes – blurring of their contours, lysis of many nuclei. Violation of the trabecular structure in some areas. A small number of granular reticuloendotheliocytes. Hematoxylin and eosin.

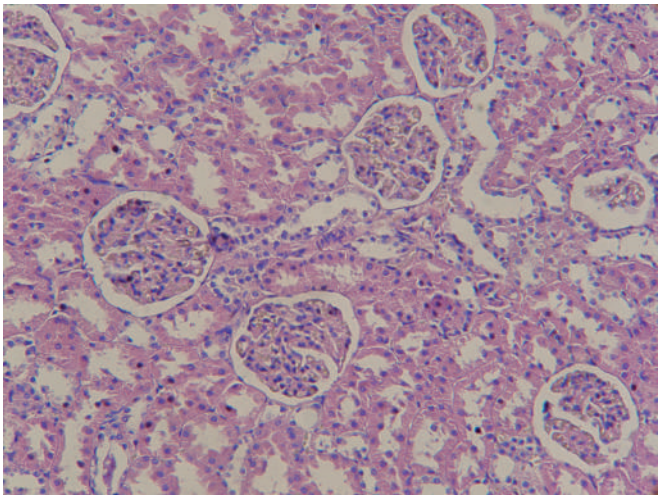

Fig. 4. Histological changes in rat kidneys after oral subchronic administration of acetamiprid at a dose of 12 mg/kg. Cellular infiltration in the intertubular stroma and between the glomerular capillaries. Hematoxylin and eosin.

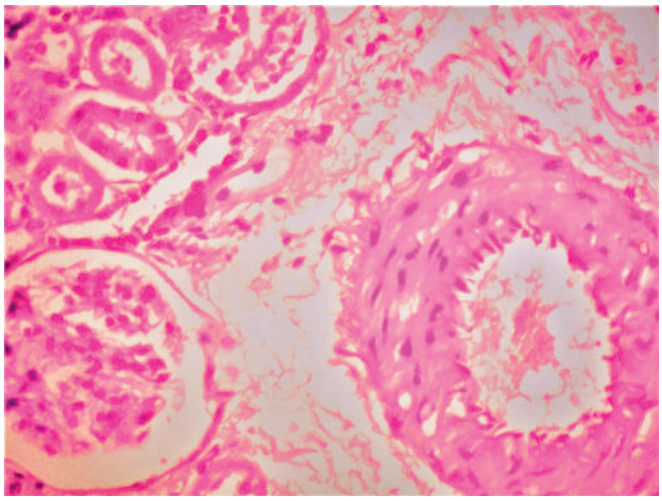

Fig. 5. Histological changes in the kidneys of rats by oral subchronic administration of acetamiprid at a dose of 60 mg/kg. Full blood vessels, thickening of their endothelium, perivascular edema. Exfoliation of epithelial cells of the convoluted tubules in their lumen. Hematoxylin and eosin.

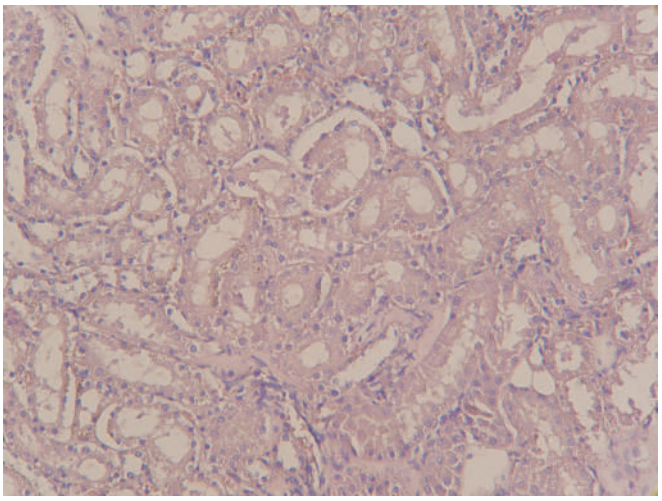

Fig. 6. Histological changes in the kidneys of rats by oral subchronic administration of acetamiprid at a dose of 60 mg/kg. The presence of loose protein eosinophilic masses in the lumen of the distal tubules and collecting tubules. Swelling of the cytoplasm of cells, blurring of their contours and lysis of many nuclei. Hematoxylin and eosin.

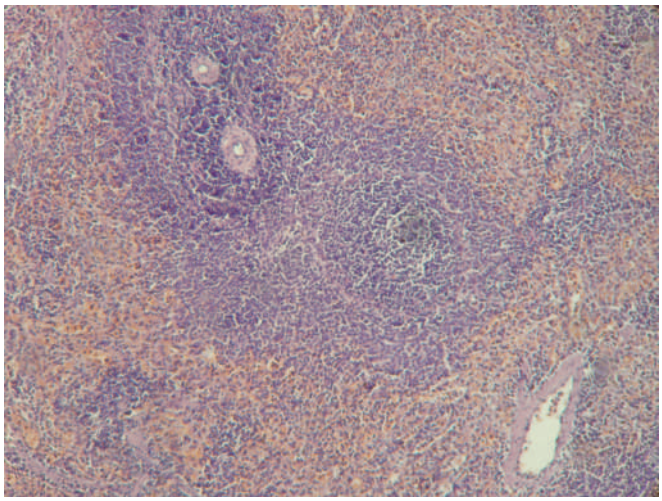

Fig. 7. Histological changes in the spleen of rats during oral subchronic administration of acetamiprid at a dose of 12 mg/kg. Hyperplasia of lymphoid nodules, determination of the contours of the central and peripheral zones. Pronounced blood supply to the vessels and capillaries of the red pulp. Hematoxylin and eosin.

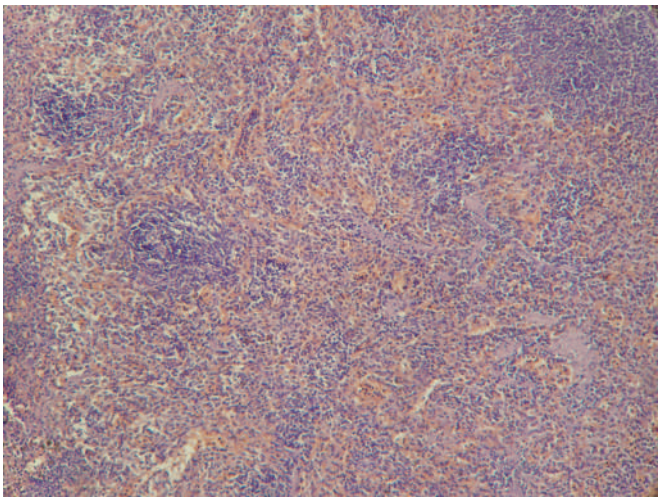

Fig. 8. Histological changes in the spleen of rats during oral subchronic administration of acetamiprid at a dose of 60 mg/kg. The white pulp is represented by small lymph nodes. Red pulp predominates over white, numerous hemorrhages, leukocyte infiltration, dilation and edema of the trabeculae. Hematoxylin and eosin.

RESULTS

It was established that under the influence of acetamiprid in doses of 6 and 12 mg/kg in the dynamics of the subchronic experiment, no animal deaths or clinical manifestations of intoxication were detected during all periods of research. At the maximum dose (60 mg/kg), individual animals were observed to have reduced mobility, depression, unkempt fur, serous and hemorrhagic discharge from the nose, discharge from the oral cavity, and tremors. One female died on the 83rd day of the experiment without obvious clinical signs of intoxication. No macroscopic changes in internal organs were found at autopsy. It was concluded that there is no connection between the death of the animal and exposure to the substance.

In the animals of this group, at the end of the experiment, a simultaneous statistically significant decrease in body weight and absolute weight of the spleen and brain, as well as the relative weight of the spleen, was observed in comparison with the control (Table I).

The morphological studies of internal organs showed the following.

BRAIN

In the microscope`s field of view were seen isolated moderately or partially hyperchromic neurocytes and neurocytes with peripheral chromatolysis of basophilic substance, with preservation of nucleus and nucleolus, when oral

acetamiprid was used at a dose of 6 mg/kg. There was a proliferation of neuroglial cells, which generally indicated the development of compensatory-adaptive processes.

In terms of oral acetamiprid administration at a dose of 12 mg/kg there were dystrophic changes in neurons, which were manifested by different types of chromatolysis, vacuolation of the cytoplasm in combination with ectopia of the partially hyperchromic nucleus of single cells.

In terms of chronic oral acetamiprid administration at a dose of 60 mg/kg, in comparison with the above groups revealed a significant expansion of the lumen of blood vessels and their blood supply, which contributed to the formation of perivascular and pericellular edema (Fig. 1). Along with this, there was a different functional state of neurons, a significant number of which were characterized by suppression of their function. This was manifested by hyperchromia of the cytoplasm of cells, their nuclei, a decrease in their size and lack of contouring of the nucleolus. Neurocytes with chromatolysis of basophilic substance (perinuclear, peripheral) and vacuolation of cytoplasm with nucleus ectopia were often found in the field of view. There were single wrinkled cells and a small number of neuroglial cells, which indicates a pronounced toxic effect of the drug on brain tissue (Fig. 2).

LIVER

At acetamiprid administration at a dose of 6 mg/kg the structure of a liver is close to rats of control group, only the large number of stellate reticuloendotheliocytes attracts attention.

Administration of acetamiprid at a dose of 12 mg/kg leads to small foci of lympho-macrophage infiltration around blood vessels and capillaries.

In the liver of rats, administration of the drug at a dose of 60 mg/kg contributed to the expansion and blood supply of the lumen of the central veins and sinusoidal capillaries, accompanied by significant perivascular, cellular and intercellular edema, with disturbances in some areas of the trabecular structure. Dystrophic and necrobiotic changes of many hepatocytes were determined by coarse-grained cytoplasm, blurred hepatocyte contours and lysis of individual nuclei. The number of granular reticuloendotheliocytes (Kupffer cells) was insignificant (Fig. 3).

KIDNEYS

The drug at a dose of 6 mg/kg did not caused changes compared with the control.

When acetamiprid was administered at a dose of 12 mg/kg, moderately pronounced dystrophic changes of proximal tubular epithelial cells were observed, which were characrerized by granular dystrophy, both with preservation of the cell nucleus and its lysis. There was cellular infiltration in the intertubular stroma and in the glomeruli (Fig. 4).

Acetamiprid administration at a dose of 60 mg/kg led to full blood vessels, thickening of their endothelium and perivascular edema. In addition, there was an expansion of the lumen of the renal corpuscles (Shumlyansky capsule), the plethora of capillaries of the glomeruli, which indicates an increase in activity of glomerular filtration processes (Fig. 5).

In the tubular apparatus of the kidneys there was an increase in the linear size, expansion of their lumens, swelling of the cells and blurring of their contours, lysis of many nuclei and a significant number of squamous cells in the lumen of the tubules. It should be especially noted the presence of loose protein eosinophilic masses in the lumen of the distal tubules and collecting tubules, which indicates a violation of renal reabsorption processes (Fig. 6).

SPLEEN

The drug at a dose of 6 mg/kg did not lead to significant changes in the structure of the organ.

When the drug is administered at a dose of 12 mg/kg, the white pulp predominated over the red due to the hyperplasia of lymphoid nodules, in which the contours of the central – B and peripheral – T zones are clearly defined. There was a moderate blood supply to the vessels and capillaries of the red pulp (Fig. 7).

When the drug is administered at a dose of 60 mg/kg there is a thickening of the serous membrane. Red pulp predominates over white, it reveals the expansion of the trabeculae, the lumen of the capillaries, their blood supply. There are small numerous hemorrhages and leukocyte infiltration. White pulp is represented by lymph nodes of small size, without definition of the light germinative centers that defines decrease in its activity (Fig. 8).

DISCUSSION

Morphological studies of neurocytes made it possible to determine various forms of compensatory and adaptive rearrangements, the nature and degree of which depends on the administered dose of acetamiprid. These changes characterize both the increased functional state of the cytoplasm and the nucleus of cells (12 mg/kg) and their inhibition (60 mg/kg). The latter was accompanied by the presence of irreversible necrobiotic changes and the appearance of shrunken cells, which indicates a more pronounced toxic effect of the substance in this dose.

The morphological studies of the liver showed that under the action of the substance at a dose of 12 mg/kg, small foci of lympho-macrophage infiltrates are observed around vessels and capillaries, which indicates an increase in metabolic processes. Dystrophic changes were not determined. When acetamiprid was administered at a dose of 60 mg/kg, hemodynamic disturbances were observed, which led to various types of edema with deformation of the trabecular structure along with dystrophic and necrobiotic signs of hepatocytes, which, in general, indicates a significant hepatotoxic effect of the drug in this dose [4].

In the kidneys, when the substance was administered at a dose of 12 mg/kg, there was leukocyte infiltration of

the intertubular stroma and around the capillaries of the glomeruli, which indicates the presence of inflammatory processes. At a dose of 60 mg/kg, a more significant toxic effect was determined, which is indicated by changes in the renal corpuscles (expansion of their space, fullness of capillaries), which is characterized by the activation of glomerular filtration processes. There is also a violation of tubular reabsorption, which is evidenced by an increase in the linear dimensions of the tubules and their lumens with the accumulation of exfoliated epithelial cells and loose acidophilic protein masses in them [6].

The above-mentioned morphological changes in the spleen testify to the development in the organ of the immune system of compensatory-adaptive changes, which in this case develop in response to the subchronic effect of the substance and determine the different nature of the functional state of the white pulp (activation at 12 mg/kg and inhibition at 60 mg/ kg) against the background of hemodynamic disturbances in the red pulp.

CONCLUSIONS

1. The conducted in-depth morphological studies showed the dose-dependent nature and degree of expressiveness of the toxic effect of acetamiprid on the body of rats in the mode of subchronic oral administration. The introduction of the substance at a dose of 6 mg/kg does not lead to significant morphological changes in the structure of all the internal organs studied in comparison with the control.
2. Morphological changes in the brain under the action of the substance at a dose of 12 mg/kg indicated an increased functional state of neurocytes, and the introduction of a dose of 60 mg/kg indicated inhibition of their activity along with irreversible cell damage (shrinkage), which indicates a pronounced toxic effect on brain tissue.
3. The effect of the substance in doses of 12 mg/kg and 60 mg/kg indicates a different degree of formation of the effect of toxic action in the liver. This effect at 12 mg/kg was determined by a disturbance in the microcirculation system and the presence of various cellular infiltrates, which characterizes adaptive changes. And the presence of dystrophic or necrobiotic changes and inflammatory processes at a dose of 60 mg/kg indicate a hepatotoxic effect.
4. In the kidneys, depending on the administered doses, there is inflammation in the intertubular stroma at 12 mg/kg and disruption of the processes of glomerular filtration and tubular reabsorption at 60 mg/kg, which indicates a nephrotoxic effect.
5. In the spleen, acetamiprid at a dose of 12 mg/kg led to the activation of the functional state of the white pulp of the organ, and at a dose of 60 mg/kg - to its suppression against the background of hemodynamic disturbances in the red pulp.
6. Based on the totality and nature of the changes detected in the conditions of the subchronic experiment on rats, the minimum NOAEL of acetamiprid is set at the level of 12 mg/kg, and a dose of 6 mg/kg can be taken as the inactive level (NOEL). The hygienic regulations, that have been developed for acetamiprid, guarantee its safe use by humans.

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ORIGINAL ARTICLE

GUT MICROBIOTA COMPOSITION AND ITS METABOLITES CHANGES IN PATIENTS WITH ATHEROSCLEROSIS AND ATRIAL FIBRILLATION

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ABSTRACT

The aim: To check changes of gut microbiota composition and its metabolites in atherosclerosis (AS) patients with or without atrial fibrillation (AF) and special connections between them and important clinic and laboratory features of investigated groups.

Materials and methods: 300 patients were investigated. All investigated were divided into 3 groups: control group (CG) – 27 patients without AS and arrhythmias; mean group – 149 patients with AS but without arrhythmias; comparable group – 124 patients with AS and AF paroxysm. By 16-S rRNA sequencing was checked gut microbiota composition. The level of trimethylamine-N-oxide (TMAO), trimethylamine (TMA) plasma was determined by gas chromatography with mass electron detection.

Results: The mean and comparable groups have the significant abundance of total bacterial mass, Bacteroides Spp., Faecalibacterium Prausnitzii, Actinobacter Spp. and decreasing Ruminococcus Spp. In the comparable group to the mean significant increasing of Actinobacter Spp. and decreasing Eubacterium Rectale, Ruminococcus Spp. were checked. Bacteroides Fragilis Group/ Faecalibacterium Prausnitzii ratio was significantly higher than in patients' comparable group. In the mean group patients compared with CG significant abundance of Streptococcus Spp. was checked. In the comparable group compared with CG significant leak of Eubacterium Rectale was checked. The highest amount of correlations was between Lactobacillus Spp., Streptococcus Spp. and clinic-laboratory changes. The mean and comparable groups the significant increasing of TMA ta TMAO plasma levels were checked. In patients of comparable group compared with patients mean group the significant increasing of TMAO plasma level was revealed.

Conclusions: We checked special bacterial changes of gut microbiota that are common for patients with AS and AF comparable with AS patients. TMAO plasma levels are increased significantly for patients with AS and AF comparable with AS patients. Connections between AS and AF with TMAO plasma levels are confirmed by reliable correlations between TMAO and age, BMI, GFR, HDL levels. Special bacterial species are closely connected with age, BMI, GFR, HDL, LDL, plasma TMA and TMAO levels.

KEY WORDS: atherosclerosis, atrial fibrillation, gut microbiota composition, trimethylamine-N-oxide, trimethylamine

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INTRODUCTION

The role of the gut microbiota and its metabolites in human health become more and more popular during last ten years. Especially occurred a lot of new data about links between gut microbiota composition and metabolic disorders. Condition of gut microbiota is an important component of human health in general. It influences on cardiovascular health directly by regulating intestinal digestion and absorption and indirectly through its metabolites as trimethylamine (TMA), trimethylamine-N-oxide (TMAO), short chain fatty acids (SHFA) [1].

Human gut microbiota is defined as complex of microorganisms, with includes bacteria and archea that lives into digestive tract. Gut microbiota changes are closely linked with metabolic disorders as obesity, type 2 diabetes mellitus, hyperlipidemia, arterial hypertension, chronic kidney diseases, fatty liver etc., which are the known risk factors of atherosclerosis (AS) and atrial fibrillation (AF) [2, 3]. But no special microbial pattern for atherosclerotic changes are not defined yet. On the one hand it is described the prevalence of Bacteroides, Escherichia coli and low level of Proteobacteria and Haemophilus for patients with AS [4]. Other authors reported abundance of *Streptococcus salivarius, Klebsiella pneumoniae and leak of Roseburia hominis in AS patients [5]. One more datum reported higher level of Proteobacteria and* Enterobacter, Escherichia/Shigella, Klebsiella [6]. *Differences in studies results and little groups of investigated patients shows us an importance of further gut microbiota composition features investigations in AS patients.*

Moreover, it is no exact data about gut microbiota composition in patients with AF. But not far away occurred a lot of information about links between AF and gut dysbiosis [7, 8]. As we know increasing TMAO plasma levels become an evidence risk factor of AS, but by the latest data TMAO level can be the important risk factor of AF occurrence also [10]. The role of SHFA in pathogenesis of AS and AF is also stayed questionable. By some data their changes can provoke AF paroxysm [11]. *Important that AF and AS can be the cause and the reason of each other in the same time. Together they form the viscous circle that's lead for increasing risk of cardiovascular events. So, their pathogenesis can be analyzed together [9].*

Table I. Clinical and laboratory characteristics of investigated groups, mean ± standard error

Characteristic /group	AS	AS+AF	CG	P1-2	P2-3	P1-3
Age (years)	67,71±3,90	67,96±0,94	56,25±2,18	P>0,05	P>0,05	P>0,05
Men (%)	48,99	47,97	48,15	P>0,05	P>0,05	P>0,05
BMI (kg/m^2)	27,02±0,33	26,93±0,43	28,12±2,10	P>0,05	P>0,05	P>0,05
GFR (ml/min)	62,03±2,31	67,73±1,98	84,01±5,48	P>0,05	P<0,05	P<0,05
Smoking (%)	51,01	41,46	40,74	P>0,05	P>0,05	P>0,05
Total cholesterol (mmol/l)	5,73±0,37	6,18±0,31	4,32±0,21	P>0,05	P<0,05	P<0,05
Triglycerides (mmol/l)	2,02±0,18	1,74±0,14	1,12±0,09	P>0,05	P<0,05	P<0,05
LDL (mmol/l)	2,63±0,29	2,66±0,24	1,54±0,11	P>0,05	P<0,05	P<0,05
HDL (mmol/l)	1,46±0,13	1,23±0,14	1,74±0,12	P>0,05	P<0,05	P<0,05

According the listed it is very important to understand if there are special changes of gut microbiota composition and its metabolites in AS patients with or without AF and also are there special connections between them and important clinic and laboratory features of investigated groups.

THE AIM

To check changes of gut microbiota composition and its metabolites in atherosclerosis patients with or without atrial fibrillation and special connections between them and important clinic and laboratory features of investigated groups.

MATERIALS AND METHODS

300 patients were investigated. All investigated were divided into 3 groups: control group – 27 patients without AS and arrhythmias; mean group – 149 patients with AS but without arrhythmias; comparable group – 124 patients with AS and AF paroxysm. We excluded patients with valvular atrial fibrillation, heart failure from Class III to IV (by New York Heart Association), reported malignancies, chronic kidney disease (Glomerular Filtration Rate, GFR <60 mL/min), thyroid pathology, inflammatory bowel disease, irritable bowel syndrome, pregnancy, taking probiotics and antibiotics for a month before the study. There were no vegetarians or vegans among the examined. Informed consent was obtained from all subjects in accordance with the Declaration of Helsinki. The study was conducted at the base and was approved by the ethical commission of the Kiev City Clinical Hospital No. 12. Clinical and laboratory characteristics of investigated groups are performed in table I.

Carotid ultrasound and Holter ECG monitoring was used for diagnosis verification. Toshiba Aplio 400 color Doppler ultrasound system was used to scan the neck blood vessels by a linear probe and a frequency of 5–12 MHz. Bilateral common carotid arteries, internal and external carotid artery, vertebral artery were investigated. Cardiosens K Holter monitor was used during 24 hours. 16-S rRNA sequencing checked gut microbiota composition, that include such bacterias: Lactobacillus spp., Bifidobacterium spp., Escherichia coli, Bacteroides spp., Faecalibacterium prausnitzii, Bacteroides thetaiotaomicron, Akkermansia muciniphila, Enterococcus spp., Blautia spp., Acinetobacter spp., Streptococcus spp., Eubacterium rectale, Roseburia inulinivorans, Prevotella spp., Methanobrevibacter smithii, Methanosphaera stadmanae, Ruminococcus spp. The level of TMAO, TMA plasma was determined by gas chromatography with mass electron detection. They were extracted from blood plasma into acid by adding internal standards. Blood sampling from patients was performed on an empty stomach from the cubital vein on the day of hospitalization. Results were presented as mean ± standard error or [95% confidence interval (CI)] for continuous variables or as a number for categorical variables. Data were compared using Wilcoxon signed-rank test or Student t-test with two critical regions by the type of distribution and Spearman's rank correlation coefficient.

RESULTS

During our work gut microbiota composition in patients with AS without arrhythmias, patients with AS and AF and control group was compared. Result or gut microbiota comparison is shown in table II.

As we can see in the table II patients of the mean (AS) and comparable (AS+AF) groups have the significant abundance of total bacterial mass, Bacteroides Spp., Faecalibacterium Prausnitzii, Actinobacter Spp. and decreasing Ruminococcus Spp. Also comparing the comparable (AS+AF) and the mean (AS) groups significant increasing of Actinobacter Spp. and decreasing Eubacterium Rectale, Ruminococcus Spp. were checked. Bacteroides Fragilis Group/ Faecalibacterium Prausnitzii ratio was significantly higher than in patients' comparable group (AS+AF). In the mean group patients (AS) compared with control group significant abundance of Streptococcus Spp. was checked. In the comparable group (AS+AF) compared with control group significant leak of Eubacterium Rectale was checked.

The correlation analysis between components of gut microbiota and the clinical and laboratory characteristics

Table II. Gut microbiota composition changes in patients with AS and patients with AS and AF compared with control group, mean [95% CI], copy/ml

Characteristic /group	AS	AS+AF	CG	P1-2	P2-3	P1-3
Total bacterial mass	7,00E+13 [5,00E+13; 1,00E+14]	1,00E+14 [8,00E+13; 7,00E+14]	7,00E+11 [1,00E+11; 1,00E+12]	P>0,05	P<0,05	P<0,05
Lactobacillus Spp.	3,00E+06 [2,00E+06; 8,00E+06]	9,00E+05 [8,00E+05; 3,00E+06]	1,00E+08 [7,00E+07; 1,00E+08]	P>0,05	P<0,01	P<0,01
Bifidobacterium Spp.	8,00E+08 [5,00E+08; 1,00E+09]	1,00E+08 [6,00E+07; 3,00E+08]	6,00E+09 [1,00E+09; 3,00E+10]	P>0,05	P<0,01	P<0,05
Escherichia Coli	7,00E+06 [4,00E+06; 3,00E+07]	9,00E+06 [3,00E+06; 4,00E+07]	8,00E+06 [6,00E+06; 3,00E+07]	P>0,05	P>0,05	P>0,05
Bacteroides Spp.	3,50E+13 [1,00E+13; 6,00E+14]	9,00E+13 [7,00E+13; 3,00E+14]	7,00E+08 [1,00E+08; 1,00E+09]	P>0,05	P<0,01	P<0,01
Faecalibacterium Prausnitzii	2,50E+12 [1,00E+12; 8,00E+12]	8,00E+12 [4,00E+12; 5,00E+13]	1,00E+08 [1,00E+07; 6,00E+09]	P>0,05	P<0,05	P<0,05
Bacteroides Thetaiotaomicron	4,00E+10 [2,00E+10; 9,00E+10]	8,00E+09 [1,00E+09; 1,00E+10]	1,00E+09 [7,00E+07; 4,00E+09]	P>0,05	P>0,05	P>0,05
Akkermansia Muciniphila	7,00E+09 [6,00E+09; 8,00E+09]	4,00E+09 [5,00E+08; 9,00E+09]	1,00E+08 [6,00E+07; 2,00E+08]	P>0,05	P>0,05	P>0,05
Enterococcus Spp.	8,50E+05 [2,00E+05; 1,00E+06]	7,00E+05 [4,00E+05; 1,00E+06]	7,00E+05 [5,00E+05; 4,00E+07]	P>0,05	P>0,05	P>0,05
Blautia Spp.	4,00E+10 [5,00E+09; 7,00E+10]	4,00E+09 [7,00E+08; 8,00E+09]	3,00E+09 [1,00E+08; 8,00E+09]	P>0,05	P>0,05	P>0,05
Actinobacter Spp.	3,00E+07 [1,00E+07; 3,00E+08]	5,00E+09 [9,00E+08; 1,00E+10]	4,00E+05 [8,00E+04; 4,00E+05]	P<0,05	P<0,01	P<0,05
Streptococcus Spp.	6,00E+07 [9,00E+06; 1,00E+08]	8,00E+06 [6,00E+06; 4,00E+07]	8,00E+05 [1,00E+05; 1,00E+06]	P>0,05	P>0,05	P<0,01
Eubacterium Rectale	5,50E+07 [2,00E+06; 4,00E+08]	8,00E+06 [1,00E+06; 5,00E+07]	4,00E+08 [3,00E+08; 1,00E+10]	P>0,05	P<0,05	P>0,05
Roseburia Inulinivorans	5,00E+08 [8,00E+07; 1,00E+10]	6,00E+07 [3,00E+06; 5,00E+08]	3,00E+08 [8,00E+06; 5,00E+08]	P>0,05	P>0,05	P>0,05
Prevotella Spp.	7,50E+08 [2,00E+08; 4,00E+10]	7,00E+08 [2,00E+08; 3,00E+09]	3,00E+08 [1,00E+07; 6,00E+09]	P>0,05	P>0,05	P>0,05
Methanobrevibacter Smithii	4,00E+06 [1,00E+06; 7,00E+06]	3,00E+06 [1,00E+06; 7,00E+06]	5,00E+07 [1,00E+07; 3,00E+08]	P>0,05	P>0,05	P>0,05
Methanosphaera Stadmanae	9,50E+02 [0,00E+00; 1,00E+04]	1,00E+03 [9,00E+02; 4,00E+03]	8,00E+02 [0,00E+00; 4,00E+03]	P>0,05	P>0,05	P>0,05
Ruminococcus Spp.	5,00E+06 [8,00E+05; 2,00E+09]	9,00E+04 [8,00E+04; 1,00E+06]	9,00E+08 [1,00E+07; 1,00E+09]	P<0,05	P<0,01	P>0,05
Bacteroides Fragilis Group/ Faecalibacterium Prausnitzii	10,00 [8,75; 100]	11,25 [4,29; 35]	5,5 [0,12; 10]	P<0,05	P<0,05	P>0,05

Table III. Gut microbiota correlations with clinical and laboratory changes, P<0.05

Gut microbiota/ clinical and laboratory changes	Age (years)	BMI (kg/m²)	GFR (ml/min)	Total cholesterol (mmol/l)	Triglycerides (mmol/l)	LDL (mmol/l)	HDL (mmol/l)	TMA (mmol/l)	TMAO (mmol/l)
Total bacterial mass	++	++	0	+	0	+	-	+	+
Lactobacillus Spp.	-	--	0	-	-	--	+	--	--
Bifidobacterium Spp.	-	--	0	-	0	-	0	-	-
Escherichia Coli	0	0	0	0	0	+	-	0	0
Bacteroides Spp.	+	--	+	0	0	0	-	+	++
Faecalibacterium Prausnitzii	+	--	0	0	0	-	0	+	+
Bacteroides Thetaiotaomicron	0	0	0	0	0	0	0	0	0
Akkermansia Muciniphila	0	0	0	0	0	0	0	0	0
Enterococcus Spp.	0	0	0	0	0	0	0	0	++
Blautia Spp.	0	0	0	0	0	0	0	0	0
Actinobacter Spp.	+	+	+	0	+	+	-	+	+
Streptococcus Spp.	+	++	0	+	+	++	--	+	++
Eubacterium Rectale	-	0	-	-	0	--	++	--	-
Roseburia Inulinivorans	0	0	0	0	0	0	0	0	0
Prevotella Spp.	0	0	0	0	0	0	0	0	0
Methanobrevibacter Smithii	0	0	0	0	0	0	0	0	0
Methanosphaera Stadmanae	0	0	0	0	0	0	+	0	0
Ruminococcus Spp.	--	--	-	0	0	--	++	--	--

*Note: + – moderate positive correlation, 0.3< r<0.7; ++ – strong positive correlation, r>0.7; 0 – no significant correlations; - – moderate negative correlation, -0.3> r>-0.7; -- – strong negative correlation, r<-0.7

Table IV. TMA and TMAO plasma levels in patients with AS and patients with AS and AF compared with control group, mean ± standard error, mmol/l

Characteristic /group	AS	AS+AF	CG	P1-2	P2-3	P1-3
TMA	23,94±1,56	25,85±1,18	19,84±1,72	P>0,05	P<0,01	P<0,05
TMAO	2,64±0,19	4,22±0,30	2,13±0,22	P<0,01	P<0,01	P<0,05

of the examined groups was done. Spearman's correlation analysis was used to explore their correlations with species abundance. It was found that the most gut microbes enriched in the mean (AS) and comparable (AS+AF) groups had significant positive correlations with clinical and laboratory characteristics, whereas those enriched in control group had significant negative correlations with clinical and laboratory characteristics. All correlations are shown in the table 3. The largest amount of correlations was checked between gut microbiota composition and such clinical characteristics as TMAO (total number = 10), Age (total number = 9), TMA (total number = 9), BMI (total number = 8), LDL (total number = 9) and HDL (total number = 9) levels. The highest amount of correlations was between Lactobacillus Spp., Streptococcus Spp. and clinic-laboratory changes.

For analysis of gut microbiota metabolites changes plasma TMA and TMAO levels were investigated. Results of plasma TMA and TMAO levels in investigated patients are shown in table IV.

As we can see from the table 3 patients of the mean (AS) and comparable (AS+AF) groups the significant increasing of TMA ta TMAO plasma levels compared with control group were checked. Also, in patients of comparable group (AS+AF) compared with patients mean group (AS) the significant increasing of TMAO plasma level was revealed.

In our work, a correlation analysis was carried out between the levels of TMA and TMAO in the blood and the clinical and laboratory characteristics of the examined groups. Correlations were found between the level of TMA in blood plasma and the age of patients (r=0.584; p<0.01), BMI (r=0.351; p<0.01), LDL (r=0.320; p<0.05) and GFR (r=0.239; p<0.05). Also, blood plasma TMAO and age of patients (r=0.634; p<0.01), BMI (r=0.522; p<0.01), GFR (r=0.327; p<0.01), and more levels of HDL (r=-0.411; p<0.01) and K+ plasma levels (r=0.761; p<0.01).

DISCUSSION

AS - the formation of fibro fatty lesions in the artery wall. It is a multifocal, smoldering, immune inflammatory disease of medium-sized and large arteries fueled by lipids. Endothelial cells, leukocytes, and intimal smooth muscle cells are the major players in the development of this disease [12]. AF is the most common arrhythmia in the world. It is known connection between AF and AS occurrence, but no exact pathogenetic explanations of them has not still present. AF by itself is both the cause of atherosclerotic changes and their consequences. The incidence of AF in patients with AS is almost three times higher than in the general population. AF and AS have many common risk factors: hypertension, obesity, dyslipidemia, age, type 2 diabetes, sleep apnea, smoking and others. Also, the basic pathogenetic mechanisms underlying AS and AF are common: subclinical permanent inflammation and endothelial dysfunction [9]. Gut microbiota changes are potentially closely link with subclinical inflammation. Moreover, it is a evidenced cause of most metabolic disorders, which are known risk factors of AF and AS [4]. The gut microbiota is a promising target for the management or prevention of inflammatory and metabolic disorders in humans.

In our work we determine the composition of gut microbiota of healthy controls and patients with AS and AF or without it. In the bough investigated groups increased the total bacterial mass, Bacteroides Spp., Faecalibacterium Prausnitzii, Actinobacter Spp. and decreased Ruminococcus Spp. We compared our results with previous studies of gut microbiota composition in patients with AS [4-6] – they are not equal due to differences in their results. Decreased Ruminococcus Spp. are special for our group. It is known that Ruminococcus Spp. plays an important role in permeability features of intestinal membrane, that why their leak can be a cause of chronic inflammation [13].

We did not find the special data about gut microbiota composition in AS with AF. In our investigation patients with AS and AF were characterized by the significant increasing of Actinobacter Spp., Bacteroides Fragilis Group/ Faecalibacterium Prausnitzii ratio and decreasing Eubacterium Rectale, Ruminococcus Spp. Also, for this group was leak of Eubacterium Rectale compared with healthy controls. By the literature date Eubacterium Rectale plays the mean role in synthesis bytirates, which has probiotic effects and also can decrease the levels of proinflammatory cytokines [14]. For patients with AS without arrhythmia were significant abundance of Streptococcus Spp. compared with AS and AF group. By the literature data Streptococcus Spp. is the one of the mean bacteria that characterized atherosclerotic proses. It was found in atherosclerotic plaques, thrombus in case of myocardial infarction [15].

TMA and TMAO level were both increased in all investigated groups compared with controls, that is matches to previous literature data [1, 6, 8, 10]. Also, TMAO level was significantly higher in patients with AF comparing group without it. That can be explained of proinflammatory effects of TMAO and its ability to influence of lipid metabolism and processes of connecting tissue formation [10].

Correlations between gut microbiota components, its metabolites and clinical and laboratory patients' characteristics were checked and analyzed. The presence of strong negative connections between the TMAO plasma levels and Lactobacillus Spp., Ruminococcus Spp. and positive connections between the TMAO plasma levels and Bacteroides Spp., Enterococcus Spp., Streptococcus Spp. are interesting. It shows us what bacteria can be the cause of increasing TMAO through investigative groups.

CONCLUSIONS

Metagenomic analysis between the gut microbiota composition and its metabolites in patients with AS and AF was done in our study. We checked special bacterial changes of gut microbiota that are common for patients with AS and AF comparable with AS patients: increasing of Actinobacter Spp. and decreasing Eubacterium Rectale, Ruminococcus Spp., Bacteroides Fragilis Group/ Faecalibacterium Prausnitzii ratio was significantly higher. Also, TMAO plasma levels are increased significantly for patients with AS and AF comparable with AS patients. Connections between AS and AF with TMAO plasma levels are confirmed by reliable correlations between TMAO and age, BMI, GFR, HDL levels that are known risk factors of AS and AF. Moreover, special bacterial species, as Lactobacillus Spp., Actinobacter Spp., Streptococcus Spp., Eubacterium Rectale, Ruminococcus Spp. are closely connected with age, BMI, GFR, HDL, LDL, plasma TMA and TMAO levels. In further research, will be interesting to checked the ways for correction described gut microbiota composition and its metabolites changes and their influence at the risks of occurrence AF in AS patients.

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ORIGINAL ARTICLE

EVALUATION OF EFFICIENCY OF CHEWING IN PATIENTS WITH ONCOPATHOLOGY OF THE ORAL CAVITY

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ABSTRACT

The aim: The aim of this work is assessment of masticatory efficacy in patients with oral tumors with different localization and severity.

Materials and methods: The analysis of masticatory efficiency in dynamics was carried out with the help of two-color chewing gum in 29 patients with tumors of the tongue, oral mucosa and mandible. The study was performed at the time of hospitalization and on day 7 after surgery.

Results: After surgery, masticatory parameters decreased compared to baseline and did not recover on the 7th day after surgery. In patients diagnosed with stage I-III mandibular cancer, masticatory values are below 0.5 (0.34 ± 0.04; 0.28 ± 0.03; 0.24 ± 0.03), indicating poor food bolus formation ($p<0.05$).

Conclusions: This method is informative for the formation of the food bolus, which takes into account not only the presence of teeth, but also the function of the muscles involved in chewing. The extent of soft tissue defects plays a more important role in chewing than the presence of dentition and jaw defects. The presence of even 8-10 teeth on the upper and lower jaws may be sufficient for satisfactory grinding and chewing, with a mixed fraction of up to 0.75.

KEY WORDS: masticatory efficiency, defects of the oral cavity, tumors of the tongue, the bottom of the oral cavity, the jaw

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INTRODUCTION

Surgical treatment of patients with tumors of the oral cavity, regardless of its type (surgical, radiation, chemotherapy) is quite traumatic. This leads to damage to surrounding tissues, disruption of their functions - chewing and swallowing [1].

The function of chewing plays an important role in maintaining normal living conditions of the human body. To assess the function of chewing use the concept of «chewing efficiency». It evaluates the function of grinding and preparation of food for the swallowing stage [2]. It is the methods of determining masticatory efficiency that remain the most informative for assessing masticatory function depending on different degrees of severity [3]. Dysfunction of chewing leads to changes in many body systems, affects the general health of patients and quality of life.

Masticatory efficiency depends on various factors. Such factors may be the condition of the teeth, the integrity of the dentition, the intensity of occlusal surfaces of the teeth, the presence and type of dentures in the mouth, performance of masticatory muscles, the amount and viscosity of saliva, the nature of food, consistency, volume of postoperative defect [4].

There are a number of methods for static and dynamic measurement of masticatory efficiency. When using static methods, the coefficients of functional significance of each tooth are used. Dynamic methods involve direct chewing tests by SE. Gelman, IS. Rubinov, chewing effect by OM Ryahovsky [5]. Accordingly, the use of such direct methods is time-consuming but is not always justified. This has led to the development of indirect methods for evaluating the eficiency of chewing, including methods of computer evaluation of test material. With the application of the latest technical advances in the medical field, there appeared methods based on the analysis of occlusiograms processed in a certain way in computer programs - graphic editors, such as Adobe Photoshop, etc. [6]. Typically, these methods are also time consuming and do not always lead to an accurate result, as there is no clear correlation between the area of occlusal contact which they estimate and the value of masticatory efficiency, they do not take into account the function of the masticatory muscles either.

THE AIM

The aim of this work is improving the definition of masticatory efficiency in patients with oncopathology of the oral cavity before and after surgery.

MATERIALS AND METHODS

The study included 29 patients aged 38-55 years (men) with malignant tumors of the tongue, oral mucosa and cancer of law jaw who were treated in the Department of Head and Neck Tumors of the Podolsk Regional Oncology Center. Among them, there were 12 patients with cancer of the tongue, 9 - with cancer of the oral mucosa and 8 - with cancer of the law jaw (Table I). The diagnosis was

Table I. Distribution of patients depending on diagnosis and stage of disease, n=29

Stage of disease	Ca of the tongue (n)	Ca of the oral mucosa (n)	Ca of the law jaw (n)
I	6	2	2
II	4	4	4
III	2	3	2

Table II. Value of masticatory indices (MI) for 20 masticatory cycles n=29, M±m.

Diagnosis	Tongue cancer			Cancer of oral mucosa of mouth floor			Law jaw cancer		
Stage of disease	I	II	III	I	II	III	I	II	III
MI before surgery, k-means (M±m)	0,89±0,04	0,78±0.04	0,73±0,04	0,92±	0,87±0,04	0,82±0,04	0,76±0,04	0,72±0,04	0,69±0,03
MI 7 days after surgery, k-means (M±m.)	0,78±0,04	0,61±0,03	0,42±0,04	0,75±0,04	0,72±0,04	0,68±0,03	0,34±0,04	0,28±0,03	0,24±0,03

Note: * - Statistically significant difference before and after treatment ($p < 0.05$).

Fig. 1. Mixed gum with thickness of 1mm.

established on the basis of clinical data and additional research methods.

To study masticatory efficiency, a chewing test with Orophys Hue-chek gum (Switzerland) was used [6, 7]. The test has features that distinguish it from other methods, it provides universality, lower time costs, as well as ease of research and high individualization for each subject. All tests were performed with the consent of each subject and in accordance with the above principles.

The study was performed at the time of hospitalization and on day 7 after surgery.

The chewing test with Orophys Hue-chek gum (Switzerland) is based on mixing two-color gum in 20 chewing movements. Patients were allowed to change the chewing side during the test. Chewing movements were counted and patients were asked to stop during a certain chewing cycle.

Software evaluates each pixel in a specific area according to its properties, such as color, intensity, or texture, to distinguish it from neighboring areas.

According to the proposed computer analysis, the mean value is 0.5-0.9, the values less than 0.5 indicate poor mixing and food bolus formation, from 0.5 to 0.75 - moderate, from 0.75 to 0.9 - good and more than 0.9 - excellent mixing of gums and food bolus formation.

Statistical processing of the obtained data was performed using a mathematical statistical method on a PC using Excel software from Microsoft Office 2003, STATISTICA 5.5 (owned by of VNMU named after MI Pirogov, licensed № AXXR910A374605FA) according to Mann–Whitney U test. Differences between groups were considered statistically significant at $p<0.05$.

RESULTS

The gum is placed in a plastic bag and leveled to a thickness of 1 mm (Fig. 1). In the future, a color gradation scale (Fig. 2) or software that runs for 24 hours was used to assess masticatory efficiency.

The results of the analyzed image are automatically transmitted to Exel, where the mean value just for this image is specified (Fig. 3).

Images of samples were analyzed, a total of 29 images. For each patient, the mixed proportion of the two colors was calculated after 20 cycles of chewing (Table II).

Analyzing the obtained data, a decrease in masticatory indices in oncopathology of the oral cavity was noted, but all of them corresponded to good and moderate food bolus formation. Thus, in cancer of the tongue stage I-II, cancer of the oral mucsa of mouth floor stage II-III and cancer of the low jaw stage I before surgery, food bolus formation is good. In cancer of the tongue stage III and cancer of the low jaw stage II-III - mixing of the food bolus is moderate.

After surgery, masticatory indices decreased compared to baseline and did not recover on day 7 after surgery. In patients diagnosed with the law jaw cancer stage I-III, masticatory indices are below 0.5 (0.34 ± 0.04; 0.28 ± 0.03; 0.24 ± 0.03) which indicates poor food bolus formation ($p < 0.05$). This is due to the volume of postoperative defects, where the low jaw is resected together with the teeth. Patients with cancer of the tongue stage III on day 7

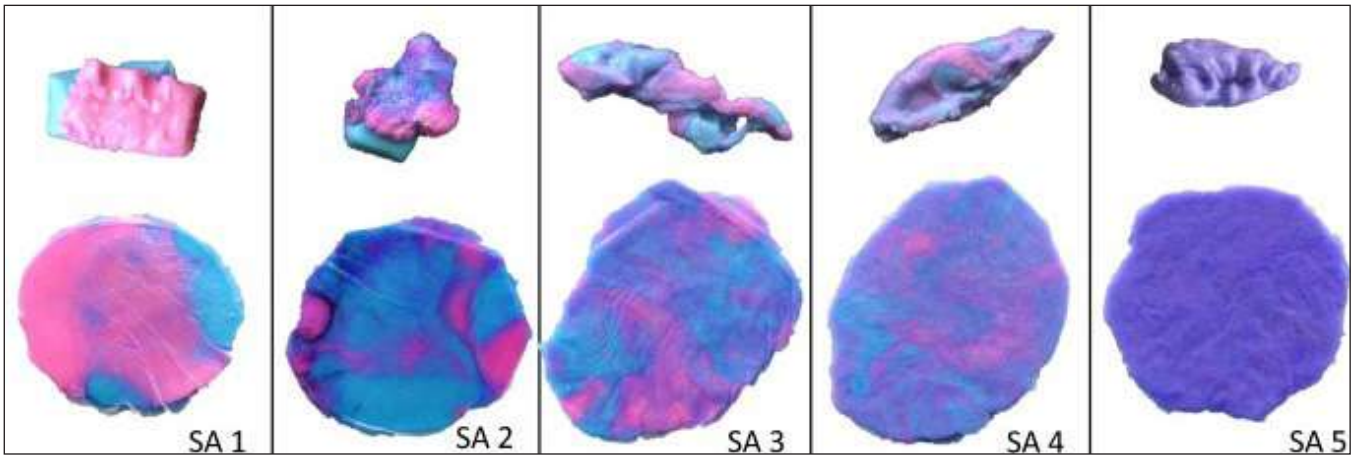

Fig. 2. Assessment Scale (SA) of chewing efficiency: SA 1 - chewing gum is not mixed, there are impressions of dental cusps; SA 2 - large parts of the chewing gum are not mixed; SA 3 – food bolus is slightly mixed, but the color is not uniform; SA 4 - food bolus is well mixed, but the color is not uniform; SA 5 – food bolus is perfectly mixed, color is uniform.

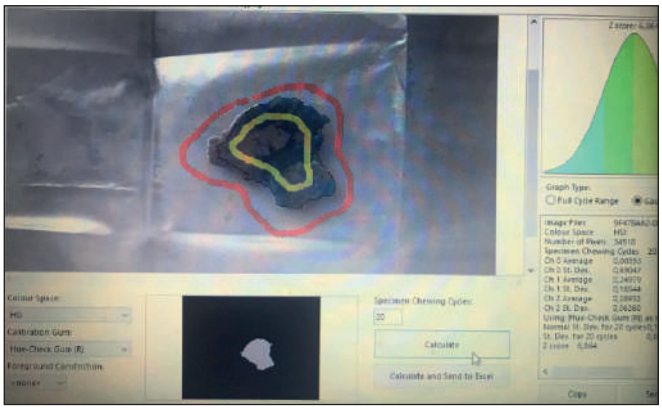

Fig. 3. Software study of mixed gum

after surgery were also unable to form a bolus, masticatory parameters being 0.42 ± 0.04, which corresponds to poor food bolus formation ($p < 0.05$). Patients with cancer of the tongue stage I and cancer of the oral mucosa of moth floor were able to mix chewing gum and the masticatory index corresponded to good food bolus formation (0.78 ± 0.04; 0.75 ± 0.04). Patients with cancer of the tongue stage II, cancer of the oral mucosa of mouth floor stage III did not completely mix chewing gum and masticatory indices corresponded to moderate bolus mixing (0.61 ± 0.03; 0.72 ± 0.04; 0.68 ± 0.03).

DISCUSSION

The study proposed and described a new method for assessing masticatory efficiency. Masticatory efficiency was evaluated by mixing two-color chewing gum for 20 chewing cycles. There are a number of methods for determining masticatory efficiency. However, all the proposed evaluation methods described in the literature require laboratory equipment, sieves, software for digital images. In addition, not all methods for determining masticatory efficiency can be used in patients with postoperative oral defects.

In our study, masticatory efficiency was assessed using k-means parameters of mixing different parts of the food bolus. Mixed areas were evaluated for each chewing gum plate. This segmentation system with using color information provided high resolution for the different areas present in each image, reducing errors caused by manual segmentation. In particular, this method expresses masticatory efficiency as the percentage of mixed areas, where masticatory efficiency 1 indicates optimal chewing, and masticatory efficiency 0 indicates its complete absence. Using the k-means clustering method, masticatory efficiency was assessed for each food bolus by evaluating mixed and unmixed sites. This clustering system can provide high resolution of the areas present in each image using color information. In addition, one can reduce errors caused by manual segmentation [2].

In general, the results of the study showed that the proposed software could objectively and automatically assess masticatory efficiency. However, this study has some limitations. Low quality images may affect the accuracy of the results of this study, which will not allow to make definitive statements about the control values of masticatory efficiency The algorithm is freely available, and the user only needs to download the food bolus image [8].

In addition, this method is informative as for the formation of the food bolus, it takes into account not only the presence of teeth, but also the function of the muscles involved in chewing. This is of importance for patients with postoperative defects of the oral cavity (incised jaw, incised muscles), as well as in the preoperative period, because all patients had reduced masticatory indices due to the presence of a tumor process.

CONCLUSIONS

1. This method is able to automatically quantify the percentage of mixed color area, providing quantitative data with minimal human interaction.
2. The method makes it possible to determine the change in masticatory parameters in the dynamics of patients with tumors of the oral cavity with different localization and varying degrees of importance.

3. The extent of soft tissue defects plays a more important role in chewing than the presence of dentition and jaw defects.
4. The presence of even 8-10 teeth on the upper and lower jaws may be sufficient for satisfactory grinding and chewing, with a mixed fraction of up to 0.75.

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ORIGINAL ARTICLE

EFFECT OF HIGH-FREQUENCY CHEST WALL OSCILLATION ON CLINICAL INDICES OF COMMUNITY-ACQUIRED PNEUMONIA IN CHILDREN

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ABSTRACT

The aim: To study the effect of high-frequency chest wall oscillation (HFCWO) on clinical indices of community-acquired pneumonia (CAP) in children.

Materials and methods: The main clinical symptoms were assessed in 107 children (girls - 45.79% and boys - 54.21%) aged 6 to 17 years with acute and uncomplicated course of CAP of moderate severity. The main group (MG) consisted of 55 children who were prescribed basic therapy (BT) in combination with HFCWO procedures. The control group (CG) comprised 52 children who received BT exclusively.

Results: In the children of MG, the intensity of cough decreased to 0.28 ± 0.06 points compared with children of CG - 0.5 ± 0.07 points ($p < 0.05$) on the 10th day of treatment. A positive dynamics of CAP in the form of the amount of sputum reduction was revealed in the MG children up to 0.06 ± 0.03 points compared with the CG children - 0.42 ± 0.07 ($p < 0.05$). On the 10th day of therapy the MG children with CAP had decrease in the number of râles in the lungs up to 0.08 ± 0.04 points compared with those of CG - 0.4 ± 0.07 points ($p < 0.05$).

Conclusions: High efficacy of HFCWO method in complex treatment of CAP in children is confirmed by the dynamics of the main clinical symptoms, such as reduction of intensity and productivity of cough as well as absence shortness of breath and moist râles in the lungs. The data obtained indicate recovery of mucociliary clearance (MCC) functions and the bronchopulmonary system as a whole.

KEY WORDS: high-frequency chest wall oscillation, community-acquired pneumonia, children

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INTRODUCTION

Pneumonia is one of the most common nosologies of the respiratory tract and remains the leading cause of morbidity and mortality in children worldwide, including Ukraine [1-4].

According to current data on the pathogenesis of pneumonia, effective airway protection is provided by MCC, which provides rehabilitation of the respiratory tract and necessary potential for the barrier, immune and cleansing function of the bronchopulmonary system [5-7].

MCC dysfunction leads to changes in the drainage function of the bronchial tree, which in turn leads to the manifestation of clinical symptoms in the form of cough, shortness of breath, infection and inflammation in children with pneumonia [8-10].

A modern method of restoring bronchial tree drainage is high-frequency chest wall oscillation based on the vibrational-compression effect of The Vest Airway Clearance System [11-14]. The Vest device has proven its effectiveness in the treatment of children with cystic fibrosis (CF) who had improvement of MCC of the airways [15-18].

THE AIM

The aim of the study was to investigate the effect of high-frequency chest wall oscillations on the clinical indices of community-acquired pneumonia in children.

MATERIALS AND METHODS

There were examined 107 children aged 6 to 17 years (11.73 ± 0.53 years) with community-acquired pneumonia of acute and uncomplicated moderate severity in the open, comparative clinical study who were treated in the pulmonology department of the Communal non-profit enterprise "Odessa Regional Children's Clinical Hospital" of Odessa regional council. The diagnosis of pneumonia was in conformity with modern standards for the diagnosis of pneumonia, was based on radiological confirmation of focal infiltrative process in the lungs and was established in accordance with the criteria approved by Order N 18 of the Ministry of Health of Ukraine [19]. The examined patients comprised 58 boys (54.21%) and 49 girls (45.79%) who were divided into groups depending on treatment. The main group (MG) consisted of 55 children (30 - boys and 25 - girls) who received basic therapy (BT) [19] with additional administration of HFCWO procedures using 1, 2, 3, 4, 5 and 6 modes of The Vest, model 105, (Hill-Rom, USA) [20]. The control group (CG) consisted of 52 children (25 boys and 27 girls) who were prescribed only basic therapy (BT).

The main clinical symptoms of pneumonia were evaluated on the 1st, 3rd, 5th, 7th and 10th days of inpatient treatment. The main clinical signs assessed included axillary body tem-

Table I. Comparative characteristics of body temperature in children during 10 days of treatment.

Days of treatment	Groups of children		p-value
	Основна група (n=55)	Контрольна група (n=52)	
1	1.08±0.06	1.1±0.06	0.91
3	body temperature was normalized	body temperature was normalized	-
5			
7			
10			

Table II. Comparative characteristics of symptoms of intoxication in children during 10 days of treatment.

Days of treatment	Groups of children		p-value
	Основна група (n=55)	Контрольна група (n=52)	
1	1.04±0.06	1.08±0.07	0.89
3	symptoms of intoxication disappeared	symptoms of intoxication disappeared	-
5			
7			
10			

Table III. Comparative characteristics of shortness of breath in children during 10 days of treatment.

Days of treatment	Groups of children		p-value
	Main group (n=55)	Control group (n=52)	
1	2.12±0.1	2.1±0.09	0.91
3	1.36±0.09*	1.94±0.09	0.001
5	0.72±0.08*	1.3±0.08	0.001
7	0.26±0.06	0.42±0.08	0.05
10	shortness of breath was not observed	shortness of breath was not observed	-

perature, manifestations of intoxication, shortness of breath at rest, intensity and productivity of cough, auscultatory changes in the lungs (the presence of moist râles).

The severity of these criteria was evaluated on a scale from 0 to 3 according to the generally accepted gradations, indicating an integer from 0 (absent), 1 (slightly expressed), 2 (moderately expressed) and up to 3 (significantly expressed) [21]. The data obtained were presented in the form of arithmetic mean (M) and standard error (m) using Microsoft Excel 2010, online calculator SISA (Simple Interactive Statistical Analysis). Comparative analysis between groups was performed on the basis of analysis of variance (ANOVA). Statistically significant indices were considered at $p < 0.05$.

The study was carried out in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the Local Ethics Committee of the institution. Informed consent of parents and children was obtained for the study.

RESULTS

According to the results of the study the body temperature in the MG children was estimated as 1.08 ± 0.06 points on the 1st day of treatment compared with those of CG - 1.1 ± 0.06 points (p=0.91). On the 3rd day of treatment, positive clinical dynamics was determined: the body temperature response was normalized in all children under study (Table I.).

In the children of MG the symptoms of intoxication were estimated as 1.04 ± 0.06 points on the 1st day of therapy compared with the CG children - 1.08 ± 0.07 points (p=0.89). On the 3rd day of therapy, the manifestations of intoxication syndrome disappeared in children of both study groups (Table II).

On the 1st day of monitoring shortness of breath was estimated as 2.12 ± 0.1 points in the MG children, compared with those of CG - 2.1 ± 0.09 points (p=0.91). On the 3rd day of treatment shortness of breath decreased to 1.36 ± 0.09 points in the MG children, compared with those of CG - 1.94 ± 0.09 points ($p < 0.001$). On the 5th day of therapy, positive clinical dynamics was revealed: dyspnea decreased in the children of MG to 0.72 ± 0.08 points compared with the children of CG - 1.3 ± 0.08 points ($p < 0.001$). On the 7th day of therapy, a decrease in dyspnea to 0.26 ± 0.06 points was noticed in the MG children compared to the children of CG - 0.42 ± 0.08 points ($p < 0.05$). All children did not have shortness of breath on the 10th day

Table IV. Comparative characteristics of intensity of cough intensity in children during 10 days of treatment.

Days of treatment	Groups of children		p-value
	Main group (n=55)	Control group (n=52)	
1	2.4±0.09	2.44±0.08	0.89
3	1.86±0.11*	2.26±0.1	0.01
5	1.38±0.09*	1.9±0.08	0.001
7	0.8±0.09*	1.08±0.08	0.01
10	0.28±0.06	0.5±0.07	0.05

Note: * - differences between MG and CG ($p < 0.05$).

Table V. Comparative characteristics of productivity of cough in children during 10 days of treatment.

Days of treatment	Groups of children		p-value
	Main group (n=55)	Control group (n=52)	
1	2.48±0.08	2.4±0.09	0.76
3	1.44±0.07*	2.08±0.09	0.001
5	0.94±0.09*	1.72±0.09	0.001
7	0.4±0.07*	0.92±0.06	0.001
10	0.06±0.03	0.42± 0.07	0.05

Note: * - differences between MG and CG ($p < 0.05$).

Table VI. Comparative characteristics of râles in children during 10 days of treatment.

Days of treatment	Groups of children		p-value
	Main group (n=55)	Control group (n=52)	
1	2.32±0.1	2.24±0.1	0.76
3	1.76±0.1*	2.06±0.1	0.01
5	1.22±0.11*	1.78± 0.08	0.001
7	0.58±0.09*	0.94±0.1	0.01
10	0.08±0.04	0.4± 0.07	0.05

Note: * - differences between MG and CG ($p < 0.05$).

of treatment (Table III) (Figure 1).

According to the results of the study, the severity of cough was estimated as 2.4 ± 0.09 points in the children of MG on the 1st day of monitoring compared with those of CG - 2.44 ± 0.08 points (p=0.89). On the 3rd day of therapy, the intensity of cough decreased to 1.86 ± 0.11 points in the children of MG compared with the CG children - 2.26 ± 0.1 points ($p<0.01$). On the 5th day of treatment cough decreased to 1.38 ± 0.09 points in the MG children compared with the children of CG - 1.9 ± 0.08 points ($p < 0.001$). It was found that the severity of cough in the children of MG decreased to 0.8 ± 0.09 points from the 7th day of therapy compared with the CG children - 1.08 ± 0.08 points ($p < 0.01$). On the 10th day of treatment, a positive dynamics of CAP as of a decrease in the intensity of cough in the MG children to 0.28 ± 0.06 points was noted compared with those of CG - 0.5 ± 0.07 points ($p < 0.05$) (Table IV) (Figure 2).

On the 1st day of treatment, the productivity of cough (sputum discharge) was estimated as 2.48 ± 0.08 points in the children of MG compared with the CG children - 2.4 ± 0.09 points (p=0.76). On the 3rd day of therapy, the amount of sputum in the children of MG decreased to 1.44 ± 0.07 points compared with those in CG - 2.08 ± 0.09 points ($p < 0.001$). From the 5th day of treatment, the improvement of clinical symptoms was revealed, namely in the children of MG the productivity of cough decreased to 0.94 ± 0.09 points compared with those of CG - 1.72 ± 0.09 points ($p < 0.001$). On the 7th day of therapy there was a decrease in the amount of sputum to 0.4 ± 0.07 points in the MG children compared with the CG children - 0.92 ± 0.06 points ($p < 0.001$). According to the results of dynamic monitoring, it was found that discharge of sputum decreased to 0.06 ± 0.03 points in the MG children on the 10th day of treatment compared with the CG children - 0.42 ± 0.07 points ($p < 0.05$) (Table V) (Figure 3).

According to the results of the study, the number of râles in the lungs was estimated as 2.32 ± 0.1 points on the 1st day of monitoring in the MG children compared with the children of CG - 2.24 ± 0.1 points (p=0.76). On the 3rd day of treatment, the number of râles in the lungs decreased to 1.76 ± 0.1 points

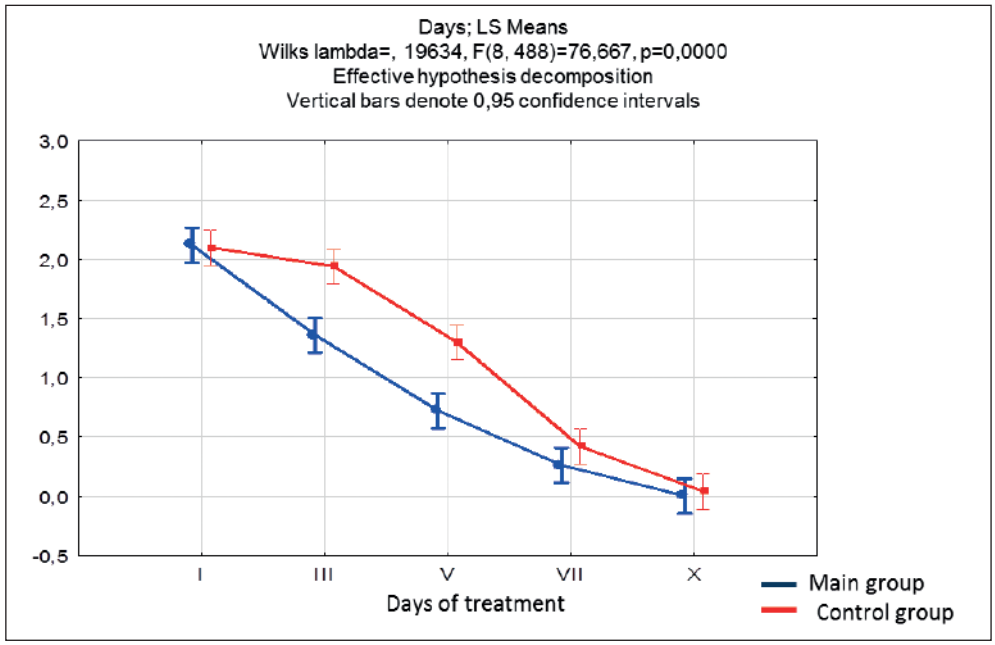

Fig. 1. Shortness of breath in the children during 10 days of treatment

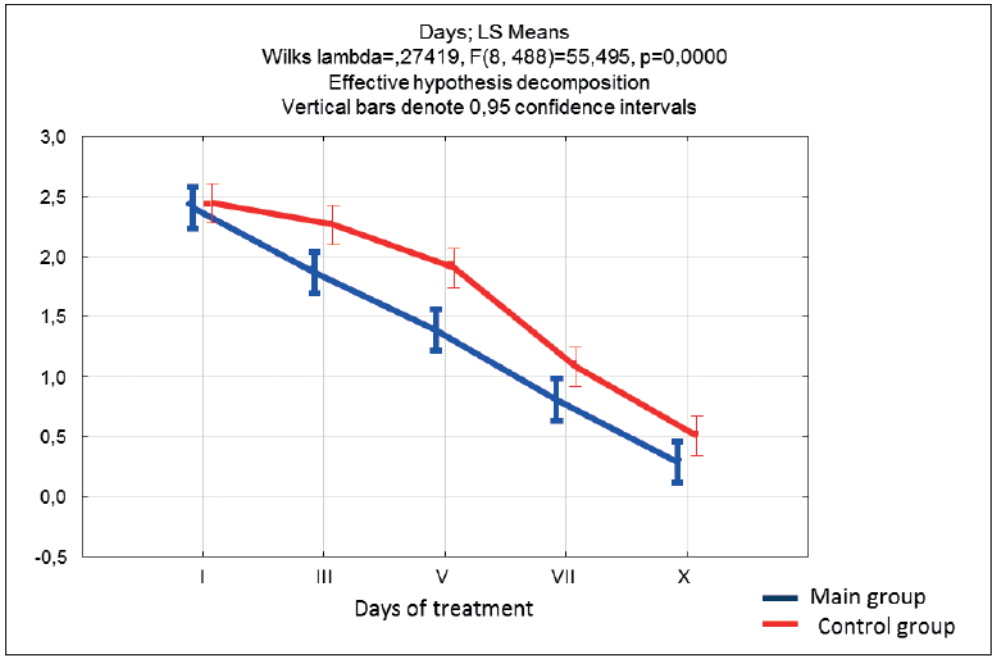

Fig. 2. Intensity of cough in the children during 10 days of treatment

in the MG children in comparison with the children of CG - 2.06 ± 0.1 points ($p < 0.01$). On the 5th day of therapy there was a decrease in the number of râles to 1.22 ± 0.11 points in the MG children compared with those of CG - 1.78 ± 0.08 points ($p < 0.001$). On the 7th day of treatment the number of râles in the lungs decreased to 0.58 ± 0.09 points in the MG children compared with the CG children - 0.94 ± 0.1 points ($p < 0.01$). On the 10th day of therapy, the positive dynamics of CAP in the MG children was proved by reducing the number of râles in the lungs to 0.08 ± 0.04 points compared with the children of CG - 0.4 ± 0.07 points ($p < 0.05$) (Table VI) (Figure 4).

DISCUSSION

Pneumonia is one of the most common diseases of the respiratory system in children and remains an important medical and social problem of modern pediatrics. Insufficient effectiveness of the applied methods of drug therapy is stated, which is confirmed by a constant tendency to a steady increase in the number of children with pneumonia [1,4].

Effective solution of the problem of basic treatment of CAP in children is associated with the development of new methods of non-drug therapy. One such method is high-frequency chest wall oscillation using the airway cleaning system The Vest Airway Clearance System [11,13,15]. The influence of The Vest device on the respiratory tract is achieved due to the oscillation parameters - vibration frequency, pressure, duration of the procedure, which the modes of HFCWO form [20].

The HFCWO system is a modern, highly effective and easily feasible method of the bronchial tree drainage, which aims to stimulate the clearance of the respiratory tract, improve lung ventilation and pulmonary gas exchange [9].

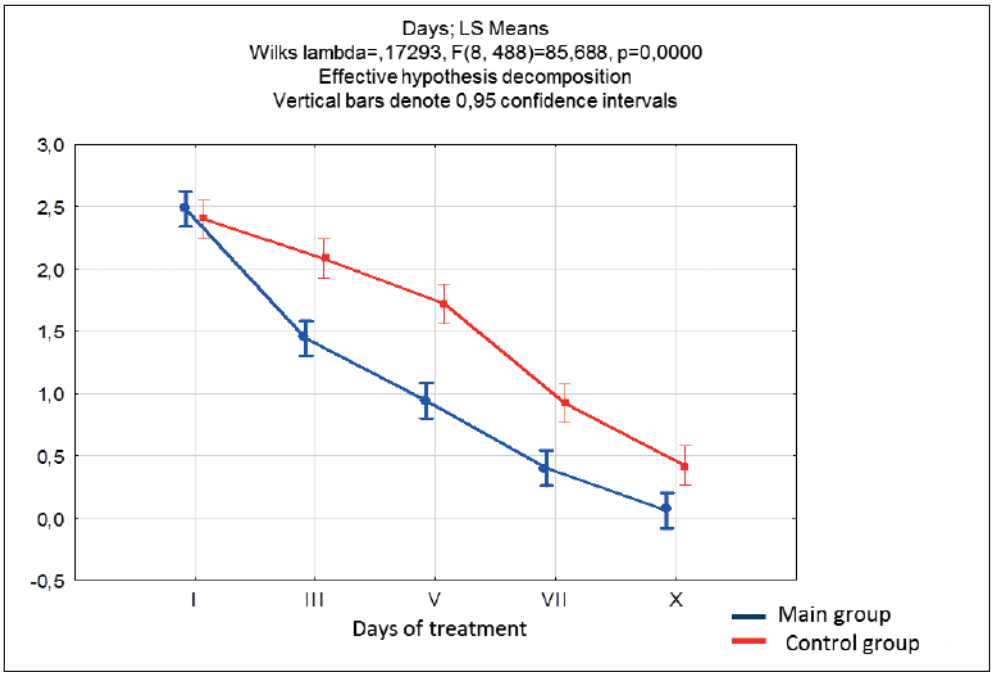

Fig.3. Productivity of cough in the children during 10-day treatment

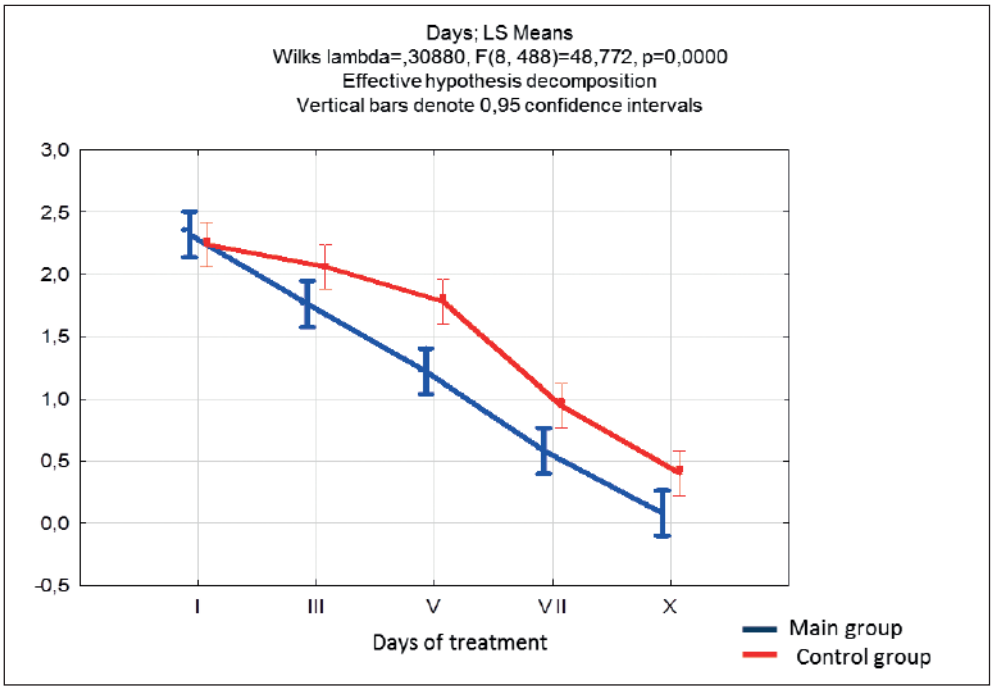

Fig.4. Râles in the children during 10 days of treatment

Thus, in order to optimize the complex therapy of CAP in children, it is necessary to include the method of airway cleansing, namely HFCWO in the daily therapy by using the airway cleansing system The Vest Airway Clearance System. Based on the study, the data obtained confirm the beneficial effect of the HFCWO method on the positive dynamics of clinical symptoms of pneumonia in the form of a decrease in the intensity and productivity of cough, the number of râles and shortness of breath.

CONCLUSIONS

1. Bronchial tree drainage procedures based on HFCWO with the use of innovative and modern airway cleaning system The Vest Airway Clearance System increase the effectiveness of the basic CAP therapy in children.
2. High efficacy of HFCWO as a part of complex treatment of CAP in children is confirmed by positive dynamics of clinical symptoms in the form of decrease in the amount of sputum by 2.42 ± 0.45 points in the children of MG, in comparison with the control group children – 1.98 ± 0.61 points, reduction of cough intensity by 2.12 ± 0.52 points compared with the control group of children - 1.94 ± 0.68 points, the number of râles in the lungs by 2.24 ± 0.48 points compared with children in the control group – 1.84 ± 0.61 points, shortness of breath by 2.12 ± 0.51 points compared with the control group of children - 2.1 ± 0.50 points.
3. The results of studies indicate the feasibility of prescrib-

ing oscillation procedures in the daily comprehensive treatment of CAP, which improves drainage function of the bronchial glands, improves pulmonary gas exchange, which in turn leads to rapid recovery of the respiratory system in children.

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Conflict of interest:

The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

THE RISK OF ACUTE KIDNEY INJURY AFTER ELECTIVE VERSUS EMERGENCY CORONARY INTERVENTION

DOI: 10.36740/WLek202212120

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ABSTRACT

The aim: A serious and common complication after percutaneous coronary intervention is acute kidney injury, which is associated with an increased risk of renal, cardiovascular and even mortality; therefore, early prognosis and identification of patients at higher risk are essential for early initiation of preventive measures. The aim of this study is to predict and compare the risk for the development of CI-AKI in patient with ACS who undergo emergency PCI or elective (i.e. after medical stabilization) PCI by utilizing the sensitivity of serum NGAL as an early and reliable predictor for CI-AK.

Material and Methods: The study include 37 patients with acute coronary syndrome, baseline serum creatinine, complete blood count and pre and two hours post operative serum neutrophil gelatinase-associated lipocalin were measured and all patients underwent percutaneous coronary intervention according to the standard protocol used in Al Najaf Cardiac Center.

Results: This is a Two-Arm study that included a total of 37 patients with acute coronary syndrome aged 38-83 years. Eighteen of them had emergency percutaneous coronary intervention while the remaining 19 had elective percutaneous coronary intervention (after medical stabilization). Elevation of serum neutrophil gelatinase-associated lipocalin level two hours after percutaneous coronary intervention was found to be significantly higher among emergency percutaneous coronary intervention group compared to elective group.

Conclusion: Acute coronary syndrome patients are undergoing emergency percutaneous coronary intervention are at an increasing risk for the development of contrast induced acute kidney injury than those undergoing elective percutaneous coronary intervention.

KEY WORDS: acute kidney injury, emergency coronary intervention, percutaneous coronary intervention

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INTRODUCTION

Acute kidney injury (AKI) is the new consensus term for acute renal failure (ARF), which describes the clinical syndrome in which an abrupt (hours to days) decrease in renal function leads to the accumulation of nitrogenous and other waste products normally cleared by the kidneys and, commonly, a reduction in urine output [1-3]. Several classification systems have been developed to simplify research and clinical practice with respect to AKI [4-6]. The Acute Dialysis Quality Initiative first defined AKI with the RIFLE criteria. The Acute Kidney Injury Network (AKIN) later supported the RIFLE criteria with minor modifications. In 2012, the validity of this information was confirmed on large samples of patients, and through that, the basics of guidance for kidney patients were identified, including improving KDIGO global results, including the stages of risk. We can define acute kidney injury as serum creatinine of 0.3 mg/dL or greater within 48 hours of observation or 1.5 times from baseline or more, which is known or assumed to have occurred within seven days, or a decrease in the volume of Urine less than 0.5 ml/kg/hour for six hours [1]. We would like to point out that in all the three classification systems mentioned above, patients should be classified according to criteria that lead to the highest (i.e., the most severe) stage of infection. The causes that lead to acute renal failure have been broadly divided into three categories: primary blood nitrogen. The former is renal, the second is intrinsic renal parenchymal injury and the third and final is post-renal obstruction [7-9]. We can say that AKI due to contrast iodinated administration is contrast-induced nephropathy (CIN) or contrast-induced acute kidney injury (CI-AKI) and contrast-induced nephropathy is a common complication after percutaneous coronary intervention (PCI) or imaging Coronary angiogenesis (CAG) [10] is the third leading cause of hospital-acquired acute kidney injury [11] and its incidence ranges from <1% in patients without comorbidities to more than 30% in high-risk populations [12-13]. Moreover, it prolongs hospitalization, increases hospital care expenditures, and, in some cases, can increase cardiovascular and renal disease [14] and all-cause mortality [15-16]. To standardize the definition of CIN, the acute kidney injury network requires, in order to diagnose post-contrast AKI, at least 1 of 3 cases completed within 48 hours after contrast media application: (1) an absolute increase in SCr of 0.3 mg/dL from baseline, (2) a relative increase in SCr levels by 50%

of baseline, or (3) urine output decreased to 0.5 mL/kg/hr for at least 6 hours in the absence of alternative causes of acute kidney injury [17]. Iodinated contrast agents promote nephrotoxicity by two major mechanisms: (1) vasoconstriction and ischemic injury, and (2) direct toxicity to renal tubular cells [18]. The strongest risk factor for the development of CIN is the pre-existing chronic kidney disease [19]. Other independent risk factors include increasing age (age >75 years), diabetes mellitus, peripheral vascular disease, advanced cardiac failure (NYHA class III,VI), anemia, use of non iso-osmolar contrast agents, high or repetitive doses of radio contrast agent, concomitant exposure to other nephrotoxins, hypotension and dehydration [20-23]. There are no current specific therapy for CIN and the dealing with this condition rely wholly on the very few available strategies that have been proven effective for prevention of CIN (the most important of which are pre- and post-hydration with intravenous fluids and choice of contrast agent) [24-25]. Therefore, early identification of patients at high risk of CIN is important in order to guide prompt therapeutic decisions and improve outcomes. Although currently the diagnosis of CIN relies mainly on serum creatinine, it has many limitations. Serum creatinine can vary with age, gender, race, muscle mass, diet and certain drugs [26], and its rate of change after the initial insult is low [27]. These limitations make SCr concentration an unreliable and inadequate biomarker in diagnosing or predicting CIN [28]. In light of this inadequacy and given that most subjects undergoing PCI are typically discharged within 24 hours and only occasionally after 48 hours, there is a compelling need for a real-time, specific renal biomarker that will allow early prediction of CIN. One of the first and most extensively studied biomarkers for AKI to emerge was Neutrophil gelatinase–associated Lipocalin (NGAL), also known as human Neutrophil Lipocalin or Lipocalin 2 [29]. NGAL is a 25 kDa protein produced by Neutrophils, binds and traffics free iron [30]. It is expressed at low concentrations in several tissues, including the kidney [30]. NGAL is significantly expressed in renal tubular epithelial cells in response to nephrotoxicity or ischemic insults, and easily detected in the blood or urine [31]. Many studies including meta-analysis have been confirmed the sensitivity of blood or urinary NGAL as an early biomarker for prediction of CIN after PCI [32-36].

THE AIM

The aim of this study is to predict and compare the risk for the development of CI-AKI in patient with ACS who undergo emergency PCI or elective (i.e. after medical stabilization) PCI by utilizing the sensitivity of serum NGAL as an early and reliable predictor for CI-AKI.

MATERIALS AND METHODS

STUDY DESIGN

A Two-Arm study that conducted at Al-Najaf Center for Cardiac Surgery and Transcatheter Intervention during the periods from February to April 2019

ETHICAL AND LEGAL ISSUES

The study was conducted after permission from the scientific council of Arab board for health specialization and Al-Najaf Center for Cardiac Surgery and Transcatheter Intervention with an informed consent have been taken from the patients.

INCLUSION CRITERIA

- Patients with ACS (STEMI, Non-STEMI and UA) who underwent PCI
- Aged >18 years

EXCLUSION CRITERIA

- CKD Patients (eGFR <60 mL/min/1.73 m^2)
- Anemia Patients with anemia (Hb < 11.5 g/dl in male, Hb < 11 g/dl in female)
- Sepsis (fever, WBC count > 12,000/μL)
- Hemodynamic instability (BP < 90/60 mmHg)
- Iodinated contrast exposure within the last 2 weeks
- Patients with active cancer

DATA COLLECTION

Demographic information such as age, gender, blood pressure, past medical history (DM, HTN, heart failure) and drug history was recorded, as well as the volume and type of iodinated contrast used in the procedure.

A 43 ACS patients who underwent PCI was evaluated, among them six patients were excluded from the study (two with eGFR <60 mL/min/1.73 m^2, one with Hb of 9 g/dl, one with WBC count of 13.500/μL and two with BP <90/60 mmHg) and the remaining 37 patients were enrolled, their age ranged from 38-83 years, 27 of them are males and 10 are females. Eligible patient were divided into two groups: Group A (emergency PCI group) consist of 18 patients who had emergency PCI, and Group B (elective PCI group) consist of 19 patients who had elective PCI after medical stabilization. Serum NGAL was evaluated in blood samples taken pre and 2 hours post PCI, centrifuged at 2000 RPM for 20 minutes, then the supernatant aliquoted in sterile tubes and stored at -20°C until assayed. Serum NGAL level was measured at Bielsa-reader ELX800 device using the Human Neutrophil gelatinase–associated Lipocalin ELISA kit (bioassay technology laboratory, Cat. No.E1719Hu). Also patients send on admission for CBC (to exclude anemia and sepsis) and serum creatinine (to estimate GFR). SCr is measured by DRI-CHEM NX500i FUJIFILM device using CRE-P III kit (FUJIFILM, CODE: 1750 LOT: 296009), eGFR calculated by using CKD-EPI Collaboration Equation [36] where:

$$eGFR = 141 \times \min(SCr/\kappa, 1)^{\alpha} \times \max(SCr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018 \text{ [if female]} \times 1.159 \text{ [if black]}$$

α=-0.329 for females, -0.411 for males; κ = 0.7 for females, 0.9 for males; min = minimum of SCr/κ or 1; max = maximum of SCr/κ or 1. All patients underwent PCI according to the standard protocol used in Al Najaf Cardiac Center

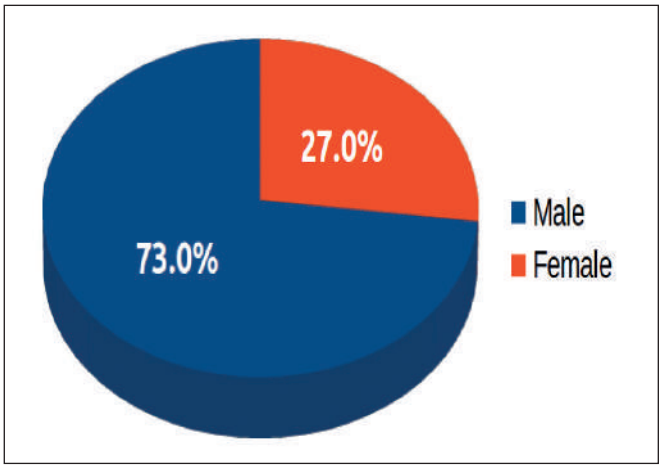

Fig. 1. Gender distribution among study population.

without significant complications and the contrast agent used in all procedures was Ultravist® 370 (Iopromide) injection, 370 mg Iodine per mL, it has osmolality of 774 m Osmol per kg of water which is hypertonic compared to plasma (approximately 2.7 times the osmolality of plasma).

STATISTICAL ANALYSIS

The software used in the present study was SPSS® (version 23.0 for Linux®), qualitative data are used, and meanwhile a continuous numerical data are presented as mean standard deviation. Comparisons of study groups performed by using chi-square test and using Student's t-test for continuous data. Correlations assessed by using Pearson's product-moment correlation coefficient. P value of < 0.05 considered as statistically significant.

RESULTS

A 37 patients with ACS who underwent percutaneous coronary intervention (PCI), the patients divided into two groups: Group A consist of 18 patients who had emergency PCI, and Group B consist of 19 patients who had elective PCI (after medical stabilization).

Study age of participants ranged was between 38 and 83 years, mean age of 60.7±11.4 and median of 62, no statistically significant difference was found between the two study groups regarding age, P-value = 0.157. Table I compares the ages of the two study groups in details.

Regarding gender, males formed 72.97% of the total study population while females formed 27.03% as illustrated in figure 1, no significant difference was observed between Group A (emergency) and Group B (elective) in regard of gender, chi-square=1.91, P-value=0.167, as detailed in table II.

Medical history and medication history of the study participants are compared between the two study groups in details in table IV. Hypertension was found to be significantly higher in Group B patients, and proportion of patients in Group B who used renin-angiotensin-aldosterone system (RAAS) inhibitor was significantly higher than those in Group A. No significant difference was observed in any of the remaining variables (table 3).

Comparison between the two study groups regarding serum NGAL level performed by using of Student's t-test. Pre-PCI level of NGAL had no significant difference between the two study groups, P-value=0.683 (Table IV), however, post-PCI level of serum NGAL was significantly higher in Group A (cases with emergency intervention) than in Group B, with a mean difference of 82.41, P-value = 0.046 (Table V).

Mean elevation in serum NGAL level between emergency and elective groups was found to be statistically significant, with P-value = 0.025. Comparison of the change in serum NGAL level during PCI is performed for both the emergency and elective groups using paired samples t-test. There was significant increase in serum NGAL level between pre-PCI status and post-PCI status for each of the two study groups as well as for the entire study population as a single group. Mean elevation in serum NGAL level in emergency group was found to be 110.83 while in elective group it was found to be 33.45. Overall mean elevation in serum NGAL level in the entire study population was found to be 71.09. Table VI describes the findings in details.

Furthermore, estimated glomerular filtration rate (eGFR) was also significantly higher in Group A (emergency) when compared to group B (elective), with P-value = 0.004. Table VII details the findings.

Pearson's product-moment correlation coefficient was utilized to assess the correlation between contrast volume and the elevation in serum NGAL level for each of the two study groups. In emergency PCI group, no significant correlation was observed between contrast volume and elevation of serum NGAL level, with correlation coefficient (R)=0.299 and P-value of 0.228. Similarly for the controls group, no significant correlation was observed, with R=-0.411, P-value=0.081. Table VIII summarize these findings.

Elevation in serum NGAL level was also compared to certain clinical variables in order to identify factors related to its elevation. No significant relationship was observed between mean elevation in serum NGAL and each of diabetes mellitus, hypertension, heart failure, RAAS inhibitor intake, or nephrotoxicity medications intake; P-value=0.133, 0.258, 0.214, 0.939, and 0.417, respectively (Table IX describes the comparisons in details).

DISCUSSION

This study compare the risk for the development of CI-AKI in patients with ACS who undergo emergency PCI versus those who undergo elective PCI (i.e. after medical stabilization) by utilizing serum NGAL as an early predictor for the development of CI-AKI, since the reliance on serum creatinine in the diagnosis or prediction of AKI has many limitations, most important of them are the facts that sCr in AKI will start to increase up to 72 hours after the initial renal insults. Moreover, kidney injury can presents without changes in sCr because of renal reserve or tubular secretion of creatinine; this means that changes in sCr will underestimate the true fall in eGFR [27]. The study

Table I. Age characteristics of the study groups.

Group	Age (years)			P-value
	Mean ± SD	Median	Range	
Group A (cases)	58.0 ± 11.3	58.5	38 – 80	0.157
Group B (controls)	63.3 ± 11.1	67.0	38 – 83	
Total	60.7 ± 11.4	62.0	38 – 83	

Student's t-test = 1.45, P-value = 0.157

Table II. Comparison of study groups regarding gender.

Group	Gender		Total	P-value
	Male	Female		
Group A (emergency)	15(83.33%)	3(16.67%)	18(100%)	0.167
Group B (elective)	12(63.16%)	7(36.84%)	19(100%)	
Total	27(72.97%)	10(27.03%)	37(100%)	

Table III. Comparison of medical and medication history between the study groups.

Variables Group A (Emergency)		Group Group B (Elective)		Total	P-value
Diabetes Mellitus	No	10 (55.56%)	10 (52.63%)	20(54.05%)	0.858
	Yes	8 (44.44%)	9 (47.37%)	17(45.95%)	
Hypertension	No	11 (61.11%)	4 (21.05%)	15(40.54%)	0.013
	Yes	7 (38.89%)	15 (78.95%)	22(59.46%)	
Heart Failure	No	16 (88.89%)	16 (84.21%)	32(86.49%)	0.677
	Yes	2 (11.11%)	3 (15.79%)	5(13.51%)	
RAAS inhibitor intake	No	16 (88.89%)	10 (52.63%)	26(70.27%)	0.016
	Yes	2 (11.11%)	9 (47.37%)	11(29.73%)	
Nephrotoxicity medication* use	No	16 (88.89%)	18 (94.74%)	34(91.89%)	0.512
	Yes	2 (11.11%)	1 (5.26%)	3(8.11%)	

*nephrotoxicity medication includes non-steroidal anti-inflammatory drugs, amino glycosides and calcineurin inhibitors.

Table IV. Pre-PCI serum NGAL levels.

Group	Serum NGAL level (µg/L) Mean ± SD	P-value
Group A (emergency)	49.82 ± 42.83	0.683
Group B (elective)	44.79 ± 30.87	
Total	47.24 ± 36.73	

Student's t-test = 0.45, P-value = 0.683

Table V. Post- PCI serum NGAL levels.

Group	NGAL level (µg/L) Mean ± SD	P-value
Group A (emergency)	160.65 ± 160.43	0.046
Group B (elective)	78.24 ± 28.98	
Total	118.33 ± 119.65	

Student's t-test = 2.15, P-value = 0.046

included two groups that had no significant differences in age or gender, making the two groups comparable. The results had demonstrated a significant increase in the serum Neutrophil gelatinase associated Lipocalin (NGAL) level two hours after performing percutaneous coronary intervention (PCI) for both groups: group A (emergency PCI group) and group B (elective PCI group). This increase had occurred despite having relatively low values before

Table VI. Serum NGAL level before and after PCI.

Group	Serum NGAL level (µg/L)			P-value
	Before PCI	2 hours after PCI	Mean difference	
Group A (emergency)	49.82 ± 42.83	160.65 ± 160.43	110.83	0.002
Group B (elective)	44.79 ± 30.87	78.24 ± 28.98	33.45	< 0.001
Total	47.24 ± 36.73	118.33 ± 119.65	71.09	< 0.001

Table VII. eGFR levels of study groups.

Group	eGFR(mL/min/1.73 m2) Mean ± SD	P-value
Group A (emergency)	99.61 ± 12.06	0.004
Group B (elective)	82.05 ± 21.15	
Total	90.59 ± 19.27	

Student's t-test = 3.12, P-value = 0.004

Table VIII. Correlation between contrast volume and elevation of serum NGAL.

Group	Correlation Coefficient (R)	Coefficient of Determination (R2)	P-value
Emergency PCI	0.299	0.089	0.228
Elective PCI	-0.411	0.169	0.081
Total	0.155	0.024	0.360

Table IX. Comparison between elevation in NGAL and certain variables.

Variables		Elevation in serum NGAL		P-value
		Mean	Standard Deviation	
Diabetes Mellitus	No	45.90	29.89	0.133
	Yes	100.74	140.81	
Hypertension	No	93.97	131.91	0.258
	Yes	55.50	70.73	
Heart Failure	No	62.92	88.40	0.214
	Yes	123.44	161.07	
RAAS inhibitor intake	No	70.26	104.48	0.939
	Yes	73.07	94.29	
Nephrotoxicity medications* use	No	75.14	103.65	0.417
	Yes	25.27	15.79	

PCI. Group A (emergency PCI) had a mean serum NGAL level of 160.65 after 2 hours, with a mean elevation of 110.83. While group B (elective PCI) patients had a mean serum NGAL level of 78.24 with mean elevation of only 33.45. These findings are consistent with the findings by Bachorzewska-Gajewska et al. in their study on 35 consecutive patients, which revealed a significantly elevated NGAL level in serum 2 hours after performing PCI, with a mean of 128.88 after two hours [37]. Mean elevation in serum NGAL level was found to be significantly higher in group A (emergency PCI) compared with group B (elective PCI). This suggests that emergency PCI is associated with higher increase in NGAL level, which in turn reflects higher renal damage in emergency cases compared to elective cases. Nusca et al. concluded in their study that serum NGAL measurement after PCI has significant importance in the prediction of contrast-induced renal impairment [38]. Valero et al. performed a study on 105 patients with acute coronary syndrome who underwent coronary angiography [39]. This study revealed that serum NGAL level is a useful predictor of changes in serum creatinine of patients with acute coronary syndrome, and can detect cases with contrast-induced acute kidney injury even when no prominent symptoms are present An interesting point to note is that group A (emergency PCI group) had significantly higher level of estimated glomerular filtration rate (eGFR) when compared to group B (elective PCI group), which reflects a better renal function among those cases. However, after PCI, group A patients had significantly higher serum NGAL level compared to group B patients, reflecting more renal impairment which mean that despite the initially higher renal function indicated by eGFR; there had been

a significant rise in NGAL, reflecting a possibly higher rate of renal damage and impairment. The same thing applied to the fact that the prevalence of hypertension and the use of RAAS inhibitor (which is risk factor for AKI) are more in the elective PCI group, their mean raise in serum NGAL level is less compared with the emergency PCI group, reflecting the higher risk of renal damage in the emergency PCI group. Assessment of correlation between contrast volume and mean elevation in serum NGAL revealed no significant correlations, neither in the emergency group nor in the elective group. Bachorzewska-Gajewska et al. described a similar finding in their study, and reported that serum NGAL level 2 hours after PCI had no significant correlation with contrast volume [37], also Alharazy et al have concluded in their study that the volume of contrast media was not associated with the development of CIN [40], however, Shaker et al. had observed in their study that NGAL level in serum was positively correlated to the volume of contrast 4 hours after PCI [41]. This may reflect a prolonged and gradual effect of contrast volume on NGAL level which may not be evident early after the procedure. Similarly, comparison of mean elevation of serum NGAL did not reveal any relationship with diabetes mellitus, hypertension, heart failure, intake of RAAS inhibitors, or intake of nephrotoxicity medications. Goo et al. had found that contrast induced acute kidney injury in patients undergoing coronary angiography is significantly higher in those treated with RAAS blockers [42]. These contrasting results may be due to the early measurement in the present study of serum NGAL level (2 hours post-PCI) while Goo et al. used a retrospective approach. Regarding nephrotoxicity medications; the non-significant finding in the present study cannot be reliably considered for clinical consideration, since the number of patients who had been taking nephrotoxicity medications is small (only three patients), therefore a larger study sample is required in order to perform reliable and accurate statistical analysis. Malyszko et al assessed the impact of diabetes mellitus in patients with normal baseline renal function undergoing angiography [43]. Interestingly they found similar rates of CI-AK in patient with diabetes and in patient without diabetes (14% *vs.* 10%) which is compatible with our results. Regarding the non significant relationship between the presence of heart failure and mean raise in serum NGAL, this is justified by the point that only one of the heart failure patients in our group has NYHA class III which regard as risk factor for CI-AKI so it is statistically no significant. Helanova et al. had also described in their prospective cohort study that serum NGAL level elevation after primary PCI is an indicator of possible future renal damage, and that this elevation reflects higher risk for mortality in patients of acute coronary syndrome undergoing PCI [44]. Serum NGAL level 2 hours after PCI is reported to be adequate predictor for acute kidney injury [45], and increased level of serum NGAL even without having positive creatinine-based criteria of acute kidney injury may reflect a higher risk of renal complications, which may necessitate renal replacement therapy, and may also be associated with higher mortality [38]. To sum up the findings in the study; emergency PCI was found to have significantly higher risk for renal damage compared to elective PCI as indicated by the elevation in serum NGAL level. This is supported by the fact described by Yang et al. who mentioned that primary PCI poses higher risk for contrast induced acute kidney injury when compared to elective PCI, possibly due to hemodynamic instability and inadequate renal prophylactic therapy [46].

CONCLUSION

Patients with acute coronary syndrome undergoing emergency PCI are at an increasing risk for the development of CI-AKI than those undergoing elective PCI. So preventive strategies should be instituted to those patients to decrease that risk as possible, which is summarized by the followings:

- Using iso-osmolar contrast media in patients undergoing emergency PCI instead of the used hypo-osmolar media
- Reducing the volume of contrast used in patients undergoing emergency PCI to the least volume possible
- Using prophylactic measures (for example intravenous saline infusion peri-operatively) for high risk patients
- Patients undergoing emergency PCI and especially those with high risk for the development of CI-AKI should have their serum creatinine measured at least 48 hours post procedure.

LIMITATIONS AND RECOMMENDATIONS

Many limitations in our study have been found and warrant mention. First, our study was not powered to detect the exact occurrence of CI-AKI as we don't have the facilities to measure serum creatinine 48-72 hours post PCI (majority of the patients discharged from the hospital within 24 hours) second, this is a single center study with small sample size. third, because of the financial issues we measure serum NGAL only pre and 2 hours post PCI so we don't have a serial measurement of its level to correlate it with multiple variable and assess its level at least 24 hours post procedure.

RECOMMANDATIONS

- Performing this study in another center in the country with larger sample size
- Serial measurement of serum NGAL (2, 4, 8, 24 and 48 hours) post PCI along with at least single measurement of sCr at 48 hours post PCI
- Assessment the time of the procedures to correlate it with the mean elevation of serum NGAL

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ORIGINAL ARTICLE

EMOTIONAL INTELLIGENCE AS A FACTOR OF PRESERVING MENTAL HEALTH AND ADAPTATION OF STUDENTYOUTH TO CRISIS SITUATIONS

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ABSTRACT

The aim: Organization of research work to substantiate and verify the potential development of emotional intelligence (EI) among student youth as a factor in preserving their mental health (MH) and adapting to crisis situations, in particular, those caused by military actions in Ukraine.

Materials and methods: The experimental base was 54 student-teachers of Vasyl Stefanyk Precarpathian National University (Ivano-Frankivsk, Ukraine). They were divided into a control group (34 people) and an experimental group (20 people), who participated in training according to the author's program. The questionnaire "Self-assessment of adaptability and well-being in crisis conditions of martial law" became the main prognostic tool of the research work. It was developed on the basis of D. Lusin's EI measurement technique, the MSCEIT test; the method of differential diagnosis of depressive states by V. Zung.

Results: The research work consisted of two parts: theoretical (definition of terminology, conceptual concepts, hypotheses of research work; development of a structural model of EI) and experimental, which was implemented in three stages: ascertainment (determination of the initial level of EI of the participants of the experiment), formative (organization of training according to the author's experimental program), control (determination of changes in the level of proficiency of EI students). The positive influence of the author's program on the increase in the indicators of EG members' possession by four components of EI (informational; adaptive behavior; self-control; empathy) at three levels is substantiated: high – an increase from 12.25% to 25%; average decrease from 43.75 to 47%; low – a decrease from 44% to 28.75%. The common, special, excellent results of our research work and experimental studies of other authors are shown.

Conclusions: The conducted study generally confirmed the hypothesis that thanks to the training of EI (in particular, its four components: informational; adaptive behavior; self-control; empathy) can become an effective factor in preserving a person's MH and adapting to crisis situations. The prognostic toolkit presented in the work, the procedure for formalizing the results of the experiment, and the methodology of the author's training program for the formation of EI are extremely representative, understandable, and valid. Since the research is based on materials that are publicly available on the Internet, its results can be used and developed by either scientists or medical and social workers, practicing teaching. All life crises, including those caused by military events, mass casualties and large-scale destruction, get to the end sooner or later. Therefore, their experience should be carefully studied in order to effectively use the potential of EI in increasing human life resources.

KEY WORDS: emotional intelligence, mental health, crisis situation, adaptive behavior, self-control, empathy, students of education

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INTRODUCTION

Initially, this study was planned as a continuation of previous studies [1] and was supposed to relate to the formation of emotional intelligence as a factor in the adaptation of student youth in the crisis conditions of the COVID-19 pandemic. However, the large-scale military operations that began in Ukraine in February 2022 and fundamentally changed its social development forced the subject of the study to be adjusted. Although the main participants of the experiment remained students, the representativeness of its results expanded, so they can be applied to other categories of the population, taking into account their psychophysiological and social characteristics.

Conducting such a study is actualized by official government data, according to which in the fourth month of the war, about a third of the population of Ukraine needed psychological support. According to scientists' forecasts, mental disorders will inevitably lead to the spread of cardiovascular diseases, diabetes, arthritis, asthma, cancer and other diseases. This situation is typical and is confirmed by the experience of other countries affected by armed conflicts [2].

THE AIM OF THE STUDY

The organization of scientific research work to substantiate and verify the potential development of emotional intelli-

gence (EI) among student youth as a factor in preserving their mental health (MH) and adapting to crisis situations, in particular, those caused by military actions in Ukraine.

MATERIALS AND METHODS

The experimental base was 54 student-teachers of Vasyl Stefanyk Precarpathian National University (Ivano-Frankivsk, Ukraine). They were divided into a control group (34 people) and an experimental group (20 people), who participated in training according to the author's program. The questionnaire "Self-assessment of adaptability and well-being in crisis conditions of martial law" became the main prognostic tool of the research work. It was developed on the basis of D. Lusin's EI measurement technique, the MSCEIT test; the method of differential diagnosis of depressive states by V. Zung.

RESULTS

The design of research work determines its division into theoretical and experimental parts.

The theoretical part involves the definition of terminology, ideological concepts, the hypothesis of research work and the development of a structural model of EI in the context of the investigated problem.

Based on international scientific experience [3], we interpret a crisis situation as an extreme aggravation of contradictions, destabilization of the situation in various spheres of a person's social and personal life in a certain region, country or the world as a whole. Its most dangerous and complex type is a military-political crisis, which creates an atmosphere of incomplete and inconsistent information, uncertainty and unpredictability of the situation, general fear, confusion, and panic. A sharp increase in destabilizing factors in the environment leads to an increase in the number of people suffering from stress, behavioral and mental disorders, and exacerbation of chronic diseases [4].

Analysis of scientific research [2; 5-8] about the impact of a crisis situation (in particular, caused by COVID-19) on MH allows to consider it as a state of a person who functions at a satisfactory level of emotional and behavioral adaptation; the ability of an individual to realize himself as a subject of interaction with the surrounding world; mental mechanisms that determine a person's social activity. Mental health plays a decisive role in overcoming "normal life stresses", coping with life's stresses and ensuring productive social work. Fear, anxiety, and stress are normal reactions to the perception of real and imagined threats, however, during crisis situations they significantly increased, because the person was faced with the unknown, uncertainty due to new realities.

In our context, we should note that, according to American scientists, problems with mental health have become one of the main obstacles to the successful study of students, because they negatively affect their motivation, social interaction, concentration of attention, and aggravate various mental illnesses [5].

Under such circumstances, the relevance of research aimed at studying effective ways of preserving MH and human adaptation in crisis situations, in particular, caused by military actions, is increasing. Representative scientific studies [9; 10; 11; 12; 13; 14; 15; 16; 17; 18] testify that one of the promising ways and means of solving this problem is the formation of EI. Based on their analysis, a complex model of EI formation was developed as the ability to perceive adequately and process information critically and to exercise self-control and emotional self-regulation of behavior in crisis situations.

This model synthesizes four main theoretical concepts. The first defines EI as a cognitive ability aimed at processing information through the prism of emotional perception. The cognitive ability of EI consists of four "branches": recognition and expression of emotions; using emotions to solve specific tasks; understanding the emotions that preceded and followed the events; regulation of one's own and others' emotions for successful interaction with the external environment [17].

The second concept characterizes EI as a person's ability to recognize and understand emotions, intentions, desires of other people and their own as well as to manage them to solve practical tasks; as a unity of intellectual processes and a tool for effective interaction with the social environment, which allows one not to succumb to stress [4; 15; 18].

The third concept structures the EI model as levels of a person's possession of 15 abilities: self-assessment of capabilities and limitations; awareness of one's emotional state; self-expression of feelings, thoughts; emotional stability; empathy; self-identification; social contacts; resistance to stress; control of emotions; assessment of realities; adjusting thoughts, ideas, behavior according to circumstances; effective problem solving; self-actualization; optimism and emotional balance; satisfaction with life [10].

The fourth concept contains two aspects related to: a) an organic combination in the structure of EI of personal and interpersonal components, which are aimed at one's own emotions and the emotions of others (according to Goleman, 1998) [12]; b) a clear fixation of the influence of EI on adaptation, which includes assimilation – reproduction in the cognitive activity of the subject of the main characteristics of the object of knowledge and accommodation – adaptation of the subject to new realities (according to Piaget, 1966) [19].

The synthesis of these concepts allows us to present an integrated model of the formation of EI as a vital resource for the preservation of MH and adaptation to a crisis situation (in our case, caused by military actions in Ukraine). It consists of four main components that outline the essence and indicators of a person's possession of EI as a factor in the regulation of behavior, emotions and adaptation to the challenges of a crisis situation (table I).

On the basis of the specified theoretical provisions, a hypothesis is put forward, according to which the organization of targeted training on the formation of specified components of EI can become an effective factor (vital resource) in preserving the MH of a person and ist adap-

Table I. Components and indicators of determining the levels of formation of EI as a vital resource of adaptation to a crisis situation

EI component	Indicatorsof EI components
Informational component reflects psycho-emotional perception	Ways of receiving information; subjects of information; nature of informatio nperception; ist effect on the psyche
Adaptive behavior is adaptation to new living conditions	Adaptability – correspondence between goals and the result of life activities, determined by a person's attitude to the surrounding world and himself; non-adaptability – a perceived inconsistency between goals and the result of life activities; maladaptability – disharmony between goals and activity results, which causes mental tension, weakening of immunity
Self-control is the conscious control of behavior and emotions. Mental state, reactions to events	The ability to realize and manage feelings, emotions; tolerance; positive thinking; optimism; ability to psychological adaptation
Empathy is sympathy, perception of another's feelings	The ability to understand the feelings and emotional state of another person; adequacy of perception of the social environment; development of social compassion; the ability for sincere emotional relationships; willingness to help others

tation to a crisis situation. An experiment was conducted to test the hypothesis.

The experimental part was implemented in three *stages*: ascertaining, formative, and control. *Their experimental base was* 54 student-teachers of Vasyl Stefanyk Precarpathian National University (Ivano-Frankivsk, Ukraine). They were divided into an experimental group (EG – 20 people), who participated in training according to the author's program, and a control group (CG – 34 people), who were involved in the experimental work for the validity of its results.

The aim of the ascertainment stage is to determine the initial level of ownership of the CG and EG members by the specified components of EI, which are important factors of adaptation to difficult life situations. For this, the scientific materials of two groups were determined and used. The first is prognostic tools for measuring EI indicators related to adaptive behavior in stressful situations: D. Lusin's EI measurement technique [13]; the method of measuring EI is the MSCEIT test, which is modeled after a traditional intelligence test [14]; The method of differential diagnosis of depressive states by V. Zung [20]. The second group consists of the results of sociological research on the psychological state of the population of Ukraine during the war [21-23].

Based on the synthesis of the mentioned materials and the experience of experimental work of scientists studying EI as a factor of psychological adaptation and health preservation of a person to changing living conditions [11; 12; 16; 18], the questionnaire "Self-assessment of adaptability and well-being in crisis conditions of martial law" was developed. It consists of 52 questions formulated in a closed form, divided into four groups corresponding to the specified components of EI. Respondents could choose several answer options for individual questions (marked with "*") and recorded their attitude to the problem at the beginning of the war and at the time of the survey (for example, 68 – 44%). Here are some examples of proposed questions and answer options that were formulated in the affirmative form.

Questionnaire "Self-assessment of adaptability and well-being in crisis conditions of martial law" (generalized data of answers in percentages are rounded for their expressiveness)

I. Informative component:

*Sources of obtaining information about the social situation in Ukraine**: a) Ukrainian official (state) mass media (68 – 44%); b) Ukrainian social networks (43–54%); c) Ukrainian Internet websites (36%); d) Russian mass media (8%); e) Western mass media (2%); f) friends, acquaintances, family members (77%); g) participants in hostilities, volunteers (24%).

The frequency and nature of receiving information about the military-political situation in Ukraine: a) constantly and purposefully (51%); b) eriodically, but purposefully (27%): c) accidentally, unplanned (22%); d) I do not receive information because I am not interested (0%); e) I avoid information (0%).

*Priorities of interest in information content**: a) any information (38%); b) about the course of military operations (54 – 36%); c) about human and material losses, destruction (54 – 46%); on military and humanitarian aid to Ukraine and its relations with other countries (22 – 18%); d) about the situation in the occupied territories of Ukraine (32%); e) about the cultural and educational life of Ukraine (14%);

Reaction to information about the social situation in Ukraine: a) makes a strong impression, causes nervous excitement, anxiety, paralyzes life (88 – 26%); b) perceived calmly, does not cause a state of anxiety, nervous excitement, depression (6 – 56%); c) does not have any impact on my life (6%); e) does not care what is happening around (0%).

II. Adaptive behavior:

Attitudes towards restrictive measures (curfews, air raid signals, movement restrictions, etc.): a) fully approve (62%); b) approve more than reject (25%); c) I object more than I approve, because they limit personal freedom and the right to choose (13%); d) I completely deny the expediency (0%);

Adherence to the requirements of restrictive measures: a) adhere steadfastly, deliberately under any circumstances (72 – 27%); b) observe only under certain circumstances and in public places (24-41%); c) follow formally (4 – 32%); d) completely ignore (0%).

Self-assessment of changes in the exponential curve of worry and anxiety in measurements: a) increased (9%); b) decreased (68%); d) did not change (15%); e) no worries (8%);

*Dominance of emotions caused by the war in Ukraine**: anger, indignation, hatred (53%); pride in Ukraine, its army, people, volunteers (52%); anxiety, fear, confusion, unpredictability (38%); depression, numbness (22%); no emotions (0%);

Changes in mental health: a) sleep disturbance – yes (12%), no (88%); b) manifestations of a depressive state – constantly (yes – 9%, no – 91%); periodically (yes – 34%; no – 66%); became more frequent (yes – 36%, no – 64%); manifestations of aggressiveness: increased (18%); b) has not changed (64%); c) it is difficult to give an answer (18%);

III. Self-control:

Social activity: a) increased (52%); b) has not changed (26%;); d) decreased (22%);

Passivity, apathy: a) increased (8%); b) decreased (18%); c) no changes (54%); d) difficult to answer (20%);

Life goals: a) changed (12%); b) has not changed (72%); c) it is difficult to answer (16%);

Cheerfulness and optimism: a) increased (6%); b) did not change (62%); c) decreased (14%); c) difficult to answer (8%);

Confidence in one's own strengths and capabilities: a) increased (32%); b) has not changed (52%); c) decreased (6%); d) difficult to answer (10%);

Adaptability to stress: a) increased (34%); b) has not changed (44%); c) decreased (8%); d) it is difficult to answer (14%);

Internal nervous tension: a) increased (46%); b) has not changed (40%); c) decreased (6%); d) difficult to answer (8%);

Emotionality (violent reaction to changes in the environment): a) increased (34%); b) has not changed (42%); c) decreased (6%); d) difficult to answer (8%);

Impulsivity of behavior: a) increased (32%); b) has not changed (42%); c) decreased (6%); d) difficult to answer (20%);

IV. Empathy:

Social openness: a) increased (42%); c) decreased (47%); d) did not change (11%):

Reaction to nervousness of another person: a) increase in nervousness, loss of balance (32%); b) physical and spiritual distancing (8%); c) sympathy (32%); c) trying to help (18%); d) indifference (4%); f) contempt for "weak-spirited" (4%);

Reaction to reports of military operations, victims, destruction: a) strong emotion (22%); b) sympathy, willingness to help, if it does not threaten one's own safety (26%); c) anxiety, willingness to help despite the threat to one's own safety (22%); d) abstraction from external events, concentration on one's own affairs (30%).

For statistical processing of the survey results, the parametric Student's t-test for independent and dependent samples and cluster analysis using the EI measurement technique [18] were used. Thus, among the participants of the experiment (n=54), three cluster groups were identified, which according to four components (informative; adaptive behavior, self-control; empathy) had the following levels of EI possession: high (12.3%), medium (48.7%), low (39%). Detailed data on individual components of EI are presented below in terms of their comparison with the results of the control experiment (table 2).

The results of the survey showed that the level of students' mastery of certain components of EI does not correspond to its potential as a factor of MN preservation and adaptation to crisis situations. This determined the expediency and focus of conducting the formative stage of the experiment.

The aim of the formative experiment is to test and verify the effectiveness of the author's educational program for the development of EI, which, according to our hypothesis, can increase the ability to adapt and contribute to the preservation of a person's MN in crisis situations. It was implemented with 20 members of the EG in the format of an educational training (32 hours) based on the use of various methods and forms of training (role games, mini-training, coaching techniques, project method, "brainstorming", "case studies", etc.). Participants performed special tasks, tests, exercises, kept personal diaries. The content of the curriculum is presented in terms of the development of four defined components of EI.

The development of the informative component of EI involved the formation of: a) knowledge and skills regarding critical perception, analysis of various information (negative, positive; official, manipulative, fake) about the course and nature of the military-political situation; b) understanding the impact of information on the psychophysiological state of a person; c) the ability to avoid or displace emotional experiences that arise under the influence of informational and psychological pressure and cause groundless experiences, stress, which negatively affects the general state of human health.

For this, the members of the EG studied and analyzed the problems related to: "infodemic" (incitement, intimidation), which significantly affects and complicates human life in difficult crisis periods [35]; disinformation (types, tools, methods of protection), which is used to manipulate consciousness, destabilize life, incite panic [24]; the interpretive function of EI as the ability to critically perceive and productively decipher emotional information (facial expression, body movements, tone of voice, etc.) based on special knowledge and individual experience [9; 25]; the spread of fake news that distorts reality and negatively affects a person's psycho-emotional state [26].

The development of the informative component of the EI turned out to be the most productive component of the formative experiment, due to the fact that it was concrete and understandable to the members of the EG, who had their own experience of working with various sources of information (collection, analysis, interpretation). On this basis, the ability to self-correct the perception of diverse information was developed. This created the basis for the development of other components of EI, because if the level of cognitive capabilities is insufficient, a person develops a line of behavior characterized by stereotyped thinking, difficulties with self-control, indifference to one's health and environment, etc.

The theoretical basis for the development of *adaptive behavior* was the understanding by students of EI as a set of mental abilities that contribute to the understanding of one's own emotions and the emotions of others [12; 14]. The phenomenon of adaptive behavior was studied in depth as a set of causes and consequences of an individual's life activities, which depend on external circumstances and the possession of self-survival and self-preservation strategies [27].

The practical improvement of adaptive behavior involved the formation of students' knowledge about strategies for coping with stress and the skills of their application, as well as the ability to perceive a stressful situation adequately, changes in

Table II. The results of the ascertainment and control experiment regarding the diagnosis of levels of mastery of EI components

Levels of EI possession	EI components							
	Informative		Adaptive behavior		Self-control		Empathy	
	1*	2**	1*	2**	1*	2**	1*	2**
High	18 %	34 %	11 %	20 %	7 %	25.4 %	13 %	20.6 %
Medium	42 %	44 %	49 %	48 %	47 %	44 %	37 %	52 %
Low	40 %	24 %	40 %	32 %	46 %	31 %	50 %;	28 %

* Data of the ascertainment experiment on 54 surveyed members of the CG and EG.
** Data of the control experiment on 20 interviewed members of the EG

health, and the skills of assessing them as a personal and collective threat. For that purpose special techniques and exercises were used to regulate psychophysiological tone, normalize the functioning of the body (breathing, cardiovascular system), reduce neuropsychological tension, counteract mental stress, and increase the body's adaptability. Autogenic training has proven to be effective, helping to regulate emotional states, eliminate unwanted experiences and anxieties, restore work capacity in case of physical and nervous fatigue, and improve sleep [28; 29].

For the development of *self-control abilities* and skills, methods of coping strategies and tests, tasks, meditative exercises are used, which form the ability to control emotions and new stereotypes of behavior in crisis situations [28; 30]. Students showed significant interest in the ideas of the philosophy of Stoicism, which are based on the postulates of free will as the highest mental function that determines a person's ability to make conscious decisions and implement them in life. EG members mastered the practical exercises of the Stoics, which increase a person's ability to self-control and self-regulation [31; 32].

The development of empathy as a component of EI involved a shift in emphasis from the formation of the ability to "passive empathy" to the readiness to provide real psychological and other help to persons who need it in critically difficult situations. For this, students mastered special methods and techniques of self-improvement and working with other people: mindfulness [33]; art therapy and fairy-tale therapy [34; 35]; nonviolent communication and nonviolent listening [36].

The implementation of the author's educational program made it possible to proceed to the final stage of the experiment.

The purpose of the control experiment is to determine the changes in the students' mastery of the EI components that occurred after the educational training and their comparison with the data of the ascertainment experiment. Repeated measurements were performed using the same prognostic toolkit and included only EG members (Table II). This approach is acceptable, because according to the data of the ascertainment experiment, the difference in the level of mastery of the specified components of EI by members of EG and CG was at the level of statistical error.

Despite the objectively determined relativity, the presented data are quite indicative and prove the real positive impact of the author's program on the growth of the indicators of EG members' possession in all four components of EI. This is evidenced by the general increase in EI indicators at the high level from 12.25% to 25% (2 times) and their decrease at the average level from 43.75 to 47% (almost 1.1 times), and at the low level from 44% to 28.75% (1.5 times).

DISCUSSION

The presented research has a lot in common and special, compared to other theoretical and experimental studies on the raised problem, and fills some existing gaps in them.

In the study, where the ECI (Emotional Intelligence Inventory) questionnaire was used as a tool for measuring EI, experiment participants were trained using the "Mastering Emotional Intelligence" program (the content is not specified), which involved two-day training sessions. The authors recorded an increase in the level of EI by 11% in 20 people from Brazil and by 24% in 19 people from the USA. It is recognized that due to the small number of samples of experiment participants, these data should be considered "preliminary" [16].

The sample of the experimental study conducted by Belgian scientists was composed of psychology students who belonged to the study (19) and control (18) groups. Their mastery of individual components of EI was assessed by a number of methods (TEIQue, ERP-Q, EMA, DOE, TAS-20, STEU) three times: before training, immediately after its completion and 6 months after training. The authors noted a "significant increase in indicators" in such components of EI as "identification of emotions" and "management of emotions" and "improvement of indicators" according to the criteria of "understanding emotions" and "expression of emotions". Fixing the preservation of positive changes 6 months after training, scientists claim that they can be increased by the efforts of the experiment participants themselves due to the knowledge and skills acquired during training [15]. These methodologically important conclusions will be taken into account in our further work.

It is worth to note the developed prognostic toolkit and the conclusions of the authors of the experimental study, who measured the development of three "branches" of EI ("identification of emotions", "use of emotions in solving problems", "conscious management of emotions"), which took place thanks to the implementation of a specially developed educational program [15].

In the researches on the raised problem [4; 6; 9; 10; 13-18, 33] a common position can be traced, according to which individual components of EI can be developed and improved in the process of learning according to special training programs

that are based on modern methodology and scientific research. However, the content of training programs, in particular, experimental studies, as a rule, is not specified or presented in a fragmentary, superficial way, because the main attention is focused on the presented formalized results of the research work.

Our study is somewhat different from the mentioned studies. We showed in detail the content and methodology of the author's training program and tried to make it representative for use and development by other scientists and social and medical workers. Taking into account all mentioned above, such prognostic tools and educational materials were selected that are publicly available on the Internet and can be used in work with different categories of the population.

Another aspect of the discourse is the comparison of the results of our research with the data of sociological surveys, which reflect changes in the emotional state and mental health of Ukrainian citizens under martial law conditions. In April 2022, the Sociological Group "Rating" conducted a nationwide survey "Ukraine in conditions of war" using the method of Computer Assisted Telephone Interviews. Its results show that 80% of respondents representing different population groups over the age of 18 felt proud of their country along with such emotions as sadness (32%), joy (20%), fear (16%), and anger (11%). However, such emotions as indifference and shame were almost absent. The growth of empathy is evidenced by data according to which almost 80% of respondents in one way or another participated in the defense of the country: provided financial assistance (45%); volunteered and helped internally displaced persons (35%), worked in critical infrastructure (13%), etc. About 2/3 of forced migrants were elderly people who had difficulty adapting to new living conditions [23].

According to a survey conducted by the Kyiv International Institute of Sociology in May 2022, the war evoked the following basic emotions in Ukrainians: anger, indignation, hatred, disgust (53%); pride in the state and the army (52%); anxiety, fear, horror (38%), shock (19%), depression, numbness (7%), shame (6%), etc. [22]. Such a deterioration of important indicators of MH correspondingly affects the entire psychophysiological state of a person.

The results of these and other [21] sociological studies indicate that in order to preserve MH and adapt people to crisis situations effectively, special educational programs for the formation of important components of EI can be developed.

CONCLUSIONS

The conducted study generally confirmed the hypothesis that thanks to the training of EI (in particular, its four components: informational; adaptive behavior; self-control; empathy) can become an effective factor in preserving a person's MH and adapting to crisis situations. The prognostic toolkit presented in the work, the procedure for formalizing the results of the experiment, and the methodology of the author's training program for the formation of EI are extremely representative, understandable, and valid. Since the research is based on materials that are publicly available on the Internet, its results can be used and developed by scientists, medical and social workers, practicing teachers as well. All life crises, including those caused by military events, mass casualties and large-scale destruction, always end sooner or later. Therefore, their experience should be carefully studied in order to use the potential of EI in increasing human life resources effectively. As the famous Sir Winston Churchill said, "Never let a good crisis go to waste."

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ORIGINAL ARTICLE

PROGNOSTIC ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN THE CARDIOVASCULAR COMPLICATIONS DEVELOPMENT IN PATIENTS WITH POLYMORBID PATHOLOGY: THE COMBINED COURSE OF HYPERTENSION, TYPE 2 DIABETES MELLITUS AND SUBCLINICAL HYPOTHYROIDISM

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ABSTRACT

The aim: To determine the prognostic value of vascular endothelial growth factor (VEGF) levels for the development of cardiovascular complications in patients with a combined course of hypertension, type 2 diabetes mellitus and subclinical hypothyroidism.

Materials and methods: 93 patients (mean age 61,71±0,87 years) with the combined course of hypertension, type 2 diabetes mellitus and subclinical hypothyroidism were examined. Parameters of lipid, carbohydrate metabolism, plasma insulin, VEGF (by ELISA), blood pressure levels were measured. Observation period was12 months.

Results: VEGF levels in the patients group were significantly higher than in the controls (482,77±21,34 pg/ ml vs. 121,84±11,66 pg/ ml, p <0,001). The results of the ROC analysis made it possible to propose the level of VEGF ≥ 512.31 pg/ml as an identifier for the cardiovascular complications development in patients with studied comorbidity. VEGF levels in patients who developed cardiovascular complications during observation period were significantly higher the VEGF threshold levels (650,76 ± 52,04 pg / ml vs. 512,31 pg/ml, respectively, p = 0,038) and VEGF levels in patients without cardiovascular complications were significantly lower the threshold values (420,47± 21,67 pg/ml vs. 512,31 pg/ml, respectively, p = 0,047).

Conclusions: Determination of the vascular endothelial growth factor plasma level allows to evaluate the long-term prognosis in comorbid course of hypertension, type 2 diabetes mellitus and subclinical hypothyroidism.

KEY WORDS: hypertension, type 2 diabetes mellitus, subclinical hypothyroidism, vascular endothelial growth factor, cardiovascular complications

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INTRODUCTION

Morbidity, mortality and disability rates associated with cardiovascular diseases (CVD), particularly those with hypertension (H), continue to be a leading affecting 26,4% of the world's adult population [1] and in low-income countries -39.2% [2]. Currently, there is an increase in the comorbidity of H with endocrinopathies, a clear but poorly studied example of which is a combination of H, type 2 diabetes mellitus (T2DM) and subclinical hypothyroidism (SH). In some studies SH was diagnosed in 28.9% patients with simultaneous presence of H and T2DM [3]. If in some cases such combination has random character, in others it is a commonality of pathogenetic processes, interrelation of the changes influencing the prognosis of patients, tactics of management and efficiency of therapy.To prevent the development of cardiovascular events and their complications in patients with a combined course of H, T2DM and SH it is essential to identify early predictors, which may include markers of endothelial dysfunction (ED). It is important that the risk of ED in patients increases with an increase in the total number of risk factors and their combination [4, 5]. Comorbidity of H, T2DM and SH has a negative impact on each other and significantly worsens the course of these diseases, which can also be associated with ED.

Now a great attention has been paid to the study of vascular endothelial growth factor (VEGF-A) as one of the earliest ED biomarkers which is activated in the formation of atherosclerosis, H, T2DM [6]. A number of studies provide data that may indicate the relationship of VEGF levels with lipid metabolism, inflammatory factors, and its important role in the vascular wall remodeling [6, 7]. Of particular interest is the study of comorbid conditions, each of which is characterized by the presence of ED in the pathogenesis and factors that exacerbate ED, which

may be manifested in increased expression of VEGF-A and obviously plays an important role in understanding the common pathogenetic processes and developing more effective preventive and curative measures.

THE AIM

The aim was to determine the prognostic value of vascular endothelial growth factor levels for the cardiovascular complications development in patients with a combined course of H, T2DM and SH.

MATERIALS AND METHODS

The study involved 93 patients (78 females and 15 males) aged 44–75 (mean age 61,71±0,87 years) with H stage II, T2DM and SH, which developed as a result of autoimmune thyroiditis (AIT). Non-inclusion criteria were symptomatic H, diabetes type 1 and other endocrine disorders, clinical signs of coronary heart disease or severe concomitant chronic diseases, pregnancy, manifested hypothyroidism or treated SH, surgical treatment of thyroid gland. The control group consisted of 30 age - and sex matched volunteers without cardiovascular diseases and endocrinopathies. Against a background of dietary recommendations, all patients received basic antihypertensive, antidiabetic and hypolipidemic therapy in individually selected doses in accordance with international and national Guidelines [8, 9] not less than 6 months before including in study. Period of observation was 12 months.

Blood pressure (BP) levels were assessed by standard procedure in a sitting position. Body mass index (BMI) was measured by formula: kg/m^2 where kg is a person›s weight in kilograms and m^2 is their height in meters squared. Assessment of carbohydrate metabolism (fasting glucose and glycosylated hemoglobin level (HbA1c)) and lipid metabolism (total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-cholesterol), very low density lipoprotein cholesterol (VLDL-cholesterol), low density lipoprotein cholesterol (LDL-cholesterol)) was made by standard methods; plasma insulin concentration – by enzyme immunoassay. To determine insulin resistance (IR) the HOMA-IR index was used according to the formula: (fasting glucose) x (fasting insulin)) mmol/ml/22.5). For verification of SH and AIT the concentration of thyroid stimulating hormone (TSH), free thyroxine (fT4) and antibodies to thyroid peroxidase (AT-TPO) in serum with enzyme immunoassay were measured. Ultrasound examination of the thyroid gland was performed according to the standard procedure on the device "LOGIQ5". Plasma levels of VEGF-A were studied by an immunoassay (ELISA) kit IBL International Gmbh (Germany) on a semiautomatic immunoassay microplate analyzer "ImmunoChem-2100", HighTechnology, Inc. (USA).

The analysis of the studied parameters for normality of distribution was carried out using the Shapiro-Wilk test. Quantitative variables are presented as M ± m (M is the mean value, m is its standard error), qualitative characteristics were described as the frequency of events (% of the observations normal number). The Student criterion was used to estimate the differences between groups in the distribution close to normal. The differences were considered statistically significant at $p<0.05$. To determine the presence and nature of the links between different manifestations and pathogenetic factors of different processes, correlation analysis was performed using the Pearson test (r) and the Chaddock scale. ROC analysis was used to determine the identifier of the development of a cardiovascular event.

Study was performed in compliance with the basic provisions of the World Medical Association (WMA), Helsinki Declaration on ethical principles for medical research involving human subjects (1964–2000) and MOH of Ukraine Order No. 690 dated September 23, 2009. The study was approved by the Bioethics Commission of Kharkiv National Medical University in accordance with the principles set forth in Helsinki Declaration. All the patients signed the inform consent.

RESULTS

Despite modern antihypertensive, antidiabetic and hypolipidemic therapy, there were signs of atherogenic dyslipidemia, hyperglycemia, IR and significantly higher BP levels in the patients with comorbid pathology compared with the control group (Table I). Plasma VEGF levels in the patients group were significantly higher than in the control group (482,77±21,34 pg/ ml vs. 121,84±11,66 pg/ ml, p <0,001).

During the observation period cardiovascular events (acute myocardial infarction, acute coronary syndrome, ischemic stroke, transient ischemic attack, heart failure progressing) were observed in 15 patients (16,13±3,81%).

To find the optimal threshold value of VEGF or the cut-off-value of this indicator as a predictor of the cardiovascular complications (CVC) in patients with H, T2DM and SH a ROC (Receiver operating characteristic) analysis was performed. Area Under an ROC Curve (AUC) is used to assess the quality of the diagnostic value of a predictor for the CVC development. The higher the AUC, the better predictive power this predictor has. In this study, the AUC is 0.821 ± 0.049, which indicates a very good quality of an identifier for the CVC development (fig. 1).

In this work, the optimal point on the ROC curve is considered which has the highest sensitivity (Se) and specificity (Sp):

Cutt-off-value = max $\left| Se + Sp \right|$.

VEGF level = 512.31 pg/ml was the optimal cut-off with the maximum values of sensitivity (Se) 84.5% and specificity (Sp) 70,2 %: in 84,5% patients with H, T2DM and SH with CVC during observation period VEGF levels were more than 512,31 **pg**/ml, and 70,2% of patients without CVC had VEGF levels less than this level. Thus, the conducted ROC-analysis allowed to determine the "cut-off " value of VEGF in patients with combined course of H,T2DM and SH with the most likely development of CVC .

Analysis of metabolic parameters and BP levels depending on the level of VEGF proposed as a predictor of CVC in studied comorbidity was carried out (Table II). Signifi-

Table I. Lipid, carbohydrate, thyroid metabolism and anthropometric characteristics, (M±m).

Parameter	Control group (n=30)	H, T2DM and SH (n=93)	p
Age, years	58,40±1,80	61,71±0,87	p>0,05
BMI, kg/m2	26,85±0,36	28,56±0,38	p=0,031
SBP, mm Hg	127,23±1,24	142,39±1,28	p=0,001
DBP, mm Hg	78,67±0,97	87,12±0,88	p=0,010
PBP, mm Hg	48,57±1,34	55,40±0,97	p<0,001
TSH, μIU/ml	2,10 ± 1,11	6,03±0,16	p=0,001
T4 free, pmol /l	12,20±2,46	14,32±0,29	p>0,05
T3free, pg/ml	4,22±0,95	5,02±0,16	p>0,05
TC, mmol/l	4,65± 0,33	5,78±0,13	p=0,002
TG, mmol/l	0,94 ±0,03	2,01±0,13	p=0,004
HDL –cholesterol, mmol/l	1,53 ± 0,02	1,27±0,03	p=0,009
VLDL-cholesterol, mmol/l	0,43± 0,01	0,88±0,05	p=0,009
LDL-cholesterol, mmol/l	2,68±0,04	3,54±0,11	p=0,002
Fasting glucose, mmol/l	4,28±0,05	6,98±0,22	p<0,001
HbA1c, %	4,24±0,08	7,19±0,11	p<0,001
Fasting insulin, μIU/ml	9,44±0,39	20,30±1,03	p=0,001
HOMA-IR	1,81±0,13	6,42±0,43	p<0,001
VEGF-A, pg/ ml	121,84±11,66	482,77± 21,34	p<0,001

Notes: SBP- systolic blood pressure, DBP- diastolic blood pressure, PBP- pulse blood pressure, TSH- thyroid stimulating hormone, p – the level of significance difference vs. the control group

Table II. Lipid, carbohydrate, thyroid metabolism and anthropometric parameters in dependence on VEGF levels in patients with H, T2DM and SH comorbidity

Parameter	VEGF plasma levels up to 512,31 pg/ ml (n=72)	VEGF plasma levels more then 512,31 pg/ ml (n=21)	p
TC, mmol/l	5,67±0,15	6,31±0,22	0,034
VLDL-cholesterol, mmol/l	0,89±0,06	0,83±0,10	>0,05
HDL –cholesterol, mmol/l	1,27±0,04	1,28±0,06	>0,05
LDL-cholesterol, mmol/l	3,45±0,12	3,99±0,20	0,035
TG, mmol/l	2,03±0,15	1,95±0,26	>0,05
TSH, μIU/ml	5,79±0,17	6,84±0,35	0,006
Fasting glucose, mmol/l	7,10±0,26	7,92±0,29	0,039
Fasting insulin, μIU/ml	20,98 ± 1,25	17,96 ±1,50	>0,05
HOMA-IR	6,74±0,52	7,96±0,30	0,046
HbA1c, %	7,25±0,12	6,99±0,21	>0,05
BMI, kg/m2	28,60±0,34	28,34±0,63	>0,05
SBP, mm Hg	141,89±1,55	146,71±1,35	0,022
DBP, mm Hg	87,25±1,06	86,67±1,45	>0,05
PBP, mm Hg	54,81±1,18	57,43±1,44	>0,05

Notes: TSH- thyroid stimulating hormone, HbA1c- glycosylated hemoglobin, SBP- systolic blood pressure, DBP- diastolic blood pressure, PBP- pulse blood pressure

cantly higher levels of TC, LDL-cholesterol, fasting glucose, HOMA-IR, SBP at VEGF values exceeding the threshold were found. It is noteworthy that at higher VEGF levels significantly higher TSH levels were noted (p = 0,006).

The presence of reliable correlations between the VEGF level below the threshold level relatively to the CVC risk and such risk factors as TG (r=0,311, p=0,008) and fasting glucose (r=0,333, p=0,004), as well as between the level of VEGF above the threshold level and TC level (r=0,392, p=0,005) and SBP (r=0,410, p=0,003) indicates a close relationship of VEGF levels with certain CVR factors (Table III).

Also Xiaomeng Sun at al. (2019) found that increased serum VEGF level was positively associated with the HOMA-IR value [10].

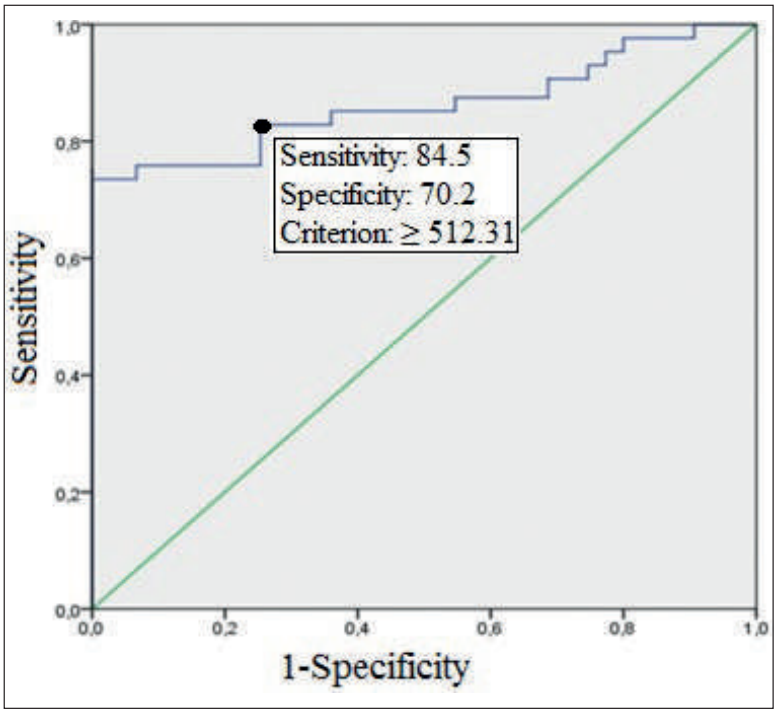

Fig.1. Receiver operating characteristic (ROC) curve of VEGF for the prediction of CVC in patient with comorbid course of H, T2DM and SH.

It was found that the level of VEGF in patients who developed CVC during observation period was statistically significantly higher the "cut-off " value of VEGF (650,76 ± 52,04 pg / ml vs. 512,31 pg/ml, respectively, p = 0,038) and VEGF levels in patients without CVC was statistically significantly lower the "cut-off " value (420,47± 21,67 pg/ml vs. 512,31 pg/ml, respectively, p = 0,047). The difference in VEGF levels between these subgroups was also significant (p=0,001) (Table IV). There were statistically significantly higher levels of TC, LDL-cholesterol, TG, HbA1c, PBP levels in patients with a complicated course of the studied comorbidity. TSH levels in patients who developed CVC during the observation period were significantly higher than in uncomplicated course (p=0,002).

DISCUSSION

Experimental data indicate a number of both favorable and unfavorable biological effects of VEGF and as a result of which we can say that VEGF is not only a leading factor in angiogenesis, but also a criterion for predicting vascular accidents in humans [11]. Earlier, A.D. Blann et al. (2002) demonstrated an increase VEGF plasma concentration in atherogenic dyslipidemia [12], Dirk C Felmeden et al. (2003) showed an increased VEGF levels in hypertensive patients [13] which was further confirmed in the others researches [11, 14].

There is abundant evidence to support that poor glycemic control in diabetic patients is associated with increased plasma VEGF, which in turn may cause several vascular complications in diabetic patients [10, 15]**.** Data on the influence of thyroid dysfunction on background of SH on endothelial status and ED formation are controversial and are not fully understood. Many studies have shown the relationship of SH with CVR factors [5, 16]. However, studies devoted to the role of VEGF in the comorbid course of H and endocrinopathies such as T2DM and SH are not enough. In our earlier studies were obtained data indicating the mutually aggravating effect of ED, dyslipidemia, hyperglycemia and IR on the course of H, T2DM and SH comorbidity [17, 18]. The presence in this study a significantly more pronounced dyslipidemia, hyperglycemia, HOMA-IR index and SBP level at VEGF levels exceeding the "cut-off " value relative to the CVC risk is an evidence of the mutually aggravating influence of these CVR factors and ED on the course of the studied comorbidity. Plasma levels of TC and SBP are included in the most scales used to assess CVR (SCORE, Framingham scale) [19, 20]. In this study the presence of a positive relationship between VEGF levels, proposed as a predictor of CVC for studied cohort of patients, with the TC levels and SBP can also be regarded as an evidence of the prognostic significance of this ED marker.

It is considered that SH has been linked to CVR factors, dyslipidemia and increased atherosclerosis. TSH level seems to be predictor of CVD, in particular when its levels are above 10,0 μIU/ml [16], but with TSH levels in the range of 4,5-10,0 μIU/ml, the data are contradictory. The data obtained in our work revealed a reliable relationship of TSH levels in the range of 4,0-10,0 μIU/ml not only with other risk factors, but also significantly higher TSH levels were revealed in persons with developed CVC on the background of H, T2DM and SH comorbidity. Presence of significantly higher TSH levels in patients with VEGF levels, corresponding to an increased CVC risk according to the ROC analysis, is a premise for identifying a significant TSH level in the range of 4,0-10,0 μIU/ml for risk of CVC in this category of patients.

CONCLUSIONS

Determination of the plasma VEGF level allows to evaluate the long-term prognosis of H, T2DM and SH comorbidity. Identification of VEGF values that are significant in relation

Table III. Correlations between metabolic, anthropometric parameters and VEGF in dependence on its level in patients with H, T2DM and SH comorbidity.

Parameter	VEGF plasma level up to 512,31 pg/ ml (n=72)		VEGF plasma level more then 512,31 pg/ ml (n=21)	
	Pearson, r	p	Pearson,r	p
TC, mmol/l	0,057	0,633	0,392	0,005
VLDL-cholesterol, mmol/l	0,259	0,028	-0,192	0,405
HDL –cholesterol, mmol/l	-0,004	0,973	-0,118	0,611
LDL-cholesterol, mmol/l	0,034	0,778	0,231	0,314
TG, mmol/l	0,311	0,008	-0,175	0,447
TSH, μIU/ml	0,548	0,001	-0,023	0,920
Fasting glucose, mmol/l	0,333	0,004	-0,198	0,389
Fasting insulin, μIU/ml	-0,028	0,815	-0,268	0,241
HOMA-IR	0,215	0,070	0,002	0,994
HbA1c, %	0,351	0,003	0,290	0,202
BMI, kg/m2	0,129	0,278	0,166	0,473
SBP, mm Hg	-0,073	0,545	0,410	0,003
DBP, mm Hg	-0,101	0,396	0,257	0,262
PBP, mm Hg	-0,034	0,778	0,094	0,686

Table IV. Lipid, carbohydrate, thyroid metabolism and anthropometric parameters in dependence on cardiovascular complications in patients with H, T2DM and SH comorbidity.

Parameter	Patients with CVC (n=15)	Patients without CVC (n=78)	p
TC, mmol/l	6,48±0,34	5,74±0,15	0,047
VLDL-cholesterol, mmol/l	0,87±0,06	0,88±0,11	>0,05
HDL –cholesterol, mmol/l	1,24±0,04	1,28±0,06	>0,05
LDL-cholesterol, mmol/l	4,13±0,30	3,49±0,12	0,035
TG, mmol/l	2,89±0,35	2,04±0,15	0,022
TSH, μIU/ml	7,53±0,47	5,74±0,15	0,002
Fasting glucose, mmol/l	6,76±0,32	7,02±0,25	>0,05
HbA1c, %	7,79±0,26	7,20±0,24	0,045
BMI, kg/m2	28,89±0,90	28,48±0,31	>0,05
HOMA-IR	7,79±0,72	6,01±0,35	0,044
Fasting insulin, μIU/ml	21,41±1,71	20,09±1,19	>0,05
SBP, mm Hg	147,80±2,05	141,99±1,46	0,028
DBP, mm Hg	85,07±1,86	87,51±0,99	>0,05
PBP, mm Hg	60,73±2,27	54,37±1,04	0,015
VEGF, pg/ ml	650,76±52,04	420,47±21,67	<0,001

Notes: CVC - cardiovascular complications

to the CVC risk, their relationship with accepted CVR factors are extremely important for effective prevention of CVC in different cohort of patients.

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ORIGINAL ARTICLE

EVALUATION OF TWO CRYOPROTECTANTS USED IN A NEW HUMAN SPERM CRYOPRESERVATION TECHNIQUE

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ABSTRACT

The aim: To examine the efficiency of different concentrations of Dimethyl sulfoxide (DMSO) and glycerol as a cytoprotectants in protection of human sperms during cryopreservation in this technique.

Material and Methods: Thirty oligozoospermic semen samples were used in this study. Samples diagnosed according to WHO 2010 criteria. Sheep's ovarian follicles obtained from local slaughterhouse and prepared by slicing the ovaries and evacuating the follicular fluid and oocyte. Each semen sample divided into six equal parts, and diluted 1:1 with cryosolution contains 5%, 10%, 15% DMSO or glycerol and injected within the emptied follicles. After freezing and thawing, the semen mixture aspired outside the follicles and sperm concentration, progressive motility, total motility, and normal morphology were examined.

Results: The best recovery rate of progressive and total motility post-thawing were with the use of 5% glycerol, and the lowest recovery rate of progressive and total motility and normal morphology were with the use of 15% DMSO.

Conclusions: In this technique, glycerol was more efficient than DMSO regarding sperm motility. The best concentration of glycerol for cryopreserve human spermatozoa is 5%.

KEY WORDS: cryopreservation, cryoprotectants, DMSO, glycerol, human spermatozoa, ovarian follicles

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INTRODUCTION

Cryopreservation of human spermatozoa become a routine work in Assisted Reproductive Technologies (ART) clinics [1]. It is used for preserve male fertility in many cases such as prior to undergoing chemo- or radiotherapy, vasectomy and other activities that may affect male fertility [2]. Sperm cryopreservation also can used in case of sperm donors to prevent the distribution of infectious diseases from donor to recipient couples [3]. Cryopreservation found to cause damage to spermatozoa and decrease its motility and viability and this may decrease the fertilization ability of the spermatozoa [4]. During cryopreservation, the ice crystals that formed intracellularly changes the integrity of sperm plasma membrane and cause damage in sperm function [5]. About 30-40% of sperm motility loss after cryopreservation [2], this may due to the damage of plasma membrane, loss of acrosome function and/or DNA fragmentation [6]. The freezing outcome can be optimized in the presence of a cryoprotective agent (CPA), which decrease the rate of dehydration during freezing to minimize the chance of intracellular ice formation [7], and improves the survival rate of the cells or tissues during cryopreservation [8]. Both glycerol and dimethyl sulfoxide (DMSO) are membrane penetrating cryoprotectant and they are widely utilizing in cryopreservation of sperms [9]. Cryopreservation of sperms not only affect sperm motility, but, the sperm concentration also found to decreased post-thawing due to the dilutions and washing steps [10], and this may not suitable for low concentrations of sperms because it may loss during washing steps [11-12]. This reason spur many researchers to experimented different techniques for cryopreserve low concentrations of sperms [11-16]. In our recent unpublished study [17], we were tried to use emptied sheep's ovarian follicles as a container for spermatozoa during cryopreservation, and we succeeded in cryopreserving different concentrations of human spermatozoa without using cryoprotectants, but we found that the best recovery rate was with the uses of glycerol-based cryosolution. This study builds on our prior results to investigate the effect of three concentrations (5%, 10% and 15%) of DMSO and glycerol on the outcome of human sperm cryopreservation using emptied sheep's ovarian follicles technique.

THE AIM

The aim of research is examination of the efficiency of different concentrations of Dimethyl sulfoxide and glycerol as a cytoprotectants in protection of human sperms during cryopreservation in this technique.

MATERIAL AND METHODS

SUBJECTS AND SAMPLES COLLECTION

Thirty semen samples were collected from patients with oligozoospermia (sperm concentration ≤15 x 10^6 mL^{-1}); their ages ranged from 21 to 55 years old. They attended to the Fertility Center Clinic at Al-Sadr Medical City, Najaf, Iraq, during the period from March 2019 to November 2019. All samples were collected from the subjects by masturbation after 3 days sexual abstinence and analyzed according to the guidelines of World Health Organization (WHO) 2010 by using a light microscope (Optica, Italy) to determine the sperm parameters, involving sperm concentration, total motility, progressive motility, normal morphology. Each sample was analyzed twice by one experienced biologist only to avoid any personal variations.

ETHICAL APPROVAL

This study was ethically approved by the medical ethics committee in Jabir ibn Hayyan Medical University, Iraq (Approval No: 19-0002). All the patients gave their informed agreement for research before they gave the semen samples.

EXPERIMENTAL DESIGN

After seminal fluid analysis (SFA), each sample divided into six equal parts and diluted 1:1 with cryosolution contains 5%, 10% and 15% glycerol or DMSO and injected in the emptied sheep's ovarian follicles and cryopreserved for two months.

PREPARATION OF CRYOPROTECTANTS

Two cryoprotectants were used in this study: DMSO and glycerol. The cryosolution of each cryoprotectant prepared in three concentrations 5%, 10% and 15% of simple medium for ART (SMART). SMART medium prepared by professor Dr. Muhammad-Baqir Fakhrildin [18].

COLLECTION AND PREPARATION OF SHEEP OVARIAN FOLLICLES

A total of 720 ovarian follicles were sliced from 612 sheep's ovary used in this study. The sheep ovaries were collected from local slaughterhouse in Najaf city. The ovaries were collected directly from the ewes after slaughtered and kept at 32-35°C with normal saline solution (0.9%NaCl) supplemented with two types of antibiotics (100mg/ml penicillin and 100mg/ml streptomycin). The ovaries then, transported to the Laboratory within 1 hour. In the Laboratory, ovaries washed three times using normal saline solution (37°C) to remove the clotted blood and reduce contamination on the ovarian surface [19]. After washing, the ovaries differentiated according to the size of ovarian follicles. The ovaries that contain follicles less than 0.3 mm in diameter were excluded, and those contain follicles larger than 0.3 mm in size sliced to remove the medulla and allows the follicles to be fit inside the cryotube. Then, the ovarian pieces that contain the follicles were stored at 4°C till the semen prepared.

PROCESS OF SPERM CRYOPRESERVATION

The prepared ovarian follicles emptied from the follicular fluid using 23-gage sterile hypodermic needle with a disposable 2 mL syringe. Then, each part of all the semen samples injected in 4 emptied follicles and inserted into cryotubes (Thermo-scientific 1.8 mL) and covered with cryosolution. The cryotube then, exposed to Liquid Nitrogen (LN_2) vapor for 15 minuets (2 cm above the surface of LN_2), then, the cryotubes plunged inside LN_2 and stored at -196°C for two months using cryopreservation LN_2 tank (MVE SC series LN_2 tank 40L).

THAWING PROCESS

After two months of cryopreservation, each cryotube was taken out from the LN_2 and immersed inside water bath at 35°C for 5 minutes, then, by using forcipes, the ovarian follicles transferred from the cryotube to a clean Petri dish and the samples withdrawn from the follicles using 23-gage sterile hypodermic needle with a disposable 3 mL syringe. The volume measured and the sample diluted 1:1 volum with the thawing solution (SMART medium plus 0.25 M sucrose) and then utilized for analyzing the sample's parameters (sperm concentration, motility, and normal morphology).

STATISTICAL ANALYSIS

The statistical analysis system (SAS) program (2012) was used to analyzed the data. Paremeters in this study were expressed as the means and standard deviations (mean ± SD) and the recovery rate of post-thawing groups expressed as percentage. The least significant difference (LSD) test and analysis of variation (ANOVA) were used to analyze the differences among groups. The p-value of less than 0.01 was considered significantly different.

RESULTS

Sperm concentration was significantly ($p<0.01$) decreased post-thawing in all groups when compared to pre-freezing, meanwhile, no significant difference was observed in sperm concentration among all three concentrations of DMSO and glycerol post-thawing. Progressive sperm motility (%) and total motility (%) significantly ($p<0.01$) decreased in all post-thawing groups when compared to pre-freezing. Post-thawing progressive motility and total motility in 5% glycerol group were significantly ($p<0.01$) higher than other groups, while the differences in progressive and total motility were non-significant between 10% and 15% DMSO groups and between 10% DMSO and 15% glycerol (Table I). Notably, the best recovery rate of progressive motility and total motility were achieved with the use of 5% glycerol

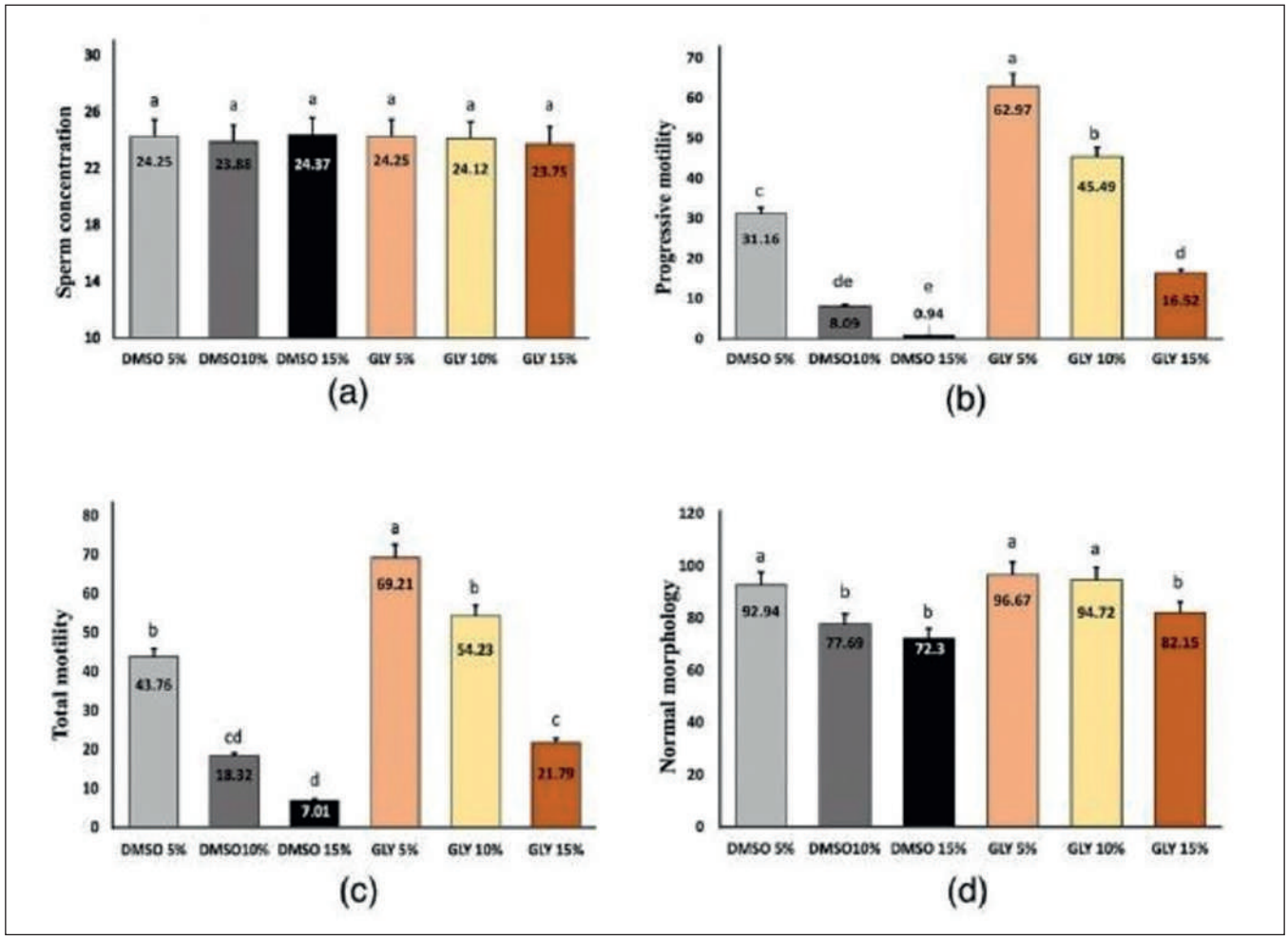

Fig. 1. Recovery rates (%) of human sperm parameters after freezing-thawing using sheep's ovarian follicles and three concentrations of DMSO and glycerol. (A) sperm concentration, (B) progressive motility, (C) total motility, (D) normal morphology, different letters on the percentage bars indicate significant differences $p<0.01$

Table I. Human sperm parameters in pre-freezing and post-thawing using sheep's ovarian follicles and three concentrations of two cryoprotectants.

Parameters	Pre-freezing	DMSO 5%	DMSO 10%	DMSO 15%	Gly 5%	Gly 10%	Gly 15%
Con	8.04±3.81^{a}	1.95±0.85^{b}	1.92±0.49^{b}	1.96±0.32^{b}	1.95±0.98^{b}	1.94±0.85^{b}	1.91±0.76^{b}
PM	26.44±5.89^{a}	8.24±1.55^{d}	2.14±0.97^{ef}	0.25±0.04^{f}	16.65±3.61^{b}	12.03±2.79^{c}	4.37±0.67^{e}
TM	38.04±9.48^{a}	16.65±3.81^{c}	6.97±1.43^{de}	2.67±0.93^{e}	26.33±5.98^{b}	20.63±4.34^{c}	8.29±2.46^{d}
NM	24.66±13.54^{a}	22.92±12.05^{a}	19.16±11.37^{b}	17.83±11.92^{b}	23.84±13.08^{a}	23.36±12.14^{a}	20.26±8.09^{b}

Data are presented as the mean ± SD, Gly: glycerol, Con: sperm concentration, PM: Progressive motility (%), TM: total sperm motility (%), NM: normal morphology (%). Different letters (a, b, c, d, e, and f) in the same row indicate significant differences ($p < 0.01$).

62.97% and 69.21%; respectively while the lowest recovery rate of progressive motility and total motility were with the use of 15% DMSO 0.94% and 7.01%; respectively (Fig. 1). Normal morphology (%) of sperms significantly ($p<0.01$) decreased post-thawing in 10%, 15% DMSO and 15% glycerol groups when compared with pre-freezing, meanwhile, no significant difference ($p<0.01$) were observed in sperm normal morphology among pre-freezing, 5% DMSO, 5% glycerol and 10% glycerol groups (Table I). The best recovery rate of sperm morphology was in 5% glycerol 96.67%, and the lowest recovery rate of sperm morphology was in 15% DMSO 72.30% (Fig. 1).

DISCUSSION

In our previous study, the emptied sheep's ovarian follicles used as a carrier for human sperm during cryopreservation. We tried to cryopreserve sperms in this carrier without using cryoprotectant to avoid the loss of sperms through dilutions and washing steps, and the results compared with the use of glycerol as a cryoprotectant in same technique. Consequently, we succeeded to cryopreserve sperms using this technique without use of cryoprotectant. But, the better recovery rate for sperms motility post-thawing found with the use of glycerol [17]. In this study, the efficiency of glycerol and DMSO as a cryoprotectants was evaluated in protection of sperms during

cryopreservation in emptied sheep's ovarian follicles technique. Glycerol is a permeable cryoprotectant widely used for cryopreservation of human sperms [20] and nonhuman primate sperms [9]. It reduces the stress of freezing through 'salt-buffering' mechanism [21], dehydrating the cells, binding with the metallic ions and reduces the expansion of total ice volume during freezing [22]. DMSO is a penetrating cryoprotectants, like glycerol, it is reducing the concentration of salt at given temperature to minimize the cell injury at that temperature [21]. Due to its lower molecular weight, DMSO penetrate into cells faster than glycerol [22]. However, both of DIMSO and glycerol have a toxic and osmotic effects on the cryopreserved cell [23]. The results of this study indicated the predomination of glycerol in the protection of cryopreserved sperms, and this result is in concordance with previous studies performed on sperms from human [24], rhesus monkey [9] and buffalo [22]. Our finding indicates that DMSO was more toxic than glycerol for cryopreserved sperms in this technique. Since the penetration of DMSO into cells faster than glycerol, Rasul et al. [22] attributed the lethal effect of DMSO to its toxic effects rather than osmotic. However, DMSO found to give better cryosurvival rate than glycerol and propanediol in cryopreserving human testicular tissue [25]. It was mentioned previously, that different cell type may needs different cryopreservation protocol and different cryoprotectant to achieves optimal survival rate [26]. The results of this study denoted that the best concentration of glycerol for cryopreserve human sperm in emptied sheep's ovarian follicles technique is 5%, and this indicates that the toxicity of glycerol increases in concentrations above 5%. Previous studies indicated that the suitable concentration of glycerol for cryopreserve rhesus monkey sperms was 5% [9] and human sperms was 7% [27]. In this study, we found that the normal sperm morphology was not affected significantly during cryopreservation with 5%, 10% of glycerol and 5% of DMSO While, the progressive motility and total motility decreased significantly in all post-thawing groups. Watson in 2000 [28] when he explained the causes of reduced fertility with cryopreserved semen, he mentioned that about 50% of sperms cannot survive cryopreservation even with optimized protocols. It was mentioned previously that cryopreservation has potential effects upon the sperm motility, viability and morphology [29]. Our finding indicates that the harmful effect of freezing with 5% glycerol in this technique restricted to plasma membrane which led to reduce the motility but not normal morphology. Our study concentrated on sperm motility and morphology, thus, further studies required to confirm these results and to evaluate the integrity of plasma membrane, acrosome and DNA in human sperm after cryopreservation in this technique.C

ONCLUSION

In conclusion, in technique of emptied sheep's ovarian follicles, sperms cryopreserved with 5% glycerol showed good post-thawing recovery of motility and morphology.

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Conflict of interest

The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

THE VALUE OF SERUM SEROMUCOID IN THE DIFFERENTIAL DIAGNOSIS OF BACTERIAL PNEUMONIA AND TUBERCULOSIS IN HIV-POSITIVE PATIENTS

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ABSTRACT

The aim: To optimize the differential diagnosis of bacterial pneumonia and tuberculosis in HIV-positive patients based on the value of serum seromucoid.

Materials and methods: The study included 77 HIV-positive patients with lung pathology. The 1st group consisted of 44 HIV-infected patients with BP; the 2nd group – of 33 patients with HIV/TB co-infection. Level of SSM, CD4+ T-lymphocytes, HIV-1 RNA viral load was determined. Clinical, laboratory, microscopic, radiological, microbiological, and statistical methods were used in the research.

Results: In patients with HIV/TB co-infection CD4+ T-lymphocyte level was lower, and viral load was higher than in HIV-infected patients with BP. The level of SSM was statistically significantly elevated in patients of both groups compared with the control ($p<0,001$), but in patients with HIV/TB co-infection the values were statistically significantly higher ($p<0,001$). In patients with BP, the content of SSM≤15,95 TU occurred statistically significantly more often than in patients with TB **($\chi 2= 65,5$; $p <0,001$).** No statistically significant relationship between SSM content and CD4+ T-lymphocyte levels was found.

Conclusions: The content of SSM in patients with HIV/TB co-infection is statistically significantly higher than in the group of HIV-infected patients with BP. Determination of SSM level can be used as a rapid method of differential diagnosis of BP and TB in HIV-positive patients that will allow to optimize the diagnostic algorithm at the early stage of hospitalization and to receive the necessary timely treatment for HIV-infected patients.

KEY WORDS: HIV infection, bacterial pneumonia, tuberculosis, HIV/TB co-infection, seromucoid

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INTRODUCTION

The problem of infection caused by the human immunodeficiency virus (HIV) continues to be one of the most relevant and important at the present stage of human development. There are more than 38 million people living with HIV and more than 155,000 people died from HIV/AIDS in the world nowadays [1]. Ukraine is considered to be the country with the heaviest burden of HIV infection in Europe. In December 2020 alone, more than 1000 newly diagnosed HIV cases were registered in Ukraine, including 281 AIDS patients, and 176 people died of AIDS [2].

Respiratory diseases, especially of the lower respiratory tract, are observed in 25–60% of HIV-positive patients, and rank second after the nervous system pathology. Lung diseases, in particular pneumonia, are the reason of death of about 85% of HIV-infected people. On the background of immunodeficiency deepening the clinical picture of classic bacterial and viral infections acquires signs of generalization. Superinfection with other opportunistic pathogens also changes the clinical picture, complicates diagnosis and treatment that results in an increase of mortality rate from community-acquired pneumonia in the general population [3-5].

Determining the etiology and prescription of appropriate antipneumonic treatment in HIV-infected patients is an extremely difficult task due to problems in the lifetime verification of the pathogen. Even the result of such a highly sensitive test as PCR depends on the type of the patient's sample (nasopharyngeal lavage or induced sputum). Sometimes the only method to confirm the etiology of BP in patients with HIV infection is a bronchoscopy, but it has certain limitations in use, such as the severity of the condition with the presence of contraindications to it [6].

One of the most serious problem of HIV infection is tuberculosis (TB) that can disguise oncological, autoimmune diseases on the background of deep immunodeficiency, and in the case of a rapid and aggressive course, it also can mimic the community-acquired bacterial pneumonia [7]. It is known that HIV is a major risk factor for the TB transition from latent to clinically pronounced form, which may be not only due to depletion of CD4+ T-cells, but also due to their functional change under the influence of *Mycobacterium tuberculosis*, significantly impairing the ability of the immune system to resist this infection even against the background of successful antiretroviral therapy (ART) [8,9]. According to official statistics, more than 30%

of HIV-infected people have TB, and almost 80% of them die from HIV/TB co-infection. Diagnosis of TB at the stage of deep immunodeficiency caused by HIV is quite difficult, so the laboratory methods currently used are not always able to confirm the diagnosis in time [10, 4]. The advantage of bacterioscopic and molecular genetic (Xpert MTB RIF) methods of sputum research is speed, the result is obtained within a day. But *Mycobacterium tuberculosis* may not be isolated due to its low concentration or in case of extrapulmonary TB. The most reliable method of TB diagnostics is bacteriological, but the result can be expected within 3 months, that can delay etiotropic treatment. Therefore, there is currently a need to develop new markers of HIV-associated TB or improve existing ones.

Factors of nonspecific resistance of the organism mediate the mechanism of their action through the activation of acute phase proteins (APPs). The APPs respons is universal to any tissue damage or the action of an infectious agent. The APPs are among the oldest components of the immune homeostasis system in the human body, able to increase their level in response to a foreign agent (necrosis, inflammation, tumor) that leads to tissue damage [11, 12]. The APPs system includes more than 30 different proteins. One of the most important of them is seromucoid (SM) (alpha-1-acid glycoprotein, orosomucoid). SM is synthesized mainly by liver cells and macrophages under the influence of steroids, proinflammatory cytokines (interleukin-1, interleukin-6, tumor necrosis factor-alpha) during the action of the damaging factor. The concentration of this protein in blood plasma increases 2-4 times during the day after the onset of the damaging agent action and correlates with the content of other reactants of the acute phase of inflammation. The anti-inflammatory functions of SM are realized during its close interaction with the cells of the leukocyte chain. Leukocytes, especially monocytes, are able to synthesize SM. The content of SM also increases in case of chronic systemic inflammatory processes on the surface of leukocytes, mainly monocytes, that actually makes the definition of SM, along with C-reactive protein, one of the most important approaches in assessing and differentiating between acute and chronic inflammation [13-15].

THE AIM

To optimize the differential diagnosis of BP and TB in HIV-positive patients based on the value of serum SM (SSM).

MATERIALS AND METHODS

The research was carried out at the Department of Infectious Diseases of Kharkiv National Medical University (KhNMU), which based on Kharkiv Regional Clinical Infectious Diseases Hospital during 2018-2020. 77 HIV-positive patients who were hospitalized with a diagnosis of community-acquired pneumonia were examined. The design of the study corresponded to a retrospective study (case – control). The object of the study was the content of the SSM in HIV-infected patients with pulmonary pathology. The subject of the study was the clinical course of pulmonary pathology in HIV-infected patients with BP and co-infection HIV/TB. Inclusion criteria: HIV-positive patients, aged from 19 to 65 years, the presence of intoxication syndrome, symptoms of lower respiratory tract affection, signs of lung infiltration. Exclusion criteria: HIV-negative patients, CNS lesions, liver cirrhosis, acute and exacerbation of chronic viral hepatitis B and C, exacerbation of CMV and EBV infection with lung damage, chronic renal failure, pneumocystis pneumonia, systematic use of alcohol and drugs, pregnant women and children, the elderly people. The age of the patients ranged from 22 to 60 years, the mean age was 38,96±0,88 years. By gender, men predominated: 51 (66,2%) men, 26 (33,8%) women. All patients signed informed consent to research. The research protocol was approved by the KhNMU Commission on Ethics and Bioethics on May 2, 2018.

Depending on the clinical-laboratory and instrumental diagnostics data, patients were divided into two groups. The 1st group consisted of 44 HIV-infected patients with BP; the 2nd group – of 33 patients with HIV/TB co-infection. The control group consisted of 31 healthy individuals. Clinical, laboratory, microscopic, radiological, microbiological, including bacteriological examination of a sputum to isolate the pathogen and determine its sensitivity to antibiotics, and statistical research methods were used.

The quantitative parameter of SSM was determined in units of turbidity (TU) on the day of hospitalization before treatment by the turbidimetric method. Reagents of LLC "Filicit-Diagnostics" were used.

Statistical analysis of the obtained data was performed by traditional methods of analytical statistics using software. The mean value of the series (M), the error in the arithmetic mean (m), the standard deviation (σ) were calculated. Statistically significant differences between the mean values (p) were calculated by Student's t-Test (t). Pearson's $\chi 2$ test was also used to assess differences between groups. The correlation coefficient (r) was used to determine the relationship between the studied indicators. Statistical data processing was performed using a PC with the application package «Microsoft Excel 2007» (Microsoft Corporation) and an additional set of statistical analysis programs (Statistica v 6.0 «StatSoft»). Differences were considered statistically significant at $p<0,05$.

The diagnosis of HIV infection was established on the basis of epidemiological, clinical and laboratory data, confirmed by determination of the total spectrum of antibodies to HIV (ELISA) with subsequent confirmation of their specificity by immunoblotting. The level of CD4+ T-lymphocytes was determined by immunocytometry, the level of the HIV-1 RNA viral load – by quantitative PCR.

The diagnosis of pneumonia was established in the presence of radiologically confirmed focal infiltration of lung tissue and at least two clinical signs of the following: acute onset of the disease with a fever above 38°C; cough with sputum; physical signs (dull percussion sound, weakened and/or bronchial breathing, focus of sonorous small-bubble

Table I. Characteristics of the content of the SSM in HIV-infected patients with BP and patients with HIV/TB co-infection (M±m)

Indicator	1st group (n=44)	2nd group (n=33)	Control group (n=31)
SSM, TU	9,99±0,45 $p^1 < 0,001$ $p^2 < 0,001$	94,71±4,21 $p^1 < 0,001$	4,05±0,17

Note. In the presence of statistically significant differences: p^1 – with the indicator in the control group; p^2 - between 1st and 2nd groups.

rales and/or crepitation); leukocytosis (more than $10x10^9$/l) and/or left shift (more than 10% bands).

The diagnosis of TB was established in accordance with the decision of the Central Medical Advisory Commission based on the Kharkiv Regional TB Dispensary № 1 on the basis of clinical-epidemiological and laboratory-instrumental methods (bacterioscopic, bacteriological, molecular genetic, radiological).

RESULTS

In 30 (68,18%) HIV-infected patients with BP the duration of the disease before hospitalization was up to 10 days, in 14 (31,82%) – 10-14 days. Patients with HIV/TB co-infection had a gradual development of the disease with subsequent hospitalization within 1 to 6 months. Oral candidiasis was microscopically confirmed in 77 (100%) patients; anemia – in 63 (81,8%); chronic viral hepatitis C with minimal activity – in 26 (33,8%) patients. Asthenic syndrome (increased fatigue, reduced efficiency and general weakness) was present in 77 people (100%), weight loss up to 10% of baseline body weight – in 43 (55,8%), more than 10% – in 34 (44,2%) patients.

15 (34,1%) HIV-infected patients with BP received ART, 5 (11,36%) of them – within 3 months, 10 (22,72%) – more than six months. In the HIV/TB co-infection group, only 5 (15,15%) patients received ART that was started within the last month.

Chest X-ray examination revealed findings corresponding to pneumonia in all patients of the 1st group – 44 (100%) persons, in 21 (47,7%) – bilateral, and in 23 (52,3%) – unilateral, including 2 (4,6%) – with pleurisy. The X-ray picture of HIV/TB co-infection was more diverse: in 2 (6,1%) patients there were signs of disseminated pulmonary TB, in 14 (42,4%) – of bilateral pneumonia, in 17 (51,5%) – unilateral, including 2 (14,3%) patients with bilateral pneumonia with pleurisy, and 1 (7,1%) – with bilateral pneumonia with pleural empyema, 2 (11,8%) – with unilateral pneumonia with pleurisy.

The microbiological examination of patients with BP revealed the broad spectrum of pathogenic and opportunistic bacteria: *Pr. mirabilis, S. epidermidis, E. coli, Kl. pneumoniae, Ps. aeroginosa, S. pneumoniae, S. aureus, S. pyogenes*.

The diagnosis of TB was confirmed by bacterioscopy, by Xpert MTB RIF and by bacteriology – in 9 (27,3%); Xpert MTB RIF and bacteriologically – in 6 (18,2%) patients; Xpert MTB RIF – in 2 (6,1%); Xpert MTB RIF, bacteriologically, and radiologically – in 1 (3,1%); bacteriologically – in 2 (15,2%); radiologically and bacteriologically – in 1 (3,1%); by the method of CT diagnostics – in 1 (3,1%); bacteriologically and CT – in 2 (6,1%) cases. In 9 (27,3%) patients, the diagnosis of TB was established on the basis of anamnestic, clinical and laboratory data, including the ineffectiveness of antipneumonic therapy in an HIV-infected person on the background of severe immunosuppression (CD-4+ T-lymphocytes <50 cells/μl).

Immunological study in the 1st group revealed values of CD4+ T-lymphocytes from 3 to 994 cells/μl, the average value was 219,07±37,82 cells/μl, while in the 2nd group the number of CD4+ T-lymphocytes ranged from 1 to 356 cells/μl, in average – 96,24±14,66 cells/μl. The average value of viral load in the 1st group was 810525,91±382183,38 RNA copies/ml, while in the 2nd group was much higher – 1720607,82±446783,73 RNA copies/ml.

The level of SSM was statistically significantly elevated in patients of the 1st and 2nd groups compared with the control group (p<0,001). The values of SSM in patients of the 1st group ranged from 5,96 to 19,14 TU, the average value was 9,99±0,45 TU, while in the 2nd group the values were statistically significantly higher (p<0,001) and ranged from 60,24 to 150,14 TU, and the average value was 94,71±4,21 TU. The data about the content of SSM obtained during the study are represented in table I.

The analysis of this indicator according to Pearson's $\chi 2$ test allowed to establish that in patients with BP the content of the SSM, equal to or less than 15,95 TU, occurred statistically significantly more often than in patients with TB ($\chi 2$= 65,5; p <0,001).

The levels of SSM were also compared with the number of CD4+ T-lymphocytes in patients of both groups. The studied parameteres changed chaotically, the correlation coefficient was statistically insignificant, which meant the lack of relationship between the indicators. Thus, the value of SSM does not depend on the number of CD4+ T-lymphocytes in HIV-infected patients.

DISCUSSION

Our retrospective longitudinal study (case-control) using a turbidimetric method for determining the content of SSM provides new information about the diagnostic value of this APP in HIV-infected patients with BP and HIV/TB co-infection. The study was conducted due to the insufficient effectiveness of traditional methods for the detection of TB in HIV-infected patients with lower respiratory tract lesions [16]. We have demonstrated the following conclusions. According to the obtained data, HIV-infected patients with BP had an acute onset of the disease with earlier terms of

hospitalization (2 to 14 days) [17], in contrast to patients with HIV/TB co-infection, in which the disease developed subacutely with subsequent clinical progression within 1-6 months [18]. Patients in both groups had clinical manifestations of opportunistic infections and chronic diseases such as oral candidiasis, anemia, chronic viral hepatitis etc. Clinically, the course of lung affection on the background of HIV infection was similar. The adherence to ART was low so insufficient number of patients received it. Chest X-ray in HIV-infected patients with BP and TB had similar signs in the form of unilateral or bilateral lung infiltration; in two cases disseminated lesions were revealed. Microbiological assay revealed a wide range of pathogenic and opportunistic microorganisms in HIV-infected patients with BP. Microbiological confirmation of pulmonary TB was obtained in 72,7% of cases by a combination of bacterioscopic, bacteriological, molecular genetic and instrumental methods, but in 27,3% of cases the diagnosis of TB in HIV-positive people had no laboratory and instrumental confirmation, that confirms the imperfection of standard methods of TB diagnostics in HIV-infected people [17]. Pulmonary TB in HIV-infected people more often than pneumonia occurred against the background of a more pronounced immunodeficiency. With a decrease in CD4+ T-lymphocyte levels below 200 cells/μl, extrapulmonary manifestations in the form of pleurisy appeared in both groups [16]. For the first time, an increase in the level of SSM was detected in groups of HIV-infected patients with BP and co-infection HIV/TB. Unprecedented levels of SSM have been demonstrated, which differed significantly in patients of the compared groups (significantly higher value in the group of patients with HIV/TB was revealed), that probably is associated with different types of inflammation, which are the pathogenetic basis of these diseases [19]. The limitations identified in our study include the relatively small sample size and the lack of correlation with CD4 + T lymphocyte levels. Thus, the obtained results demonstrate the necessity to take into account when examining HIV-infected patients with lower respiratory tract affection clinical differences in the initial period of the disease development and determination of SSM as APP, the level of which can be an additional differential diagnostic marker of active TB in HIV-infected people.

CONCLUSIONS

1. The statistically significant increase in the level of SSM in HIV-infected patients with pulmonary pathology was revealed.
2. The content of SSM in patients with HIV/TB co-infection was statistically significantly higher than in the group of HIV-infected patients with BP.
3. No statistically significant relationship between SSM content and CD4+ T-lymphocyte levels was found.
4. Determination of SSM can be used as a rapid method of differential diagnosis of BP and TB in HIV-positive patients that will allow to optimize the diagnostic algorithm at the early stage of hospitalization and to receive the necessary timely treatment for HIV-infected patients.

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ORIGINAL ARTICLE

PLACENTAL BIOMARKERS: PP13, VEGF IN DIAGNOSTICS OF EARLY AND LATE PREECLAMPSIA

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ABSTRACT

The aim: To investigate role of CD23, VEGF and PP13 in diagnostics of early and late preeclampsia, and their benefit for prediction of preeclampsia.

Materials and methods: Investigation included 40 placentas from deliveries in women with preeclampsia (main group) and 40 placentas from physiological delivery in somatically healthy women, who had no complications during pregnancy (control group). Placentas in the main group were devided into two sub-groups (20 in each) – with early and late preeclampsia. Each group underwent both hystomorphometrical and immunohystochemical investigation with biomarkers CD23, VEGF and PP13.

Results: Positive immunohystochemical reaction to PP13 was determined in all samples of syncitiotrophoblast of villi of chorion. Investigations showed that expression of PP13 in sub-groups with early and late preeclampsia was a lot lower comparing to control group (normal pregnancies). Positive immunohystochemical reaction to VEGF was determined in all samples of endothelia of the capillaries of the villi of chorion. Our investigation showed that expression of VEGF in sub-groups with early and late PE was a lot lower comparing to a control group. Immunohystochemical reaction to CD23 was comperatively lower in all samples in endothelia of the capillariesof the villi of chorion and cyncithiotrophoblast.

Conclusions: Determined specialties of the expression of angiogenic factors (PlGF, VEGF, endoglin) and production of PP13, by altered expression of VEGF, PlGF in first trimester of pregnancy, which is associated with lowest production of PP13, accompanied by placental dysfunction and preeclampsia.

KEY WORDS: biomarkers, placenta, early and late preeclampsia, endotheliopathy, PP13, VEGF

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INTRODUCTION

Preeclampsia is one of the leading causes of maternal death – 60 000 annually, and it complicates from 5 to 8% of all pregnancies. It has been proven, in clinical and experimental researches that gestational endotheliopathy is a basic mechanism in the development of hypertensive disorders during pregnancy [1-4]. Gestational endotheliopathy results into ischemia, hypoxia and oxidative stress, and plays the leading role in development of preeclampsia. Preeclampsia that develops in terms less than 34 weeks is called early preeclampsia, after 34 weeks – late preeclampsia. Origin of early preeclampsia is related to inadequate invasion of trophoblast, hypoxia in placenta and release of biologically-active substances, that in future will influence endothelia [5]; in case of late preeclampsia it`s mostly connected with maternal cardiovascular system, that will influence integration of endothelia [1].

Angiogenesis-related factors, including sFlt-1 (soluble fms-like tyrosine kinase 1) and PlGF (placental growth factor), soluble endoglin (sENG), are produced by abnormal placentas in higher than normal quantities and released into maternal circulation and play an important role in placental dysfunction; altered levels are detectable several weeks before onset of pregnancy complications. In vitro diagnostic tests for these biomarkers can improve early diagnosis and facilitate prediction of maternal and fetal outcomes [6,7]. Pooled information on placental perfusion (ultrasonography, mean arterial pressure), clinical characteristics, and biomarker levels (PlGF) can improve first-trimester prediction and preeclampsia diagnosis. Angiogenic factors with or without clinical characteristics can facilitate second-/third-trimester prediction of early-onset and late-onset preeclampsia. Analysis of angiogenic factors with or without uterine Doppler substantially improves sensitivity and specificity for predicting adverse outcomes and iatrogenic preterm delivery [6]. The imbalance of proangiogenic and antiangiogenic factors in circulation is thought to trigger the onset of PE by inducing microangiopathy in target organs such as the kidney, liver, or brain [7].

THE AIM

The aim is to investigate role of CD23, VEGF and PP13 in diagnostics of early and late preeclampsia, and their benefit for prediction of preeclampsia.

MATERIALS AND METHODS

Investigation included 40 placentas from deliveries in women with preeclampsia (main group) and 40 placentas from physiological delivery in somatically healthy wom-

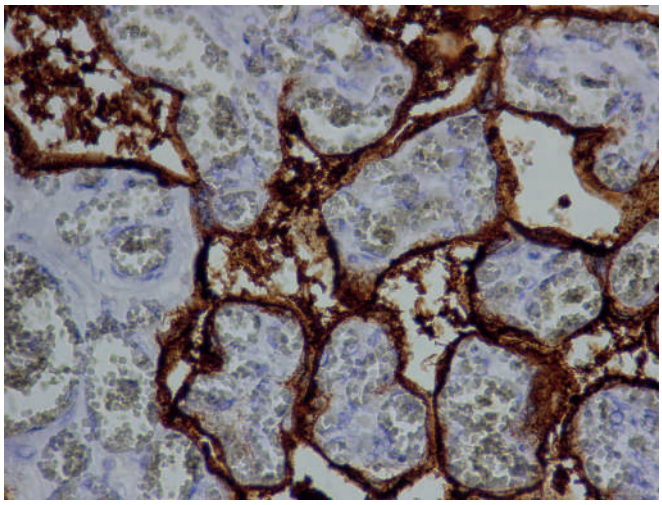

Fig. 1. PP13, control group, positive reaction to biomarker

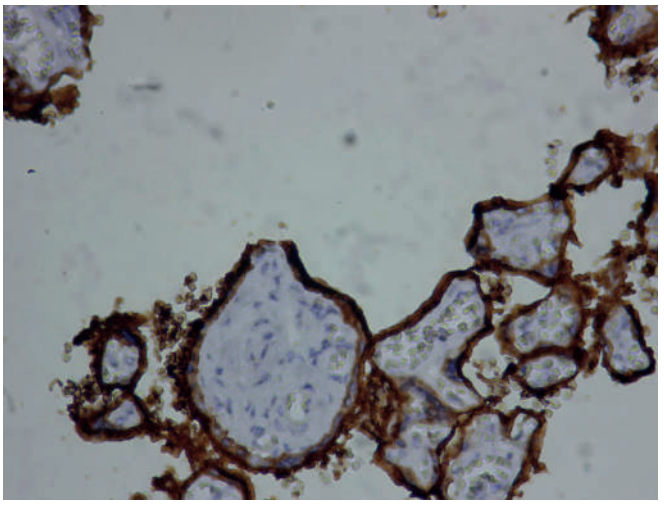

Fig. 2. PP13, early preeclampsia, low positive reaction to biomarker

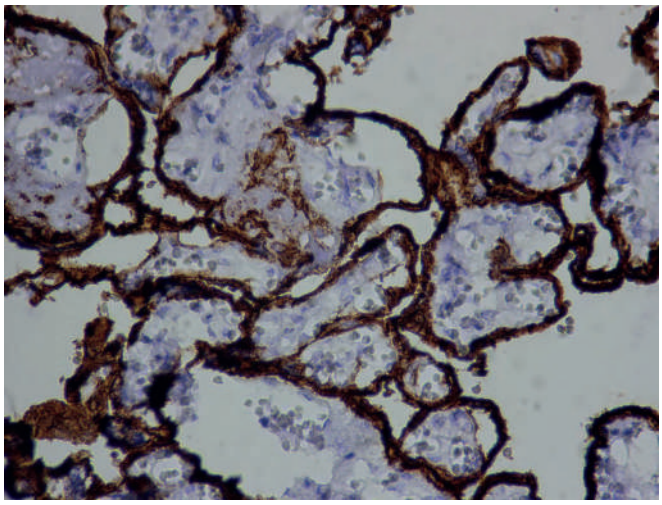

Fig. 3. PP13, late preeclampsia, moderate postive reaction to biomarker

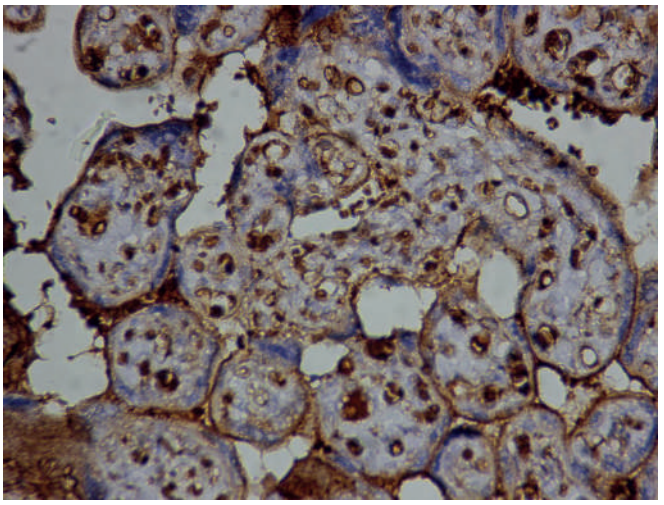

Fig. 4. VEGF, control group, the biggest area of expression

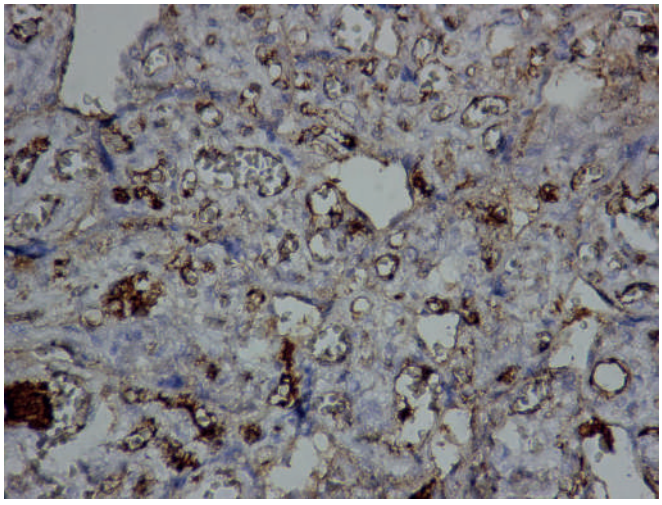

Fig. 5. VEGF, early preeclampsia, the smallest area of expression

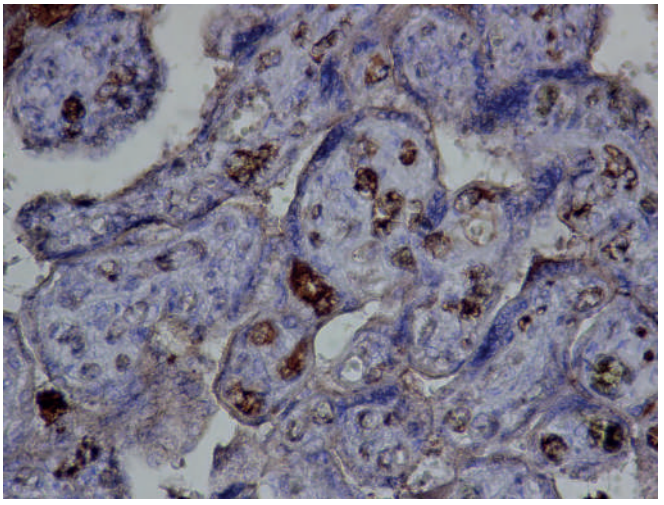

Fig. 6. VEGF, late preeclampsia, moderate index of area of expression

en, who had no complications during pregnancy (control group). Placentas in the main group were devided into two sub-groups (20 in each) – with early and late preeclampsia. Each group underwent both hystomorphometrical and immunohystochemical investigation with biomarkers CD23, VEGF and PP13. Expession of antigens of CD23, VEGF and PP13 was conducted by immunohystochemical method on generally accepted methodology with the

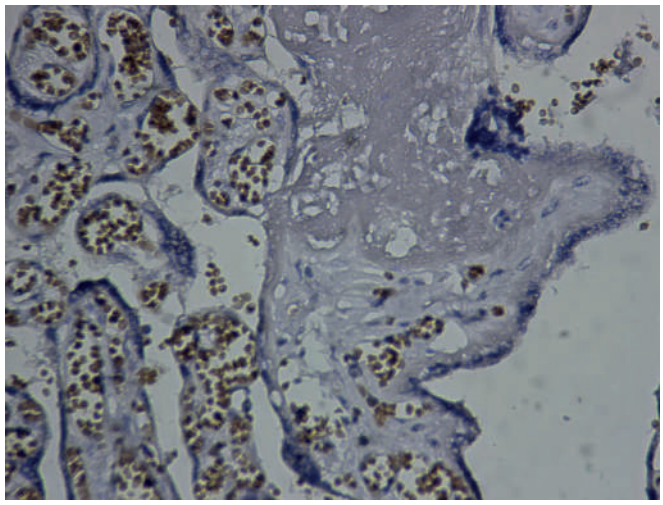

Fig. 7. CD23, control group

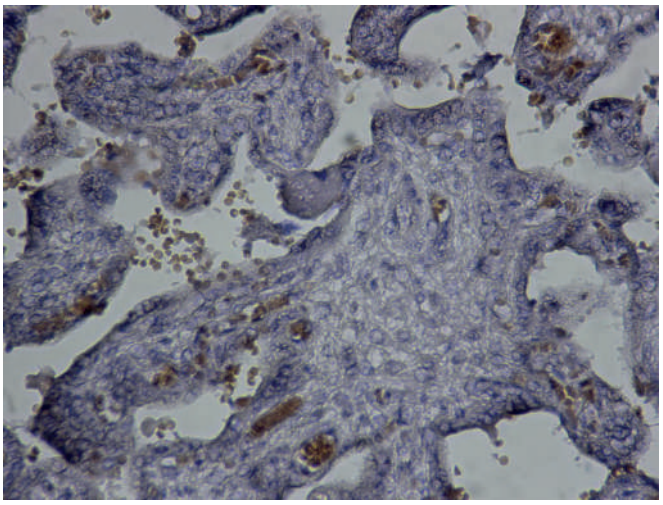

Fig. 8. CD23, early PE

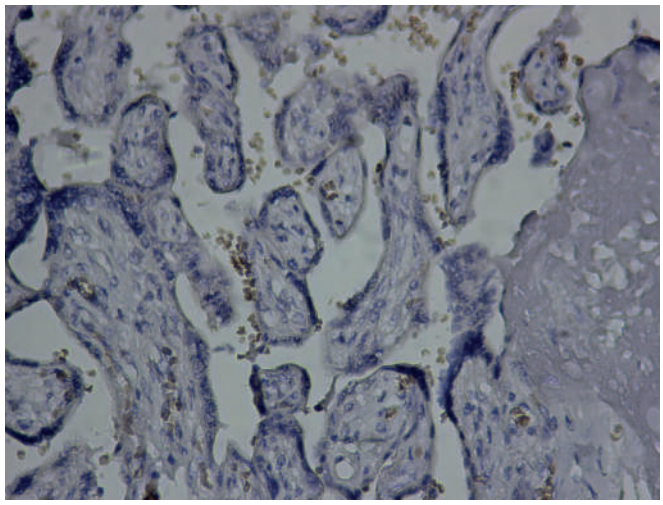

Fig. 9. CD23, late PE

decamouflage of antiangiogens in microwave oven or in citrate buffer (pH 6,0), on water bath during 30 minutes in serial paraffin cuts of placenta, by determination of monoclonal antibodies of class II Clone QBEnd 10 with system of visualization K 801221 EnVision FLEX (universal set EnVision Flex, High pH, Dako).

Microslides were investigated under the microscope Olympus BX 46 with illumination mode according to Keller with ×400 sampling, in order to receive general imagination about the results of immunohystochemical investigation. The quantitative estimation of the results was conducted by microphotos received by the mean of microscopic images fixation system, that consists of microscope Olympus BX 46, digital chamber Olympus UC 30, personal computer on base of Intel Pentium 4 and «Cells entry» software. Photos were made with ×40 sampling (eyepiece 10×, lens 40×), with complete closing of aperture diaphragm, with lifted capacitor in Photo mode and time of display 1/20 sec. Chamber sensitivity-maximal, size of the image-1280×1024 pixels and JPEG graphic picture size (normal). Photography was made from 5 fields of vision for each microslide. Estimation of the expression of investigated biomarker was conducted by systems of computer analysis of microscopic images Morphology 5.2.

After conducting the stages of reactions we estimated brown membrane and cytoplasmic colouring (for biomarker CD23, VEGF, PP13 (clons ab-1, TermoScientific, solution 1:200, Dako Autostainer Instruments). Estimation of expression levels was carried by semiquantitative method on such parameters, as degree of mark spread and colour intensity.

We estimated optical density and area of expression in the entervillious space of placentas. Calculation of relative area of expression was conducted as a relation between area, occupied by immune-positive cells, and general area of cells in vision field; it was expressed in percentage. Optical density of investigated objects was measured in standard units. First index was showing the expression of investigated marker population' cells, second – in separate cells.

The degree of spreading was determined by counting of number of coloured cell nucleus comparing to total quantity of cells in percentage. Intensity of colouring was estimated by semiquantitative method: 1 point – weak colouring of nucleus, 2 points – moderate colouring of nucleus, 3 points – intense colouring of nucleus.

RESULTS

PP13 is located in syncitiotrophoblast of the villi of chorion and in multinuclear luminal trophoblast in transformed decidual spiral arterioles. PP13 is secreted by syncitiotrophoblast, and along with decreased levels of expressed PP13 gestational period was complicated by preeclampsia.

Positive immunohystochemical reaction to PP13 was determined in all samples of syncitiotrophoblast of villi of chorion. Investigations showed that expression of PP13 in sub-groups with early and late preeclampsia was a lot lower comparing to control group (normal pregnancies). The smallest area of expression of PP13 biomarker in villi chorion of placenta was determined in subgroup with early preeclampsia, a little bigger it was in late preeclampsia and the biggest area was determined in control group (Fig 1,2,3).

Table I. Specialties of expression of PP13, VEGF, CD23 in early, late preeclampsia, and normal pregnancies

	Early PE	Late PE	Normal pregnancy	P
PP13	1.54±0.13	3.78±0.22	7.97±0.64	<0.001
VEGF	2.56±0.32	7.24±0.67	12.45±0.82	<0.001
CD23	0.18±0.02	0.34±0.04	0.52±0.05	<0.001

Table II. Placental biomarkers

Biomarker	Early PE (n=20)	Late PE (n=20)	Normal pregnancy (n=40)
PP13	2	4	16
VEGF	9	6	4
CD23	3	2	1

Estimation of the degree of vascularization of villi of chorion was conducted with the help of VEGF biomarker, which is expressed by endothelial cells. It`s an important parameter of functional activity of placenta. Positive immunohistochemical reaction to VEGF was determined in all samples of endothelia of the capillaries of the villi of chorion. Our investigation showed that expression of VEGF in sub-groups with early and late PE was a lot lower comparing to a control group.

Though, the smallest area of expression of VEGF was determined in sub-group with early preeclampsia, it was bigger in group with late PE, and the biggest area of expression was in control group (Fig 4,5,6).

Expression of CD23. Immunohystochemical reaction to this biomarker was much lower in all samples in endothelia of the capillaries of the villi of chorion and cyncithiotrophoblast (Fig 7,8,9).

During immunohystochemical investigation of placenta of two main groups we found decreased expression of biomarker VEGF in endothelia of vessels of chorion, that can point out on violation of function of vascular system and increased vascular resistance of placental blood stream. The results testify to complicated motion of pregnancy, with the development of preeclampsia (Table I).

In early onset preeclampsia evaluated biomarkers PP13 (OR 0.2, 95% CI 0.03, 0.8), VEGF (OR 6.2, 95% CI 1.6, 23.7), CD23 (OR 6.9, 95% CI 0.7, 71.0). In late onset preeclampsia odds were for PP13 (OR 0.4, 95% CI 0.1, 1.3), VEGF (OR 3.9, 95% CI 0.9, 15.8), CD23 (OR 4.3, 95% CI 0.4, 50.9). Obtained data is significantly associated with early onset preeclampsia (Table II).

DISCUSSIONS

In normal pregnancies, serum levels of PP13 slowly rise with gestational age. Several studies have reported that decreased serum levels of PP13 in the first trimester increase the risk of subsequently developing preeclampsia. Measurement of PP13 levels as a first trimester screening marker for preeclampsia may provide an opportunity for identification of women destined to develop early-onset preeclampsia [8].

Moslemi Zadeh et al. conducted a prospective nested case-control study that recruited 1500 pregnant women and 100 women developed preeclampsia and represented the case group. Of 100 women with preeclampsia, 66 cases have mild preeclampsia, while 34 cases have severe pre-eclampsia. Serum PP13 levels along with PAPP-A were measured in the first and second trimesters and were significantly lowered in women who developed preeclampsia ($p< 0.001$). The cumulative value of all the four variables with cut-off points of 238.5 has sensitivity and specificity of 91.0% and area under curve of 0.968. The study concluded that measuring PP13 with PAPP-A in both trimesters of pregnancy is advantageous for the prediction of the incidence of preeclampsia [8,9].

Andraweera P.H. et al. summarized the current knowledge of the roles of the VEGF family in early placentation and of the abnormalities in maternal plasma and placental expression of angiogenic proteins in adverse pregnancy outcomes compared with normal pregnancy. PlGF and sFLT-1 in combination with other clinical and biochemical markers in late first or second trimester appear to predict early-onset preeclampsia with a high sensitivity and specificity. However, VEGF family proteins do not have sufficient power to accurately predict late-onset pre-eclampsia, small-for-gestational age pregnancies or preterm birth. Functional polymorphisms in these angiogenic genes are implicated in pregnancy complications, but their contribution appears to be minor [10].

According to Adi L. Tarca et al. investigation which included 90 patients with a normal pregnancy and 33 patients with early preeclampsia. Two to six maternal plasma samples were collected throughout gestation from each woman. As a result at 22.1-28 weeks of gestation, lower abundance of placental growth factor (PlGF) and vascular endothelial growth factor A, isoform 121 (VEGF-121), as well as elevated sialic acid binding immunoglobulin-like lectin 6 (siglec-6) and activin-A, were the best predictors of the subsequent development of early preeclampsia (81% sensitivity, FPR = 10%); the increase in siglec-6, activin-A, and VEGF-121 at 22.1-28 weeks of gestation differentiated women who subsequently developed early preeclampsia from those who had a normal pregnancy or developed late preeclampsia (sensitivity 77%, FPR = 10%) [11].

Rebecca E. Allen et al. identified 30 studies (65,538 women) for inclusion. Twenty four studies assessed pre-

eclampsia of any onset, 10 studied early onset preeclampsia and seven evaluated late onset preeclampsia (after 34 weeks of gestation). The biomarkers PAPP-A (OR 2.1, 95% CI 1.6, 2.6), PP13 (OR 4.4, 95% CI 2.9, 6.8), sFlt-1 (OR 1.3, 95% CI 2.9, 6.8), pentraxin (OR 5.3, 95% CI 1.9, 15.0) and inhibin-A (OR 3.6, 95% CI 1.7, 7.6) were significantly associated with any preeclampsia. The odds of early onset preeclampsia were significantly increased when the biomarkers PlGF (OR 3.4, 95% CI 1.6, 7.2), PAPP-A (OR 4.8, 95% CI 2.5, 22.5), PP13 (OR 7.5, 95% CI 2.5, 22.5), soluble endoglin (OR 18.5, 95% CI 8.4, 41.0) and inhibin-A (OR 4.1, 95% CI 1.9, 8.8) were abnormal. Two biomarkers, soluble endoglin (OR 2.1, 95% CI 1.9, 2.4) and inhibin-A (OR 1.9, 95% CI 1.4, 2.8) were significantly associated with late onset preeclampsia. Obtained data indicates that abnormal maternal blood biomarkers in early pregnancy are significantly associated with preeclampsia, particularly early onset disease [12].

CONCLUSIONS

Determined specialties of the expression of angiogenic factors (PlGF, VEGF, endoglin) and production of PP13, by altered expression of VEGF, PlGF in first trimester of pregnancy, which is associated with lowest production of PP13, accompanied by placental dysfunction and preeclampsia.

To our opinion biomarkers can not be estimated by themselves, it would be more useful to evaluate them together with Doppler monitoring and pregnancy anamnesis, along with other laboratory tests.

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ORIGINAL ARTICLE

ORIGANUM MAJORANA ATTENUATES CIPROFLOXACIN-INDUCED NEPHROPATHY IN RATS

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ABSTRACT

The aim: The researchers wanted to discover if *Origanum majorana* (O. M.) has any renoprotective qualities in a CIN rat model.

Material and Methods: Control, ciprofloxacin (ciprofloxacin-induced CIN), two *O. majorana* groups (rats treated with *O. majorana* 30 mg and 45 mg), and two ciprofloxacin Plus *O. majorana* groups (n = 8) were randomly assigned to rats (CIN rats treated with *O. majorana* at 30 mg and 45 mg). Renal function tests were performed, as well as histological investigation.

Results: The levels of serum blood urea nitrogen (BUN) and creatinine increased after ciprofloxacin treatment. The serum BUN and creatinine levels in the ciprofloxacin + *O. majorana* groups were lower as well as in *O. majorana* groups, however, kidney damage was higher in the ciprofloxacin group and reduced tissue damage in combination groups and *O. majorana* groups rats.

Conclusions: *O. majorana* decreases experimental CIN *in vivo*. This effect is thought to activate the antioxidant defenses pathway.

KEY WORDS: *Origanum majorana*, ciprofloxacin, urea, creatinine, and anti-oxidants

Wiad Lek. 2022;75(12):3046-3049

INTRODUCTION

Origanum majorana L. is an aromatic and medicinal plant found throughout the Mediterranean region. Traditional medicine has long utilized this species to treat a variety of ailments, including allergies, hypertension, respiratory infections, diabetes, stomach pain, and intestinal antispasmodic [1]. Nephrotoxicity protective effect due to contain thymol, carvacrol, tannins, hydroquinone, arbutin, methyl arbutin, vitexin, orientin, thymosin, triacontane, sitosterol, cis-sabinene hydrate, limonene, terpinene, camphene, and flavonoids including diosmetin, luteolin, and apigenin are among the phytochemicals found in *Origanum majorana* [2]. When the ratio of pro-oxidant species to anti-oxidant defense systems in a cell is out of balance, oxidative stress occurs, causing damage to three primary macromolecules in cells: DNA, lipids, and proteins [3]. Antioxidants include free radical scavengers including glutathione (GSH), ascorbic acid and tocopherol. Detoxifying enzymes such as glutathione-S-transferases (Gsts), UDP-glucuronyl transferases (Ugts), NAD(P)H: quinone oxidoreductase 1 (Nqo1), and catalytic and modifier subunits of -glutamyl cysteine ligase (Gclc, Gclm), which manufacture GSH, also contribute to antioxidant status [4]. Renal toxicity reactions could be caused by drug-induced reactive oxygen species (ROS), which is prevalent during the drug research and development process. The importance of ROS in cellular damage is well established, and it has been proposed that the covalent binding of ROS and reactive intermediates to macromolecules may play a role in severe adverse drug reactions [5]. Ciprofloxacin induce kidney damage effects may be due to induce oxidative stress generation in the renal cells which causes protein depletion in hepatocytes due to nucleic acid loss and DNA damage. This could lead to a significant decline in mitochondrial number and degeneration, which is the source of energy [6]. Depending on quantities and duration, *O. majorana* essential oil and its oxygenated monoterpene component linalool displayed antioxidant and cytotoxic action. These findings suggest that concentrations are crucial in their application. The anti-oxidant activities of *O. majorana* and linalool suggest that they could be used to treat renotoxicity [7]. As a result, activating the anti-oxidative pathway to stimulate the production of cytoprotective genes could be employed to treat renal diseases. The goal of the study was to see if *O. majorana* could protect the kidney against cipro-induced renal damage in an acute chemical model. The renal protective properties of *O. majorana* against ciprofloxacin were studied because it has been proven to activate anti-oxidative system. Renal toxicity was investigated using rats.

THE AIM

The aim of the research wad to discover if *Origanum majorana* has any renoprotective qualities in a CIN rat model.

Table I. Distribution of kidney`s makers which measure in serum among study groups.

Groups/Markers	Urea (mg/dl)	Creatinine (mg/dl)	*p-value
Control	24.50 ± 4.370	0.88 ±0.05	0.00#
Ciprofloxacin	46.00 ± 3.162	1.98 ± 0.57	
O. majorana - 50	25.16 ± 4.35	0.88 ± 0.38	0.96
O. majorana - 100	23.83 ± 2.63	0.81 ± 0.58	
Combination-1	34.83 ± 3.06	1.35± 0.1	0.75
Combination-2	32.23 ± 2.36	1.21 ± 0.23	

Each value is a mean of eight animals ± SD, *=one-way Anova, #= significant

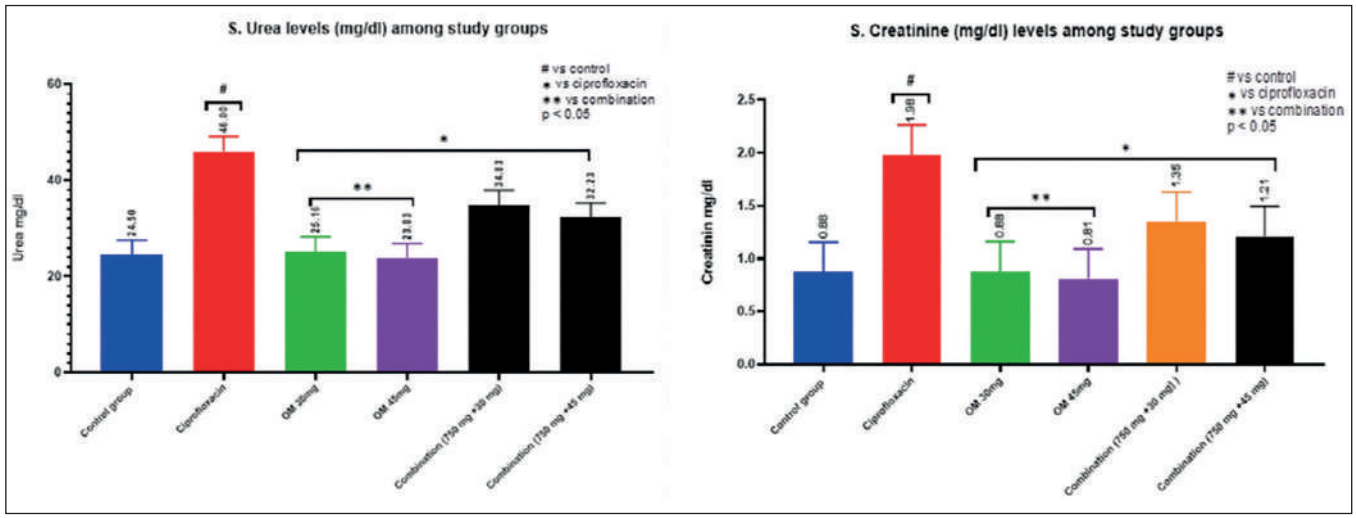

Fig. 1. Representation of the urea and creatinine levels (mg/dl) among study groups.

MATERIALS AND METHODS

ANIMALS AND STUDY DESIGN

Six groups of 48 Sprague Dawley male rats will be formed. For a period of 30 days, each group will contain 8 rats. Drug preparation: *O. majorana* was dissolved in water and delivered orally at doses of 30, and 45 mg/kg, respectively. The animals were maintained in the animal house at the University of Karbala's Faculty of science. The University of Karbala-Animal Care and Research Committee accepted the experiment, and the investigation followed the Laboratory Animals Guide Care. The animals were in full access to clean water, and they will be divided into the following groups: the control group: were given water for 30 days; the ciprofloxacin group: were given drug at a concentration of 100 mg/kg once daily by oral administration for 30 days [8]. The *O. majorana* - 50 group: given *O. majorana* at a concentration of 30 mg/kg once daily by orally administration for a period of 30 days [9]. The *O. majorana* - 100 group: given *O. majorana* at a concentration of 100 mg/kg once daily by orally administration for a period of 30 days [10]. The Combination-1 group: given at ciprofloxacin 100 mg/kg once daily by oral administration for 30 days plus *O. majorana* orally with a concentration of 30 mg/kg [10-11]. The Combination-2 group: given at ciprofloxacin 100 mg/kg once daily by oral rout for 45 days plus *O. majorana* orally with a concentration of 100 mg/kg. At end of experiment, animals were killed by heart puncture under ketamine (25 mg/kg) and xylazine (5 to 10 mg/kg) anesthesia [12]. The animals were sacrifice for collection of blood and liver tissues for further analyses [13].

MEASUREMENT OF OXIDANT PARAMETERS

According to the manufacturer's procedure and a recent study, the levels of urea and creatinine were measured using two commercial detection kits.

STATISTICAL ANALYSIS

The data is presented as averages with standard deviations (SD). The significance of differences in multiple group comparisons was established using the one-way analysis of variance in SPSS software 26.0. (ANOVA).

RESULTS

O. MAJORANA PREVENTS CIN - IN RATS

The serum blood urea nitrogen (BUN) and creatinine values in each group are shown in table I and figure 1. The ciprofloxacin group had significantly higher serum BUN and creatinine levels than the control group (P=0.05). The *O. majorana* alone or in combination with ciprofloxacin re-

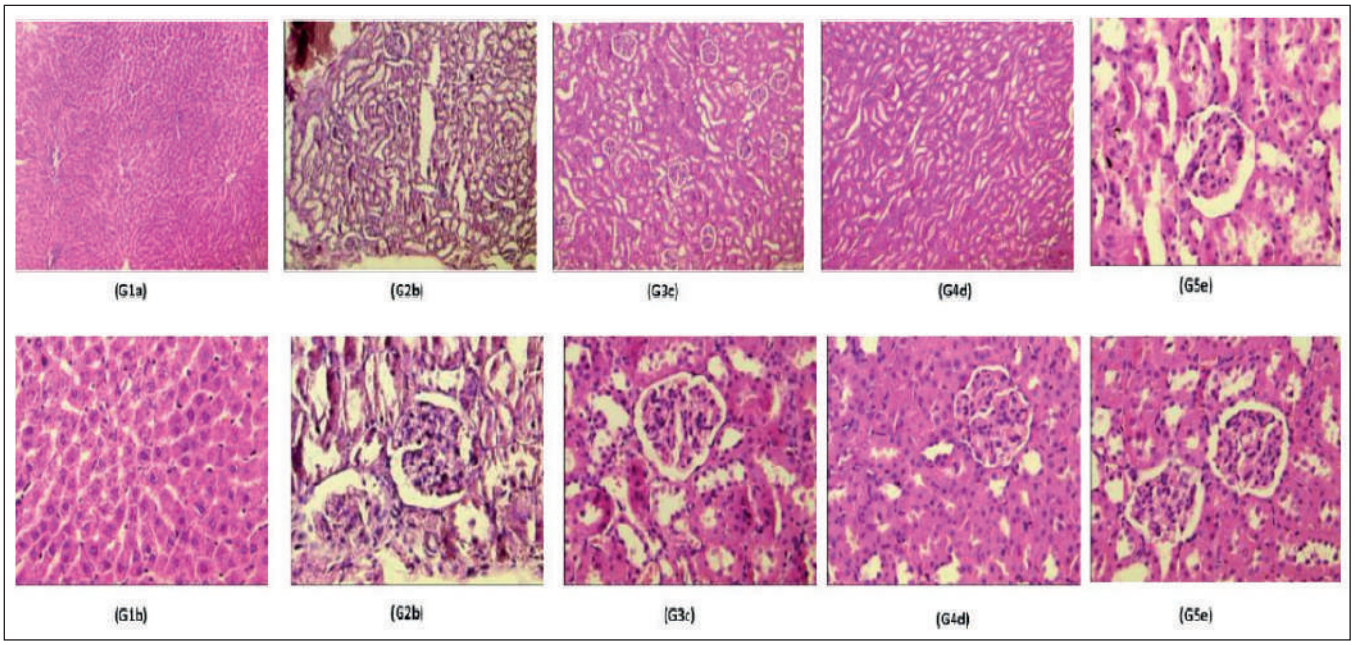

Fig. 2. Representative histologic samples from several groups: control group (a1, 1b), ciprofloxacin group (b1, b2), O. majorana group (b1, b2) (c1, c2), and ciprofloxacin + O. majorana group (d1, d2) (e1, e2). Magnification: X400.

sulted in lower urea and creatinine levels in the blood than the ciprofloxacin group. The ciprofloxacin + *O. majorana* group had significantly lower blood BUN and creatinine levels than the ciprofloxacin group (P=0.05), indicating that *O. majorana* may have a reno-protective effect.

***O. majorana* and ameliorated renal histological damage**

The degenerative alterations in kidney slices from all groups are depicted in figure 2. The kidney sections of the control and *O. majorana* groups of animals showed no notable histological changes. Kidney portions from the ciprofloxacin group had substantial damage, including lesions, tubular necrosis, and hemorrhagic foci. The development of these lesions and tissue damage is greatly reduced in the ciprofloxacin + *O. majorana* group. According to this kidney pathological finding, *O. majorana* may prevent CIN rats from renal histological injury.

DISCUSSION

In human patients, the incidence of raised blood creatinine and urea levels linked to ciprofloxacin prescription ranges from 0.2% to 1.3%, according to published statistics. Furthermore, incidences of ciprofloxacin-related azotemia have been found to range between 1.8 and 13.1 per 1000 patients who have been given the antibiotic. A high index of suspicion should be maintained in the absence of any distinguishing clinical symptoms of nephrotoxicity other than an increase in urea and creatinine serum values after treatment of a fluoroquinolone [14]. *O. majorana* has a cytoprotective effect of against cell injury due to flavonoids, carotenoids, phenolic compounds, vitamins, and antioxidant enzymes are part of the antioxidant defense system that protects against oxidative damage caused by ROS. Although synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxy anisole (BHA) are routinely utilized in processed foods, they have been linked to some negative side effects [15-16]. According to our findings, ciprofloxacin administration resulted in acute renal impairment. Renal function and histological damage have both decreased in the animals. Furthermore, the ciprofloxacin group had significantly higher ROS levels in renal tissue. Administering *O. majorana* without an inducible agent, on the other hand, boosted anti-oxidative enzyme activity via increasing the expression of the antioxidant factors, demonstrating that *O. majorana* plays a role in renal protection. This protection demonstrated after CIN induction greatly reduced renal damage and decreased ROS levels in the ciprofloxacin + *O. majorana* group. The presence of oxidative damage in the CIN rats was also revealed by the lower urea, and creatinine in the renal tissues of the ciprofloxacin group in this investigation. Treatment with *O. majorana* boosted the renal protective activities to protect against oxidative damage in rats without CIN, as demonstrated in figure 2. Furthermore, *O. majorana* therapy immediately scavenged ROS and increased anti-oxidant enzymes in rats with CIN. *O. majorana's* reno-protective action can be related to the elimination of excess ROS directly. Previous research [17-18] have reported *O. majorana's* antioxidant properties, which are consistent with our findings. In addition, *O. majorana* improved the viability of ciprofloxacin-induced injuries.

CONCLUSIONS

O. majorana improves CIN as assessed by renal function and kidney pathology, according to our findings. *O. majorana* significantly decrease the levels of urea and creati-

nine, as well as improve histopathological tissue damage. The increased antioxidant defense in the kidney and the lowering of the urea and creatinine are primarily responsible for these positive benefits. *O. majorana* may thus be a useful medication in the prevention of CIN, although more research and randomized clinical studies are needed to confirm its preventive role.

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ORIGINAL ARTICLE

CRITERIA FOR ASSESSING ENDOGENOUS INTOXICATION IN PATIENTS WITH MULTIPLE PERITONITIS

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ABSTRACT

The aim: To determine the diagnostic significance of the level of malondialdehyde (MDA) in various biological media for RP for assessing and predicting the course of the disease.

Materials and methods: Our study included The work was based on the results of surgical treatment of 60 patients with RP: according to MPI I - in 17 (28.3%), MPI II - in 23 (38.4%) and MPI III - in 20 (33.3%) patients. Surgical intervention for RP was aimed at sanitation and drainage of the abdominal cavity. The control group included 15 practically healthy people, whose blood and urine biochemical parameters served as the norm. According to the clinical course of the disease, the patients were divided depend on admission, The secondary product of lipid peroxidation - MDA was studied in blood serum, peritoneal exudate and urine in patients with RP, depending on the severity of the pathological process according to the Mannheim peritoneal index (MPI) - I severity (I), II severity (II), III severity (III). The work was based on the results of surgical treatment of 60 patients with RP: according to MPI I - in 17 (28.3%), MPI II - in 23 (38.4%) and MPI III - in 20 (33.3%) patients.

Results: Analyses of results showed that the average value of MDA in various biomedicals of recovered (n = 18) and subsequently died (n = 5) patients with MPI II are given in Table. 2. As can be seen from the presented data, the average value of MDA in blood serum and peritoneal exudate in patients with a lethal outcome is significantly higher than in those who have recovered. This fact indicates a more pronounced endogenous intoxication in patients who died from RP.

Conclusions: The content of MDA in the blood serum before surgery in patients with a lethal outcome was 190% ($p < 0.001$). The level of MDA in urine in patients according to MPI II who subsequently died before surgery was 110% ($p < 0.001$), and in those who recovered by 300% ($p < 0.001$) it was higher than the norm. exudate taken during the operation averaged 4.14 ± 0.33 nmol / ml, then in the recovered - 2.89 ± 0.08 nmol / ml. A high level of MDA in the blood serum, peritoneal exudate with a decrease in the elimination of MDA in the urine in the postoperative period are prognostically unfavorable signs in patients, indicating the continuation of endogenous intoxication and a possible death.

KEY WORDS: endogenous intoxication, generalized peritonitis, malondialdehyde (MDA), inflammation

Wiad Lek. 2022;75(12):3050-3054

INTRODUCTION

Treatment of patients with generalized peritonitis (RP) continues to be one of the most pressing problems of abdominal surgery. According to observations, in 20 - 30% of all cases associated with acute surgical pathology of the abdominal organs, peritonitis develops [1,2]. Mortality in RP in Europe reaches from 9.79 to 84% and averages 38.6-45%, which indicates the absence of a single algorithm for the treatment of this complication [3,4]. Along with the systemic inflammatory response and hypoxia, one of the leading places in the development of disorders in the homeostasis system in RP is occupied by disorders of free radical processes and the development of endotoxicosis, which are the main causes of metabolic disorders [5,6].

Increased attention to the problem of RP is due to the accompanying serious complications: extremely severe endotoxicosis, the development of abdominal sepsis, multiple organ failure and pyoinflammatory complications [7,8]. Along with the systemic inflammatory response and hypoxia, one of the leading places in the development of disorders in the homeostasis system in RP is occupied by disorders of free radical processes and the development of endotoxicosis, which are the main causes of metabolic disorders [9,10].

In the implementation of metabolic processes, lipid peroxidation (LPO) plays a leading role [11,12]. LPO processes as a necessary metabolic link proceed normally in a healthy organism, however, under certain conditions (trauma, inflammation, ischemia, hypoxia, etc.), a sharp intensification of lipid peroxidation occurs with the formation of an excess amount of free radicals, which leads to the development of oxidative stress, and LPO becomes a universal mechanism of damage to both a single molecule and cells at the level of membranes and organ tissue [13-15]. An increase in the intensity of LPO processes plays a triggering role in the development of multiple organ failure [16,17]. To neutralize excess lipid peroxidation and maintain the LPO processes at a stationary level in the body, there is an antioxidant defense system (AOD), which normally provides a balance between LPO and AOD [18-20]. Violations of the antioxidant status of the body with a sharp intensification of lipid peroxidation and AOD deficiency reduce the detoxification of the body and lead to the development of poisoning by the type of oxidative distress [21].

Table I. Comparative assessment of MDA parameters in blood serum, peritoneal exudate and urine (M ± m, min-max (in nmol / ml).

Test material	Index	Research time,day					Norm value
		Before operation	1	3	5	7	
Blood serum	I	6,31±0,25 4,5–7,9	8,58±0,23 5,6–9,6	6,17±0,18 5,2–8,3	5,68±0,18 4,7–7,5	5,3±0,18 4,3–7,4	3,79±0,5 3,4–4,1
	II	9,77±0,34 6,8–11,9	10,73±0,36 7,7–13,9	9,1±0,31 6,8–12,1	8,23±0,32 6,2–11,5	7,26±0,3 5,7–11,1	
	III	13,08±0,42 9,5–16,5	15,58±0,47 10,6–18,8	12,01±0,5 8,5–15	11,24±0,52 7,5–14,4	10,55±0,44 7,1–13	
Abdominal exudate	I	2,45±0,07 2,1–3,1	2,88±0,88 2,4–3,5	2,19±0,08 1,6–2,8	2,11±0,08 1,6–2,7	-	
	II	3,16±0,14 2,2–4,81	3,99±0,18 2,6–6	3,32±0,12 2,4–4,81	3,06±0,11 2,2–4,25	2,81±0,11 2,2–3,87	
	III	4,43±0,22 3,1–6,1	5,05±0,25 3,5–6,91	4,34±0,19 3,5–6	4,13±0,15 3,5–5,6	3,72±0,09 2,9–4,1	
Urine	I	0,043±0,003 0,015–0,065	0,051±0,04 0,02–0,07	0,048±0,03 0,02–0,065	0,042±0,03 0,015–0,06	0,033±0,02 0,02–0,05	0,2±0,02 0,01-0,03
	II	0,072±0,005 0,038–0,11	0,112±0,08 0,043–0,16	0,098±0,07 0,03–0,13	0,084±0,07 0,02–0,12	0,057±0,05 0,02–0,1	
	III	0,107±0,009 0,04–0,18	0,152±0,04 0,05–0,23	0,136±0,01 0,07–0,2	0,111±0,01 0,06–0,18	0,105±0,09 0,05–0,155	

Table II. The average value of MDA in various test materials in patients with RP according to MPI II, (M ± m, min-max (in nmol / ml)

Outcome of the disease	Research material	Research time, day				
		Before operation	1	3	5	7
Favourable (n-18)	Blood serum	9,42±0,39 6,8–11,9	10,32±0,39 7,7–12,8	8,93±0,34 6,8–12,1	8,08±0,35 0,2–11,5	7,11±0,31 5,7–11,1
	Abdominal exudate	2,89±0,08 2,2–3,5	3,59±0,09 2,6–4,1	3,14±0,08 2,4–3,6	2,93±0,08 2,2–3,5	2,7±0,09 2,2–3,3
	Urine	0,8±0,004 0,05–0,11	0,13±0,004 0,09–0,16	0,109±0,005 0,07–0,13	0,094±0,005 0,06–0,12	0,06±0,005 0,04–0,1
Unfavourable (n-15)	Blood serum	n = 5 11,04±0,30 10–11,6	n = 5 12,24±0,5 10,9–13,9	n = 3 10,07±0,46 9,3–10,9	n = 3 9,17±0,46 8,4–10	n = 2 8,2; 9,1
	Abdominal exudate	n = 5 4,14±0,33 3,17–4,81	n = 5 5,41±0,27 4,61–6	n = 3 4,35±0,32 3,73–4,81	n = 3 3,84±0,37 3,11–4,25	n = 2 3,7; 3,87
	Urine	n = 5 0,043±0,2 0,038-0,051	n = 5 0,048±0,03 0,043–0,06	n = 3 0,037±0,003 0,03–0,04	n = 3 0,025±0,003 0,02–0,03	n = 2 0,02; 0,027

In our opinion, the study of lipid peroxidation processes in peritoneal exudate, blood serum and urine is of great importance [22].

There are practically no works in the literature that provide comparative assessments of the levels of lipid peroxidation metabolites in blood serum, peritoneal exudate and urine.

THE AIM

The aim of the work was to determine the diagnostic significance of the level of malondialdehyde (MDA) in various biological media for RP for assessing and predicting the course of the disease.

MATERIALS AND METHODS

All patients had been admitted to the SI «Zaycev V. T. Institute of General and Urgent surgery of National Academy of Medical Sciences of Ukraine», Kharkiv, Ukraine for surgical treatment for generalized peritonitis (RP) underwent standard clinical and laboratory tests. Patients who were treated at the Department of Surgical Infections.

According to the clinical course of the disease, according to severity the patients, according the level of MDA were divided into the groups. The secondary product of lipid peroxidation - MDA was studied in blood serum, peritoneal exudate and urine in patients with RP, depending

on the severity of the pathological process according to the Mannheim peritoneal index (MPI) - I severity (I), II severity (II), III severity (III).

The work was based on the results of surgical treatment of 60 patients with RP: according to MPI I - in 17 (28.3%), MPI II - in 23 (38.4%) and MPI III - in 20 (33.3%) patients. ... Surgical intervention for RP was aimed at sanitation and drainage of the abdominal cavity. The control group included 15 practically healthy people, whose blood and urine biochemical parameters served as the norm.The concentration of MDA in all three biological media was determined by the method of L.I. Andreeva et al. It is necessary to find a foreigh method before the operation, during the operation in the taken exudate, on the 1st, 3rd, 5th and 7th days after the operation.

The authors declare that all procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008 (5), as well as the national law. Informed consent to participate in the study was discussed and signed by all study participants. Meeting of the Bioethics Commission (protocol №1 06.09.2021) of the State Institution "Zaycev V.T. Institute of General and Urgent Surgery of the National Academy of Medical Sciences of Ukraine".

There was not found a significant difference in the number of patients, average age, gender composition, or body mass index (BMI) found between OA groups ($p>0.05$).

Ethics review. All patients signed a voluntary informed consent for the examination and treatment, as well as publication of anonymized personal medical information for academic purposes.

Statistical analysis. Determine the diagnostic significance of the level of malondialdehyde (MDA) in various biological media for RP for assessing and predicting the course of the disease using non-parametric statistical techniques (analysis of two-dimensional frequency distributions based on the chi-squared criterion and relative risk) in free-access software for statistical calculations and MS Excel spreadsheet processor. The differences between the main and control groups are statistically significant ($p<0.05$).

RESULTS

The level of MDA in peritoneal exudate, blood serum and urine directly depended on the severity of RP according to the MPI. It was found that in patients with MPI I in the blood serum, the concentration of MDA was 66.5% ($p <0.001$), in urine - 2.1 times ($p <0.001$) more than in healthy people. In peritoneal exudates obtained during the operation, the concentration of MDA was 2.45 ± 0.07 nmol / ml.

The results of the study as a whole are presented in Table I.

In patients with MPI II, the MDA level in the blood serum was 2.6 ($p <0.001$), in the urine - 3.6 times ($p <0.001$), according to MPI III - 3.5 ($p <0.001$) and 5.3 times ($p <0.001$), respectively, higher than the normal value. In the peritoneal exudate taken during the operation, the content of this metabolite was 3.16 ± 0.14 according to MPI II, and 4.43 ± 0.022 nmol / ml according to MPI III.

The average value of MDA in various biomedicals of recovered (n = 18) and subsequently died (n = 5) patients with MPI II are given in Table II. As can be seen from the presented data, the average value of MDA in blood serum and peritoneal exudate in patients with a lethal outcome is significantly higher than in those who have recovered. This fact indicates a more pronounced endogenous intoxication in patients who died from RP. So, if in patients with a fatal outcome the average value of MDA in peritoneal exudate taken during the operation averaged 4.14 ± 0.33 nmol / ml, then in those who recovered it was 2.89 ± 0.08 nmol / ml. The content of MDA in blood serum before surgery in patients with a lethal outcome was 2.9 times ($p <0.001$), and in recovered patients - 2.5 times ($p <0.001$) more than in healthy people.

The level of MDA in urine in patients according to MPI II who subsequently died before surgery was 2.1 times ($p <0.001$), and in those who recovered it was 4 times higher ($p <0.001$) compared to the norm. The average MDA value of blood serum and peritoneal exudate on the 3rd and 3rd day of the postoperative period in recovered patients and patients with lethal outcome increased. Subsequently, the value of MDA in peritoneal exudate and blood serum in recovered patients significantly differed, having a clear tendency to decrease.

During the entire postoperative period, there was a higher level of MDA in the urine with a maximum value on day 3 and up to a level of 0.130 ± 0.004 nmol / ml in recovered patients. The consistently high level of MDA in the urine was accompanied by a natural decrease in the MDA content in the blood serum in these patients. In patients with a lethal outcome throughout the entire postoperative period of observation, the level of MDA in the blood serum and exudate remained high. Against this background, the level of MDA in the urine was lower than in the recovered patients. Thus, a weakly expressed tendency towards a decrease in the level of MDA in peritoneal exudate and blood serum is accompanied by a progressive decrease in the elimination of this metabolite in the urine in patients with a fatal outcome.

In patients with MPI III, in general, the maximum values of the MDA level in the peritoneal exudate and blood serum were recorded. At the same time, an increase in the intoxication of MDA values in the exudate was observed from 4.43 ± 0.22 nmol / ml on the day of surgery to a maximum of 5.05 ± 0.25 nmol / ml on the first day after surgery. In the blood serum before the operation, the MDA level was 13.08 ± 0.42 nmol / ml with a maximum value on the 3rd and 3rd day of the postoperative period. In these patients, there was a distinct tendency towards a decrease in the level of MDA in the peritoneal exudate and blood serum with increased elimination of MDA in the urine.

DISCUSSION

Many pathophysiological events develop due to generalized peritonitis. Free oxygen radicals caused by inflammation damage the cell membrane. Free oxygen radicals increase

lipid peroxidation. The final product of lipid peroxidation, malondialdehyde (MDA) indirectly shows the amount of free oxygen radicals [23].

It should be noted, the comparative analysis of the researches showed that the study of MDA in peritoneal exudate, blood serum and urine in the postoperative period in patients with RP is one of the objective criteria for assessing endogenous intoxication [24]. The relatively rapid resolution of the phenomena of peritonitis in patients was accompanied by a regular drop in the level of MDA in the exudate and serum with enhanced elimination of this metabolite in the urine. A high level of MDA in the blood serum and peritoneal exudate with a decrease in the elimination of MDA in the urine in the postoperative period are prognostically unfavorable signs in patients, indicating the continuation of endogenous intoxication and possible death [25].

Intraabdominal complications, infections, microorganisms and toxins in abdominal cavity due to generation of inflammatory response in the peritoneum is defined as the accumulation of purulent exudat [26]. This inflammatory response may be local or generalized on the surface of the peritoneum. At the present time in Ukraine the statistics of mortality of generalized peritonitis are very high and amounts to 41,3-71,7% whereas postoperative mortality in GP varies in the range of 41,8-72,5% [22]. It is very important to notice that in elderly patients the mortality rate increases to 99 %. Mortality in GP in Europe reaches from 9.79 to 84% and averages 38.6-45%, which indicates the absence of a single algorithm for the treatment of this complication [28,29].

Generalized peritonitis is one of the reasons that increase oxidative stress and marks such as lipid peroxidation, an indicator of oxidative stress, is mediated the harmful effects of oxidative stress. There are many ways of mechanisms, are known as antioxidants, against free oxygen radicals occurring in the body. If the concentration of free oxygen radicals released is much more than the antioxidant capacity of the cleaner system, irreversible cell damage occurs. In a healthy cell, there is a balance between the formation and removal of free radicals. Oxidative stress occurs if the balance is disrupted for any reason and more free radicals are formed or the level of antioxidants decreases. This stress causes serious cell damage if prolonged and severe [26,27].

CONCLUSIONS

1. The content of MDA in the blood serum before surgery in patients with a lethal outcome was per 2,9 times more ($p < 0.001$), and in those who recovered - was per 2,5 times ($p < 0.001$) more than in healthy people 3,79±0,5 nmol / ml ($p < 0.001$). The level of MDA in urine in patients according to MPI II who subsequently died before surgery was per 2,9 times more ($p < 0.001$), and in those who recovered per 4 times more ($p < 0.005$) it was higher than the norm. exudate taken during the operation averaged 4.14 ± 0.33 nmol / ml, then in the recovered - 2.89 ± 0.08 nmol / ml.
2. Further rapid resolution of the phenomena of peritonitis in patients was accompanied by a regular drop in the level of MDA in the exudate and blood serum with enhanced elimination of this metabolite in the urine.
3. A high level of MDA in the blood serum, peritoneal exudate with a decrease in the elimination of MDA in the urine in the postoperative period are prognostically unfavorable signs in patients, indicating the continuation of endogenous intoxication and a possible death.

STUDY LIMITATIONS

This study containes the research of determination and diagnostic significance of the level of malondialdehyde (MDA) in various biological media for RP for assessing and predicting the course of the disease. Since studying and control these marks we improve results in the post- and preoperative periods and realize the full effect of treatment in patients.

PROSPECTS FOR THE FUTURE RESEARCH

Since the careful study the level of malondialdehyde (MDA) in patients with generalized peritonitis in surgical treatment and conservative treatment patients can notice to improving of surgical results, we plan to develop an algorithm that will reduce the number of postoperative complications.

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ORIGINAL ARTICLE

ANALYSIS OF THE ADVISABILITY OF USING DIFFERENT TYPES OF BASE PLASTICS BY STUDYING THE NEEDS OF THE POPULATION IN REMOVABLE PROSTHESIS

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ABSTRACT

The aim: To determine the needs of the population of the Ternopil region in prosthetics with removable orthopedic dentures and to study the physical and technological properties of different types of base plastics.

Materials and methods: The needs of Ternopil and Ternopil region population in removable prosthetics were studied on the basis of consolidated annual reports of city and district dental clinics of Ternopil region. Base dental plastics «Ftoraks» (Stoma, Ukraine) and «Villacryl H Plus» (Zermapol, Poland) were used to study the physical and mechanical properties (deformations during compression and tension).

Results: As a result of our research, we found that the need of the Ternopil region population for removable prosthetics is quite high. When analyzing the choice of designs of removable laminar prostheses, it was found that 64.99 ± 0.14% of patients required the manufacture of partial removable dentures, and 35.01 ± 0.18% – complete removable dentures. A significant difference was found in the physical and technical characteristics of the samples of the studied plastics, which were polymerized by various methods.

Conclusions: Dental base plastic «Villacryl H Plus», which was packaged and polymerized by casting, has significantly higher physical and mechanical properties than dental base plastic «Ftoraks».

KEY WORDS: physical and technological properties, partial or complete adentia, partial and full laminar dentures, base dental plastics

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INTRODUCTION

Sufficient attention is paid to the study of the needs of the population of Ukraine in dental orthopedic care. Large-scale preventive measures, improvement of known methods and approaches to treatment, unfortunately, did not significantly reduce the percentage of patients with defects of teeth and dentition. According to the scientific literature, the number of such patients from the total population of Ukraine is 70-95% [1-4].

Moreover, it should be noted that the problem of partial loss of teeth today is considered not so much as an aesthetic defect, but as a complex damage to the organism, which significantly affects the quality of patient's life. The population's needs for removable prosthetics, according to of authors who have studied this problem is quite high and directly proportional to the age groups of patients [5-7].

Partial or complete adentia is the most common pathology of the dental system. Despite the wide range of preventive and modern treatment measures the problem of tooth loss remains extremely relevant [8-12]

According to the modern scientific literature, the number of patients with defects of the dentition from the total population of Ukraine is from 70% to 95%, which causes a high need for rational prosthetics [13-15].

According to some authors, the overall needs for orthopedic dental care in Kyiv are on average 75%, especially in the age group 35-55 years. The needs for permanent prosthetics in this region are 67.80%, which is especially typical for the age group 35-45 years [16].

According to the analysis of the prevalence of dentition defects in the Lviv region, it was found that 71.63 ± 1.70% of the examined persons have partial adentia of the dentition, among which the included defects are 71.74 ± 1.16%, and the end defects are 28.26 ± 1.16% [16].

Some researchers have determined the needs of Poltava region population in prosthetics with removable orthopedic structures. It is established that the need in this region for removable prosthetics is 36.75 ± 0.14%. During the detailed analysis of the choice of designs of removable dentures it was found that in 56.00 ± 0.30% was indicated production of partial removable laminar prostheses, and in 44.00 ± 0.30% – complete removable laminar prostheses [13, 17].

It is known that the needs for different types of dentures depend on age [18]. The adult population up to 40 years needs non-removable types of orthopedic dentures, at the age of 40-59 years – combined types (non-removable and removable), at the age of 60 years and older – mostly removable (partial and full laminar) dentures.

The problem of prosthetics with partial loss of teeth is especially relevant. Removable laminar prostheses, restoring the lost functions of chewing and speech, simultaneously have a number of disadvantages: unstable fixation, especially on the lower jaw, uneven distribution of masticatory pressure and, most importantly, cause psycho-emotional problems in patients who use these prostheses [19-21].

It should be noted that in the clinic of removable dentures great importance is attached to the mucous membrane of the prosthetic bed and adjacent tissues, because there is direct contact with the base of the removable prosthesis, which irritates it. The mucous membrane of the prosthetic bed and the surrounding part of the oral cavity, being in abnormal conditions, separated from moisture by saliva, is subjected to constant load, pressure and mechanical irritation by the prosthesis, exfoliated epithelium and food debris [22, 23]

Today there is a lot of data to study the condition of the mucous membrane of the prosthetic bed and buccal epithelium against the background of the use of partial removable laminar prostheses, however, there is no data on treatment and prevention of infectious and inflammatory processes on the mucous membrane of the prosthetic bed by developing and putting into practice new solutions to increase the time of adaptation [24].

Current trends in the development of orthopedic dentistry are closely related to the use of new materials and technologies for the manufacture of dentures, which significantly increases their functional value.

98% of acrylic plastics are used to make removable plate prostheses. They are the main structural material for the bases of removable dentures due to good aesthetic, hygiene and high technology properties [25, 26].

Among the main disadvantages of acrylic plastics are insufficient strength and fragility. Insufficient strength of prosthesis bases made of acrylic plastics is one of the reasons for frequent breakages of removable laminar prostheses. Low indicators of physical and mechanical parameters of acrylic plastics are closely related to the technology of their polymerization [27-29].

Thus, we can conclude that the need of the adult population of Ukraine in prosthetics with removable dentures is extremely high. However, the issue of the prevalence of dentition defects in the population of the Ternopil region is still unexplored.

THE AIM

The aim of the study is to determine the needs of the population of the Ternopil region in prosthetics with removable orthopedic dentures and to study the physical and technological properties of different types of base plastics.

MATERIALS AND METHODS

This study was conducted at the orthopedic dentistry department I. Horbachevsky Ternopil National Medical University, Ukraine, and was approved by the ethics committee of the I. Horbachevsky Ternopil National Medical University, which determined that the general ethical rules of humane treatment of patients were observed when working with patients in accordance with the requirements of the Tokyo Declaration of the World Medical Association and the International Recommendations of the Helsinki Declaration of Human Rights.

The needs of Ternopil and Ternopil region population in removable prosthetics were studied on the basis of consolidated annual reports of city and district dental clinics of Ternopil region, which were compiled annually by the departments of practical health care according to statistical form 039 - 4/u, from 2017 to 2021.

The number of primary patients who had removable laminar prostheses was taken into account.

During this period, 385,502 patients were examined in a planned and preventive manner.

To study the quality of polymerization of base acrylic plastics, the methods of polymerization on a «water bath» in a cuvette with a gypsum mold, in the apparatus for dry polymerization under pressure and the method of polymerization using cast pressing.

Base dental plastics «Ftoraks» (Stoma, Ukraine) and «Villacryl H Plus» (Zermapol, Poland) were used to study the physical and mechanical properties.

Samples of base plastics «Ftoraks» and «Villacryl H Plus», made by different methods of polymerization were divided into six groups. The I group included samples of base plastic «Ftoraks», the polymerization of which was carried out in a "water bath"; II group – samples of base plastic «Ftoraks», the polymerization of which was carried out in an apparatus for dry polymerization under pressure; III group – samples of base plastic «Ftoraks» which polymerization was carried out in the improved device for casting pressing; IV group – samples of base plastic «Villacryl H Plus», the polymerization of which was carried out in a "water bath"; V group – samples of base plastic «Villacryl H Plus», the polymerization of which was carried out in an apparatus for dry polymerization under pressure; VI group – samples of base plastic «Villacryl H Plus», the polymerization of which was carried out in an advanced apparatus for casting pressing.

There were five samples of these base plastics in each of these groups. The result of the study was the arithmetic mean of the values of the destructive stress on compression (tension) of five test specimens from each group.

The deformation characteristics of all groups of test samples were studied during their deformation under compression and tension.

Statistical processing of the obtained results was performed using a program for statistical analysis of data from biomedical research «Statistica 6.0» (Stafsoft, USA).

The following methods were used for statistical analysis of the obtained data:

- analysis of variation series – calculation of arithmetic mean and its average mistake (M + m);
- assessment of the reliability of the difference between the results obtained in the compared groups using Student's t-criterion.

Table I. The average values of the elastic limit of dental base plastics during compression deformation «Ftoraks» and «Villacryl H Plus»

№ of sample	I group mPa	II group mPa	III group mPa	IV group mPa	V group mPa	VI group mPa
1.	18.4	58.7	67.8	17.7	57.4	67.1
2.	26.1	61.6	69.7	18.8	61.0	67.6
3.	21.4	59.2	68.9	17.3	58.8	68.1
4.	21.3	54.7	68.4	18.1	62.6	68.3
5.	20.8	52.1	69.3	16.8	64.7	67.5
ε M±m	21.6± 1.25	57.3± 1.70	68.8± 0.33	17.7± 0.34	60.9± 1.30	67.7± 0.22

Table II. The average values at deformation on stretching of dental base plastics «Ftoraks» and «Villacryl H Plus»

№ of sample	I group mPa	II group mPa	III group mPa	IV group mPa	V group mPa	VI group mPa
1.	62.2	59.8	73.3	58.8	74.0	76.4
2.	58.5	71.2	75.1	60.2	64.7	75.9
3.	58.1	58.7	73.7	64.6	66.8	75.6
4.	59.4	63.2	74.4	60.2	67.2	76.7
5.	59.8	63.2	74.9	62.2	69.6	76.9
ε M±m	59.6± 0.72	63.1± 2.19	74.28± 0.34	61.2± 1.01	68.5± 1.59	76.3± 0.24

Before comparing the obtained data, diagrams with a normal distribution curve (Gaussian dome) and the Shapiro-Wilk test were used to determine the type of data distribution.

Subject to the hypothesis of the normality of the distribution of data, the comparison of indicators was carried out using the Student's t test. Differences were considered significant with a probability level of at least 95% ($p<0.05$).

RESULTS

It was found that of the total number of examined in 281,416 people there is a partial secondary adentia, which is 72.99 ± 0.14%. Among them, 129,451 patients (45.99 ± 0.05%) required removable prosthetics.

In a detailed analysis of the choice of designs of removable laminar prostheses, it was found that 84,143 patients (64.99 ± 0.14%) required the manufacture of partial removable prostheses, and 45,308 patients (35.01 ± 0.18%) – complete removable prostheses.

One of the indicators of the quality of manufactured removable laminar prostheses is their resistance to mechanical stress. An analysis of the statistics of the annual reports showed that a significant number of removable laminar prostheses, which were manufactured during the five years of observation, were subject to repair every year.

We think that the method of polymerization of dental base plastics in a «water bath», which is used for the manufacture of removable laminar prostheses, is not perfect, which leads to frequent breakdowns of dentures made by this method.

Violation of the polymerization regime and imperfection of technological methods leads to frequent breakdowns of removable laminar prostheses. In this regard, we conducted experimental studies of plastic samples «Ftoraks» (Stoma, Ukraine) and «Villacryl H Plus» (Zermapol, Poland) which were polymerized in a «water bath», in the apparatus for dry polymerization under pressure and in the apparatus for casting pressing.

The results of the study of the elastic limits of the base dental plastics «Ftoraks» and «Villacryl H Plus» during compression deformation are shown in Table I, and the average values of tensile deformation - in Table II.

As a result of the study for each of the six types of samples of base plastics «Ftoraks» and «Villacryl H Plus» it was found that polymerization in an advanced apparatus under pressure gives higher values of the elastic limit than polymerization in a «water bath» and in the apparatus for dry polymerization under pressure.

It was found that the values of the compressive strength under deformation for samples «Ftoraks» (group III) and for samples «Villacryl H Plus» (group VI), the polymerization of which was carried out in an apparatus for casting pressing, approximately by 3.1 times ($p < 0,05$) more than the samples, the polymerization of which was carried out on a «water bath» (I and IV groups) and by 1.2 times ($p < 0,05$) more than the samples, the polymerization of which was carried out in the apparatus for dry polymerization under pressure (II and V groups) ($p <0,05$).

DISCUSSION

As a result of our research, we found that the need of the Ternopil region population for removable prosthetics is quite high. It was found that in 72.99 ± 0.14 have partial secondary adentia. Among them, 45.99 ± 0.05% required removable prosthetics.

When analyzing the choice of designs of removable laminar prostheses, it was found that 64.99 ± 0.14% of patients

required the manufacture of partial removable dentures, and 35.01 ± 0.18% – complete removable dentures.

The differences in mechanical properties obtained as a result of the study in the experimental groups are due to the fact that in the samples of base plastics «Ftoraks» and «Villacryl H Plus», the polymerization of which was carried out in a «water bath», there are no cross-links between linear molecules, ie no mesh structure is formed, which is much less susceptible to deformation. In samples, the polymerization of which was carried out in the apparatus for dry polymerization under pressure, this process is also not complete [19, 26]. As for the samples, the polymerization of which was carried out in the apparatus for casting pressing, they have the best performance. This may be due to the greater compaction of the base material during casting, streamlining of the polymer structure, as well as the lack of negative effects of water on dentures base plastics [29].

Samples of base plastics «Ftoraks» and «Villacryl H Plus», which were polymerized in a dry polymerization apparatus under pressure, have slightly better indicators in comparison with samples, which were polymerized in a «water bath». This is due to the positive effect of the pressure created on the plastic in the apparatus, in which the placement of polymer macromolecules is more ordered [6, 20].

When stretched after plastic deformation within 1%, the samples of base plastics «Ftoraks» and «Villacryl H Plus» are destroyed, which makes it possible to determine the tensile strength of the materials studied [28].

Researches have shown that the elastic limits of the samples of base plastics «Ftoraks» and «Villacryl H Plus», polymerized in the apparatus for casting pressing, are equal to 68.8 ± 0.33 mPa and 67.7 ± 0.22 mPa, respectively, which is approximately by 3.2 times ($p < 0,05$) more than in the «water bath» (21.6 ± 1.25 mPa and 17.7 ± 0.34 mPa, respectively) and by 1.2 times ($p < 0,05$) more than in the apparatus for dry polymerization under pressure (57.3 ± 1.7 mPa and 60.9 ± 1.3 mPa), respectively.

When conducting studies of the samples of base plastics «Ftoraks» and «Villacryl H Plus» was found a significant difference in the tensile strength in the experimental groups (59.6 ± 0.72 mPa and 61.2 ± 1.01 mPa – I and IV groups, respectively, 63.2 ± 2.19 mPa and 68.5 ± 1.59 mPa – II and V groups, respectively, 74.28 ± 0.34 mPa and 76.3 ± 0.24 mPa – III and VI groups, respectively) ($p < 0.05$).

CONCLUSIONS

1. It has been established that the need of the population of the Ternopil region for removable prosthetics is quite high. As a result of the analysis of medical records, it was revealed that 45.99±0.05% of patients needed removable prosthetics. Among them, 64.99±0.14% of patients required the manufacture of partial removable prostheses and 35.01±0.18% - complete removable prostheses.
2. An important indicator of the quality of manufactured removable lamellar dentures is their resistance to mechanical stress. It has been established that the indicators of the elastic limit and tensile strength of samples of the base plastic «Villacryl H Plus» (Zermapol, Poland) significantly exceed those of the samples of the base plastic «Ftoraks» (Stoma, Ukraine).
3. It has been established that samples of base plastics made in an apparatus for foundry pressing have higher physical and mechanical properties than samples made in an apparatus for dry polymerization under pressure and in a «water bath».

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ORIGINAL ARTICLE

FEATURES OF THE FEMORAL HEAD FRACTURES COMBINED WITH ACETABULUM POSTERIOR WALL FRACTURES SURGICAL TREATMENT

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ABSTRACT

The aim: To conduct a thorough analysis of the surgical approach features in femoral head fractures combined with acetabulum posterior wall fractures; to analyze these patients' treatment results 12-36 months after the surgery; to determine the criteria affecting the satisfactory treatment results achievement in these injuries.

Materials and methods: The surgical treatment results were evaluated 13 and 36 months after the surgery. The retrospective analysis included 21 patients (17 men and 4 women) with femoral head fractures combined with the acetabulum posterior wall fractures. Pipkin and Brumback classifications were used to classify the fractures.

Results: The results of acetabulum posterior wall fractures delayed treatment are significantly different to the ones treated with early osteosynthesis. The reasons for that are difficulties in anatomical repositioning, femoral head malnutrition due to its displacement or chronic subluxation, reduced blood supply to the fragments due to surgical manipulations during the approach. Thus, according to Matta criteria anatomical reposition of the fragments was achieved in 19 (90.5%) cases, imperfect reposition in 1 (4.8%) case, unsatisfactory reposition - in 1 (4.8%) patient. The treatment results' improvement after 3 years occurred due to hip joint replacement in 5 (23.8%) patients.

Conclusions: The treatment results of femoral head fractures with acetabulum posterior wall fractures depends on the type of fracture, concomitant injuries, the timing, and method of removing the femoral head dislocation, and the surgical treatment method.

KEY WORDS: fracture, dislocation, femoral head, hip joint, aseptic necrosis, osteosynthesis

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INTRODUCTION

The femoral head fractures with concomitant acetabulum posterior wall fractures are rare injuries that usually occur due to traumas with high-energy mechanisms, such as traffic accidents, falls from heights, work-related accidents, and sports injuries. Around 5-15% of posterior femoral dislocations are associated with a fracture of the femoral head, according to statistics [1-4]. In 1869, Birkett [5] first described a fracture of the femoral head, which has always been associated with traumatic posterior hip dislocation, but the incidence was low. In 1954, Stewart and Milford described four degrees of torsion of the femoral head; wives with a fracture of the head or neck of the proximal femur were classified as grade IV [6]. In 1957, Garrett Pipkin, an orthopedic surgeon in Kansas City, Missouri, further classified Stewart and Milford grade IV injuries. This classification system for femoral head fractures became known as the Pipkin classification system [7]. Based on the location of femoral head fractures in relation to the fossa and accessory acetabular involvement, the Pipkin classification is widely used for femoral head fractures.

The femoral head fractures are intra-articular fractures, so the articular surface restoration by accurate fracture reduction is of our primary importance [8]. These fractures' prognosis depends on the repositioning accuracy and the fixation stability. Traumatic hip dislocation together with the femoral head fractures, is often associated with high damage to other organs and combined fractures and can cause such complications as femoral head avascular necrosis, hip joint traumatic arthrosis, and heterotopic ossification [9-11]. Therefore, rapid, and accurate treatment at an early stage of injury is needed as well as a constant follow-up [12-13]. The presence of a posterior acetabulum wall fracture complicates the fracture reduction and worsens the treatment prognosis.

THE AIM

To conduct a thorough analysis of the surgical approach features in femoral head fractures combined with acetabulum posterior wall fractures; to analyze these patients' treatment results 12-36 months after the surgery; to de-

termine the criteria affecting the satisfactory treatment results achievement in these injuries.

MATERIALS AND METHODS

We conducted a retrospective analysis of the treatment results of the femoral head fractures combined with acetabulum posterior wall fractures, which were registered in the Kyiv Regional Clinic Hospital in 2002- 2019 years. Pipkin [7] and Brumback [14] classifications were used to classify the femoral head fractures combined with acetabulum posterior wall fractures.

A group, which included 21 patients, aged from 21 to 56 years, was formed. According to the Pipkin classification, all patients were assigned to type IV. According to the Brumback classification, all patients were divided into: 1A - 5 (23.8%) patients, 1B - 5 (23.8%) patients, 2A - 4 (19%) patients, 2B - 7 (33.4%) patients. Male patients predominated with 17 (81%) men, while the rest, 4 (19%), were female. 18 (86%) patients were injured due to a road accident, 3 (14%) due to a fall from a height.

Since hip fractures are usually a result of a high-energy trauma, it is often advised to take concomitant injuries into consideration, which can significantly affect the further treatment choice and its duration. Among all patients, 8 (38.10%) were diagnosed with polytrauma and 2 (9.5%) were diagnosed with a combined injury of pelvic bones and other organs and systems.

The primary task was to perform the closed reduction of the dislocated femoral head. The closed dislocation reduction was successfully performed on the day of the injury in 9 (43%) patients, in the first 3 days post-injury – in 7 (34%) patients, the delayed surgical treatment was performed after stabilization of the general condition in 5 (23%) cases.

At the second stage of the treatment all patients underwent surgeries: femoral head osteosynthesis with screws and acetabulum posterior wall osteosynthesis with plates and screws, using the Kocher-Langenbeck approach. These surgeries were performed 3 to 41 days after the injury, which is explained by the time of transportation from other hospitals or the severity of concomitant injuries. The postoperative reduction assessment was conducted according to Matta scale: anatomical (displacement up to 1 mm), non-ideal (2-3 mm), unsatisfactory (more than 3 mm). The treatment results evaluation was conducted according to the Thompson-Epstein scale [15]. We analyzed the clinical and radiological results after 12 and 36 months in all post-operative patients.

The treatment results descriptive statistics are presented in form of patients' distribution (number of observations and distribution in %). The subgroups comparison with an assessment of the difference significance was performed using the X-square ($\chi 2$) test and Fisher criterion.

RESULTS

We analyzed the early results of the patients' treatment - no postoperative complications were detected. Some late postoperative complications were found: heterotopic ossification was diagnosed in 1 (4.8%) patient and aseptic necrosis of the femoral head in 5 (23.8%) patients. Of the 4 (19%) patients presented with significant femoral head trauma, 2 (50%) developed aseptic necrosis. Therefore, 4 (19%) patients underwent hip arthroplasty after 4-6 months. Post-traumatic sciatic nerve neuropathy, confirmed by electroneuromyography of both lower extremities, was found in 2 (9.5%) patients, and subsequently required long-term neurological treatment.

According to Matta scale, anatomical reposition of the fragments was achieved in 19 (90.5%) cases, imperfect reposition – in 1 (4.8%) patient, and unsatisfactory reposition – in 1 (4.8%) patient. The number of imperfect and unsatisfactory repositioning cases depended in both cases on the surgical intervention timing after the injury and the late femoral head dislocation reduction.

The evaluation of the treatment results in patients with femoral head fractures combined with posterior acetabulum wall fractures was conducted in the period from 12 to 36 months after the injury according to the Thompson-Epstein scale [15]. In 12 months, excellent results were obtained in 6 (28.6%) patients, good results in 5 (23.8%), satisfactory results in 6 (28.6%) and poor results in 4 (19%) patients. In 36 months, excellent results were obtained in 9 (42.9%) patients, good results in 7 (33.3%), satisfactory results in 3 (14.3%) and poor results in 2 (9.5%) patients. The treatment results' improvement after 3 years occurred due to total hip arthroplasty in 5 (23.8%) patients.

After a statistical evaluation of the treatment results in patients with a femoral head fracture combined with the acetabulum posterior wall fractures, the correlation between the treatment results and the severity of the injury was found – the frequency of poor results is significantly higher in patients who presented with polytrauma 70%, than in the subgroup without polytrauma - 27.3% (p=0.05). The results significantly worsen in patients with the late hip dislocation reduction (more than 3 days after injury): in case of the reduction on the injury day, the share of excellent and good results is 88.9%, c, which later led to the femoral head aseptic necrosis and total hip arthroplasty. The satisfactory and poor results' share, on the contrary, is higher in case of a late hip displacement reduction, p=0.014 (Table I). In case of multifragmentary acetabulum fractures with the additional femoral head damage, poor results were found in 66.7% cases, while in the absence of such injuries in patients, poor results were not found at all ((p=0.006), considering the Haldane-Anscombe correction), which is associated with the fragments anatomical repositioning complexity and late osteosynthesis of the acetabulum. These changes are statistically significant and indicate the importance of anatomical repositioning and performing the surgery on time.

During the treatment results analysis of patients with femoral head fracture combined with the acetabulum posterior wall fractures according to the Brumback classification, the dependence of a significant increase in the frequency of excellent treatment results in patients with 1A

Table I. Evaluation of the femoral head fractures combined with acetabulum posterior wall fractures treatment results

Parameters	Groups	Patients' distribution	Treatment results		P (χ2)
			Excellent/ Good	Satisfactory/ Poor	
Brumback	1A	5 (23.8%)	4 (80%)	1 (20%)	-
	1B	5 (23.8%)	4 (80%)	1 (20%)	
	2A	4 (19%)	1 (25%)	3 (75%)	
	2B	7 (33.3%)	2 (28.6%)	5 (71.4%)	
Brumback	1 (A+B)	10 (47,6%)	8 (80,0%)	2 (20%)	0,016*
	2 (A+B)	11 (52,4%)	3 (27,2%)	8 (72,7%)	
Sex	M	17 (81%)	9 (52.9%)	8 (47.1%)	0.916
	F	4 (19%)	2 (50%)	2 (50%)	
Polytrauma, combined trauma	Yes	10 (47.6%)	3 (30%)	7 (70%)	0.05 *
	No	11 (52.4%)	8 (72.7%)	3 (27.3%)	
Multifragmentary fractures, Femoral head injuries	Yes	15 (71,4%)	5 (33,3%)	10 (66,7%)	0,006*
	No	6 (28,6%)	6 (100,0%)	0 (0,0%)	
Hip dislocation reduction (day)	Injury day	9 (42.9%)	8 (88.9%)	1 (11.1%)	0.014 *
	Up to 3 days	7 (33.3%)	2 (28.6%)	5 (71.4%)	
	>3 days	5 (23.8%)	1 (20%)	4 (80%)	

Notes: P (χ2) is an assessment of the difference probability based on the achieved treatment results for individual subgroups of parameters (* - $p<0.05$)

and 1B injuries (80.0%) according to Brumback was found together with the ascertainment of poor results as a result of type 2A and 2B injuries – 72.7% (p=0.016). This data indicates that the treatment outcome is also affected by the type of fracture according to the Brumback classification, as it emphasizes the importance of joint instability, types of femoral head dislocation, and acetabular fracture severity in predicting an adverse outcome.

DISCUSSION

The most significant problem in the treatment of such fractures is the combination of the damaged femoral head and the acetabulum wall. This usually occurs as a result of high-energy trauma or due to a number of repeated attempts to reduce the dislocation. Such attempts often lead to multifragmentary fractures of the posterior acetabulum wall and complicate further fixation.

We performed osteosynthesis of these fractures using the Kocher-Langenbeck approach as we consider it to provide the best view of all fragments in such injuries. The patient is positioned exclusively on his side with the injured limb easily accessible. This position allows us to easily control the joint area and eliminate the femoral head dislocation.

In patients with the femoral head damage combined with the acetabulum posterior wall fractures we have repeatedly observed posterior wall fragments entering the joint cavity, so the ability to access it freely is of a high importance. This can only be achieved by displacing the femoral head.

Also, one of the femoral head fractures osteosynthesis peculiarities is being ready to extract a broken head fragment from the joint cavity if it is needed, perform its osteosynthesis extra-articularly, and atraumatically reduce the dislocation after the osteosynthesis.

After the joint cavity revision and the fragments removal from it, we perform osteosynthesis of the femoral head. We used two options of such osteosynthesis. In case of a significant fragment, retrograde osteosynthesis was used and in case of a minor fragment, antegrade osteosynthesis with screws. After osteosynthesis and the reduction of femoral head dislocation, fragments were fixed using a plate placed from the ischial tubercle up to the acetabulum roof. Stable fixation was considered mandatory, therefore in multifragmentary fractures in 5 (23.8%) cases, spring plates were additionally used and installed under the main ones, which provided a needed fixation of minor fragments in the edge zone of the acetabulum. Anatomical repositioning and stable fixation of posterior wall fractures was achieved in 90.5% of cases.

The rehabilitation in all cases began the day after surgery, patients started to fully load the limb in 12 weeks after the X-Ray control. For results evaluation, we used the Thompson-Epstein scale [15], as it illustrates the results of damage to this area most objectively.

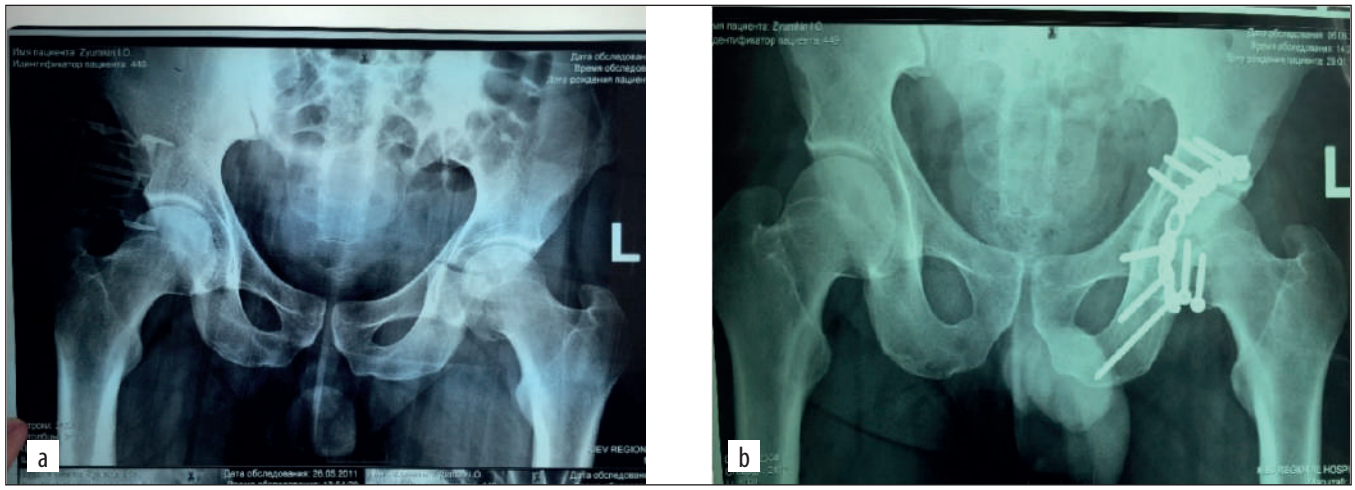

Fig. 1. X-Ray photographs of patient's A pelvic bones.
a) Left acetabulum posterior wall fracture and transverse fracture, left femoral head fracture type Pipkin IV;
b) Open osteosynthesis of the femoral head (with screws), and the acetabulum (with a plate and screws).

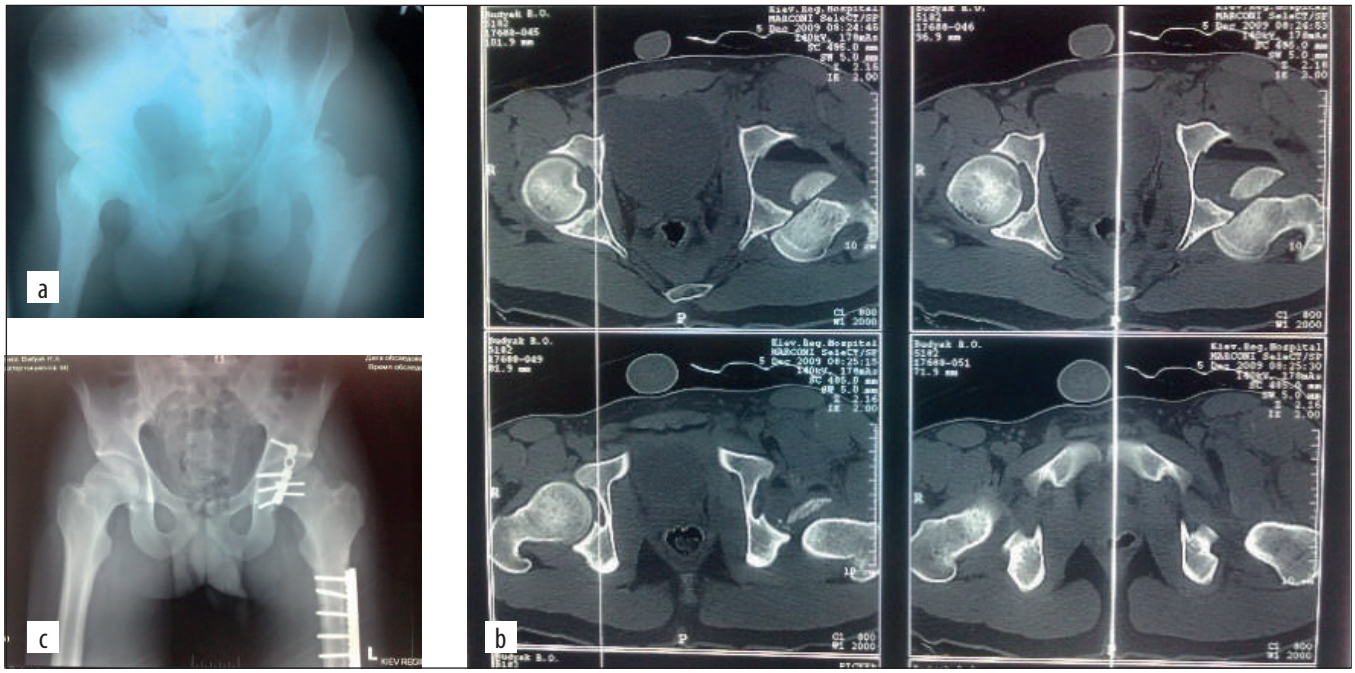

Fig. 2. X-Ray photographs and CT-scans of patient's B pelvic bones.
a), b) left acetabulum posterior wall fracture, left femoral head fracture;
c) open osteosynthesis of the femoral head (with screws), the acetabulum (with a plate and screws) and the femoral shaft.

The worst results were obtained in the group of patients with the late femoral head dislocation reduction. The reasons for the late reduction were the severity of the patients' condition, the concomitant injuries, and the technical problems in reducing the dislocation due to the interposition of fragments. In this group of patients 19% of aseptic necrosis and deforming arthrosis cases were noted. A significant number of complications was also found in patients who presented with multifragmentary femoral head fractures according to the Brumback classification.

The Pipkin classification is the most commonly used for femoral head fractures [7] but is not exhaustive; does not take into account the degree of grinding of broken fragments or the size of the fracture femoral head and acetabulum, or the stability of the joint in type 4 injuries. Thus, the classification system is not capable of serving as a guideline for surgical treatment. However, medium- and long-term studies that have evaluated the prognosis of patients with femoral head fractures have found that the Pipkin classification is prognostically useful [16,17]. The classification system of Brumback et al. [14] is more complete than the Pipkin classification system, taking into account the direction of dislocation and joint stability. Because the Brumback system emphasizes the importance of joint instability, direction of dislocation, and severity of acetabular fracture in predicting poor outcome.

CASE 1.

The patient described in the cases were treated at the Kyiv Region Clinical Hospital.

Patient A., 35 years old. The injury was obtained in a traffic accident on 05/22/2011. Diagnosis: left acetabulum posterior wall fracture, left acetabulum transverse fracture, left femoral head fracture type Pipkin IV.

During the admission to a district hospital, the femoral head dislocation was reduced. On May 25, 2011, the patient was admitted to Kyiv Regional Clinic Hospital. On May 27, 2011, a surgery was performed – open osteosynthesis of the femoral head (with screws), and the acetabulum (with a plate and screws). In 15 months, according to the Thompson-Epstein scale, the treatment result was rated by the authors as good.

CASE 2.

The patient described in the cases were treated at the Kyiv Region Clinical Hospital.

Patient B., 42 years old. The injury was obtained in a road accident on December 1, 2009. Diagnosis: left acetabulum posterior wall fracture, left femoral head fracture type Pipkin IV, left femoral shaft fracture.

During the admission to a district hospital, the femoral head dislocation was reduced. On December 4, 2009, the patient was admitted to Kyiv Regional Clinic Hospital. On December 7, 2009, a surgery was performed – open osteosynthesis of the femoral head (with screws), and the acetabulum (with a plate and screws). In 12 months, according to the Thompson-Epstein scale, the treatment result was rated by us as excellent.

CONCLUSIONS

The treatment results of the femoral head fractures combined with the acetabulum posterior wall fractures depend on the severity and type of the fracture, the concomitant injuries, the timing, and method of reducing the femoral head dislocation, and the chosen surgical treatment method.

The treatment results in our study were influenced by such factors as the severity of the injury (in patients with polytrauma the frequency of poor results is significantly higher - 70%, than in the subgroup of patients without polytrauma - 27.3% (p=0.05)), late reduction of the femoral head dislocation (in case of the reduction on the injury day, the share of excellent and good results is 88.9% and in case of a late reduction it decreases to 20%, p=0.014), as well as the type of acetabulum fractures and the femoral head damage degree (poor results were found in 66.7% cases that involved multifragmentary acetabulum fractures with the additional femoral head damage , while in the absence of such injuries, poor results were not found at all (p=0.006), considering the Haldane-Anscombe correction). The fracture type also has a prognostic effect on the outcome of the treatment. In our study the dependence of a significant increase in the frequency of excellent treatment results in patients with 1A and 1B injuries (80.0%) according to Brumback was found together with the ascertainment of poor results as a result of type 2A and 2B injuries – 72.7% (p=0.016).

The results of fracture treatment were significantly improved by us in several patients with aseptic necrosis of the femoral head by performing the hip replacement surgery on time.

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ORIGINAL ARTICLE

LUNG PROTECTIVE POTENTIAL EFFECT OF ZILEUTON DURING ENDOTOXAEMIA MODEL IN MALE MICE

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ABSTRACT

The aim: This study was undertaken to investigatethe possible lung protective potential effect of zileuton during polymicrobial sepsis, through modulation of inflammatory and oxidative stress pathway.

Material and Methods: 24 adult male Swiss-albino mice aged 8–12 weeks, with a weight of 25–35g, were randomized into 4 equal groups n=6, sham (laparotomy without CLP), CLP (laparotomy with CLP), vehicle (equivalent volume of DMSO 1 hour prior to CLP), and Zileuton (5 mg/kg 1 hour prior to CLP) group. After 24 hrs. of sepsis, the lung tissue harvested and used to assess IL-6, IL-1B, IL-17, LTB-4,12(S) HETE and F2-isoprostane as well as histological examination.

Results: Lung tissue inflammatory mediators IL-6, IL-1B, IL-17, LTB, 12 (S) HETE) and oxidative stress were carried out via ELISA. Lung tissue levels of IL-6, IL-1B, IL-17, LTB4, 12(S) HETE and oxidative stress (F2 isoprostan)level were significantly higher in sepsis group ($p<0.05$) as compared with sham group, while zileuton combination showed significant ($p<0.05$) lower level in these inflammatory mediators and oxidative stress as comparedto sepsis group. Histologically, All mice in sepsis group showed a significant ($p<0.05$) lung tissue injury, while in zileuton pretreated group showed significantly ($p<0.05$) reduced lung tissue injury.

Conclusion: The results of the present study revealed that zileuton has the ability to attenuate lung dysfunction during CLP induced polymicrobial sepsis in male mice through their modulating effects on LTB4,12(S) HETE and oxidative stress downstream signaling pathways and subsequently decreased lungtissue levelsof proinflammatory cytokines (IL-1β, and IL-6,IL-17).

KEY WORDS: zileuton, CLP, endotoxemia,sepsis, IL-6, IL-1B, IL-17, LTB-4, 12(S)HETE, F2-isoprostane

Wiad Lek. 2022;75(12):3066-3073

INTRODUCTION

One of the most complex clinical challenges in medical practice is sepsis-induced lung dysfunction, which results from polymicrobial sepsis and despite many therapeutic approaches have been used in such clinical challenges, still there is more need for a new effective therapeutic approach. Endotoxemia, a systemic inflammatory response syndrome induced by infection, is the most common condition in patients treated in critical care units [1]. A gram-negative bacterial infection causes endotoxic shock, the most dangerous form of sepsis. Sepsis, commonly known as septic shock, is a pathophysiological disease characterized by severe hypotension, increasing metabolic acidosis, SIRS, tissue destruction and multiple organ dysfunction syndrome, acute respiratory distress syndrome (ARDS), acute lung injury (ALI), and mortality [2]**.** Increased endothelial vascular permeability occurs in many organs during sepsis, leading in plasma extravasations and subsequent bacterial translocation, which may contribute to the development of severe tissue damage [3]. The finding of main products compounds such as endotoxin, cytokines, and products of arachidonic acid metabolism, as well as the manifestation that injecting these molecules into human volunteers or experimental animals could cause a clinical syndrome similar to sepsis [4]. Monocytes control the innate immune response to microorganisms by producing inflammatory cytokines such as interleukin-6 (IL-6), interleukin-17 (IL-17) and interleukin-1beta (IL-1β), SIRS (systemic inflammatory response syndrome), multiple organ failure, and death are all possible outcomes [5]. Lipopolysaccharide (LPS), a major component of gram-negative bacteria that has been studied as a critical regulator of bacterial infection pathogenesis and plays an important role in endotoxic shock, is induced by the cegal ligation and puncture model (CLP) [2], 5-lipoxygenase (5-LO) produces leukotrienes B4 (LTB4), a strong proinflammatory lipid mediator generated from arachidonic acid [6]. The recruitment of leukocytes, especially neutrophils, is one of LTB4's main functions. LTB4 employs two G protein-coupled receptors (GPCRs) to carry out its biological tasks: BLT1 and BLT2.

BLT1, a high-affinity receptor for LTB4, is expressed by inflammatory cells such as leukocytes. Unlike BLT1, BLT2 is a low-affinity LTB4 receptor that may be present in a variety of organs [7]. In contrast to LTB4's highly selective binding to BLT1, numerous arachidonic acid metabolites, such as 12(S)-hydroxyeicosatetraenoic acid (HETE) and 12-hydroxyheptadecatrienoicacid (12-HHT), have been identified as BLT2 ligands [8]. Multiorgan failure and/or decreased tissue perfusion can occur in severe sepsis, as well as equable shock [9]. It has the potential to become a life-threatening condition by causing molecular alterations that result in numerous organ dysfunctions [10].

THE AIM

This study was undertaken to investigatethe possible lung protective potential effect of zileutonduring polymicrobial sepsis, through modulation of inflammatory and oxidative stress pathway.

MATERIAL AND METHODS

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

DESIGN OF THE STUDY

24 adult male albino Swiss mice, weighing 25-35 g and aged 8-12 weeks, were purchased from the Animal Resources Centre of the College of Science at the University of Kufa for this study, which was kept at a constant temperature of 25°C and humidity of 60-65 percent, with a 12 h light: 12 h dark cycle. In this study, the mice were divided randomly in to four groups (6 mice in each group) and as the following:

- sham group: All mice in this group were given anesthesia and a laparotomy surgery, but without CLP.
- cecal ligation and puncture operated group(CLP) (sepsis group): All mice in this group had their cecums ligated and punctured.
- vehicle group: All mice in this group were given an equal volume of DMSO i.p injection 1 hour before CLP.
- zileuton pretreated group: All mice in this group received a 5-mg/kg zileuton i.p injection 1 hour before CLP.

EXPERIMENTAL PROCEDURE

Mice were anesthetized intraperitoneally with 100 mg/kg ketamine and 10 mg xylazine [11]. The cecum was revealed after an abdominal laparotomy was performed through a 1.5 cm midline incision. The cecum was then ligated right below the ileocecal valve and punctured twice with a G-20 needle before being returned to its anatomical location. After that, the abdomen was sutured with a 5.0 surgical suture. Mice were checked for various indicators of illness every four hours for 24 hours before being returned to their cages with unlimited food and drink. The surgical control group consisted of sham surgically operated mice (anesthesia and laparotomy without CLP) [12].

COLLECTION AND PREPARATION OF SAMPLES

The mice's lungs were removed 24 hours after CLP. Tissue samples were divided into two parts: half were treated in PBS (phosphate buffer solution) and immediately frozen at -80°C to be used for ELISA analysis. The remaining samples were fixed For histological analysis.

PREPARATION OF TISSUE SAMPLES FOR HISTOPATHOLOGY

Lung tissues obtained after the sacrifice of mice were washed with cold isotonic sodium chloride solution 0.9 percent to remove red blood cells or clots, then fixed in 10% formalin and processed in paraffin tissue blocks, after which microscopic sections of 5µm thickness were taken from the blocks and stained with hematoxylin-eosin dye [13]. Following the fixation of the specimens, the first stage was dehydration, which involved immersing the specimens in ethanol for two hours for each concentration 70%, 80%, 90%, and 100% to extrude any left-over formalin or H2O from the samples. Then they were cleaned with xylene (an organic solvent), which was used to remove the alcohol from the samples in order to seep with paraffin wax. Finally, the specimens were embedded in paraffin wax, which was allowed to dry and form a solid block, allowing thin pieces to be created. A rotary microtome equipped with disposable steel knives was used to section the blocks at a thickness of 5µm. The sections were then flattened on a water path at 37°C, floated on to microscope slides and allowed to dry, and then painted with Hematoxylin and Eosin stain to detect the damaging level in the lung tissue, and images were obtained using optical microscopy from each lung segment. Histological sections from all groups were evaluated to semiquantify the difference in lung damage. The histopathology test was performed at original magnifications of X400 [14] and scored by the percentage of tissue damage as follows:

Score 0: nodamage, normalarchitecture.
Score 1 (mild): less than 25%damage
Score 2 (moderate): (25-50)%damage
Score 3 (severe): (50-75)%damage
Score 4 (highlysevere): (75-100)% damage.

Statistical Analysis

Statistical analyses were done by SPSS version 26. The data were expressed as Mean ± SEM. ANOVA (analysis of variance) was used for comparisons of multiple groups followed by post-hoc test using Bonferroni correction. For the histopathological changes of lung tissue, Kruskal-Wallis test was used to determine statistical significance of the difference among the multiple groups as mean score for histopathological changes of lung tissue. Statistically in all tests, p-value ≤ 0.05 was considered significant.

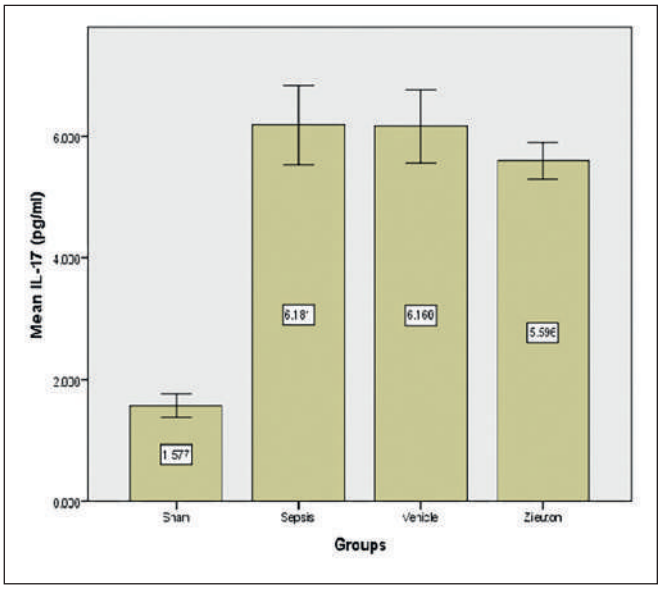

Fig. 1. Mean lung tissue level of IL-17 (pg/ml) ± SEM of the four experimental groups at the end of the experiment (number of mice in each group=6): sham vs. sepsis group, p-value =0.00001 (significant); zileuton vs. sepsis and vehicle group, p-value =0.081 (unsignificant).

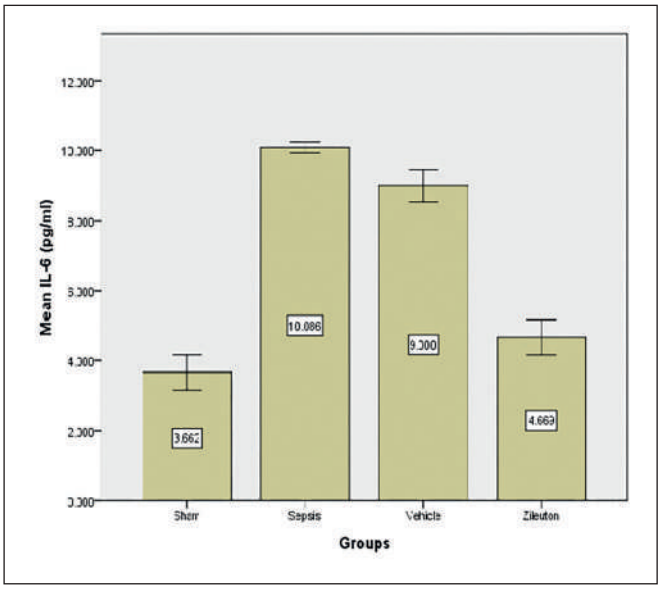

Fig. 2. Mean lung tissue level of IL-6 (pg/ml) ± SEM of the four experimental groups at the end of the experiment (number of mice in each group=6): sham vs. sepsis group, p-value =0.00001 (significant); zileuton vs. sepsis and vehicle group, p-value =0.00001 (significant).

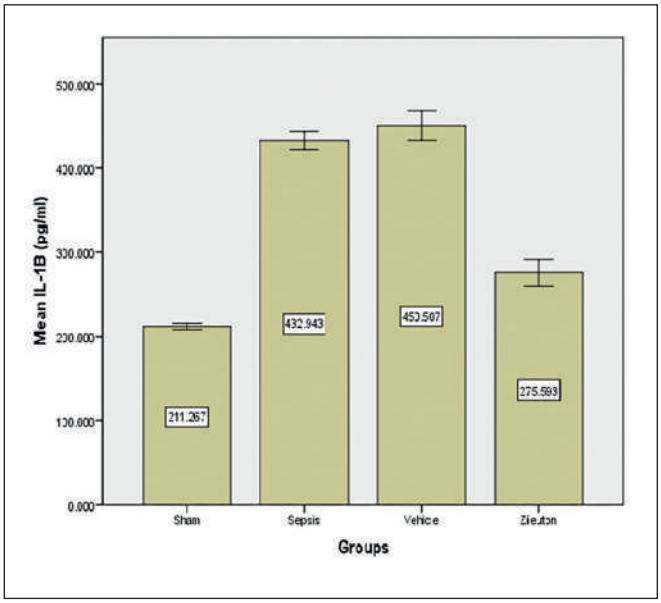

Fig. 3. Mean lung tissue level of IL-1B(pg/ml) ± SEM of the four experimental groups at the end of the experiment (number of mice in each group=6): sham vs. sepsis group, p-value =0.00001 (significant); zileuton vs. sepsis and vehicle group, p-value =0.00001 (significant).

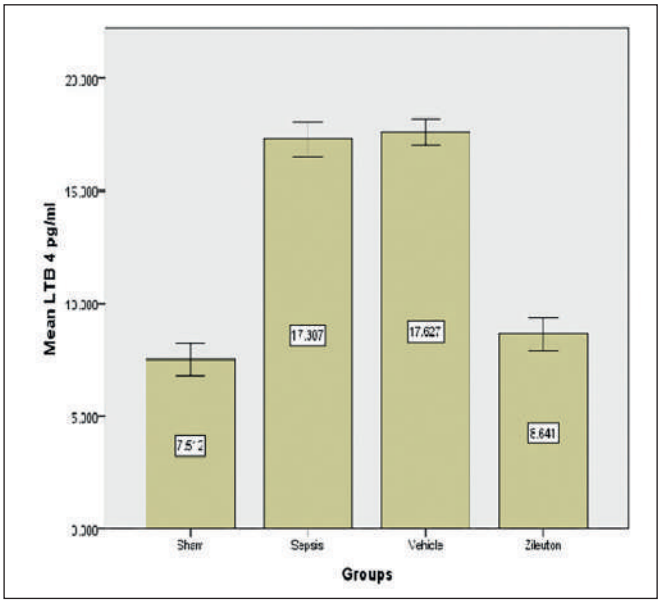

Fig. 4. Mean lung tissue level of LTB4 (pg/ml) ± SEM of the four experimental groups at the end of the experiment (number of mice in each group=6): sham vs. sepsis group, p-value =0.00001 (significant); zileuton vs. sepsis and vehicle group, p-value =0.00001 (significant).

RESULTS

ZILEUTON DECREASED INFLAMMATORY IL-6, IL-1B,IL-17 MARKERS IN LUNG TISSUE

When comparing the sepsis group to the sham group, the lung level of IL-17 was considerably ($p< 0.05$) higher in the sepsis (control) group. The lung levels of IL-17 in zileuton treated group were slightly lower (Fig. 1). Lung tissue level of IL-6 and IL-1B were significantly ($p< 0.05$) increased in sepsis group as compared with sham group.On the other hand, a 5-mg/kg zileuton pretreatmen significantly reduced the two inflammatory markers IL-6 and IL-1B level in lung tissue (Fig. 2-3).

ZILEUTON DECREASED LTB-4 AND 12(S)HETE INFLAMMATORY MARKERS IN LUNG TISSUE

Lung tissue level of LTB-4 and 12(S) HETE was significantly increased in sepsis group as compared with sham group. 5 mg/kg of zileuton pretreated group demonstrated significantly

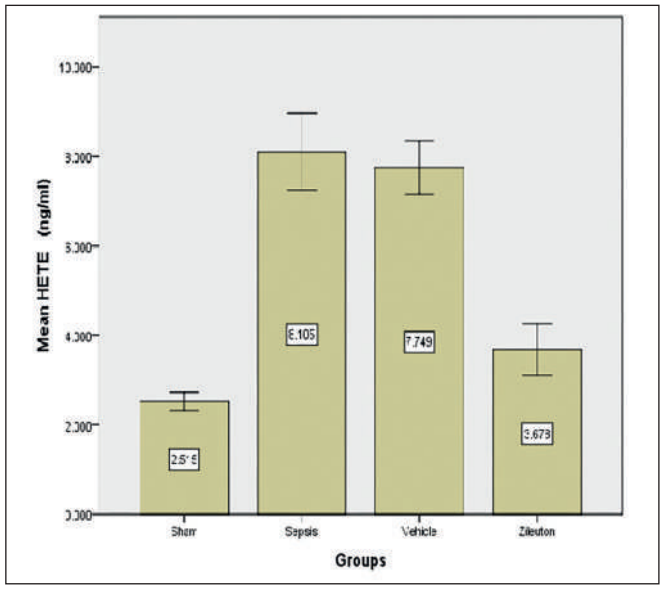

Fig. 5. At the end of the experiment, the mean lung tissue level of 12(S) HETE (ng/ml) SEM of the four experimental groups (number of mice in each group=6): sham vs. sepsis group, p-value =0.00001 (significant); zileuton vs. sepsis and vehicle group, p-value =0.00001 (significant).

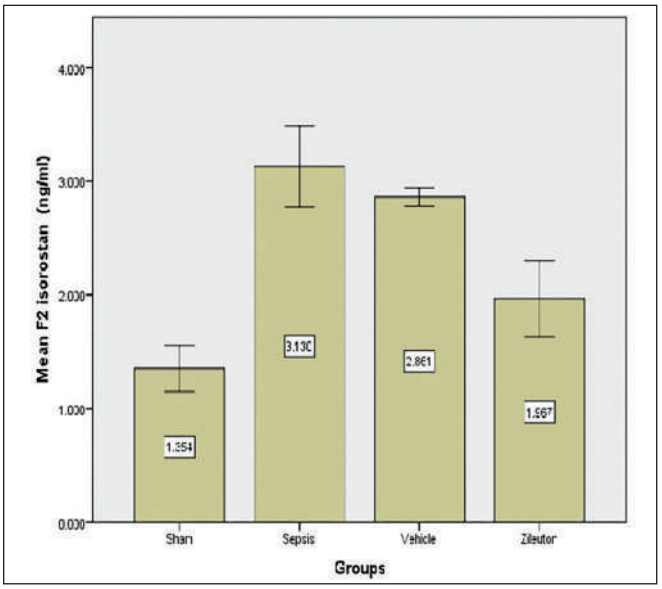

Fig. 6. Mean tissue level of F2 isoprostane (pg/ml) of the four experimental groups at the end of the experiment (number of mice in each group =6): sham vs. sepsis group, p-value =0.00001 (significant); zileuton vs. sepsis group, p-value =0.00001 (significant).

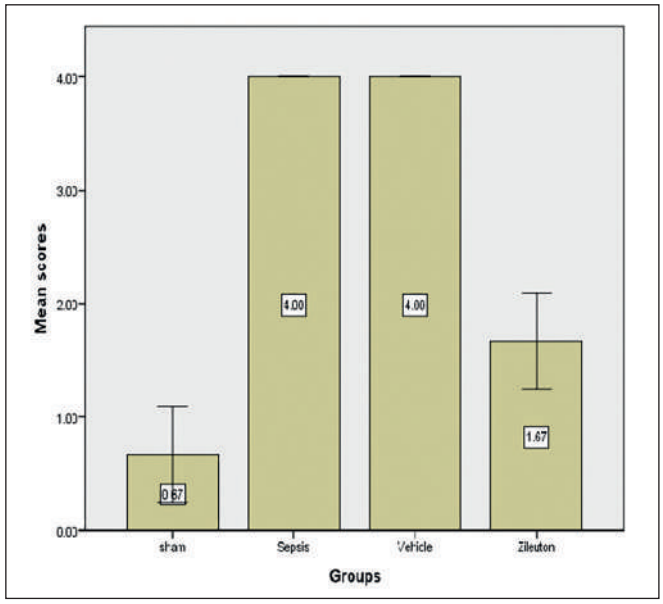

Fig. 7. Mean histopathological score of lung tissue of the four experimental groups at the end of the experiment (number of mice in each group =6): sham vs. sepsis and vehicle group, p-value =0.0001 (significant); zileuton vs. sepsis group, p-value =0.0001 (significant).

decrease the expression of inflammatory mediators LTB-4and 12(S) HETE as compared with sepsis group (Fig. 4-5).

ZILEUTON ATTENUATED OXIDATIVE STRESS (F2-ISOPROSTANE) IN LUNG TISSUE

Mice in sepsis group exhibited a significant increase in lung tissue level of F2-isoprostane comparing with sham group. Zileuton pretreatment significantly decreased the lung tissue level of F2-isoprostane compared with sepsis group (Fig. 6).

ZILEUTON MINIMIZED LUNG INJURY

The histological features of the zileuton pretreated mice group showed mild architectural alterations when compared with sham group. In sepsis and vehicle groups, the development of congestion, significant perivascular inflammation of mononuclear (lymphocytes, plasma cells) inflammatory cells, interstitial oedema, and localized extravasation of red blood cells as well as neutrophils and macrophages within alveoli (Fig. 7-8).

DISCUSSION

Sepsis is a life threatening organ dysfunction syndrome cuased by dysregulated host response to infection that leads to uncontrolled inflammatory response followed by immune suppression [15]. The mortality rate of severe sepsis is still high 20% to 65% despite the advances in critical care, among multiorgan dysfunction, there is amajor complication in sepsis is progressively impaired lung function and susceptibility to intrapulmonary infection. The development of acute respiratory distress syndrome (ARDS) is common in those cases [16]. The degree of inflammatory response and its sustained leukocyte activation may determine the clinical evolution of ARDS, the way that perpetuation of pro-inflammatory cascade activation that, due to loss of the alveolar compartmentalization in ARDS, can reach the blood stream and induced multiple organ dysfunction syndrome [17]. The studies are still insufficient to determines the role of pharmacological therapies in this cases. In the current study, we estimated the protective effect of zileuton on improving lung function following polymicrobial sepsis induced by CLP model in mice.

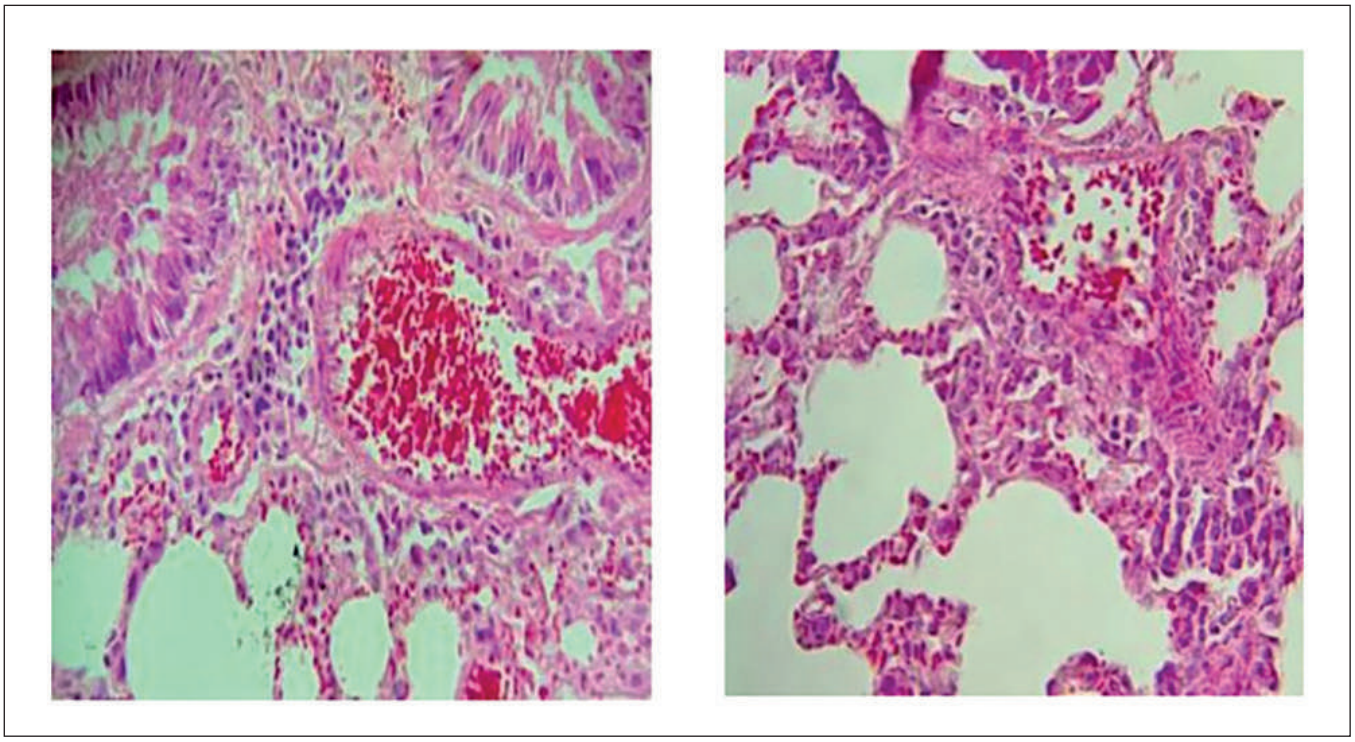

Fig. 8. Histopathological examination of lungl section. The section stained with haematoxylin and eosin (x400). A: photomicrograph of the lung section for sham group demonstrates normal regional lymph node, no inflammation, no oedema and no swelling; B: photomicrograph of the lung section for sepsis group demonstrates vascular congestion with perivascular mononuclear inflammatory cells infiltration; C: photomicrograph of the lung section for vehicle group demonstrates heavy mixed inflammation, vascular congestion and interstitial edema; D: photomicrograph of the lung section for zileuton treatment group demonstrates interstitial inflammation limited to 10% of the examined lung tissue surrounded by normal tissue.

EFFECT OF ZILEUTON ON PROINFLAMMATORY CYTOKINES(IL1B,IL-6 AND IL-17)

In this study, zileuton significantly lower the levels of pro inflammatory cytokines IL1B and IL-6, but slightly lowe the level of IL-17 when we compared with that of sepsis and vehicles groups demonstrating preservation of lung function. The hallmark of the inflammatory reaction in lung sepsis is the initial infiltration of neutrophils into the sepsis of lung parenchyma, the probabl mechanism of these effect was that zileutondepletion of neutrophil infiltration and reduced the proinflammatory markers as well as zileuton inhibited prostaglandins production by interfering at the level of arachidonic acid release, furthere more, leukotrienes considers as metabolic products of 5 lipoxygenase activation so initiate immune cell chemotaxis which are critical molecular players in the inflammatory pathophysiology of asthma and allergy [18]. This effect suggests that zileuton may improve lung injury during sepsis through its anti-inflammatory effect on IL1-B, IL-6 ana IL-17. This results is consistence with Larsson et al. [19], their result appearing that significant diminish in the levels of serum IL-6 in zileuton pretreated group as compare with the sepsis group when the mice undergo CLP that induced polymicrobial endotoxemia. Similar results obtained by Silva et al. [20] who demonstrated that zileuton improved reduction of interleukin-1B, interleukin-6 and decrease brain damage in the CNS, so improved the neurological outcome of brain ischemia.

EFFECT OF TREATED ZILEUTON ON PRO-INFLAMMATORY LEUKOTRIENE-B4(LTB4)

The present study was found that there is a significant decrease in lung tissue level of LTB4 for zileuton pretreated group in comparison to sepsis and vehicles groups**.** The probable mechanism of zileuton is that formation of leukotrienes depends on the lypoxygenation of the arachidonic acid by 5-lypoxygenase. Zileuton is an active and powerful inhibitor of the activity of 5-lypoxygenase and as such inhibits generation of its products, Collin et al. [21] indicated that LTB4 after endotoxemia induced in rats by LPS model, the 5-lipooxygenase inhibitor has significantly attenuated LTB4 synthesis on multiple organ injury\dysfuction caused by sever endotoxemia, Abueid et al. [22] demostrated that zileuton was found to be effective to suppress increased LTB4 on ischemia/reprefusion induced myocardial damage in rats. These findings suggest that zileuton plays a fundamental role in the lung protective effects after sepsis.

EFFECT OF SEPSIS ON 12(S)-HYDROXYEICOSATETRAENOIC ACID (12(S) HETE)

The current study has showed significant higher lung tissue level of 12(S) HETE in sepsis and vehicles groups as compared with sham group. Similar result obtained by Kwon et al. [3] where significantly increased serum 12(S) HETE level in lipopolysaccharide (LPS) idnduced endo-

toxic shock. Acute pulmonary inflammation can caused by CLP induced polymicrobial sepsis leads to immune-cell infiltration, mucuse production, vascular leak into the airways, and epithelial cell damage, so unregulated inflammationis an underlying cause of many chronic obstructive pulmonary disease, and fibrosis [23]. Elevated of eicosanoid 12 (S) HETE level are known to increased in response to inflammatory stimuli in the lung, and neutrophiles infiltration into the pulmonary space is a halmark feature of lung injury [24]. One of explanation that interprests association of 12 (S) HETE and lung injury, is that in an acute lung injury (ALI) mouse model undergo CLP induced sepsis led to induction of inflammation,increased vascular permeability, and upregulation of lipooxygenase, So production of lipooxygenase in alveolar macrophages and fibroblasts leads to bronchial epethelial injury via 12(S) HETE with leukotriene and pro-inflammatoru cytokines dependent mechanism.

EFFECT OF ZILEUTON ON 12(S) -HYDROXYEICOSATETRAENOIC ACID (12(S) HETE)

The present study has demonstrated that zileuton pretreated group significantly downregulation the expression of 12 (S) HETE. In this study, zileuton inhibitory effect against 5-lipoxygenase (5-LO) protected mice against overload lung injury, the improving of lung function by inhibitory effect of arachidonic acid metabolite. The role of 5- lipoxygenase inhibitor zileuton in sepsis induced by CLP has not yet been studied. Our current study is the first report on the roles of zileuton on reduction 12 (S) HETE level. On the other hand Wu et al. [25] showed that role of 5-LOX inhibtor zileuton in cardiac remodeling and protected mice against cardiac hypertrophy, fibrosis, and oxidative stress in response to LV pressure overload by reduction role of 12(S)-HETE and 15 (S)-HETE of activating PPARα/NRF2 signaling in cardiomyocytes.

EFFECT OF SEPSIS ON OXIDATIVE STRESS MARKER (F2 ISOPROSTANE)

In this current study, it has been observed that it is significantly increased in F2 isoprostane level in the sepsis group as compared to the sham group.Increased oxidative stress and production of reactive oxygen species play a key role in triggering and retaining the inflammatory response. Measurement of F2-isoprostanes, has been proven to be one of the maximum consistent processes to measure oxidative stress condition [26]. In sepsis conditions, an over-production of ROS takes place, both in the circulation and in the affected organs [27]. This is probably caused by the high levels of cytokines LTB-4 that stimulate the activation of endothelial and immune cells, increasing their formation of ROS [28]. In addition, hypoxia produced in more severe forms of sepsis has been correlated with an increase of ROS production in cells [29]. The outcomes within the present study are in settlement with the ones stated by means of [30] who exhibited that oxidative stress has a great influence in sepsis in both mitochondrial and endothelial dysfunction as well as oxidative stress biomarkers would be expected to be useful in an optimal sepsis diagnosis and prognosis. Reference showed that plasma levels of F2-isoprostanes and isofurans are associated with renal, hepatic, and coagulation failure but not with circulatory or pulmonary failure in severe sepsis suggesting that lipid peroxidation is a prominent feature of septic multi-system organ failure [31].

EFFECT OF ZILEUTON ON OXIDATIVE STRESS MARKERS F2 ISOPROSTANE

zileuton treated group showed a higher decrease in the tissue lung level of oxidative stress F2 isoprostane as compared with sepsis and vehicle group, zileuton pretreated group showed significantly (p-value ≤ 0.05) lower level of lung tissue F2-isoprostane as compared with sepsis group. Hadi et al. [32] demonstrated that zileuton treatmenthad significantly reduced of oxidative stress parameters and retard the progressionof atherosclerosis. However, the molecular mechanism of antioxidant effect of 5-Lipoygenase inhibitors has not yet been fully investigated, Kanaoka et al. [18] showed that the important source of reactive oxygen species are lipoxygenase enzymes responsible for the metabolism of arachidonic acid and other unsaturated fatty acid. On the other hand Czapski et al. [33] demonstrated that 5-Lipoxygenase promotes lipid peroxidation in vitro as well as in brain tissue. To the best of our knowledge, there is no data available about the effect of zileuton on F2-isoprostne in lung injury sepsis.

EFFECT OF SEPSIS ON THE HISTOPATHOLOGY OF LUNG TISSUE

The present study has showed that sepsis and vehicles groups had significant higher degree of lung tissue injury as compared with sham group. The histopathological damage score were mostly highly severe for sepsis and vehicle groups. Histopathological findings in sepsis and vehicles groups were associated with extensive extravasation of red blood cells with heavy perivascular mononuclear inflammation cells and interstitial edema, similar results also experienced by Rojas et al. [34] that found there were a congestion and infiltration of inflammatory cells predominantly neutrophils in endotoxin (LPS)-induced lung injury.

EFFECT OF ZILEUTON ON THE HISTOPATHOLOGY OF LUNG TISSUE AFTER SEPSIS

Treatment with zileuton significantly reduce the lung tissue injury as compared with sepsis and vehicles groups. The histopathological damage scores were mostly mild for zileuton pretreated group. Furthermore; zileuton pretreated group has more resemble to normal tissue architecture than montelukast pretreated group, Alabbassi et al. [35] demonstrated thatzileuton histologically reduced the num-

ber of inflammatory cells and ameliorate the destruction of lung architecture and pulmonary fibrosis induced by amiodarone.

CONCLUSION

The results of the present study revealed that zileuton has the ability to attenuate lung dysfunction during CLPinduced polymicrobial sepsis in male mice through their modulating effects on LTB4,12(S)HETE and oxidative stress downstream signaling pathways and subsequently decreased lungtissue levelsof proinflammatory cytokines (IL-1β, and IL-6,IL-17).

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Conflict of interest
The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

EFFECTS OF ALPHA-LIPOIC ACID ON GLYCEMIC STATUS IN 2 TYPE DIABETES PATIENTS WITH CHRONIC CORONARY SYNDROME

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ABSTRACT

The aim: To study the possibilities of alpha-lipoic acid (ALA) to control the parameters of carbohydrate metabolism.

Materials and methods: We examined 80 people with type 2 DM and coronary heart disease who suffered non-Q-myocardial infarction (non-Q-MI). All patients at the time of inclusion in the study received oral hypoglycemic agents, ACE inhibitor, β-blocker, statin and antiplatelet agent. 600 mg of ALA per day for 4 months was added to this treatment. After checking the patients for compliance with the criteria, they were divided into the main and experimental groups. The dosage of alpha-lipoic acid was determined for each of the groups. The results of the treatment were analyzed by determining the mean and standard deviations.

Results: At the end of the observation period, a significant decrease in the level of fasting glucose (FG) by 11.6% was found, which corresponded to the average size of the clinical effect. The values of glycosylated hemoglobin (HbA1c) and the insulin resistance index HOMA (HOMA-IR) showed only a tendency to decrease on the background of treatment. The effect of ALA on postprandial glycemia (PPG) and insulin levels was not detected in this study.

Conclusions: An additional 4-month dose of ALA in addition to baseline therapy showed a moderate effect on the decrease in FG concentration in the absence of significant dynamics in other parameters of glycemic control in the examined patients.

KEY WORDS: diabetes mellitus, coronary disease, myocardial infarct, lipoic acid

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INTRODUCTION

A sign of modernity is the increasing comorbidity of diseases, which requires the combined efforts of various professionals to solve the most urgent medical problems. One of the applications of this comorbidity is the combination of diabetes mellitus (DM) and chronic coronary syndrome (CCS). In particular, blood clotting has a significant influence on the development of cardiovascular system diseases. It should also be noted that patients with MD coronary heart disease (CHD) have a worse prognosis. Epidemiological studies have established that under such conditions the rate of repeated myocardial infarctions (MI), mortality due to the development of complications and the risk of coronary death increases [1-3].

Diabetology and cardiology have the significant successes and achievements in the last ten years but the results of treatment in everyday clinical practice, although they reflect the progress of science in general, still they do not look very optimistic because of rather high mortality rate among such patients [1, 2, 4, 5]. In addition to the classic risk factors of cardiovascular mortality in diabetic patients, there are specific factors for CD duration and importance of diabetes, control of glucemia, presence of hypoglycemic conditions. All this points to the importance of metabolic factors in the formation, progression and consequences of CHD [6, 7].

Therefore, the current problem of current clinical medicine is the search for optimal and effective pharmacotherapy in the context of comorbidity. The clinical course occurs pathomorphosis and often due to the presence of diabetes there is refractoriness to cardioprotective treatment strategies or appearance of uncomfortable drugs effects [8].

The key role in the pathogenesis of diabetes and its complications belongs to oxidative stress (OS). In vivo studies have proved the role of hyperglycemia in the generation of OS, which causes endothelial dysfunction, contributes to the development of macroangiopathies, progression of atherosclerosis [9, 10].

In view of the above, the study of the effect of alpha-lipoic acid (ALA) under conditions of DM and SS combination is of interest due to the positive effects of the product such as antioxidant properties [10, 11], the presence of antiplatelet effect that was also demonstrated in our previous studies [12], and the potential effect on carbohydrate metabolism [10]. A number of experimental studies demonstrated the ability of ALA to increase glucose consumption in insulin-sensitive and insulin-resistant muscle tissues [13]. It increases the content of glucose transporters [14], stimulates glucose granulation [15, 16], inhibits the accumulation and secretion of insulin-induced by high glucose content [17].

However, the number of clinical studies on the efficacy of the drug in influencing carbohydrate metabolism in

patients with type 2 DM and in comorbidity with CCS is limited. Moreover, their results are often inconsistent. So, today, it is not possible to identify clearly the place of ALA in the treatment of comorbid pathology and to make residual conclusions about the drug's effect on metabolism disorders in these patients.

THE AIM

This study aimed to evaluate the effects of ALA on parameters of carbohydrate metabolism, including glucose, glycosylated hemoglobin (HbA1c), insulin, and insulin resistance index HOMA (HOMA-IR) in patients with type 2 diabetes who underwent non-Q-MI.

MATERIALS AND METHODS

In this clinical study, 80 patients (mean age was 63.37 ± 7.59 years) with type 2 MD with non-Q-MI, including 49 men and 31 women, and 40 healthy individuals (control group - CG), comparable in age and gender. CG subjects did not have a history of DM and CCS, as well as other chronic diseases, they did not record abnormalities on objective examination, according to general laboratory parameters, ECG, sonography of the heart and treadmill test.

This clinical study was conducted in accordance with the moral and ethical principles of the Declaration of Helsinki, the Council of Europe Convention and current legislation of Ukraine on human rights. The study was started after agreeing on the design of the work with the local commission on ethics of the National Medical University named after O. Bogomolets. Each of the subjects received informed consent to participate in the study.

Inclusion criteria were: the presence of a patient with type 2 diabetes and a history of non-Q-MI, oral hypoglycemic agents, ACE inhibitor, β-blocker, statin and antiplatelet agent, baseline HbA1c level ≤9.5%.

Exclusion criteria: the presence of type 1 DM, baseline HbA1c level >9.5%, insulin therapy, congenital or acquired valvular heart disease, persistent atrial fibrillation or atrial fluttering, cardiomyopathy, symptomatic arterial hypertension, severe heart failure, corresponding to NYHA Class III-IV, infectious diseases, kidney and liver diseases, oncopathology.

Patients who met all inclusion criteria and did not have any exclusion criteria were divided into 2 groups by random sampling: the main (n = 43) and the compare group (n = 37). Patients in the main group as adjunctive therapy were prescribed 600 mg of ALA per os 30 minutes before breakfast. Compare group patients continued treatment with only basic therapy. The subjects were observed and the total duration of treatment was 4 months.

Prior to treatment and at the end of the observation period, the following were determined: the concentrations of fasting glucose (FG) and 2 hours postprandial glucose (2-hPPG) in the serum using the glucose oxidant method, the HbA1c level by the immunoturbimetric method and the insulin level by the immunochemical method with electrochemiluminescence detection on a Cobas 6000 analyzer (Switzerland). Units for glucose are mg/dL, HbA1c - % and for insulin it was used μIU/mL. HOMA (HOMA-IR) insulin resistance index was calculated according to the formula (1):

HOMA-IR = insulin intake (μIU/mL) x fasting glucose (mg/dL) / 22.5.

Venous blood samples were taken on an empty stomach (at least 10 hours after the last meal) and 2 hours after breakfast to determine postprandial blood glucose.

Descriptive statistics were used to analyze the data (means and standard deviations). To study differences before and after treatment with standardized mean difference (SMD), a paired t-test (significant level of $P < 0.05$) was used after studying the normal distribution of data using the Kolmogorov-Smirnov test. For comparing two paired proportions we used the McNemar test. Data were analyzed with STATA 12.

RESULTS

Our study assessed the indicators of carbohydrate metabolism, namely FG ($p<0.001$), 2-hPPG ($p<0.001$), HbA1c ($p<0.001$), and HOMA-IR index ($p<0.001$). 001), HbA1c ($p<0.001$), insulin ($p<0.001$), and HOMA-IR ($p<0.001$) were significantly higher in the treated patients compared to CG (Table I).

Assessment of carbohydrate metabolism indices after 4-month ALA treatment in patients of the main group showed a significant decrease of FG level ($p<0.05$). Moreover, there was a positive tendency to decrease the HbA1c level ($p<0.1$) and the insulin resistance index HOMA ($p<0.2$), without a significant effect on the 2-hPPG level and insulin concentration. However, there was no significant dynamics of the studied parameters in the comparison group (Table I).

The results of the comparative analysis revealed a significantly higher clinical effect in the main group relative to the comparison group. The standardized size of the clinical effect in the main group on the FG indicator corresponds to the average size of the clinical effect and significantly exceeds the dynamics of the indicator in patients compare group (Fig 1).

Taking into account the positive tendencies to decrease insulin resistance in the examined patients, we carried out an in-group analysis of insulin and HOMA-IR levels among the patients studied and their dynamics against the background of the treatment used.

Thus, increased insulin level was detected in 11 patients (25.6%) of the main group, and normal insulin level - in 32 patients (74.4%) of the group. ALA had a 9.3% ($p<0.2$) decrease in patients with hyperinsulinemia with an increase in the same proportion of patients with normal insulin levels (Fig 1).

Among the patients of the comparison group hyperinsulinemia was observed in 12 patients (32.4%), and normal insulin level was typical for 25 patients (67.6%). Against the background of baseline therapy there was a decrease

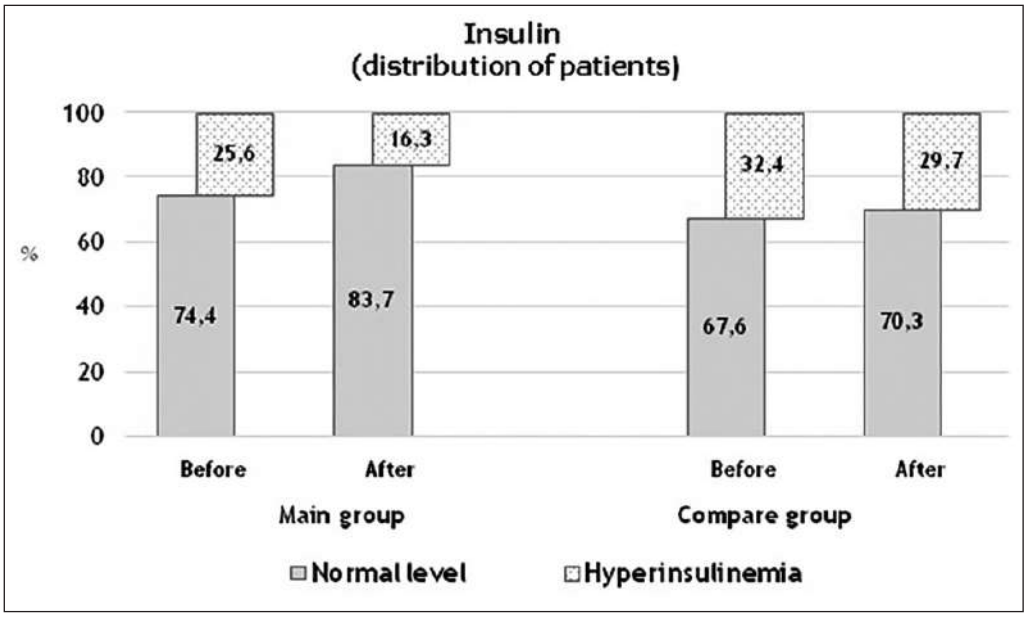

Fig. 1. Assessment of the effect size of applied treatment in the examined groups by SMD: alpha-lipoic acid with basic treatment versus only basic treatment.

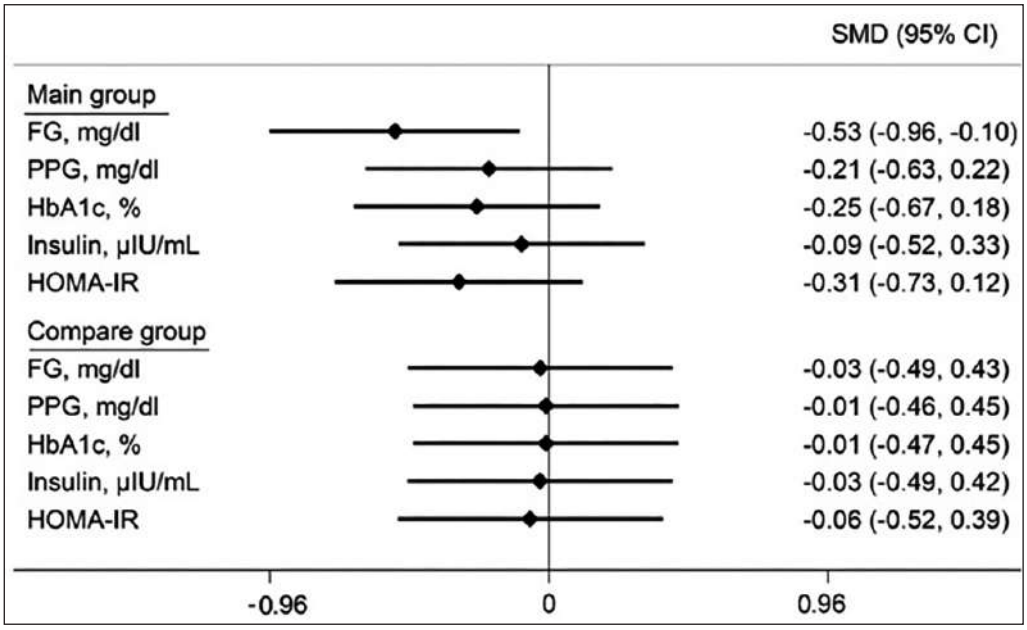

Fig. 2. Changes in the distribution of examined patients by fasting insulin level on the background of applied treatment. Note: the difference is statistically insignificant ($p>0.05$).

in the number of patients with elevated insulin levels by 2.7% ($p>0.05$) with an increase in the number of patients with normal insulin levels by the same amount (Fig 2).

The analysis of the insulin resistance index HOMA showed that the great majority of patients in the main group - 40 patients (93%) had an elevated index, 3 individuals (7%) had normal HOMA-IR. Treatment resulted in a 13.9% decrease in the incidence of insulin resistance, $p<0.1$ (Fig 3).

According to the outcome data, 33 patients (89.2%) of the comparison group had elevated HOMA insulin resistance index, only 4 patients were characterized by normal values of this index. After treatment, the number of patients with insulin resistance remained unchanged.

Therefore, the prescription of ALA causes not only a tendency to decrease the HOMA-IR index, but also reduces the prevalence of insulin resistance among the main group of patients.

DISCUSSION

According to the literature data, clinical work with the use of ALA and evaluation of the drug effects on the parameters of carbohydrate metabolism, present inconsistent results.

For instance, a randomized placebo-controlled subcutaneous blind study [18] with use of ALA at a dose of 100 mg/dobu in women with gestational SD for 8 months demonstrated a significant decrease in the level of FG. A significant decrease in glucose and HbA1c levels with an increase in insulin concentration in patients with type 2 SD [19] was observed while taking 7 mg ALA/kg body weight for 8 months. However, as a part of this randomized double-blind placebo-controlled trial, patients received carnosine and thiamine instead of ALA. Genazzani AD. et al. [20] in their work demonstrated not only a significant decrease in glucose and HbA1c levels, but also a decrease in the HOMA insulin resistance index while using 400 mg

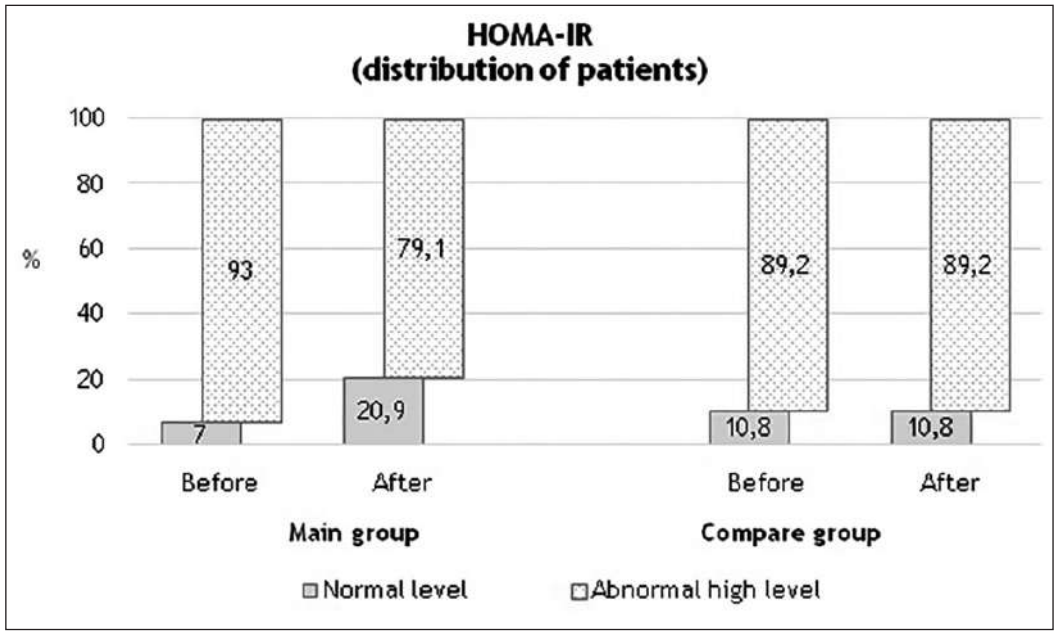

Fig. 3. Changes in the distribution of examined patients by HOMA-IR on the background of applied treatment. Note: the difference is statistically insignificant (p>0.05).

Table I. Changes of carbohydrate metabolism indices in the examined patients against the background of the applied treatment (M±SD).

Variables	Time, SMD	Main group (n=43)	Compare group (n=37)	Control group (n=40)
FG, mg/dl	Before	160.91±32.27#	156.04±36.49#	85.41±10.48
	After	145.46±25.68*	154.97±35.92#	
	SMD (95%CI)	-0.53 (-0.96--0.1)	-0.03 (-0.49-0.43)	-
2-hPPG, mg/dl	Before	192.65±34.09#	199.44±38.68#	96.50±9.23
	After	185.86±31.57	199.10±34.46	
	SMD (95%CI)	-0.21 (-0.63-0.22)	-0.01 (-0.47-0.45)	-
HbA1c, %	Before	7.47±0.96#	7.66±1.01#	4.97±0.40
	After	7.23±0.98	7.65±0.93	
	SMD (95%CI)	-0.25 (-0.67-0.18)	-0.01 (-0.47-0.45)	-
Insulin, μIU/mL	Before	16.90±6.72#	17.62±7.88#	10.32±3.10
	After	16.26±6.98	17.38±7.57	
	SMD (95%CI)	-0.09 (-0.52-0.33)	-0.03 (-0.49-0.43)	-
HOMA-IR	Before	6.87±3.63#	6.73±3.61#	2.15±0.62
	After	5.86±2.85	6.51±3.20	
	SMD (95%CI)	-0.31 (-0.74-0.12)	-0.06 (-0.52-0.39)	-

Note: #– p<0.001 compared with CG; * – p<0.05 compared with data before treatment; SMD (95%CI) for repeated measures: <0.5 – 'small' effect, 0.5-0.8 – 'medium' effect, >0.8 – 'large' effect; M±SD – Mean and Standard deviation.

of ALA for 12 months in women with polycystic ovary syndrome and obesity who have close relatives with SD.

In another randomized placebo-controlled study [21], 30-day intake of ALA at a dose of 600 mg in pre-diabetes had no effect on GN, but caused a significant decrease in insulin and HOMA-IR. In a prospective randomized double-blind placebo-controlled trial [22], when 900 mg of ALA was added to treatment for 10 days to patients in critical condition, who were treated in the intensive care unit, a significant decrease in glucose level was observed, as well as ALA interfered with FG and HOMA-IR increase. At the same time, the level of insulin did not change. According to the data of meta-analysis [23], which was based on the data of 41 articles, 16 of which had a poor quality, ALA significantly decreases HbA1c, but does not affect insulin level and insulin resistance index HOMA. The results of another meta-analysis [24] that analyzed data from 24 studies showed the efficacy of ALA in reducing not only HbA1c, but also the level of insulin and insulin resistance. In our study, ALA influenced the decrease of FG level with no significant effect on PPG, HbA1c and insulin resistance, which is consistent with a number of the presented works.

In contrast to previously conducted studies, in the work of Agathos E. et al. [25] using 600 mg of ALA for 40 days

in patients with diabetic neuropathy had no dynamics of HbA1c and HbA1c levels. Similar results were demonstrated in the study of Mendoza-Nunezh VM. et al. [26] with 6-month use of 600 mg ALA in patients with type 2 SD. No significant changes in glucose level and HbA1c were detected. When Cavestro C. et al. [27] used 400 mg of ALA twice a day for 6 months in patients with migraine and insulin resistance didn't detect changes in FG, 2-hPPG and insulin levels, also there were no changes in insulin resistance indices.

A number of experimental works investigated the mechanisms of ALA effect on carbohydrate metabolism parameters. Thus, Zhang Y. H. and co-workers [14] studied glucose metabolism in the brain of P301S mice and found that chronic administration of ALA increases glucose availability by increasing the levels of glucose transporters GLUT3 and GLUT4, vascular endothelial growth factor, mRNA, haemoxygenase-1 protein. Apart from that, activation of glucolysis was detected due to increased activity of hexokinase and increased activation of proliferator-activated gamma coactivator 1-alpha receptor - PGC-1α, and enzymes for DNA renewal – OGG1 / 2, and MTH1. It was found that the main mechanism of renewal of glucose metabolism can include activation of the neurotrophic factor – BDNF / tyrosine kinase B / hypoxia-inducible factor-1α - HIF-1α signaling pathway.

The results of Yang Y. [17] demonstrated the ability of ALA to induce apoptosis in HIT-T15 beta cells of the pancreatic gland treated with high glucose concentrations. Moreover, ALA induced oxpression and secretion of insulin in HIT-T15 cells induced by high glucose content. It was found that the above-mentioned effects were associated with increased expression of Pdx1 and Bcl2 genes, decreased expression of Bax gene and increased Akt phosphorylation in HIT-T15 cells under the influence of ALA. According to Liu Z. and co-authors. [16] ALA due to its insulin-like effect is able to inhibit the development of insulin resistance and increase glucose metabolism in the brain of C57BL/6J mice with induced high-fat dietary obesity. Konrad D. and co-workers [15] in in vitro studies established that similarly to insulin, ALA stimulates phosphorylation of p38 MAPK, significantly increasing the kinase activity of α- and β-isoforms of MAPK, by 2,4 times increases the translocation of GLUT4 molecules on the surface of the cells and causes activation of GLUT4, stimulates IRS-1-associated PI3K activity and enhances phosphorylation and activates Akt1, the key enzyme of the PI3K/Akt signaling pathway. Therefore, these results explained the insulin-promoting effects of ALA and provided a new insight into the role of ALA in the treatment of SD.

CONCLUSIONS

In addition to the basic treatment, the use of ALA for 4 months in 2 type diabetes patients with coronary heart disease who underwent non-Q-MI has a significant reduction in FG by 11.6%. Moreover, there was a favorable tendency to decrease the HbA1c and the HOMA-IR levels, as well as a reduce in the prevalence of hyperinsulinemia and insulin resistance by 9.3% and 13.9%, respectively.

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ORIGINAL ARTICLE

COMPUTED RADIOGRAPHY UTILIZATION FOR TELECOBALT^{60} TO ACHIEVE THE RADIATION CERTAINTY

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ABSTRACT

The aim: This research aimed to show the achievement of Telecobalt60 radiation certainty using computed radiography, in comparation with non-verified computed radiography.
Materials and methods: This research is a quantitative study, randomized double-blind, and consecutive sampling design. The study was conducted by observing and comparing the data of verified computed radiography (VerC) computed radiograph for Telecobalt60 compared to the non-verified computed radiography (nVerC) Telecobalt60 data.
Results: The results showed that there are significant statistical differences in several measurement characteristics between the verified computed radiography arm and the non-verified computed radiography arm. All of the value divergences of the verified computed radiography arm are less than 7 mm while the non-verified computed radiography arm are 7 mm or more ($P<0.050$). Furthermore, all of the edge aspect of measurement in the verified computed radiography arms are less than the non-verified computed radiography, all without manual block utilization ($P<0.050$).
Conclusions: We conclude that Telecobalt60 radiation certainty is significantly better achieved by using computed radiography, when compared to non-verified computed radiography Telecobalt60 use. This research contributes to provide evidence based for better Telecobalt60 radiation accuracy and quality of radiotherapy outcome by using computed radiography.

KEY WORDS: Radiotherapy, computed radiography, certainty

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INTRODUCTION

External radiation therapy remains as one of the most important modality for cancer treatment [1-23] The World Health Organization (WHO) reported constantly increasing mortality rates due to cancer. In year 2018 alone, cancer had caused deaths for 9.6 million people throughout the world [16, 24-26]. The American Cancer Society reported that in 2007, there were 10 million new cases of cancers, and this disease had taken at least 8 million lives worldwide. Scientist had predicted that by 2030 there will be an annual 26 million new cancer cases and 17 million cancer related deaths worldwide which mostly will occur in the developing countries [1,6,10,16,27-30].

Radiotherapy remains as an essential treatment for around 60% of cancer patients worldwide.(1,3–5,10,11,27,31,32) either as a curative or palliative form, and also recommended as one of the best possible treatments for every patient with cancer by the ISCRO-USA and WHO. There were few inventions of radiotherapy in the beginning of the 20th century especially for the cancer treatment of breast, stomach, skin, and nose in America, France, Austria, and Sweden. Those inventions in radiotherapy paved for the discovery of the two main techniques of radiotherapy delivery, namely teletherapy (cobalt60 or linac Moreover, WHO and the International Society for Cancer and Radiation Oncology (ISCRO) in United States of America had previously gave recommendation for radiotherapy as treatment for cancers [1, 12-23,33,34].

Radiotherapy inventions in the early 20th century had discovered two main mechanism of radiotherapy delivery, which are tele-radiotherapy (tele-therapy) and brachytherapy [1,12-21,35-37]. The invention of Computed Tomography (CT) - scan, Verification Portal Imaging (VPI), and Multi-Leaf Collimator (MLC) devices could further optimize radiation dose delivery to achieve more satisfactory therapeutic result [1,10,12-23,26,34-38].

Telecobalt^{60} remains an important tele-therapy device for cancer treatment [12-18, 21-23,26,30,32]. Telecobalt^{60} devices comprises at least 30% of global availability of radiotherapy devices, treating around 1 million cancer patients regardless the economic condition and income of the country. To date, Telecobalt^{60} is still a relevant tele-therapy device in majority of developing countries. It provides various advantages over linear accelerator tele-therapy, such as less maintenance costs and infrastructure requirements, low power demand, and much simplified quality assurance of the beam parameters [4,5,18,21,31-34].

However, radiotherapy is vulnerable to errors in its delivery process due to the complexity of the technology,

techniques, processes and human resource quality [4,11-15,17,18,21,22,26,38-46].

Errors in radiation therapy delivery is presented as radiation inaccuracies and inequality between the real and the calculated theoretical value of radiation aspects and safety. Errors which often occur in radiation therapy delivery comprise of errors in radiation dose, equality of radiation site and irradiated patient, errors in the device or software, treatment planning system (TPS), and human errors [1,11-13,17,18,21,22,26,40,42-50].

Errors in radiation site equality consist of error in defining simulator image scheme and subject positioning, determining the radiation target, and conforming body or tumor size of the patient. The factors which may contribute to errors in radiation site equality in the radiotherapy delivery process include lack of experience or competence, emotional and physical burnouts, recording and guideline malfunctions, over-confidence on automatic device system, mal-interactions or insufficiency of the radiation quality control commissioning (particularly related to radiation site equality and radiation target accuracy), staffing system, team selection, working environment, and adaptation to advances in technology [12,13,17,18,21,22,26-48].

Errors in radiation site equality can be reduced by conducting continuous and sustainable verification and validation of the previously filled standard check lists in the radiotherapy processes. The check and re-check processes can be conducted on daily, weekly, or monthly basis. This include factors affecting the radiation site equality (such as definition of simulator image scheme and subject positioning, determination of radiation target, and patient's body or tumor size conforming) and factors affecting radiation target accuracy (such as radiation target determination, tele-therapy equipments setting and preparation, and human resources). Therefore, VPI device is needed to reduce radiation error, this include gamma-graph/ SFR, computed radiography (CR), or portal image equipment (EPID).

Previous data showed that radiation site equality errors in radiotherapy delivery process were low and rarely occur, owing to the implementation of standard quality control and rigid regulation in radiotherapy delivery. This include continuous verification and recording of the standardized check lists in radiotherapy delivery processes using computerized system. Nevertheless, errors in radiation site equality during radiotherapy delivery process could always happened, creating serious dangers whenever the standard of procedures and quality controls in radiotherapy delivery were not to be undertaken seriously and continuously[11,13,17,18,21,42,43,46].

Parallel to that fact, prior evidences suggested that VPI device played an important verification role in radiotherapy delivery. Verification portal image (VPI) devices which are available for use nowadays consist of SFR/ gamma-graph (conventional form) and CR (digital form) [12–23,26,53].

Computed radiography (CR) digital VPI device was introduced in 1980 to subtitute conventional SFR/gamma-graph for radiation verification process, CR comprises of image plate (IP), imaging plate reader (IPR), and computer. The image plate contains high photo-stimulated barium-based phosphor plate which is capable to store the beamed-gamma or X - ray for further release by laser scan/optical stimulation process to produce an image. The image plate was placed in a cassette which consist of a frame (made of steel or aluminum), a tube side of carbon fiber, and a thin layer of tin on its back [13–15,17–19,21,51,53–55].

The processes of creating a digitalized image of computed radiography begins when the image plate is exposed to beamed-gamma or X - ray and displays a latent shadow. Afterward, the exposed image plate is plugged into the image plate reader and further scanned by a helium-neon laser (which is a red radiated light) until the crystals in the image plate radiates a violet-blue light. The violet-blue light is then detected a by photo-censor and converted into the computer by an analogue digital converter system to be processed. The image is further displayed to computer monitor. After obtaining and displaying the image, the image plate is transferred to the other part of the image plate reader to be wiped off [13–15,17–19,21,51,53–55].

In the past, SFR/ gamma-graph was widely applied before computer radiography was introduced. However, today this domination is rapidly decreasing, in correlation to the advanced radiotherapy technology developments. Gamma-graph was no longer used due to the high cost, labor intensive, high risk from the hazardous material for the process, low quality of radiography image, fixed grey-scale response (incapable of contrast adjustment) in its screen film, fixed latitude, fixed dose to the patient, used, inefficient storing/ archiving process, and film retrieve difficulty [13–15,17–19,21,51].

Computed radiography provides significant advantages to support Telecobalt60 as a VPI device, such as producing better image quality, instantly available for distribution to clinical image, much easier to review previous imaging, and more cost-effective to run the service, storing and archiving [12–15,17–19,21,22,26,51,56].

Furthermore, CR is superior in signal to noise ratio (SNR) or contrast to noise ratio (CNR) values. Hence, some expert refer to its better quality of image which made it worth recommended as a VPI device [12–15,17–19,21,22,57].

The weakness of CR utilization is the longer process it takes for plugging into the image plate reader. However, CR device utilization shares similar benefits as electronic portal image equipment (EPID) in the linear accelerator (Linac) tele-therapy machine [12–15,17,18,21–23,26,51,53,56]. Several evidences on CR utilization since 2005 displayed its utilization only in the field of diagnostic radiology, by which its correlation to the application technique of the exposure factors (kVp, mAs, or EI) serves as the basis for diagnostic imaging manufacture in digital image system optimalization [58].

The use of Telecobalt60 device has significant shortcomings to reach the target in achieving the expected quality and therapeutic result of cancer treatment as it imposes a substantial risk of radiation errors. The main limitation of Telecobalt60 radiation occurs due to the absence of VPI

Table I. The characteristic of the verified computed radiography (VerC) arm and non-verified computed radiography (nVerC) arm – without manual block use.

Characteristic	X+		X-		DX		Y+		Y-		DY	
	VerC	nVerC	VerC	nVerC	VerC	nVerC	VerC	nVerC	VerC	nVerC	VerC	nVerC
Median	3.58	7.00	2.76	6.11	0.54	3.70	3.38	6.60	3.35	6.01	0.38	2.55
Mean	3.55	6.97	2.93	6.36	1.00	4.35	3.51	8.31	3.39	7.35	0.80	3.41
Modus	5.21	0.04	0.04	0.20	0.23	9.03	3.38	1.03	0.10	0.24	0.00	0.23
Maximum	6.31	13.98	6.08	11.87	6.68	9.89	5.97	19.47	6.16	18.24	3.54	8.91
Minimum	0.25	0.04	0.04	0.20	0.00	0.41	0.00	1.03	0.10	0.24	0.00	0.23

X+: The edge aspects of the right axis measurement, either in the computed radiography reader or simulator image scheme; X-: The edge aspects of the left axis measurement, either in the computed radiography reader or simulator image scheme; DX: The differences between the X+ and the X-; Y+: The edge aspects of the upper ordinate measurement, either in the computed radiography reader or simulator image scheme; Y-: The edge aspects of the lower ordinate measurement, either in the computed radiography reader or simulator image scheme; and DY : The differences between the Y+ and the Y-; VerC: verified computed radiography arm; nVerC: non-verified computed radiography arm.

Table II. The normality data of the verified computed radiography(VerC) arm and non-verified computed radiography (nVerC) arm, without and with manual block use.

Normality	X+ Non-Block	X- Non-Block	DX – Non Block		Y+ Non-Block	Y- Non-Block	DY – Non Block		X+ Block	X- Block
			Insial	Transform			Insial	Transform		
Psig.KS	0.20	0.20	0.00	0.20	0.20	0.20	0.00	0.20	0.20	0.20

X+: The edge aspects of the right axis measurement, either in the CR reader or simulator image scheme; X-: The edge aspects of the left axis measurement, either in the computed radiography reader or simulator image scheme; DX: The differences between the X+ and the X-; Y+: The edge aspects of the upper ordinate measurement, either in the CR reader or simulator image scheme; Y-: The edge aspects of the lower ordinate measurement, either in the computed radiography reader or simulator image scheme; DY : The differences between the Y+ and the Y-; Non-block: without manual block use; Block: with manual block use; Psig.KS : Psig. Kolmogorov Smirnov.

device which render potential errors in radiotherapy [12–23,33,34,42,43,46,51,59]. Ideally, Telecobalt60 should always be equipped by VPI device as it is substantial to improve radiation certainty in order to achieve a better quality outcome of radiotherapy [12–15,17–19,21–23,39,42,43,46,49,53].

THE AIM

This research aims to reveal the radiation certainty achievement by utilizing verified computed radiography (VerC) for Telecobalt^{60} in compare to non-verified computed radiography (nVerC) Telecobalt^{60} use. This paper consists of several sections. The first section presents the research introduction. Section 2 explains the study materials, method, and how the study was conducted. Section 3 reveals the study results. Section 4 consisted of discussion which compares the previous evidences and theories to the evidences found in this research, and section 5 is the conclusion of this research.

MATERIALS AND METHODS

Subjects of this study were patients of Department of Radiotherapy – dr. Moewardi Hospital, Surakarta - Central Java, Indonesia who were treated from February 18th to May 31th 2019. The study involved 30 female subjects, aged 20 to 60 years old, all diagnosed with early stage (stage I) to locally advanced stage (stage IVA; FIGO's criteria) cervical cancer, all confirmed by biopsy from the cervical part of uterus. Our study aims to reveal the radiation certainty achievement by utilizing computed radiography. In this study, we used experimental method which emphasized Telecobalt^{60} pixel image display formed by the g-exposed image reader and converted to the computed radiography to present the verification.

The materials used in this research consisted of NPIC GWXJ80 Telecobalt^{60} and simulator device, computed radiography cassette and reader (CR-workstation), computed radiography (CR) cassette-seat holder, magnification software, manual beamed block, and quality control standard check lists for radiotherapy delivery.

STUDY DESIGN

This research is a quantitative study, with a randomized, double-blind, and consecutive sampling design. The study was conducted by observing and comparing the data of verified computed radiography (VerC) computed radiograph for Telecobalt^{60} compared to the non-verified computed radiography (nVerC) Telecobalt^{60} data. The sample was taken by random sampling method, using MS Excel software by selecting random numbers through integer randomization (RANDBETWEEN function).

ETHICAL APPROVAL AND PERMISSIONS

Permission to collect data from the patients was obtained in Department of Radiotherapy, dr. Moewardi Hospital, Surakarta, Central Java, Indonesia.

Table III. The non-paired T-test comparison data of the verified computed radiography (VerC) arm and non-verified computed radiography (nVerC) arm, without and with manual block use.

T-test	X+ Non Block	X- Non Block	DX Non Block	Y+ Non Block	Y- Non Block	DY Non Block	X+ Block	X- Block
Psig. Levene test	0.000	0.001	0.000	0.000	0.000	0.000	0.012	0.232
Psig. Equal variances not assumed	0.045	0.041	0.000	0.021	0.031	0.000	[assumed] 0.303	[assumed] 0.580
Mean difference	2.31	2.18	3.34	3.42	2.77	2.60	-1.31	0.65
95% - Confidence Interval	0.06 – 4.56	0.09 – 4.26	2.15 – 4.54	0.54 – 6.31	0.26 – 5.27	1.60 – 3.60	-3.83 – 1.21	-1.69 – 2.99

X+: The edge aspects of the right axis measurement, either in the computed radiography reader or simulator image scheme; X-: The edge aspects of the left axis measurement, either in the computed radiography reader or simulator image scheme; DX: The differences between the X+ and the X-; Y+: The edge aspects of the upper ordinate measurement, either in the computed radiography reader or simulator image scheme; Y-: The edge aspects of the lower ordinate measurement, either in the computed radiography reader or simulator image scheme; DY : The differences between the Y+ and the Y-; Non-block: without manual block use; Block: with manual block use.

Table IV. The paired T-test data comparison of the verified computed radiography (VerC) arm and non-verified computed radiography (nVerC) arm, without manual block use

Paired T-test	X+ Non Block	X- Non Block	Y+ Non Block	Y- Non Block
Psig. Equal variances not assumed	0.470	0.264	0.695	0.550
Mean difference	-0.296	-0.452	0.179	0.262
95% - Confidence Interval	-1.12 – 0.53	-1.26 – 0.36	-0.74 – 1.10	-0.62 – 1.15

X+: The edge aspects of the right axis measurement, either in the computed radiography reader or simulator image scheme; X-: The edge aspects of the left axis measurement, either in the computed radiography reader or simulator image scheme; Y+: The edge aspects of the upper ordinate measurement, either in the computed radiography reader or simulator image scheme; Y-: The edge aspects of the lower ordinate t measurement, either in the computed radiography reader or simulator image scheme; Non-block: In not using manual block.

STATISTICAL ANALYSIS

The collected image data of computed radiography was analyzed by using comparison independent t-test in the SPSS statistics software.

RESULTS

Study result consists of the characteristic, normality, and data comparison which were totally taken from the subjects of both study arms, which are verified computed radiography (VerC) arm and non-verified computed radiography (nVerC) arm, respectively 30 people, and there was no statistically missing data.

The characteristic data of the two study arms consisted of median, mean, modus, maximum and minimum value divergences of the computed radiography reader to the simulator image scheme in every edge aspects of measurements (X+, X-, DX, Y+, Y-, DY). Both VerC and nVerC arm were not using manual block. The data is presented in the following table below.

The data from table I reveals that there were significant differences in the statistical characteristic between the verified computed radiography arm and the non-verified computed radiography arm, such as the median, mean, modus, or maximum value divergences of the simulator image scheme between the 2 arms, in which all of the value divergences of the VerC arm on those characteristic were less than 7 mm with maximum value divergences ranges from 3.54 to 6.68 mm (the radiation certainty is considered tobe achieved when the tolerated shift to the divergence of simulator image scheme is less than 7 mm). On the opposite, some of the characteristic of the nVerC arm were 7 mm or more (especially in the edge aspects of X+, Y+, and Y-) with the maximum value divergences ranges from 8.91 to 19.47 mm (the radiation certainty is considererd not to be achieved when the tolerated shift to the divergence of simulator image scheme is more than 7 mm).

The data from table II reveals that the normality data between the verified computed radiography (VerC) arm and the non-verified computed radiography (nVerC) arm were similar (Psig. Kolmogorov Smirnov > 0.050), while the significance value was 0.20 (P>0.050). Therefore, the value divergences in all of the edge aspect of measurement (X+, X-, DX, Y+, Y-, DY) in the two study arms, either without manual block or with manual block use, can be applied to the non-paired/paired t-test.

Afterwards, the non-paired and paired T-test comparison data of the two study arms, either without manual block or with manual block use, include the analytical values

of Lavene and equal variances (either not assumed or assumed), mean difference, and 95%-confidence interval, which is presented in the following table below.

The data from table III reveals that without manual block use, the data variance between the verified computed radiography (VerC) arm and the non-verified computed radiography (nVerC) were not similar (Psig. Lavene < 0.050). However, in the non-paired t-test pattern, the variance similarity is not an absolute condition for applying non-paired t-test. This reveal a statistically significant differences between the two study arms in which the significance values range from 0.000 to 0.045 (P<0.050) and the mean differences range from 2.18 to 3.42 mm.

When the significance value is less than 0.050 (P<0.050), it can be concluded that there is a statistically significant differences between the two study arms, where the value divergences of all the edge aspect of measurement (X+, X-, DX, Y+, Y-, DY) in the VerC arm were less than the nVerC arm (without manual block use), with the 95% confidence interval range from 0.06 to 6.31.

The data from table III also reveals that with manual block use, the data variance between the VerC and the nVerC arm, were similar (Psig. Lavene > 0.050), which suggest no statistically significant differences between the two study arms in which the significance values range from 0.303 to 0.580 (P>0.050) and the mean differences range from -1.31 to 0.65 mm.

When the significance value is more than 0.050 (P>0.050), it can be concluded that there is no statistically significant differences between the two study arms, where the value divergences of two edge aspect of measurement (X+/X-) in the VerC arms were similar to the nVerC arm (with manual block use), with the 95%-confidence interval range from -3.83 to 2.99.

The data from table IV reveals that without manual block use, there were no statistically significant differences between the initial and post 20th radiation delivery with verified computed radiography, where the significance values range from 0.264 to 0.695 (P>0.050) and mean differences range from -0.452 to 0.262 mm.

When the significance value is more than 0.050 (P>0.050) it can be concluded that there is no statistically significant differences between the initial and post 20th radiation delivery with verified computed radiograph, where the diverged values in four edge aspect of measurement (X+, X-, Y+, Y-) in the initial VerC arms were similar to the post 20th radiation delivery with verified computed radiograph, and the 95%-confidence interval range from -1.26 to 1.15.

DISCUSSION

Cases of treated site errors is not unusual in radiation delivery. This errors can be reduced by using the VPI device to confirm the equality of the radiation site factors and the accuracy of the radiation target factors [11–15,17–19,21–23,26,39–43,48,49]. for each step in the radiotherapy process. Overview of the report: The report seeks to promote awareness and encourage quantification of uncertainties in order to promote safer and more effective patient treatments. The radiotherapy process and the radiobiological and clinical frameworks that define the need for accuracy are depicted. Factors that influence uncertainty are described for a range of techniques, technologies and systems. Methodologies for determining and combining uncertainties are presented, and strategies for reducing uncertainties through QA programs are suggested. The role of quality audits in providing international benchmarking of achievable accuracy and realistic action levels is also discussed. RECOMMENDATIONS The report concludes with nine general recommendations: (1 In order to reach an accurate dose delivery and achieve a more satisfactory outcome in radiotherapy, radiation delivery using tele-therapy device should be equipped by the portal imaging device for verification purpose [1,12–15,17–19,21–23,26,39,42,43].either as a curative or palliative form, and also recommended as one of the best possible treatments for every patient with cancer by the ISCRO-USA and WHO. There were few inventions of radiotherapy in the beginning of the 20th century especially for the cancer treatment of breast, stomach, skin, and nose in America, France, Austria, and Sweden. Those inventions in radiotherapy paved for the discovery of the two main techniques of radiotherapy delivery, namely teletherapy (cobalt60 or linac The absence of VPI device has become a limitation for Telecobalt^{60} radiation which may render in certainty and errors in radiation delivery, especially in determining radiation site equality or the accuracy of the radiation target [12–14,17–19,21,23,33,34,42–46,51]. { “id” : “ITEM-3”, “itemData” : { “DOI” : “10.1088/0031-9155/53/12/007”, “ISSN” : “0031-9155”, “PMID” : “18506074”, “abstract” : “In this study, we perform a scientific comparative analysis of using (60

The evidences presented in this quantitative study obtained from analysis result suggest that there are significant differences in the statistical characteristic between the verified computed radiography (VerC) arm and the non-verified computed radiography (nVerC) arm of this study. This comprise of the mean, median, modus, or maximum value divergences of the simulator image scheme between the two arms. All of the divergences value of those characteristic from the VerC arm were less than 7 mm and maximum value divergences range from 3.54 to 6.68 mm (radiation certainty is considered to be achieved when the shift toleration to the divergence of the simulator image scheme is less than 7 mm). On the other hand, some of the characteristic of the nVerC arm were 7 mm or more (particularly in the edge aspects of X+, Y+, and Y-), with maximum value divergences range from 8.91 to 19.47 mm (radiation certainty is considered not to be achieved when the shift toleration to the divergence of the simulator image scheme is more than 7 mm). The significance value is less than 0.050 (P<0.050) which imply that there are statistically significant differences between the two study arms, where the value divergences in all of the edge aspect of measurement (i.e. X+, X-, DX, Y+, Y-, DY) in the VerC arms are less than the nVerC arm (without manual block use), with the 95%-confidence interval range from 0.06 to

6.31. Hence, the radiation certainty is better obtained by utilizing computed radiography as a combined verification device to Telecobalt^{60}.

However, the computed radiography device will also need some procedures and devices as parts of its computed radiography-workstation, e.g. portal film cassette or computer. These are quite an extra expence for maintaining budget. Other problem which may rise is the scarcity of spare parts with good quality.

CONCLUSIONS

Our study conclude that the radiation certainty is significantly improving with the utilization of computed radiography in order to enhance the performance of Telecobalt^{60} device. The radiation certainty is achieved through prior improvement on the influencing factors of the Telecobalt^{60} device itself, such as the radiation site equality and the radiation target accuracy. These factors should always be maintained by performing a routine and continuous radiotherapy confirmation and verification process starting from regular filling of check lists on daily, weekly and monthly basis, check and re- check process followed by supervisor validation and proper radiation delivery assigment.

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The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

PREDICTING THE OCCURRENCE OF PRIMARY OPEN-ANGLE GLAUCOMA DEPENDING ON THE GENETIC POLYMORPHISM ENDOTHELIAL NO SYNTHASE (*NOS3*) GENE

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ABSTRACT

The aim: To develop the model for predicting primary open – angle glaucoma (POAG) depending on the presence of the genetic polymorphism in the endothelial NO-synthase (*NOS3*) gene.

Materials and methods: The results of genotyping 153 patients (153 eyes) with POAG are included in this investigation. 47 patients were in the control group. Their age was 65,0±13,1 years, duration of disease – 4,9±5,3 years. The polymerase chain reaction was carried out in the patients' blood in the real time mode (Gene Amp® PCR System 7500 amplifier; USA) with the help of the TaqMan Mutation Detection Assays Life-Technology test system (USA). The program Statistica 10 (StatSoft, Inc., USA) was used for mathematical testing of the obtained results.

Results: The regression analysis confirmed the effect of rs1799983 and rs2070744 polymorphisms of the *NOS3* gene on the development of POAG. Calculating their specific gravity based on the degree of the impact on the probability of developing the disease showed that rs2070744 – 72.2% had the greater impact than rs1799983 – 38.5%. The regression model of POAG risk depending on the genotypes of the *NOS3* gene rs1799983 and rs2070744 polymorphisms was constructed with the satisfactory quality of mathematical prediction (-2log=202.59; χ^2=28.91; P<0.001). The value of probability of developing POAG exceeded the limit value (Cut-off=0.8), respectively, OR 4.39 (95% CI 1.00-19.30; P=0.048) and OR 14.15 (95% CI 1.88-106.28; P<0.001) in carriers of the rs1799983 and rs2070744 *GT-CC* and *TT-CC* haplotypes.

Conclusions: The results of the study proved the importance of risk genotypes (*TT* rs1799983 and *CC* rs 2070744) for the development of POAG in patients from the Ukrainian population. It has been shown that the significant increase in the risk of POAG exists for carriers of the *GT-CC* and *TT-CC* haplotypes.

KEY WORDS: primary open-angle glaucoma, *NOS3*, rs1799983 (*G894T*, Glu298Asp), rs2070744 (*T-786C*)

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INTRODUCTION

Primary open-angle glaucoma (POAG) is the most common type of glaucoma. It is characterized by asymptomatic development with the gradual decrease in peripheral vision [1]. The reason for this is the development of glaucoma optic neuropathy (GON) with damage to the optic nerve, inefficiency of the eye's drainage system with fluid accumulation and increased intraocular pressure [2].

Endothelial dysfunction plays the important role in the development of GON [3]. According to modern concepts, the vascular endothelium has the number of important functions both in normal and pathological conditions through the regulation of the vascular tone, microvascular permeability, angiogenesis, activity of the dorsal and proteolytic systems, mechanisms of cell adhesion and the development of inflammatory reactions [4, 5]. Among many regulatory factors, the leading role is played by nitric oxide (NO), its formation occurs with the involvement of the universal membrane enzyme – endothelial NO-synthase (eNOS; NOS3) [6].

The polymorphic condition of this gene can be the risk factor of the POAG development, according to the polymorphisms rs1799983 (*G894T,* Glu298Asp) and rs2070744 (*T-786C*) of the *NOS3* gene [7, 10].

The rs1799983 polymorphism (chr7:150999023, GRCh38.p12) is the result of the missense mutation *89T>A,G,* the replacement of aspartate with glutamine at position 298 (Glu298Asp) is the sequent, it leads to the decrease in eNOS activity [8-10].

The rs2070744 (*T-786C*) polymorphism (7q36.1; 7:150992991; GRCh38) is localized in the intron of the *NOS3* gene [10] and it is associated with the increased risk of POAG [10-12].

THE AIM

The aim of the work was to develop the model for predicting primary open – angle glaucoma (POAG) depending on the presence of the genetic polymorphism in the endothelial NO- synthase (*NOS3*) gene.

MATERIALS AND METHODS

The results of genotyping in 153 patients (153 eyes) with the established diagnosis of POAG have been included in this investigation. The control group included 47 patients without such diagnosis. There were 57 men (37.25%) and

Table I. Correspondence of categorical variables to indicator values which were used in regression analysis

Indicator	Variable name	Categorical value	Indicator value
rs1799983 NOS3 gene	1NOS3	GG	101
		GT	102
		TT	103
rs2070744 NOS3 gene	2NOS3	TT	101
		TC	102
		CC	103
Sex	Sex	male	101
		female	102

Table II. Independent variables included in the POAG probability prediction model and their statistical characteristics

Independent variables	β	±SE	95 % BI	Wald	p
Constant	-418,700	121,564	-(643,542-183,391)	8,73	0,003
1NOS3	1,568	0,647	0,300-2,837	5,87	0,015
2NOS3	2,527	0,680	1,195-3,859	13,82	<0,001
Sex	0,443	0,244	-0,661-0,866	3,31	0,069

Notes: β-regression coefficient; SE-standard error of the regression coefficient; 95% BI – 95% probable interval for the regression coefficient; Wald – Wald's statistics; P – significance of the discrepancy with the null hypothesis.

Table III. Classification of predicted and actual data by belonging to the categories of probability and risk assessment POAG

Cut-off	Actual data		Prediction (n)		Indicators (%)		
	Категорія	n	POAG+	POAG–	Sensitivity	Specificity	Accuracy
0,5	POAG +	153	140	13	91,5	6,38	71,5
	POAG –	47	44	3			
0,8	POAG +	153	111	42	72,5	70,2	72,0
	POAG –	47	14	33			

Note: Cut – off=0.5 is the standard cut-off point of P(POAG) values, calculated by Formula 2; Cut – off=0.8 is the optimal cut-off point.

96 women (62.75%) in the POAG group. There were 22 men (46.81%) and 25 women (53.19%) in the control group. According to the stages of POAG, patients were distributed as follows: stage I – 11.76%, stage II – 17.65%, stage III – 52.94% and stage IV – 17.65%.

According to the generally accepted protocol for examining patients with POAG [13], complaints and medical history were carefully collected from each patient, visometry, Hamphrey's perimetry, refractometry, pneumotonometry, biomicroscopy, gonioscopy, ophthalmoscopy, and optical coherence tomography (OCT) were performed.

Molecular genetic testing was performed in whole venous blood samples obtained from patients in the amount of three milliliters, in accordance with their permission and in compliance with the necessary biotic standards. The genotypes of the NOS3 gene rs1799983 (*G894T*, Glu298Asp) and rs2070744 (*T-786C*) polymorphisms were determined by real-time polymerase chain reaction in the automatic Gene Amp® PCR system 7500 amplifier ("Applied Biosystems", USA). At the first stage of the investigation, genomic DNA was isolated from whole venous blood using Purelink® Genomic DNA Kit For Purification of Genomic DNA ("INVITROGEN"; USA) reagents. Genetic analysis was performed using the unified test system TaqMan Mutation Detection Assays Life-Technology (USA).

Multi-factor logistic regression technology was used to construct the model for predicting the risk of the POAG development [14]. The analysis included data from 153 patients with POAG, united by the sign of the disease's presence and data from 47 patients in the control group. The POAG presence sign was used as the resulting variable regression. If the patient had POAG, the variable was assigned the indicator value of 1, and if it was absent – 0. The last value is taken as the reference value.

Stable indicators were used as factor signs in the construction of the model, they remained unchanged throughout the patient's life, and also they do not change with the progression of the disease. These signs included categorical indicators of gender and genotyping data for *NOS3* gene polymorphisms; they were converted into indicator values.

The selection of statistically significant independent variables of regression equations (predictors) was carried out in the step-by-step exclusion mode. Regression coefficients (β), their standard errors (SE), and the significance of their

Table IV. Distribution patients with POAG and the control group depending on the combination of genotypes of NOS3 gene polymorphisms rs1799983 and rs2070744

Haplotype(rs1799983-rs2070744)	$P_{(POAG)}$	POAG (n=153)	Control (n=47)	$P_{(EFC)}$	OR	95 % BI
GG-CC	0,487	0	7 (14,89%)	-	-	-
GG-CT	0,070	21 (13,72%)	10 (21,28%)	0,249	0,59	0,25-1,36
GG-TT	0,006	24 (15,69%)	8 (17,02%)	0,822	0,91	0,38-2,18
GT-CC	0,820	25 (16,34%)	2 (4,25%)	0,048	4,39	1,00-19,30
GT-CT	0,267	47 (30,72%)	15 (31,91%)	0,859	0,95	0,47-1,91
GT-TT	0,028	0	0	-	-	-
TT-CC	0,956	36 (23,53%)	1 (2,13%)	<0,001	14,15	1,88-106,28
TT-CT	0,636	0	4 (8,51%)	-		
TT-TT	0,122	0	0	-		

Note: P(POAG) is the probability of developing POAG calculated by Formula 2; P(EFC) is the statistical significance of genotype distribution comparisons using the exact Fisher's method.

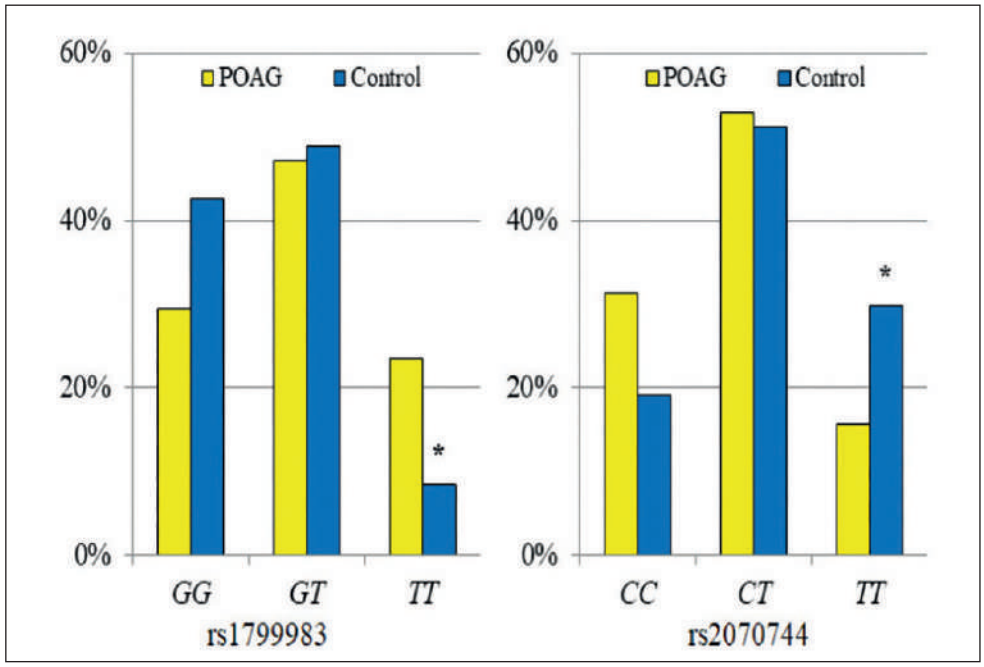

Fig. 1. Frequency of genotypes of rs1799983 and rs2070744 polymorphisms in patients with POAG and in the control group;

differences from the null hypothesis were calculated using the Wald's statistics criterion (Wald) for logistic models.

The adequacy of logistics models was judged by receiver operating characteristic (ROC), and the area under the ROC – AUC (area under curve) curve was calculated. The model was considered adequate with the statistically significant difference AUC from 0.5. Wald's statistics, maximum likelihood coefficients (- 2log), xi-square ($\chi^{2)}$, and the Hosmer-Lemeshev's consent criterion were calculated for logistic models.

The percentage of influence of the selected regression indicators on the dependent variable was calculated using the formula:

$$d_k = \frac{\beta_k^2 * 100\%}{\sum_{i=1}^{n} \beta_i^2} \quad (1),$$

where d_k is the percentage of influence of this indicator; β_k – β is the coefficient of this independent variable of the regression equation; the denominator is the sum of the squares of the β-coefficients of all independent variables.

The degree of association of haplotypes with POAG was determined by calculating the odds ratio (OR) and the 95% certain interval (95% CI). In all cases of statistical estimation, the value of P<0.05 was considered probable.

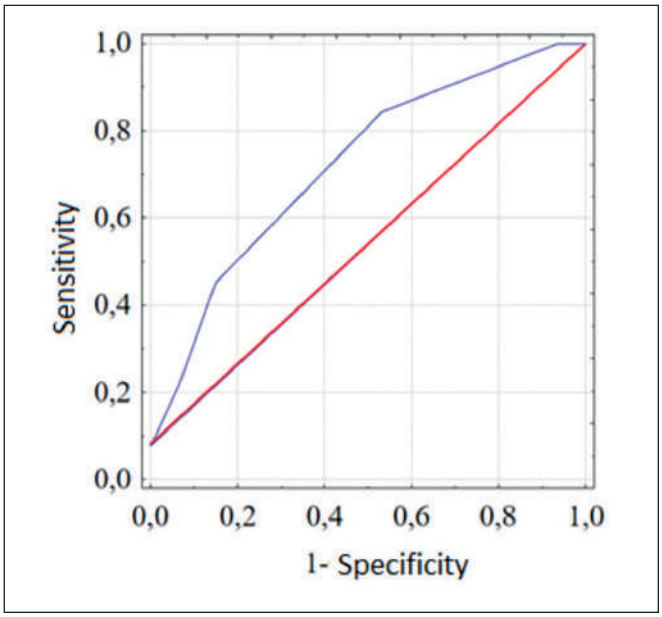

Fig. 2. ROC-diagram of the model for predicting the probability of POAG development

RESULTS AND DISCUSSION

The comparative analysis of the distribution of genotype frequency in patients with POAG and in the control group (fig. 1) showed that the certain difference was determined for both polymorphisms.

rs1799983 showed the decrease in the frequency of the hereditary homozygous *GG* genotype ((p_{Fet}=0,110)) and the increase in the frequency of the minor *TT* genotype (p_{Fet}=0,110) in patients with POAG compared to the control group. The Hardy-Weinberg's test for cases and controls showed random inheritance patterns in both POAG patients and the control group (χ^2=0,472; P=0,790 i χ^2=0,538; P=0,764, respectively).

rs2070744, the increase in the frequency of the hereditary homozygous *CC* genotype (p_{Fet} =0.139) was in the patients with POAG and the decrease in the frequency of the minor *TT* genotype (p_{Fet}=0,049) compared to the control group. The Hardy-Weinberg's test showed random inheritance in patients with POAG and in the control group (χ^2=1,119; P=0,571 i χ^2=0,051; P=0,975, respectively).

The statistical significance of independent variables included in the regression analysis is shown in Table II.

Based on the results of the analysis of Wald's statistics and interval characteristics of β-coefficients, the discrepancy between which and the null hypothesis was statistically significant, the following variables were selected as predictors for the regression model: the rs1799983 polymorphism of the *NOS3* gene (indicator value 1NOS3) and the rs2070744 polymorphism of the *NOS3* gene (indicator value 2NOS3). The following variables were selected as predictors for the regression model: rs1799983 polymorphism of the *NOS3* gene (indicator value 1NOS3) and rs2070744 polymorphism of the *NOS3* gene (indicator value 2NOS3). The following formula can be used as the mathematical expression of the developed model:

$$P(poag) = 1/(1 + e^{-(-418{,}7+1{,}568\times 1NOS3+2{,}527\times 2NOS3)}) \quad (2),$$

where: $P_{(POAG)}$) – probability of POAG developing;

1NOS3 – indicator value for the rs1799983 polymorphism of the *NOS3* gene:

GG = 101; *GT* = 102; *TT* = 103;

2NOS3 – indicator value for the rs2070744 polymorphism of the *NOS3* gene:

TT = 101; *TC* = 102; *CC* = 103.

Calculating the specific weight of predictors based on the degree of their influence on the dependent variable (Formula 1) allowed them to be arranged in the series in descending order as follows: the greatest influence was exerted by the genotype "2NOS3" (72.2%) and the smallest – by the genotype "1NOS3" (38.5%). At the same time, the value of the resulting regression variable was higher in patients with risk alleles in their genotypes: for rs2070744 of the *NOS3* allele *C* and for rs1799983 of the *NOS3* allele *T*.

Indicators that characterize the overall efficiency of the model based on the correspondence of the calculated data to the actual ones indicated the satisfactory quality of the mathematical forecast: -2log (Likelihood)=202.59; χ^2=28.91; P<0.001; Hosmer-Lemeshev's consent criterion: χ^2=12,17; p=0,144.

To evaluate the operational properties of the developed model, the ROC diagram was constructed and analyzed (fig. 2). In the developed mathematical model, the area under the ROC diagram was AUC=0.733±0.039 (95% CI 0.691-0.818; P<0.001), which satisfactorily characterized the quality of the predictive model.

Taking into account the satisfactory operational characteristics of the model as the whole, the attempt was made to find the optimal cut-off point for $P_{(POAG)}$values (Formula 2), the results of the forecast classification will be the best for this model. The search results are displayed on the frequency-probability diagram (fig. 3).

The continuous line shows the sensitivity diagram, the dotted line shows the specificity diagram, and the stipple line shows the accuracy diagram. Frequencies are plotted along the vertical axis, and probability values are plotted along the horizontal axis. The vertical line marked Cut-off=0.5 corresponds to the standard cut-off point, and the line marked Cut-off=0.8 corresponds to the optimal cut-off point.

The results of evaluating the discriminative model's ability are presented in the classification table (table III).

Analyzing the classification results at the standard point of separation of positive and negative prognostic results (Cut-off=0.5), it was found that the number of patients with the positive prognosis for the development of POAG coincided with the real condition in 91.5%. At the same time, the unmistakable negative prognosis for the absence of POAG was possible only in 6.38% of cases. This, even with the relatively high overall accuracy of the prognosis (71.5%; OR 0.73; 95% CI 0.20-2.69; P=0.641), cannot meet clinical requirements.

Analysis of the frequency-probability diagram allowed to determine the optimal threshold for cutting off positive and negative results of the prognosis Cut-off=0.8. At the

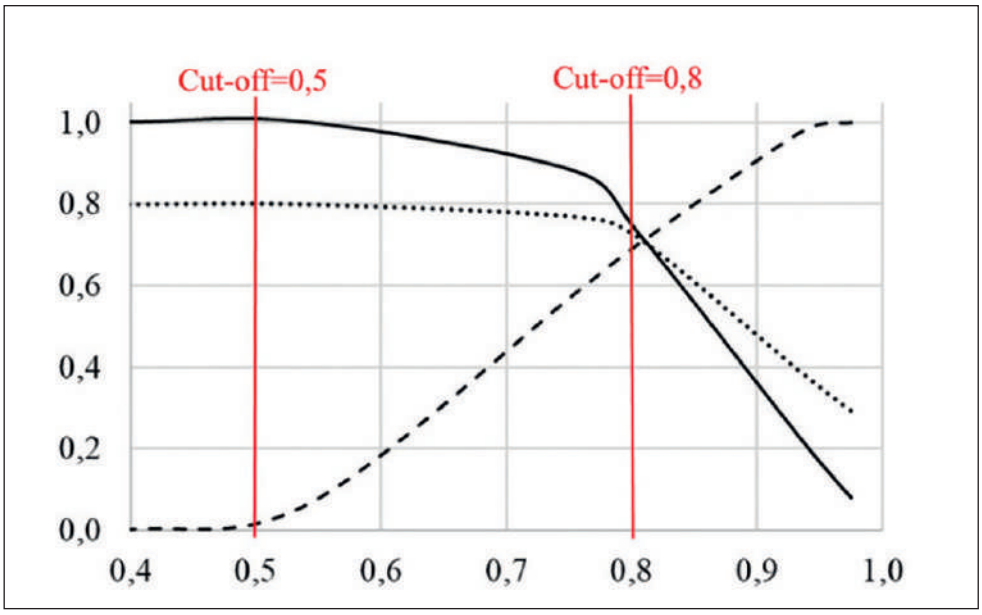

Fig. 3. Diagram of the dependence of sensitivity, specificity and accuracy on the probability of POAG development

same time, the detection of patients with the positive prognosis of POAG was less (72.5%) than the actual value, but the correct negative prognosis of POAG was increased to 70.2%. The overall accuracy of this model was 72.0% (OR 6.23; CI 3.03-12.78; P<0.001), which indicated satisfactory discriminative properties of the model.

Further, the distribution of patients with POAG was analyzed depending on the haplotype of the *NOS3* gene polymorphism rs1799983 and rs2070744 and the calculated probability of developing risk POAG (Formula 2) compared to the control group (table IV).

The actual distribution of patients with POAG and the control group confirmed the calculated data on the limit value of the developing POAG risk probability (Cut-off=0.8). In the *GT-CC* and *TT-CC* haplotypes the probability of developing POAG (Formula 2) exceeded the limit value (Cut-off=0.8). At the same time, the *GT-CC* haplotype increased the POAG risk developing with the value of OR 4.39 (95% CI 1.00-19.30; P=0.048). and the *TT-CC* haplotype increased the POAG risk with the value of OR 14.15 (95% CI 1.88-106.28; P<0.001) in the case-control study (90 patients with POAG and 127 controls).

The *T-786C* and Glu298Asp polymorphisms of the e*NOS* gene were shown to be associated with POAG [15]. Thus, the *T-786C* polymorphism was the risk factor among women (OR 2.28; 95% CI 1.11-4.70; P=0.024) and in patients over 52 years of age (OR 2.11; 95% CI 0.98-4.55; P=0.055).

CG T-786C and Glu298Asp haplotypes showed the borderline association with the POAG risk in the overall analysis (OR 1.76; 95% CI 0.98-3.14; P=0.055). among women (OR 2.02; 95% CI 0.98-4.16; P=0.052). and for the patients over 52 years of age (OR 3.48; 95% CI 1.54-7.84; P=0.002). We got the similar result – in the general analysis. the *GT-CC* haplotype (see table.4) had the positive association with POAG (OR 4.39; 95% CI 1.00-19.30; P=0.048).

It was observed also the association of the minor *TT* variant of the Glu298Asp *NOS*3 polymorphism with POAG in women in the investigation by J.H. Kang et al. (2011) [16]. In this regard, we separately analyzed the distribution of genotypes of this polymorphism by gender, but no statistical significance was found (P=0.195).

However, it was found that women had the association of allelic polymorphism with POAG: for the *G* allele OR = 0.50; CI 0.25-0.99; for the *T* allele OR = 2.00; CI 1.01-3.95 (P=0.043) when it was stratified by gender. This indicated the greater significance of the rs1799983 polymorphism for the POAG development in women compared to men. In our opinion, this observation should be confirmed in the examination of larger groups of patients.

By the way, there are investigations where the direct relationship between *NOS3* gene polymorphisms and POAG has not been established [9, 17, 18]. But the same time, it should be noted that the authors found certain associations with POAG phenotype: with low blood pressure [18] or with hemorrhages in the optic disc in normotensive glaucoma patients' groups [9].

According to these results, it is necessary to continue research in different populations and establish the link between *NOS*3 gene polymorphisms not only with the presence of the disease, but also with its manifestations and the degree of progression.

According to the case-control study (173 patients and 171 controls), the rs1799983 polymorphism was significantly associated with POAG especially in men (for the *T* allele: OR 1.77; 95% CI 1.07-2.94; P=0.025) [19]. At the same time, male-carriers of the *CT* haplotype rs2070744 and rs1799983 had the significantly increased risk of POAG (OR 2.60; 95% CI 1.16-5.82; P=0.016). In our study carriers of this haplotype had the significantly increased POAG risk developing (OR 14.15; 95% CI 1.88-106.28; P<0.001).

According to the meta-analysis the *TT* rs2070744 and *GG* rs1799983 genotypes are associated with the reduced POAG risk; it is more common in women [20]. Our investigation also showed the protective role of the *GG-TT* haplotype rs1799983 and rs2070744, which had the lowest probability of POAG developing ($P_{(POAG)}$=0.006; see table. 4).

Examining POAG patients (76 men and 84 women aged 41 to 75 years) showed the disease-related association for the *CC* genotype rs2070744 (OR 2.54; 95% CI 1.26-5.13; P=0.007), it was preserved for women when it was stratified by gender [11].

Unlike other investigations, the association of POAG with rs1799983 was not shown [11]. However, the lower total nitrate/nitrite (NOx) content was found in the blood plasma of POAG patients confirming the importance of endothelial dysfunction for the development of the disease [11].

The significance of the rs2070744 and rs1799983 polymorphisms of the *NOS*3 gene for POAG development was confirmed by meta-analysis in 2021. It covered the SID, MagIran, IranMedex, IranDoc, ScienceDirect, Embase, Scopus, PubMed, Web of Science and Google Scholar search engines with no time limit until May 2020 [10].

According to the results of 16 studies (1631 patients with POAG and 2405 controls for rs2070744 and 1456 patients and 2240 controls for rs1799983) the increased risk of POAG was found in carriers of *CC* genotypes rs2070744 (total OR 1.14) and *TT* rs1799983 (total OR 1.31). It fully coincides with the results of our investigation.

Thus, the results of the investigation are consistent with ones obtained for other patient populations and prove the importance of risk genotypes (*TT* rs1799983 and *CC* rs2070744) for the POAG development. In addition, we have shown that there is the significant increase in the POAG risk for carriers of the *GT-CC* haplotypes (OR 4.39) and *TT-CC* (OR 14.15).

CONCLUSIONS

1. Regression analysis confirmed the influence of polymorphisms rs1799983 and rs2070744 of the *NOS3* gene on the development of POAG in patients from the Ukrainian population. At the same time, rs2070744 had a greater influence on the probability of the disease developing.
2. The possibility of constructing an adequate regression model of POAG genetic risk proved the presence of rs1799983 and rs2070744 association with the disease (P<0.001).
3. Haplotypes rs1799983-rs2070744 *GT-CC* and *TT-CC* carriers had the highest probability of POAG developing, which may be the basis for the detection of genetic predisposition in the prehospital stage.

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ORIGINAL ARTICLE

POTENTIAL PROTECTIVE EFFECTS OF NIMODIPINE FROM CEREBRAI ISCHEMIA REPERFUSION INJURY IN RATS

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ABSTRACT

The aim: To see whether nimodipine had neuroprotective effects in cerebral ischemia/reperfusion injury.

Materials and Methods: A total of 28 adult male Sprauge-dawley rats weighting 200-300 g were distributed randomly into 4 groups (7 animals in each group): sham (neck dissection without bilateral common carotid artery occlusion), control (bilateral common carotid artery occlusion for 30 minutes and reperfusion for 1 hour), vehicle (7 days of daily carboxymethylcellulose by oral gavage followed by bilateral carotid artery occlusion and reperfusion), and nimodipine-treated rats (7 days of 3 mg/kg/day of oral Azelnidipine pretreatment then bilateral common carotid artery occlusion and reperfusion). Besides assessment of histological changes and brain infarct volume, the brain tissues were sectioned to estimate NF-κB p65, IL-6, IL-10, TNF-α, ICAM-1 and total anti-oxidant capacity.

Results: Cerebral NF-κB p65, IL-6, IL-10, TNF-α, ICAM-1, in addition to cerebral infarct size were markedly increased in control and vehicle related to sham rats, while total anti-oxidant capacity was considerably decreased. Treatment with nimodipine resulted in remarkable increment of total anti-oxidant capacity, while NF-κB p65, IL-6, TNF-α, and ICAM-1 showed great reduction. Cerebral IL-10 levels didn't change by nimodipine treatment. Histologically, control and vehicle rats showed severe brain ischemic changes which is dramatically reduced by nimodipine treatment.

Conclusions: Our study results revealed that nimodipine can greatly decrease cerebral infarct size and reduce histological ischemic injury in male rats subjected to cerebral ischemia/reperfusion. The neuroprotective actions of nimodipine possibly originated from its anti-inflammatory and antioxidative effects. Nimodipine protection was unrelated to IL-10.

KEY WORDS: nimodipine, CI/RI, NF-κB p65, IL-6, IL-10, TNF-α, ICAM-1, T-AOC

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INTRODUCTION

Ischemic stroke is defined as irreversible harm to brain, spinal cord, or retinal cells caused by ischemia [1]. The worldwide burden of stroke is rising considerably as the world's population grows in size and ages. The brain is highly vulnerable to ischemia. The prominent vulnerability of brain to ischemic damage reflects its high metabolic rate and its limited capacity to store glucose [2]. Nimodipine is a 2nd generation 1,4-dihydropyridine L-type calcium channel blocker [3]. Since nimodipine being the most lipophilic of all calcium channel blockers, it accumulates in cerebral arteries resulted in significant vasodilation and increased blood flow, thereby reducing brain ischemia [4].

THE AIM

The aim of this research was to see whether nimodipine had neuroprotective effects in cerebral ischemia/reperfusion injury.

MATERIALS AND METHODS

Cerebral ischemia/reperfusion injury refers to a time when the brain's blood flow is disrupted, followed by a period when perfusion is restored, while reperfusion after cerebral ischemia can restore brain flow and salvage reversibly damaged tissue, it can paradoxically precipitate more damage and necrosis. Till now, no drug has been confirmed for post-ischemic cerebral injury treatment and this comprises a great challenge. The work was carried on the Department of Pharmacology at the Kufa College of Medicine.

Twenty-eight adult male Sprague-Dawley rats weighting 200-300 g were purchased from the animal house at Kufa college of science and they were housed in the same place at 25±1°C temperature and 60-65% humidity lights on a 12-hour light/dark cycle. The rats were allowed free food and water. The study has been documented by the institutional animal care and use committee of Kufa University and the investigations were done in accordance with the llaboratory animal guide care. the rats were distributed randomly into 4 groups (7 rats in each group):

- Group 1 (sham group): Rats had been exposed to same surgical procedures, but without bilaterall common carotid artery occlusion (BCCAO);
- Group 2 (control group): Rats had been subjected to

surgery with BCCAO for 30 minutes and then reperfusion for 1 hour but with no drug;
- Group 3 (vehicle group): the rats were received oral 0.3% carboxymethyll cellulose (CMC) daily for 7 days prior to surgery. Then BCCAO for 30 minutes followed by reperfusion for 1 hour;
- Group 4 (azelnidipine-treated group): Rats were received 3 mg/kg/day azelnidipine by gastric gavage for 7 days prior to surgery, then BCCAO for 30 minutes followed by reperfusion for 1 hour.

INDUCTION OF GLOBAL CEREBRAL ISCHEMIA

BCCAO cause a diffuse ischemia model. A median incision was made in neck, and both common carotid arteries were detached from vagall nerves and blocked for 30 minutes to produce ischemia under general anesthesia with ketamine 100 mg/kg and xylazine 10 mg/kg intraperitoneally. The clamps were withdrawn 1 hour of reperfusion was allowed.

SAMPLES PREPARATION

At experiment's completion, the rats were sacrificed and the brains were isolated and preserved in ice as fast as possible and divided coronally into 3 slices for further ELISA assessment, histopathology and immunohistochemistry analysis, and 2,3,5-Triphenyltetrazolium chloride (TTC) stain.

PREPARATION OF BRAIN TISSUE SAMPLES FOR ELISA

The homogenization started by adding brain sample, in a ratio of 1:10 (w/v), to a mixture of ice-cold 0.1M PBS, containing 1x protease inhibitor cocktail and 0.2% Triton X-100 [5]. The brain samples tubes were immersed in ice and homogenized by a sonicator, then was centrifuged at 14000 xg for 20 minutes at 4°C. The supernatants were aspirated and their levels of interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor-α (TNF-α), intercellular adhesion molecule-1 (ICAM-1), and total anti-oxidant capacity (T-AOC) were estimated according to ELISA kits protocols.

PREPARATION OF BRAIN TISSUE SAMPLES FOR HISTOPATHOLOGY

The brain samples were fixed in 10% formalin, engaged with automated tissue processor, and stained with hematoxylin and eosin. The histological changes were scored as follows [6]:
- 0 (normal): normal morphology.
- 1 (mild): slight edema or dark eosinophilic neurons.
- 2 (moderate): at least two small hemorrhages.
- 3 (severe): localized necrosis.

Cerebral levels of nuclear factor kappa B (NF-κB) were analyzed by Dako Envision immunohistochemistry technique [7]. Cerebral infarct volume was measured by immersion TTC stain method [8].

PREPARATION OF NIMODIPINE

Nimodipine doses (purchased from ChemScene/USA) were prepared immediately prior to use by dissolving in 0.3% CMC and was given through oral gavage in a dose of 5 mg/kg/day.

STATISTICAL ANALYSIS

Data were analyzed by SPSS version 26. Parametric data were analyzed by using of ANOVA test followed by LSD post hoc test. Non-parametric data were assessed by using Kruskal-Wallis followed by post hoc tests. In all tests, p-value<0.05 was counted statistically significant.

RESULTS

NIMODIPINE EFFECTS ON BRAIN IL-6, IL-10, TNF-A, ICAM-1, AND T-AOC ESTIMATED BY ELISA

In the current study, we observed that the control and vehicle groups had remarkably elevated ($p<0.05$) cerebral IL-6, TNF-α, and ICAM-1related to sham rats. This increment was considerably reduced ($p<0.05$) in nimodipine-treated rats (Fig. 1-3).

Meanwhile, cerebral T-AOC levels were dramatically reduced ($p<0.05$) in the control and vehicle groups compared to the sham groups. These levels were significantly upregulated ($p<0.05$) by nimodipine treatment, compared to the control and vehicle groups (Fig. 4).

Cerebral IL-10 levels were greatly elevated ($p<0.05$) in the control and vehicle groups compared to the sham rats. This elevation didn't change by nimodipine treatment (Fig. 5).

EFFECTS OF NIMODIPINE ON CEREBRAL NF-*KB* P65 ESTIMATED BY IMMUNOHISTOCHEMISTRY

In our work, NF-κB p65 expression was remarkably higher ($p<0.05$) in the control and vehicle groups compared to the sham rats. This marked elevation was reduced dramatically ($p<0.05$) by nimodipine treatment (Fig. 6-7).

EFFECTS OF NIMODIPINE ON CEREBRAL HISTOPATHOLOGY

Histological assessment showed normal morphology in sham group. The control and vehicle groups showed markedly severe ($p<0.05$) ischemic injury. Nimodipine pretreated rats showed great amelioration ($p<0.05$) in these changes (Fig. 8-9).

EFFECTS OF NIMODIPINE ON CEREBRAL INFARCT SIZE

Our work showed that there was a significant increment ($p<0.05$) in the infarction size percentage in control and vehicle compared to sham groups. This percentage was markedly ameliorated ($p<0.05$) in nimodipine-pretreated group (Fig. 10-11).

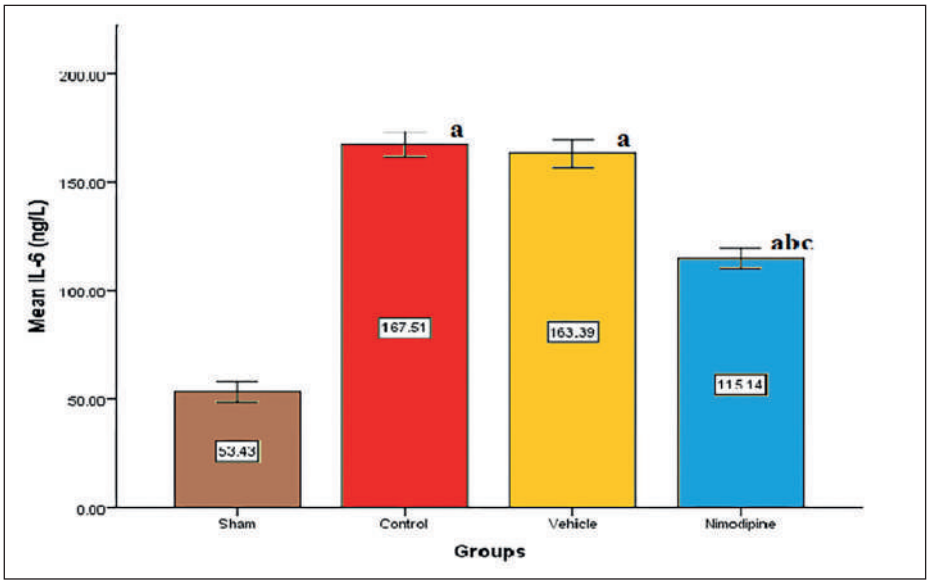

Fig. 1. The mean of cerebral IL-6 (ng/L) of the four groups (n=7), value consider significant difference at p< 0.05, significant difference when compared to: (a) sham group, (b) control group, (c) vehicle group.

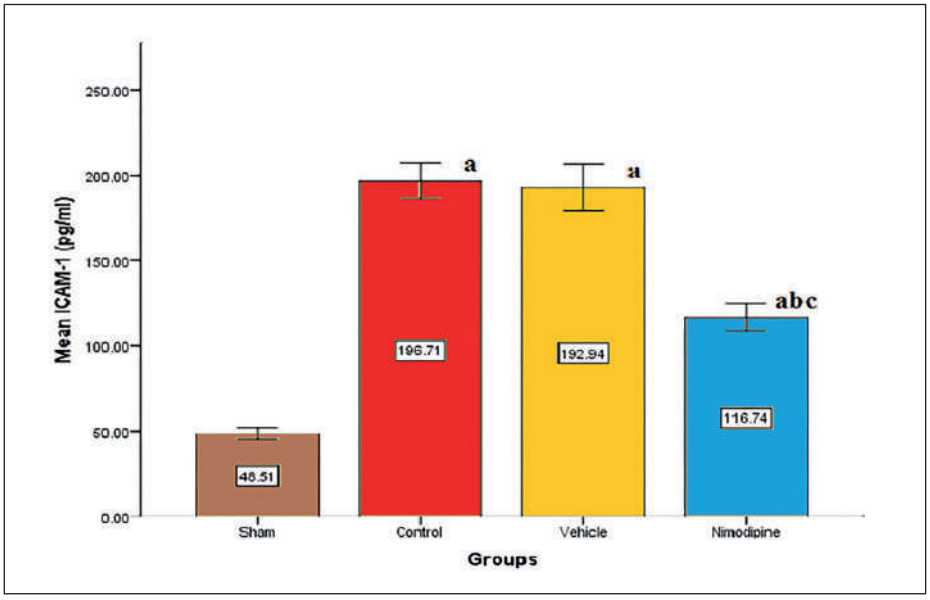

Fig. 2. The mean of cerebral TNF-α (ng/L) of the four groups (n=7), value consider significant difference at p< 0.05, significant difference when compared to: (a) sham group, (b) control group, (c) vehicle group.

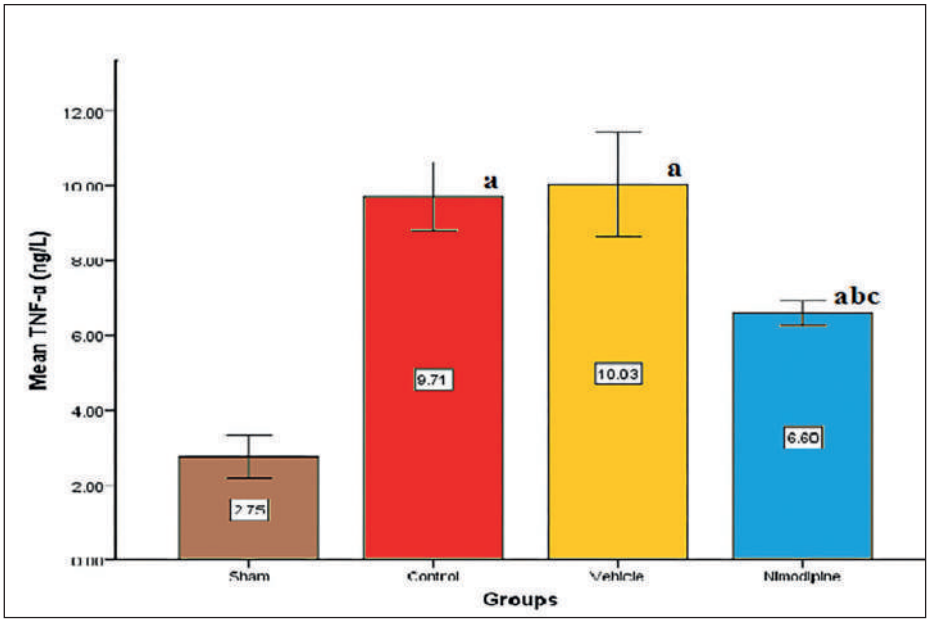

Fig. 3. The mean of cerebral ICAM-1 (pg/ml) of the four groups (n=7), value consider significant difference at p< 0.05, significant difference when compared to: (a) sham group, (b) control group, (c) vehicle group.

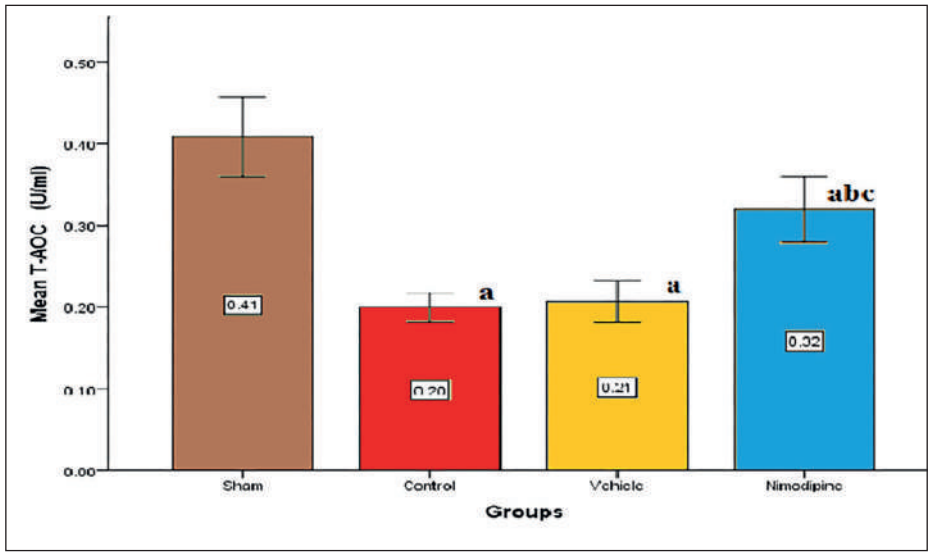

Fig. 4. The mean of cerebral T-AOC (U/ml) of the four groups (n=7), value consider significant difference at $p< 0.05$, significant difference when compared to: (a) sham group, (b) control group, (c) vehicle group.

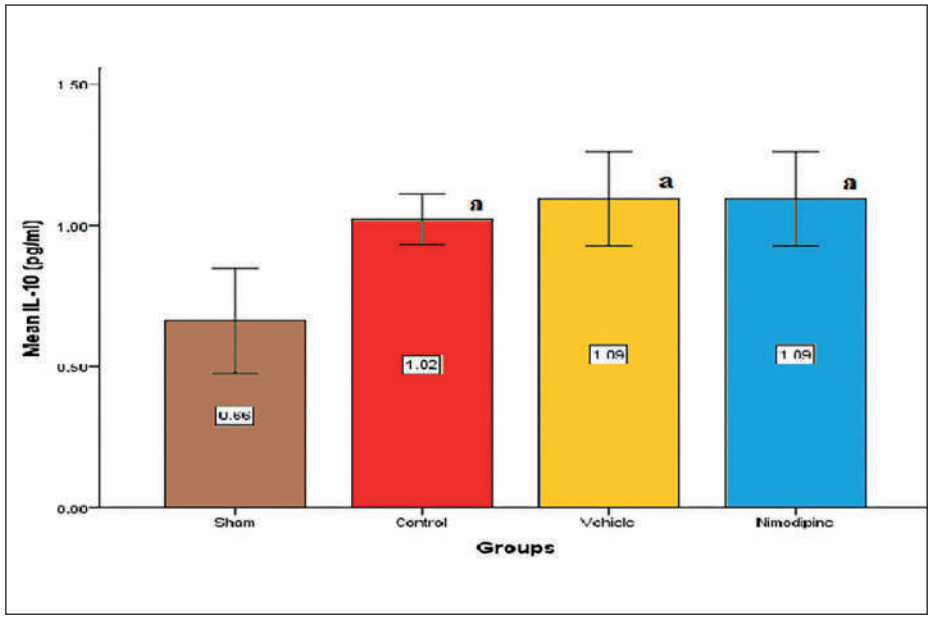

Fig. 5. The mean of cerebral IL-10 (pg/ml) of the four groups (n=7), value consider significant difference at $p< 0.05$. Significant difference when compared to: (a) sham group, (b) control group, (c) vehicle group.

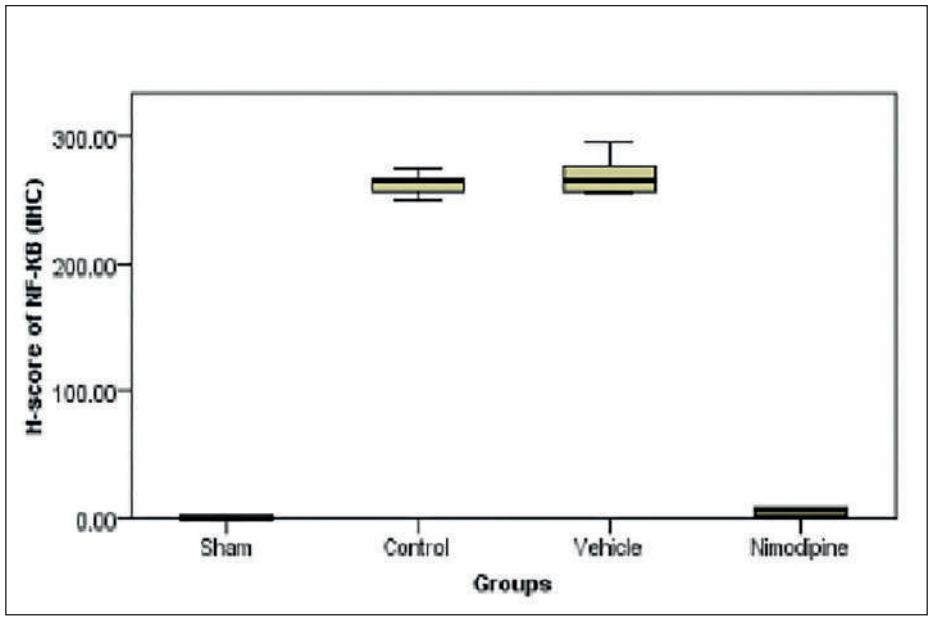

Fig. 6. Kruskal-Wallis box plot showing medians of cerebral NF-κB expression among the four groups, significant difference ($p< 0.05$) between sham vs. control and vehicle groups. Significant difference ($p< 0.05$) between control and vehicle versus nimodipine-treated groups.

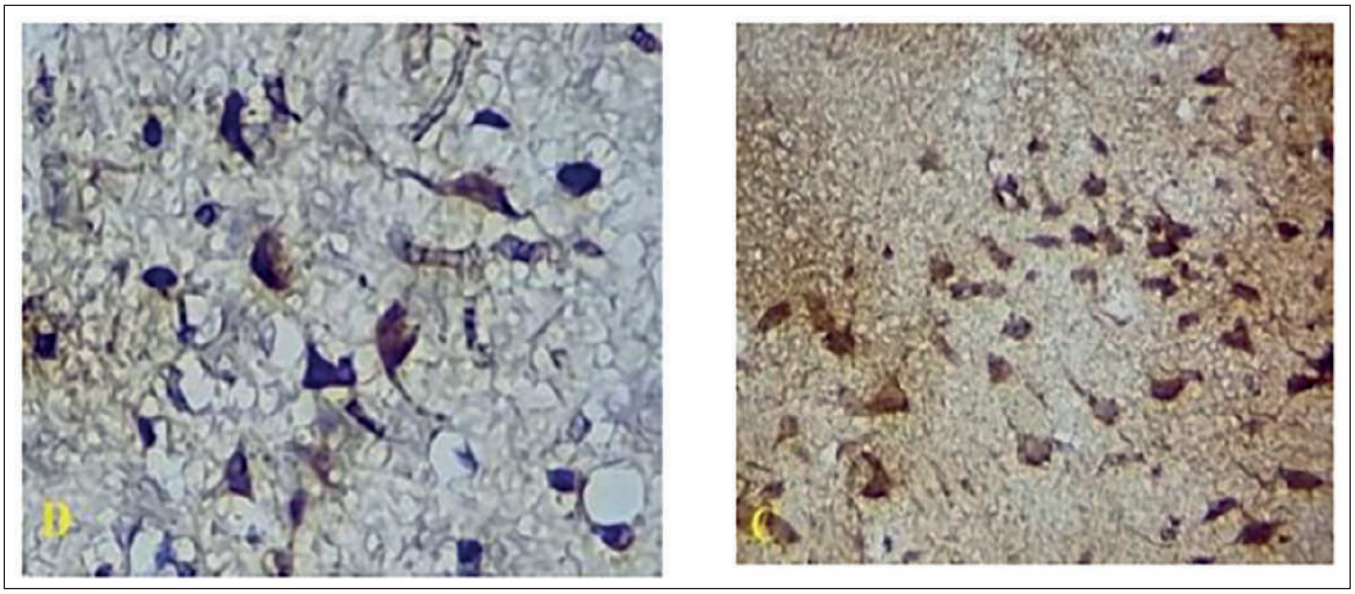

Fig. 7. Immunohistochemistry of NF-κB nuclear expression in rat brain: (A) sham group: negative expression (X100); (B) control group and (C) vehicle group: intense nuclear expression (X200); (D) nimodipine-treated group: weak expression (X200).

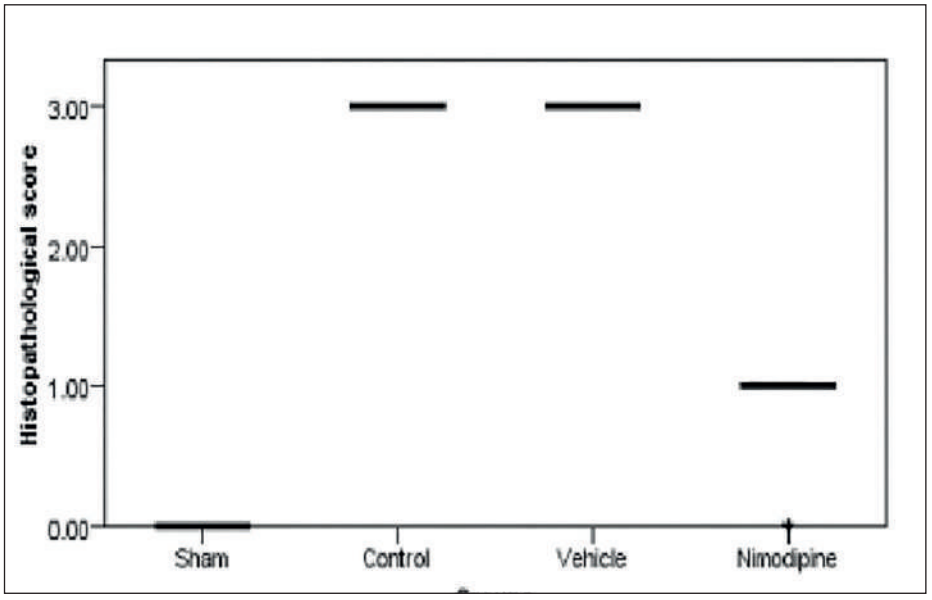

Fig. 8. Kruskal-Wallis box plot showing medians of histopathological ischemic changes among the four groups, significant difference ($p<0.05$) between sham vs. control and vehicle groups. Significant difference ($p<0.05$) between nimodipine-treated vs. control and vehicle groups.

DISCUSSION

EFFECTS OF NIMODIPINE ON CEREBRAL IL-6, IL-10, TNF-A, ICAM-1, AND T-AOC

Our study revealed that nimodipine markedly down-regulated the inflammatory mediators IL-6, TNF-α and ICAM-1and greatly elevated cerebral T-AOC levels compared with the ischemic and vehicle rats. Nimodipine didn't change cerebral IL-10 levels. These observations indicates that nimodipine may ameliorate brain damage during cerebral ischemia/reperfusion injury (CI/RI) through its anti-inflammatory and anti-oxidative effects. Nimodipine effect was unrelated to IL-10. Our results were consistent with that of Shi et al. [9], Hu et al. [10] and Cai et al. [11]. Bai et al. [12] found that CI/RI forms a vicious circle of inflammatory process that can be inhibited by nimodipine. The current study results were in line with Abdel-Fattah et al. [13] who observed insignificant elevation of cerebral IL-10 levels versus CI/RI rats. As far as we can tell, there is no studies on nimodipine effect on cerebral T-AOC of rats.

EFFECTS OF NIMODIPINE ON CEREBRAL NF-*KB* P65

In the current study, nimodipine caused marked reduction of cerebral NF-κB p65 nuclear expression compared to ischemic and vehicle groups, suggesting that nimodipine has anti-inflammatory effect through inhibition of NF-κB p65 expression. Our results are in accord with Zhang et al. [14] and Chen et al. [15] who indicating that NF-κB inactivation mechanism is related to the nimodipine protective properties.

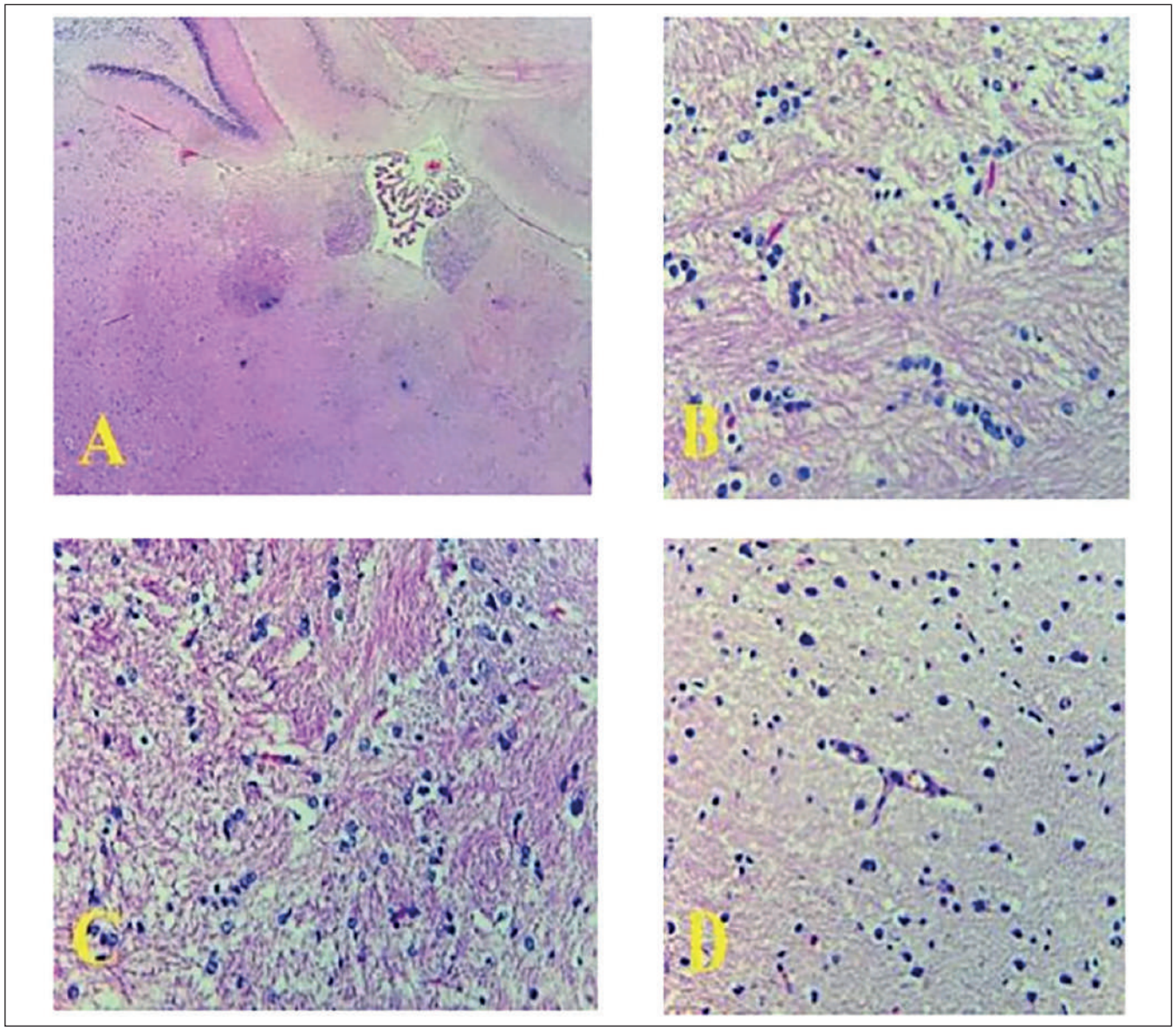

Fig. 9. Cross section of rat cerebrum stained with hematoxylin and eosin: (A) sham group: normal cerebrum, cerebellum, and choroid plexus (X400); (B) control group and (C) vehicle group: severe interstitial edema with dark eosinophilic neurons (X400); (D) nimodipine-treated group: mild interstitial edema (X400).

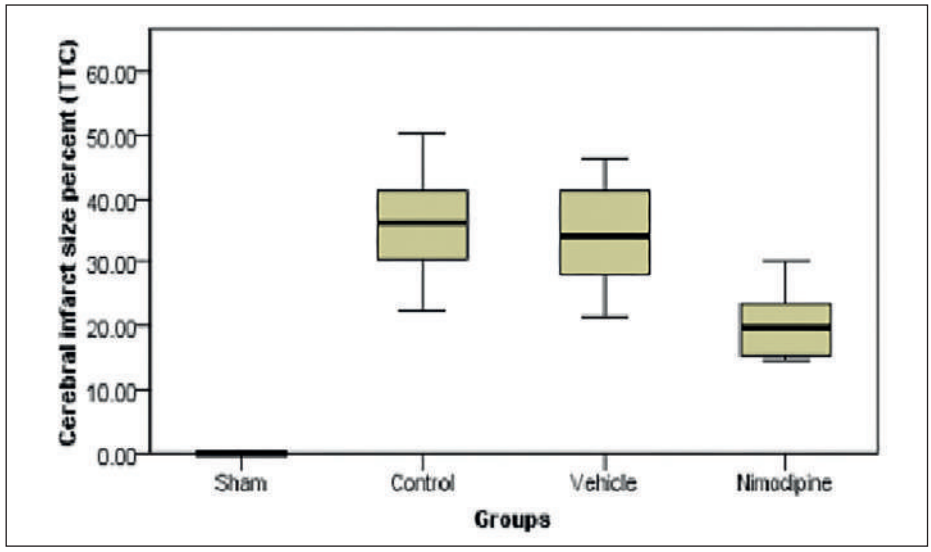

Fig. 10. Kruskal-Wallis box plot showing medians of cerebral infarct size among the four groups, significant difference ($p<0.05$) between sham vs. control and vehicle groups, significant difference ($p<0.05$) between nimodipine-treated vs. control and vehicle groups.

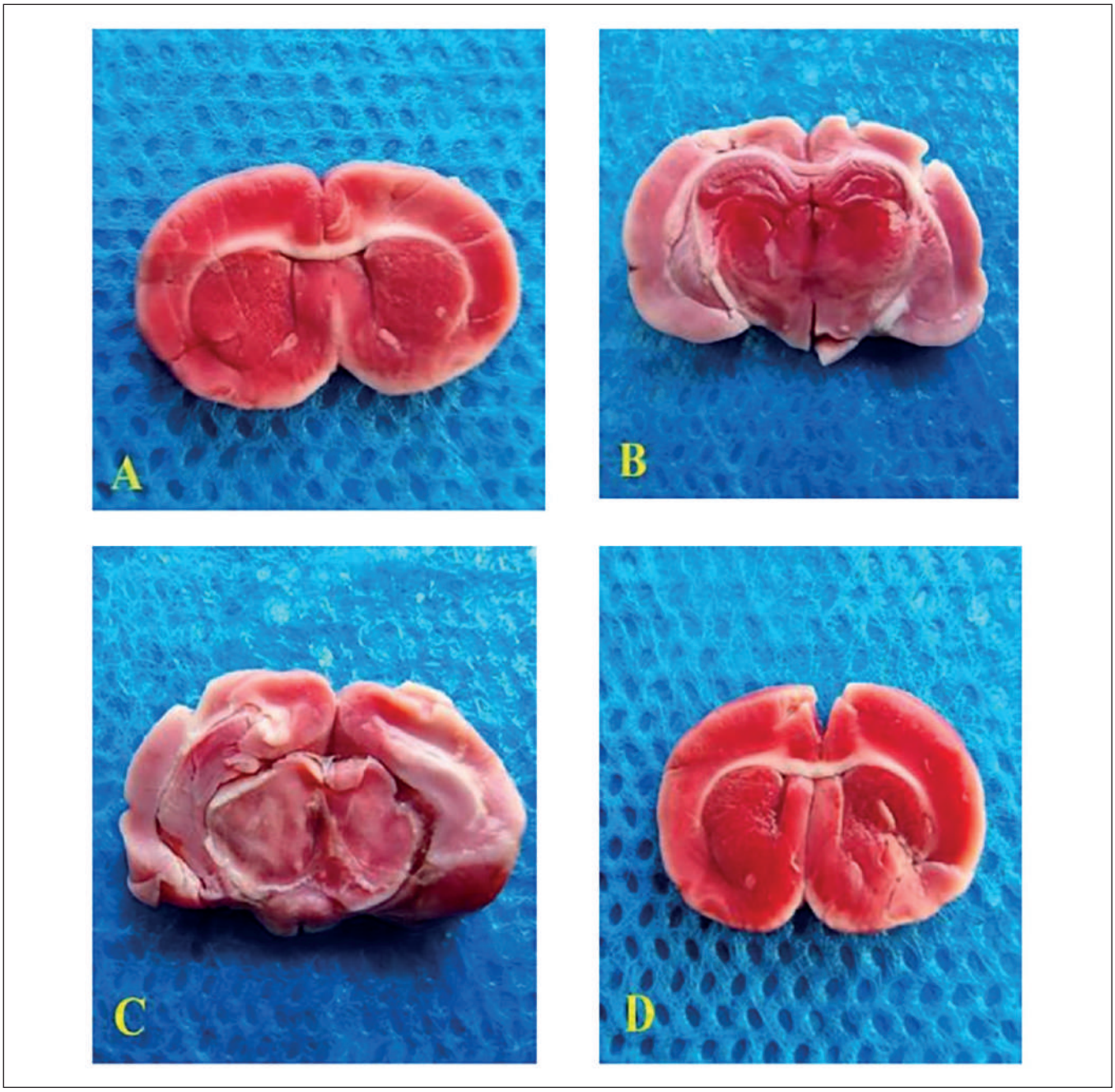

Fig. 11. Photographs of coronal brain slices stained with TTC: (A) sham group, (B) control group, (C) vehicle group, (D) nimodipine-treated group.

EFFECTS OF NIMODIPINE ON CEREBRAL HISTOPATHOLOGY

Nimodipine-treated rats showed a great amelioration in the cerebral ischemic changes compared to control and vehicle groups. Our results are consistent with that of Babu et al. [16], Zhang et al. [14] and Bai et al. [12].

EFFECTS OF NIMODIPINE ON CEREBRAL INFARCT SIZE

In the present study, cerebral infarction size was dramatically reduced in nimodipine-treated group subjected to CI/RI. Our study results are in accord with that of Sobrado et al. [17], Cai et al. [11] and Chen et al. [15].

CONCLUSION

Nimodipine has neuroprotective actions on rats subjected to CI/RI possibly through its anti-inflammatory and anti-oxidative properties. The protective effects of nimodipine was unrelated to IL-10.

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Conflict of interest

The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

MORPHOLOGICAL PECULIARITIES OF THE PANCREAS OF MALE RATS AFTER PROLONGED ADMINISTRATION OF MONOSODIUM GLUTAMATE DURING THE RECOVERY PERIOD

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ABSTRACT

The aim: To study changes in the exocrine and endocrine parts of the pancreas of rats after abolition of monosodium glutamate (MSG) administered in the diet.

Materials and methods: White male laboratory rats with a baseline weight of 120 ± 5 g were randomized into 3 groups: 1 – control, 2 – animals with daily feeding of 70 mg/kg MSG for 8 weeks, 3 – abolition of MSG with transfer of animals to a standard diet and pancreatic examination after 8 weeks. We used histological studies with morphometric analysis and statistical processing of acini and acinar cell areas, Langerhans islets, connective tissue (according to Stolte M.) and adipose tissue. Preparations of pancreas were stained with hematoxylin and eosin and azan.

Results: The animals of groups 2 and 3 showed atrophic, degenerative and inflammatory disturbances in the exocrine and endocrine parts of the pancreas, which worsened after 8 weeks of MSG withdrawal (3rd group). In the preparations, the Langerhans islets were of different shapes and sizes. Small islets predominated, as well as islets with low density of α- and β-cells, different capillary filling with blood and overgrowth of connective tissue in the capillary areas. The acinar cells and acini were reduced, and degenerative abnormalities were detected in the structures.

Conclusions: After daily administration of 70 mg/kg MSG for 8 weeks, atrophic and degenerative changes in the exocrine and endocrine parts of the pancreas were revealed. No recovery of pancreatic structures was observed 8 weeks after MSG withdrawal.

KEY WORDS: monosodium glutamate, pancreas, experiment, rats

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INTRODUCTION

To date, more than 2500 additives are added to food products to preserve their properties and extend their shelf life. One of the most widely used additives both in Ukraine and around the world is monosodium glutamate (MSG) [1]. Despite the widespread use of MSG in the food industry, some questions about its effects on the body are in the field of debate [2, 3]. The metabolic effects of MSG have been demonstrated in animal studies using different concentrations from 2 mg/kg to 6 g/kg weight [3, 4]. It has been shown that MSG even in low concentrations has a toxic effect on the central nervous system, causes disorders in the liver, kidneys, lymph nodes, spleen, pancreas and reproductive system [2, 5-9].

MSG is of great importance for pancreatic function. According to the data of various studies (molecular-biological, electrophysiological and immunohistochemical) it is established that the cells of islets of Langerhans express functional receptors of MSG [10] and glutamate transporters [11]. These data indicate that glutamate can function as a signal intercellular mediator, modeling glucagon and insulin secretion in Langerhans islets. In addition, glutamate transporters and an antiporter are present in the pancreas. Glutamine entering the pancreas through the bloodstream is metabolized to glutamate in islet cells. Relative to pancreatic cells, glutamate can enter the acinus cells through the islet-acinus ductus axis, but the transporters of this pathway are under investigation. In addition, acini cells can absorb glutamine from their environment and excrete it as glutamate into pancreatic juice [12]. Thus, as defined by the authors, the dynamics of pancreatic glutamate can potentially play a significant physiological role in the homeostasis of the entire body, as well as reflect the endocrine function of the pancreas [12]. In this regard, the administration of exogenous MSG in different concentrations can affect the state of the pancreas. However, while there are fundamental studies on the state of the body's metabolic systems after administration of MSG, studies that would address the possibility of determining the state of the body's organs and systems after its long-term action with subsequent withdrawal of use are limited.

THE AIM

To study changes in the exocrine and endocrine parts of the pancreas of rats after abolition of monosodium glutamate (MSG) administered in the diet.

MATERIALS AND METHODS

The experimental study was performed on 20 white male laboratory rats with an initial weight of 120±5 g. Animals were randomized into three groups: Group 1 (n=5) - control rats; Group 2 (n=5) - experimental animals given MSG for 8 weeks, were removed from the experiment and served as a control for Group 3, in which 5 animals received MSG for 8 weeks and were then transferred to a standard diet, and after 8 weeks these animals were removed from the experiment to evaluate the recovery process in the pancreas. The animals were housed in individual well ventilated cages in the vivarium of the Daniel Galitsky Lviv National Medical University. The experiment was conducted in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) [13] and was guided by the protocol of the Bioethics Commission of the Daniel Galitsky Lviv National Medical University.

ADMINISTRATION OF MSG

The rats were weighed before the experiment and at its stages. To assess the condition of the pancreas, the rats of the experimental group were given daily food with supplement MSG in a dose of 70 mg/kg for 8 weeks [14.]. MSG was administered orally. In conversion, our chosen dose of 70 mg/kg for the rat is equivalent to 700 or 816 mg for humans weighing 60 or 70 kg, respectively [15, 16]. The control group of animals received a standard diet without the addition of MSG. After 8 weeks, MSG was abolished and the animals were switched to a standard diet. After 8 weeks the animals were removed from the experiment under anesthesia by decapitation, which was necessary to collect blood for further biochemical studies.

HISTOLOGICAL INVESTIGATION

Rat pancreas was fixed in a 10% solution of neutral formalin, dehydrated in ascending alcohols (ethanol), cleared in xylene, and embedded in paraffin. Serial sections of 7-10 μm thickness were obtained on a Reichert microtome (Austria). Dewaxed sections were stained with hematoxylin and eosin (combination) and azan. Morphometric studies were performed using Test-5, Stepanizer, KAAPA Image, Base, and Microsoft Excel programs on a personal computer using a visual analysis system. Images of histological preparations were displayed on a computer monitor using an SEO SCAN microscope and a Vision CCD Camera.

Exocrine and endocrine parts of the pancreas were examined. The exocrine part of the pancreas was analyzed by evaluating the area of acini and acinar cells. Appearance of fibrous disorders was estimated according to Stolte M. [17]. The author classified 4 degrees of fibrotic changes depending on the location of the connective tissue in the organ (diffuse or segmental fibrosis). Grade I was defined as predominantly perilobular fibrosis with mild atrophy or without atrophy of exocrine parenchyma, i.e. mild fibrosis. If fibrosis has spread into pancreatic portions with moderate atrophy of exocrine parenchyma, is regarded as moderate fibrosis (grade II). In case of wide areas of fibrosis in intralobular sections accompanied by atrophy of exocrine parenchyma, they are defined as degree III, i.e. high degree of fibrosis. Under fibrous reconstruction of pancreas accompanied by destruction of exocrine parenchyma was defined as IV degree of fibrosis. Fat tissue was assessed according to a scoring system: 0 – single islets of adipocytes in the areas of vascular triads, 1 point –single foci of adipose tissue between lobules, 2 points – about 30% between lobule septa, 3 points – overgrowth in interlobular septa and interacinar spaces. We assessed larger diameter of Langerhans islets (μm), as well as the percentage of Langerhans islets with connective tissue overgrowth to islets without fibrosis.

STATISTICAL ANALYSIS

Two hypotheses were tested as a result of the statistical analysis of the experimental series: H_0 - indicating no difference between the averages in the compared groups and H_1 an alternative hypothesis based on the assumption that the averages in these groups are not equal. The level of statistical significance was assumed to be $p < 0.05$. Data were presented as M±m, where M is the mean value, m is the standard arithmetic deviation. Comparative analysis of the experimental data including normally distributed (diameter of Langerhans islets, acinar cell area and acini area) was performed using One Way Anova analysis of variance with the addition of Tukey's honestly significant difference test (Tukey's HSD), which is based on a studentized range distribution (Q) (this distribution is similar to the t-test). Dispersion analysis ANOVA shows the presence or absence of statistically significant differences (p) between compared variables using the F-criterion (ratio of between-group (factor) variance to within-group (residual) variance). However, ANOVA is not sufficient to decide how exactly the groups differ from each other. Tukey's HSD test provides a test of the differences between the paired means of a sample for significance, controlling for the probability of committing One Way Anova error, and also shows which means of particular groups (when compared with each other) differ in magnitude.

The non-parametric Mann-Whitney U-test was used to assess differences between two independent samples (islets with fibrotic changes). If Ukr > Uemp (Ukr is the value from the Mann-Whitney table; Uemp is the estimated value), the hypothesis of a significant difference in the compared experimental groups (H_1) was accepted; If Ucr < Ump, there is no effect (H0). (H_0).

RESULTS

After 8 weeks of feeding rats with food including MSG, degenerative and destructive disorders were found in the exocrine and exocrine parts of the pancreas. Marked edema of the organ was observed. Reduction of acini size, their delimitation from each other by layers of loose connective

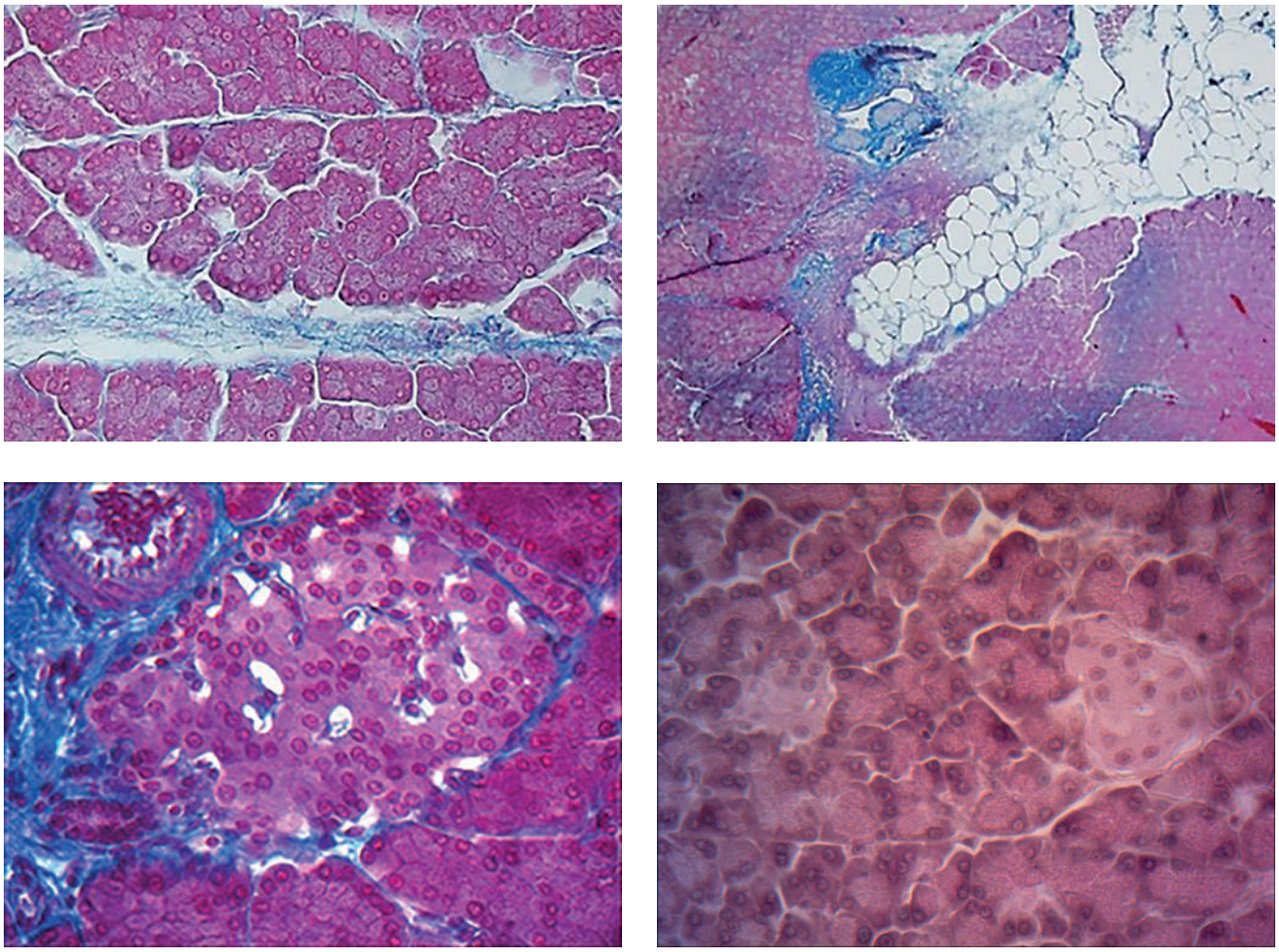

Fig. 1. 8 weeks of MSG administration. Fragments of rat pancreas. A. Acini of reduced size, overgrowth of loose connective tissue. Azan. X 200. B. Replacement of lobules with fatty tissue, edema, overgrowth of connective tissue. Azan. X 200. C. Langerhans islet. Connective tissue in capillary spaces. Adjoining acini crowded with zymogenic granules. Azan. X 400. D. Langerhans islet. Single β-cells. Hematoxylin and eosin. X400.

tissue was noted (Fig.1 A). Connective tissue overgrew between acini and in the spaces between lobules that resulted in acini deformity. According to Stolte M. [17], at 8 weeks after cessation of feeding animals with sodium glutamate a grade II fibrosis was noted.

Areas of lymphocytic infiltration located between acini and lobules were detected. Some acini and even lobules were replaced by adipose tissue, marked borders of adipose tissue, which was localized in triads, and around ducts (Fig. 1. B). The ducts, both interstitial and acini, were dilated and filled with vacuolized fluid; edematous fluid was present between the acini, leading to their separation and destruction. Vessels were sharply dilated, full of blood, small extravasations were formed.

Acini were of different shapes and sizes, but small acini prevailed. There was revealed pycnosis of exocrinocytes, more such cells were located in marginal parts of the gland and in the areas of edematous fluid accumulation. Exocrinocytes were irregularly filled with zymogenic granules, nuclei were characterized by heteromorphism, from small hyperchromic with dense chromatin to large hypochromic with diffuse chromatin distribution.

Langerhans islets in the preparations were of different shapes and sizes. Small islets prevailed, as well as islets with low density of α- and β-cells (Fig. 1. C). A peculiarity was different capillary filling with blood and overgrowth of connective tissue in the capillary sections (Fig. 1.D).

After cancellation of MSG and feeding rats with standard food for 8 weeks of the study were revealed: an increase in atrophic, degenerative and inflammatory manifestations. Signs of edema were preserved and increased degenerative changes were detected in both exocrine and endocrine parts of the pancreas. Overgrowth of loose connective and fatty tissues was revealed. The acini in most areas of the pancreas were disconnected due to oedema, small acini predominated, some with destructive changes. A pairwise comparison (One-Way ANOVA test) of the acinus area values of the experimental series yielded statistically significant results: 1:2 ($p = .00013$), 1:3 ($p = .00000$) and 2:3 ($p = .02940$) (Table I). A pairwise comparison of the series with a further validation by The Tukey's HSD test confirmed the validity of the One-Way ANOVA test, showing that the direction of destructive changes of the acini was highly significant in

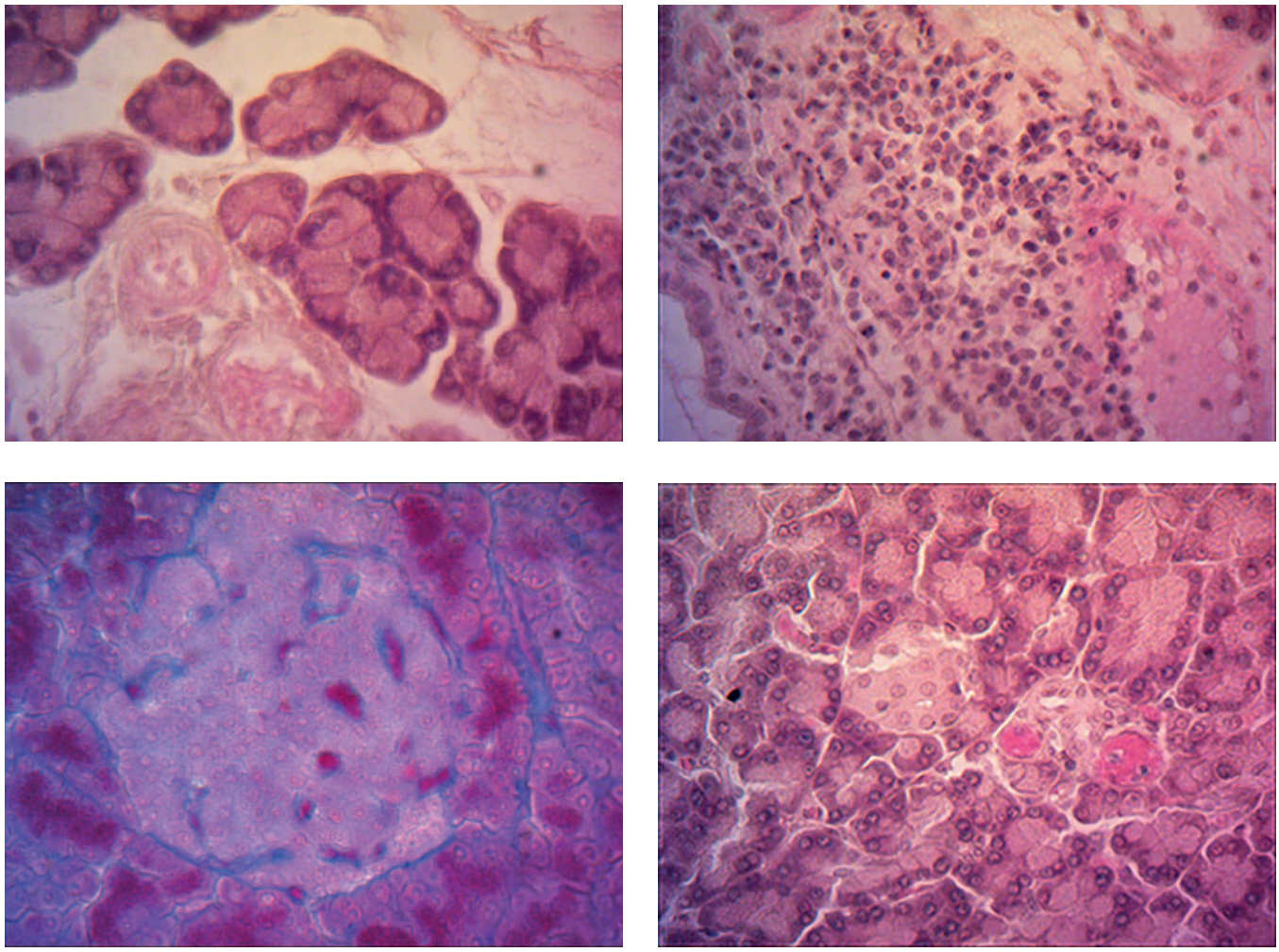

Fig. 2. 8 weeks after MSG withdrawal. Fragments of rat pancreas. A. Edema. Detachment of acini. Destruction. Remains of ductal system. Destruction of vessels. Hematoxylin and eosin. X 400. B. Lymphoplasmacytic infiltration. Edema. Disturbance of duct wall. Ductal epithelium flattened, desquamated in places. Hematoxylin and eosin. X 400. C. Langerhans islet with overgrowth of connective tissue. Azan. X 400. D. Small islets of Langerhans. Low density of α- and β-cells. Dilated vessels with stasis in and near the islet. Hematoxylin and eosin. X400.

the compared series 1:3, less so in 1:2, the lowest values were recorded in the 2:3 series, but the validity of the difference indicated a progression of the abnormalities after withdrawal of the MSG. A pairwise comparison (One-Way ANOVA test) in the experimental groups (1:2, 1:3, 2:3) showed statistically significant results for the acinar cell area in the pancreas (Table II). The Tukey's HSD test revealed that the highest rates of destruction were seen in the 1:3 series (Table II).

Areas of pancreatic ductal permeability were present, leading to oedema and accompanied by destructive changes of the acini, which were placed among plasmatic eosinophilic substance (Figure 2 A).

Areas with permeable ducts were present, resulting in edema and accompanied by destructive changes in the acini, which were placed among the plasmatic eosinophilic substance (Fig. 2 A). Some were without cells. Diffuse focal infiltration with lymphoid and plasmocytic elements was present for all terms of the study (Fig. 2 B). There was no significant difference between the index of adipose tissue in the pancreas in three series of the experiment.

A decrease in the number, size and shape of Langerhans islets was recorded. Blood supply disorders were noted due to edema and overgrowth of connective tissue in spaces between capillaries (Fig. 2 C), as well as in some islets dilated capillaries were overfilled with blood or empty (Fig. 2 D). Decreased density of **α**- and **β**-cells and their apoptosis were noted. By the end of the experiment, signs of chronic inflammatory process persisted. The count of Langerhans islets with connective tissue overgrowth showed that while this index at the end of MSG feeding (series 2) was (39.1 ± 4.09)%, 8 weeks after cancellation (series 3) it was increased (57.948 ± 3.285)% (p= 0.01208, Mann-Whitney U test).

The assessment of islets of Langerhans with connective tissue overgrowth after 8 weeks of feeding and after 8 weeks of withdrawal showed that while this index at the end of MSG feeding (2^{nd} series) was (39.1 ± 4.09) %, after 8 weeks of withdrawal (3^{rd} series) it was increased (57.95 ± 3.285)%. Assessment of series statistical significance using the Mann-Whitney U test revealed that Ukr 4 > Uemp 1; (p = 0.01208), i.e., hypothesis H1 was accepted, that is, connective tissue overgrowth in islets of

Table I. Comparative analysis of acini area in the series of experiments

Pairwise Comparisons* of the indicators of the experimental series		$HSD_{.05}$ = 93.9938 $HSD_{.01}$ = 119.1378	$Q_{.05}$ = 3.4358 $Q_{.01}$ = 4.3549
1:2	1 = 925.07 2 = 748.73	176.33	Q = 6.45 (*p* = .00013)
1:3	1= 925.07 3= 646.00	279.07	Q = 10.20 (*p* = .00000)
2:3	2 = 748.73 3 = 646.00	102.73	Q = 3.76 (*p* = .02940)

* One-Way ANOVA test used; The Tukey's HSD – honestl significant difference

Table II. Comparative analysis of acinar cell area in the series of experiments

Pairwise Comparisons* of the indicators of the experimental series		$HSD_{.05}$ = 9.5619 $HSD_{.01}$ = 12.1198	$Q_{.05}$ = 3.4358 $Q_{.01}$ = 4.3549
1:2	1= 84.59 2= 73.51	11.07	Q = 3.98 (*p* = .01989)
1:3	1= 84.59 3= 62.62	21.97	Q = 7.89 (*p* = .00000)
2:3	2= 73.51 3= 62.62	10.89	Q = 3.91 (*p* = .02231)

* One-Way ANOVA test used; The Tukey's HSD – honestl significant difference

Table III. Comparative analysis of changes in the diameter of Langerhans islets in the series of experiments

Pairwise Comparisons* of the indicators of the experimental series		$HSD_{.05}$ = 9.5619 $HSD_{.01}$ = 12.1198	$Q_{.05}$ = 3.4358 $Q_{.01}$ = 4.3549
1:2	1= 133.67 2= 104.78	28.89	Q = 4.87 (*p* = .00365)
1:3	1= 133.67 3= 79.93	53.75	Q = 9.07 (*p* = .00000)
2:3	2= 104.78 3= 79.93	24.85	Q = 4.19 (*p* = .01350)

* One-Way ANOVA test used; The Tukey's HSD – honestl significant difference

Langerhans was increased 8 weeks after discontinuation of MSG feeding.

Use of a One-Way ANOVA test showed statistical significance in pairwise comparisons of islet Langerhans diameter of experimental series 1:2 (p = .00365), 1:3 (p = .00000), 2:3 (p = .01350) (Table III). The Tukey's HSD paired series comparison analysis with additional validation confirmed the statistical significance of the increase in small islets in the pancreas of the paired series with a high value in the 1:3 series. After discontinuation of the MSG, by the end of the study (2:3), an increase in the pancreas of small-diameter islets of Langerhans was recorded in the pancreas of rats.

DISCUSSION

The use of nutritional supplements is of great interest to researchers to evaluate their various side effects on the body. A large number of experimental studies have shown negative effects of MSG (E 621) on organs and body systems [3, 6, 18,19]. In our study, the effect of MSG on the pancreas of rats at a dose of 70 mg/kg weight was evaluated using histological methods. The data obtained indicate that MSG after 8 weeks of use has an inhibitory effect on the endocrine and exocrine parts of the pancreas, and after cancellation of MSG there is no restoration of its structural organization. After MSG action there is pancreatic edema, which persists during the stages of MSG withdrawal, which leads to separation of acinar structures and their death. A decrease in the area of acini, a change in their shape and a decrease in the area of acinar cells, which is reflected in their metabolism, are noted. The revealed changes under the conditions of histological and morphometric study reflect the toxic effect of MSG, which should be considered in combination with other negative effects on other systems of the body. Thus, adipose tissue formation under the influence of MSG promotes the biosynthesis of proinflammatory cytokines by adipocytes, these molecular factors activate apoptosis, disrupt intercellular interactions and adaptive reserves of phagocytes [2, 20]. In addition, the progression of structural disorders of the pancreas after withdrawal of MSG may be associated with imbalances in the immune system, increased cytotoxic function of immunocompetent cells. In addition, potential triggers of the inflammatory process can be cellular hypoxia, mechanical stress of adipocytes, excess of free fatty acids and lipopolysaccharides [21]. It has been proved that even low doses of MSG, namely,

the administration of MSG to rats at a dose of 30 mg/kg for a short period of 4 weeks leads to an increase in the serum content of total and tyrosine-containing peptides, affects the content of low and medium molecular weight substances, and also leads to an increase in the index of intoxication factor, which indirectly indicates a violation of the detoxification of endogenous metabolites in the liver of animals [22]. It can be assumed that the toxic effect of MSG on AA transporter (EAAC), identified only in acinar cells, leads to early atrophic and destructive changes in the acini. Combined with the literature data that in the pancreas identified glutamate receptors and transporters, namely in Langerhans islets AA type L (LAT1), glutamate 1 (GLT1), glutamate-aspartate (GLAST), cystine-glutamate antiporter (xCT), the latter also identified in duct cells [23]. We can speculate that the toxic effect of MSG 70 mg/kg leads to damage to transporters in Langerhans islets and acini.

CONCLUSIONS

The nature and degree of expression of changes in histomorphometric parameters of rat pancreas after prolonged action of MSG and in 8 weeks after its cancellation indicate the presence of morphological signs of decrease in its exo- and endocrine activity. In the rat pancreas we detected progression of degenerative changes in comparison with the control series and after cancellation of MSG: enlargement of connective tissue, edema of organ parenchyma, disorders of vessels and ducts walls with fluid going out into interacinaric spaces, enlargement of diffuse-focal inflammatory infiltration area. When assessing statistical significance, a decrease in the area of acini and acinar cells, reduced diameter of Langerhans islets and increased islets with overgrowth of connective tissue were revealed. Immediately after drug withdrawal, there were no cells in some acini, acinar cells had small nuclei with dense chromatin. The data presented suggest a toxic effect of MSG on the pancreas during the intake phase and progression of degenerative changes after withdrawal.

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ORIGINAL ARTICLE

INTERNET-DELIVERED LOW-INTENSITY CBT FOR PEOPLE WITH SOCIAL ANXIETY DISORDER IN A PERIOD OF COVID-19: RESULTS OF PILOT RESEARCH

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ABSTRACT

The aim: The study aims to provide evidence of the effectiveness of online low-intensity CBT-based psychological interventions on the psychological well-being of people with social anxiety disorders and related impairments in the COVID-19 pandemic.

Materials and methods: 222 volunteers aged 18-35 years included in study: low-intensity CBT group (n=106) and control group (n=116). To assess the mental health problems were used International Neuropsychiatric Interview (MINI) and a set of IAPT scales. Analyses considered levels of pre-post intervention effect sizes and clinically significant improvement of symptoms of social anxiety disorder, generalized anxiety disorder, depression, and distress in maintaining general and work activity scores.

Results: Comparisons between the low-intensity interventions group and control (self-help guide psychological care as usual) indicated more reduction in the severity of symptoms of social anxiety disorder and comorbid impairments associated with depression or generalized anxiety disorder. Changes for social phobia and other outcomes indicate that the odds of relapse or exacerbation of symptoms in the control group are more significant than those after a CBT-based low-intensity psychosocial care program. Analysis showed a significant interaction between outcomes scores and the number of sessions: more than five online sessions and homework with a self-help guide improved outcome.

Conclusions: This pilot trial provides initial evidence that low-intensity online interventions based on CBT result in reductions in psychological problems for persons with a social anxiety disorder during the COVID-19 pandemic.

KEY WORDS: unguided online therapies; quarantine restrictions; social impairment

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INTRODUCTION

With the onset of the pandemic and the introduction of quarantine restrictions, there has been an increase in mental health problems and psychological well-being. WHO reports and research data for 2020-2021 show that the symptoms of depression and anxiety disorders are twice the prevalence compared to the pre-epidemic period [1, 2]. O. Ebrahimi and colleagues point out that people who mostly adhered to social distancing showed significantly higher symptoms and levels of psycho-emotional distress compared to others [2]. Thus, the establishment of quarantine restrictions and the global pandemic situation have become significant challenges for the psychosocial adaptation of people, and socio-economic aspects are an additional factor of vulnerability. Such as, some people with high social anxiety may feel relief by physical distancing, but a lack of interaction can also maintain social anxiety and raise the risk of relapse symptoms and associated distress after alleviated restrictions.

Research on available methods to alleviate the effects of such changes on the mental health and stress response of vulnerable groups in a pandemic can be a key component in addressing complex issues in the progression of mental illness and preventing maladaptive strategies for stress management and suicide risk [3]. In recent decades, more controlled studies have shown that online psychological treatment under the guidance of a mental health professional is as effective for a wide range of psychiatric and physical conditions as offline interventions, and leads to sustainable improvements, work in a setting with limited face-to-face meetings, and be considered cost-effective [4]. According to the proposed definition of R. Shafran and colleagues, the use of self-help materials, the total duration of contact time is six hours or less and the possibility to be provided by specialists providing supportive psychosocial care in mental health characterizes low-intensity CBT programs [5].

Concerning support for people with social anxiety disorders, there is evidence that psychological care provided online (including access to self-help manuals, weekly online meetings or expert feedback, and an online discussion forum) has had a persisted throughout the year positive effect on the severity of social anxiety symptoms, general worries, depression, and quality of life [6, 7]. Recent research demonstrates that guided and unguided self-help can increase access to social phobia treatment in the

population [8]. At the same time, there are warnings that in social anxiety disorder, concomitant comorbid anxiety and depressive symptoms and high levels of avoidance of corrective emotional experience suggest an insufficient or poor response to treatment, especially online [9].

Internet self-guided psychological support based on low-intensity CBT has already proven itself in treating depression. As one that has the potential to increase access to evidence-based psychological care and reduce the cost of treating depression [10]. Recent studies indicate that CBT effectively treats pandemic-related anxiety and depression. Studies in Australia and the United Kingdom noted that most participants found the intervention helpful. And that the intervention group showed a significant reduction in anxiety and depression compared with the control [11]. Post-treatment and follow-up outcomes in a pilot study of intensive 7-day internet-based cognitive behavioral therapy for social anxiety disorder demonstrated substantial reductions in social anxiety and depressive symptom severity and functional impairment [12].

THE AIM

The study aims to provide evidence of the effectiveness of online low-intensity CBT-based interventions on the psychological well-being of people with social anxiety disorders and related impairments in the COVID-19 pandemic.

MATERIALS AND METHODS

The search participants and studies were conducted in 2020-2021. All procedures followed the ethical standards of research with human participants and were performed according to the Declaration of Helsinki. The institutional board of the Faculty of Health Science, Ukrainian Catholic University, approved all study procedures. All participants gave informed consent to participate in the study. Participants didn't receive any financial donations. In the future, they engaged in a low-intensity CBT program free or got free psychological counseling from psychologists of the Center of mental health and trauma-therapy.

Inclusion criteria in the study were: a) persons aged 18-35 years; b) significant subjective complaints of psycho-emotional distress associated with social restrictions due to quarantine conditions and/or avoidance of social contacts due to social anxiety (including anxiety before negative evaluation and censure); c) duration at least six months.

Psychopathological conditions caused by a) chronic somatic pathology; b) use of psychoactive substances or medications; c) head injuries or the result of significant traumatic stress such as loss of loved ones or participation in hostilities; were used as general exclusion criteria. Additional criteria were d) receiving psychotherapeutic care at the time of participation in the study; e) lack of access to the Internet to receive interventions in the format of video conferencing. In addition, the risk of suicide was assessed, and psychotherapy would be recommended if necessary to prevent suicide attempts or referred to specialized psychological or psychiatric care services. All participants confirmed the absence of active COVID disease symptoms.

The first (initial) stage of the study (2020-2021) was aimed at screening and forming groups according to the criteria for inclusion among people who sought psychological help from the Center of mental health and trauma-therapy at UCU Institute of Mental Health. Respondents' participation was voluntary. All applicants who consented completed online self-report questionnaires of their social-demographic information: age, gender, employment status, marital status, education, and diagnosed mental health problems in anamnesis. After that, with eligible participants qualified clinical psychologists conducted individual structured diagnostic interviews based on MINI: International Neuropsychiatric Interview by Sheehan D.V. and Lecrubier Y. (adapted by I. Ushtan, 2011) in the Center for Mental Health UCU [13].

At the second (interventions) stage, according to the initial diagnostic interview and confirming consent about participating in the study, 106 people formed the primary sample (intervention group). Randomly, the intervention group was divided into two subgroups of 53 persons. The first subgroup engaged in low-intensity CBT program; the participants of the second subgroup were assigned to the waiting list. Upon completing the course with the first subgroup, the participants of the second subgroup received the same course of psychosocial assistance. The low-intensity psychosocial care program included reading materials and seven online sessions twice a week, lasting up to 50 minutes for four weeks (six hours of contact time). The control group (n=116) received one initial individual consultation with a psychologist and access to self-help materials. After four weeks, which corresponded to the intervention term and after one month at the "follow-up" stage, they have had one individual consultation at a time.

To achieve the goals of the study, all participants completed self-report measures prior to session 1 (initial individual consultation for control group), 4 weeks post-intervention, ("posttest") and one month later ("follow-up"). We used online questionnaires from guidance The Improving Access to Psychological Therapies (NICE, 2018): measuring the severity of depression – PHQ-9 (Kroenke, Spitzer, Williams, 2001), generalized anxiety disorders – GAD-7 (Spitzer, Kronke, Williams, et al., 2006), social anxiety disorders - SPIN (Connor, 2000), distress in maintaining general and work activity – W&SAS (Mundt, Marks, et al., 2002) [14]. The above methods were translated and adapted by the Ukrainian Institute of Cognitive Behavioral Therapy in 2006-2013.

The final stage of the study was data processing and concluding the effectiveness of the proposed program. We used a linear analysis of variance (ANOVA) to determine changes in mental health state (i.e., the dependent variable: severity of depression, generalized anxiety disorders, social anxiety disorders, distress in maintaining general and work activity) concerning the type of "psychosocial intervention" (i.e., low-intensity CBT and self-help guide psychological care as usual) and "time" (i.e., after four weeks and one

Table I. Change in baseline mental health states following posttest (4 week) and follow-up (1 month) of comparing with control

Outcomes	Time	ISgr1 (n=47)	ISgr2 (n=41)	Control (n=98)	Difference in LS mean (95% CI)[a, b]	*p* value
		M (SD)	M (SD)	M (SD)		
SPIN	Baseline	33.60 (7.04)	35.12 (11.70)	35.67 (10.69)		
	4-week	25.96 (6.15)	30.71 (9.52)	35.79 (10.94)	9.7 (8.2 to 11.1)a	0.000
					5.1 (0.6 to 9.6)b	0.027
	1 month	24.09 (6.16)	26.83 (8.32)	36.71 (12.17)	12.6 (8.6 to 16.6)a	0.000
					9.9 (5.3 to 14.4)b	0.000
PHQ-9	Baseline	13.72 (3.84)	13.47 (3.78)	14.38 (3.70)		
	4-week	10.70 (2.95)	12.04 (3.04)	14.09 (4.28)	4.2 (2.7 to 5.7)a	0.000
					2.9 (1.2 to 4.5)b	0.001
	1 month	9.85 (2.41)	10.14 (2.62)	15.16 (4.42)	5.3 (3.8 to 6.8)a	0.000
					5.0 (3.4 to 6.6)b	0.000
GAD-7	Baseline	11.82 (2.33)	12.02 (2.29)	11.95 (2.25)		
	4-week	8.40 (1.58)	9.98 (1.67)	12.00 (2.27)	3.6 (2.8 to 4.4)a	0.000
					2.0 (1.2 to 2.9)b	0.000
	1 month	8.02 (1.39)	8.46 (1.47)	12.02 (2.27)	4.0 (3.2 to 4.8)a	0.000
					3.6 (2.7 to 4.4)b	0.000
W&SAS	Baseline	25.36 (2.74)	26.27 (3.41)	26.26 (3.36)		
	4-week	19.70 (6.75)	20.85 (2.82)	26.26 (3.36)	6.6 (4.3 to 8.8)a	0.000
					5.4 (4.1 to 6.8)b	0.000
	1 month	17.68 (2.12)	17.15 (2.48)	26.36 (3.41)	8.8 (7.5 to 9.9)a	0.000
					9.2 (7.9 to 10.5)b	0.000

SPIN (total score range: 0-68; higher scores indicate more severe dysfunction by social anxiety disorder); PHQ-9 (total score range: 0-27; higher scores indicate elevated more severe depression); GAD-7 (total score range: 0-21; higher scores indicate more severe worry); W&SAS (total score range: 0-40; higher scores denoting higher levels of disability or functional impairment work and social functioning); a compared intervention subgroup 1 to control; b compared intervention subgroup 2 to control

month). Between-group differences were assessed using p-values and least-squares mean. Within-group effect sizes for the pre-post change outcome measures were calculated using a partial eta-squared model, with 95% confidence intervals from ANOVA. Statistical analyses were conducted using SPSS Version 23.0 (SPSS Inc., 2019).

RESULTS

At the start of study, the intervention group included participants aged between 18 and 33 years (M = 23,3, SD = 5,25), 72,6% (n=77) of female participants and 59,4% (n=63) were students. 60,4% (n=64) of the intervention group noted that they live alone or are not in a long-term relationship. All participants met diagnostic criteria for social anxiety disorder according to data from structured diagnostic interviews based on MINI: International Neuropsychiatric Interview, and 69,8% (n=74) had moderate or higher-level symptoms on the SPIN that were impacting their daily functioning. Eighty-four participants (79,2%) also had a comorbid symptom of Major Depressive Disorder, and eighty-one participants (76,4%) had signs of Generalized Anxiety Disorder. Clinical signs in all comorbid indicators did not exceed moderately severe severity, which allowed participants to engage in low-intensity CBT without medication. To assess compliance with the psychological support protocol, we recorded sessions: participants were given access to session records individually and were removed at the end of the intervention period. The checklist confirmed that 89% of the sessions complied with the protocol.

The initial evaluation of the results after four weeks was performed for 49 (92.4%) participants of the first subgroup of the intervention and 42 (79.2%) participants of the second, one month later: 47 (88.7%) and 41 (77.4%) respectively. At the 4-week stage, 17 people (14.6%) from the control group dropped out of the study, 11 of whom due to the need to start medical treatment in a hospital. At the follow-up stage, one more person dropped out in 1 month due to a deteriorating mental state and the need to start medical treatment. Study completion rates were 83% for a low-intensity CBT program group (n = 88) and 84% for the control group (n = 98).

Comparing pre-post change on outcomes scores, the results revealed a significant interaction effect for groups by time for interventions (ANOVA: $F_{(2, 87)} = 7,366$, $p = 0,000$,

partial eta-squared 0,36). Statistically significantly reduces were found on all outcome measures: social anxiety severity (SPIN): $F_{(2, 87)} = 11,64$, $p = 0,000$, partial eta-squared 0,15, 95% CI [4,74 to 5,76]; depression severity (PHQ-9): $F_{(2, 87)} = 12,34$, $p = 0,000$, partial eta-squared 0,16, 95% CI [1,98 to 2,95]; general anxiety severity (GAD-7): $F_{(2, 87)} = 26,66$, $p = 0,000$, partial eta-squared 0,29, 95% CI [2,00 to 2,86]; functional impairment (W&SAS): $F_{(2, 87)} = 40,13$, $p = 0,000$, partial eta-squared 0,39, 95% CI [5,14 to 5,99]).

Including the amount of attended sessions in analysis indicated significant interaction between outcomes scores and the number of sessions, $F_{(2, 87)} = 158,146$, $p = 0,000$, partial eta-squared 0,96. More than five online sessions and homework with a self-help guide generally improved outcome. In our opinion, the interaction between low-intensity CBT psychological support and number of sessions can preserve the effect directing to reduce the relapse.

After 4 weeks of low-intensity CBT program, indicates a reduction in severe dysfunction by social anxiety disorder of a SPIN point in both subgroups of interventions (ANOVA: ISgr1: mean difference 9.7 [95% CI, 8.2 to 11.1], $p = 0.000$; ISgr2: 5.1 [95% CI, 0.6 to 9.6], $p = 0.027$), compared to control participants groups (Table I). From the point of view of comorbid pathology, at the stage of "posttest" (4 weeks) the participants of the intervention subgroups achieved a reduction in the level of depression (ISgr1: 4.2 [95% CI, 2.7 to 5.7], $p = 0.000$; ISgr2: 2.9 [95% CI, 1.2 to 4.5], $p = 0.001$) and generalized anxiety (ISgr1: 3.6 [95% CI, 2.8 to 4.4], $p = 0.000$; ISgr2: 2.9 [95% CI, 1.2 to 4.5], $p = 0.001$), compared to control participants groups (Table I). Considering a need to adapt to the conditions of quarantine restrictions associated with COVID-19, the impact of the intervention showed a more significant decrease in levels of disability or functional impairment on work and social functioning than among those who were in the control group (all p-values <0.000).

After 1 month follow-up, there was a greater reduced in the primary results of severe dysfunction by social anxiety disorder (ISgr1: 3.6 [95% CI, 2.8 to 4.4], $p = 0.000$; ISgr2: 2.9 [95% CI, 1.2 to 4.5], $p = 0.001$). The intervention led to reduced negative mood and vital impairments in the clinical picture of comorbid depression, levels of worries, and disability or functional impairment in work and social interactions (Table I).

There were no side effects throughout the study. After one month (for participants who completed the assessment), fewer participants in the intervention group than those in the control group achieved or maintained the threshold values of the severity of probable mental health severe impairment and symptoms associated with adjusting for pandemic settings. Changes for social phobia indicate the odds of relapse or exacerbation of symptoms in the control group are 7.7% times greater than the odds after low-intensity CBT program. We are 95% confident that the true odds ratio (OR) is between 0.01 and 5.62. For depression symptoms 50.2% against 88.1%, odds ratio 0.21 [95% CI, 0.09 to 0.51], $p < 0.05$, and generalized anxiety disorder 19.8 vs. 85.7%, OR 0.19 [95% CI, 0.09–0.41], $p < 0.05$. There were slightly more participants in the control group than in the interventions, which reported worsening depression during the 1-month assessment and anxiety and were forced to withdraw from the study and be referred for medical treatment. However, that differences weren't significant, $p = 0.07$.

DISCUSSION

Our study is a pilot and aims to provide evidence that the use of low-intensity CBT-based psychological interventions delivered online can positively impact the psychological well-being of people with social anxiety disorders and related problems in the COVID-19 pandemic. The COVID-19 pandemic forced self-isolation and fear for one's own life, which has triggered the inability to socialize among people with social anxiety disorders. Loneliness, avoiding distress (anxiety) feelings, and depressive thoughts, as modifier factors, could decrease faith in their abilities and personal well-being, social support, and security.

Previous research suggests that low-intensity CBT may help sustain people with common anxiety disorders and depression [4, 9]. The reduction of psycho-emotional distress can be considered a transdiagnostic indicator of the effectiveness of psychological support in periods of adjustment to global or local changes or challenges. Although the conclusion of the current study has its limitations, we consider the results to be significant evidence that the organization and implementation of psychological support to people with psychiatric diagnoses during a pandemic is an urgent challenge today.

The proposed psychological care program aims to restore psychological resources during the period of adaptation to the requirements of quarantine restrictions and distancing forms of human interaction during a pandemic. The proposed program aims to help prevent the worsening comorbid impairments of adaptation and prevent relapses of social anxiety disorder after quarantine restrictions. Providing access to psychological help online and engaging in at least 5 of the seven sessions allows us to conclude about the acceptability of the proposed method of providing psychological support.

This research has shown a significant reduction in the severity of symptoms of social anxiety disorder on the SPIN and comorbid impairments associated with depression or generalized anxiety disorder (in terms of PHQ-9 and GAD-7 scores) among participants who received a low-intensity CBT program, emphasizing the potential benefits of the intervention. The modules of the online sessions covered: 1) psychoeducation of anxiety, social anxiety, and anxiety of adjustment related to the pandemic COVID-19; 2) strategies of normalization and coping worries in pandemic periods; 3) psychoeducation about the impact of maladaptive beliefs and social avoidance on anxiety; 4) training in mindfulness and problem-solving techniques on reducing using of safety behavior, including avoidance and procrastination; 5)

psychoeducation about comorbid depressive states and reducing by behavioral activation, challenging negative thinking with behavioral experiments and thought records; 6) promoting the development of skills of social support and assertive communication. Changes among the intervention group participants showed a reduction in maladaptive beliefs, the level of distress, and the tendency to avoid social situations in maintaining general and work activity (in terms of SPIN and W&SAS scores). Upon completion, conducted a session on relapse management and support for implementing corrective experience of social interaction. After one-month, fewer participants in the intervention group than those in the control group achieved or maintained the threshold values of the severity of probable mental health severe impairment and symptoms associated with adjusting for pandemic settings. Changes in social phobia scores and other outcomes indicate that the odds of relapse in the control group are more significant than after a CBT-based low-intensity psychosocial care program. Analysis showed a significant interaction between outcomes scores and the number of sessions: more than five online sessions and homework with a self-help guide, improved outcomes. The results of our study are generally consistent with the results of previous studies [6, 7, 11, 12].

Despite the results, our study has several limitations. First, the study was conducted on the Ukrainian sample under the quarantine restrictions imposed on its territory. COVID-19 infection rates (including hospitalizations and deaths) and the socio-economic aspects can also impact the severity of distress, which differs from experience in other countries. Secondly, the participants are primarily women, so expanding the sample is a prospect for further research. Limitations of diagnosis should also include using self-reported methods to track the dynamics of changes in the results of structured interviews only at the stage of involving participants. We also see the challenge that the results demonstrated had a more significant effect in the early stages of the study and see the benefits of obtaining data within six months after interventions.

CONCLUSIONS

In conclusion, the presented pilot study highlights the efficacy of using online low-intensity CBT psychological support in the context of restriction of corrective social interaction experiences and challenges to adjustment to new circumstances during the COVID-19 pandemic in people with social anxiety disorder. These data demonstrated clinically significant improvement of symptoms of social anxiety disorder, generalized anxiety disorder, depression, and distress in maintaining general and work activity scores. Initial results can be offered as a rationale for further scaling up and longer-term studies of the effectiveness of mental health interventions during life's challenges. The proposed program also will promote psychological support for people with social anxiety disorders in conditions of limited access to psychotherapy.

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ORIGINAL ARTICLE

DOES THE APPLICATION OF CONVERSION FRACTURE-TREATMENT METHOD AND THE TECHNOLOGY OF TELEMEDICAL MOVEMENT MONITORING AFFECT THE LONG-TERM RESULTS OF THE TREATMENT OF VICTIMS WITH MULTIPLE GUNSHOT LONG BONES FRACTURES?

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ABSTRACT

The aim: To improve the results of treatment of patients with multiple gunshot fractures of long bones by developing the technology of fixation method conversion with combined autoplasty and postoperative telemedical control (loading +ROM (range of motion).

Materials and methods: Two comparison groups were formed: the main (84 patients) and the control (62 patients). For the patients of this group all elements of the restorative treatment system were used (DCO, extrafocal osteosynthesis (including hinged), ultrasonic cavitation, NPWT, biochemical indicators of blood, conversion technology with usage of regenerative technologies, rehabilitation program) and telemedical control with applications (ROM+weight bearing). The control group (62 patients) - patients who received almost the same treatment, but only autoplasty with cancellous bone was included for bone plastics an telemedical counseling were not used

Results: 1 year after the final method of fixation, it was established that the relative indicators were also lower in the patients of the main group, and a statistically significant difference was found in the indicator of the frequency of contracture formation, which may indicate the timely establishment of low dynamics in increasing the amplitude of movements and appropriate response (redress, arthrolysis, tenolysis).

Conclusions: Implementation of telemedicine and combined plastic surgery of bone defects in the restorative treatment system reduce the frequency of major complications that affect the objective result, affect better physical and mental health indicators during the observation period of 12 months.

KEY WORDS: fractures, gunshot, quality of care, telemedicine, trauma

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INTRODUCTION

The ongoing armed conflict in eastern Ukraine is resulting in a large number of victims with multiple high-energy fractures of long bones. This category of patients requires the use of a large number of modern treatment methods aimed at preserving life, treating wounds, fractures, and their recovery lasts for years. Telemedicine makes it possible, in addition to quick making of highly qualified decisions during inpatient treatment, to conduct dynamic outpatient monitoring of the patient's rehabilitation progress. However, the effectiveness of telemedicine is mainly considered from an economic point of view, and its clinical impact has not been sufficiently studied.

The problematics of the issue is determined by the fact that limb injuries occupy the first place among all localizations (32-68%) in gunshot injuries [1-7]. Multiple fractures of the limbs occur in 8.5-13.7% [8]. Gunshot wounds of the limbs are often accompanied by damage to the main vessels (18-38%), which, with proven failure to apply the 3 rules (a tourniquet, urgent transfusion of blood/its preparations, victim evacuation to the hospital within 60 minutes), significantly increases mortality at the pre-hospital stage, which is key in I-II levels of medical care [6] . The problems of treatment at the hospital stage are related to the long-term hospital stay, the number of surgical interventions, early postoperative complications, economic factors. The main factors affecting the outcome of restorative treatment are: concomitant damage to main vessels, nerves, compartment syndrome, the size of the soft tissue and bone defects, combined damage to the head, chest, and abdomen [7, 9-11]. At the outpatient stage, an increased number of infectious complications (compared with closed fractures) is noted by 2.3-46.2%, depending on the input data of the patients, as well as the selected treatment tactics, the frequency of delayed consolidation (47.4-92.6%)/non-union (0-6.4%), post-traumatic osteoarthritis in intra-articular fractures (92.8%), minor/moderate/significant dysfunction of large joints (32.8-52.6%) in intra-/para-articular fractures, in diaphyseal – 13.6-24.3% [1, 4, 11-15]. In connection with a significant number of factors leading to mortality, a greater number of unsatisfactory restorative treatment results, for this patients

Fig. 1. Assessment of active flexion in the elbow of patient B., 1978. 1 week after changing the fixation method.

category implementation of modernized tactics is needed. Telemedicine is considered as a factor that makes it possible to provide full-fledged highly qualified consultation assistance at II-IV levels of evacuation. And at the I level, the implementation of telemedicine is caused by technological networks limitations and cyber security issues. Telemedicine is not a substitute for deploying predictable medical resources or optimizing training/counseling: telemedicine is Plan B, and Plan A is training, hospital deployment, and casualty evacuation. But, when network and communication resources are sufficient, telemedicine provides best practices in harsh, resource-constrained environments where timely evacuation is not possible [9, 12].

To replace bone tissue defects and to reduce the volume of surgical interventions, it is optimal to use modern matrices based on bioglass and PRP (platelets rich plasma). In our previously conducted experimental study (the experimental animal was a Chinchilla rabbit, weight=3530±72 g, age=11±4 months, the defect size of the tibia proximal metaepiphysis=2 mm, control points=4 and 12 weeks) it was established that the application of BG+PRP (bioglass+platelets rich plasma) in 12 weeks after the intervention had the highest bone tissue density in the defect area (68.5-88.1%) compared to: bioglass+PRF (platelets rich fibrin) (63.2-80.3%), bioglass and BMA (bone marrow aspirate) (50, 5-66.2%) [16].

In addition to synchronous/asynchronous "doctor-doctor" and "doctor-patient" counseling, there are a number of teletechnologies that make it possible to objectify the treatment process. E.g. ComeBackMobility™ technology for remote load monitoring. This technology makes possible to track in a real-time the operated lower limb load. Another application, DrGoniometer™ [17], with the help of a software assistant, allows you to take a picture of a patient's segment during active movements, and calculate the amplitude using an on-screen protractor. However, there are currently no clinical studies on the effect of using these technologies in the treatment of patients with gunshot fractures.

THE AIM

To improve the results of treatment of patients with multiple gunshot fractures of long bones by developing the technology of fixation method conversion with combined autoplasty and postoperative telemedical control (loading +ROM (range of motion)).

MATERIALS AND METHODS

After preliminary statistical processing and forecasting, two comparison groups were formed: the main (84 patients) and the control (62 patients). All patients were injured during the Joint Forces Operation (JFO) in the East of Ukraine. These victims were treated in 2014-2021 at the levels of medical care of the Ministry of Defense of Ukraine (military medical centers and hospitals) as well as front-line civilian hospitals (Selidovsk, Toretsk Central Hospital, Mariupol Medical Center). Inclusion criteria were: patients with multiple fractures of long bones, etiology – gunshot trauma, polytrauma type: multiple/combined, age 18-60 years, size of the bone defect is up to 3 cm^3 (CT data). Exclusion criteria were: death of the patient, non-compliance with the treatment regimen by the patient. Non-inclusion criteria were: smoking in combination with diabetes in one patient, anatomical damage of main arteries, nerves, age - less than 18 and older than 60 years, accompanying decompensated diseases, the presence of which can sig-

Fig. 2. The process of monitoring the load on the operated limb in patient S., 1991. with an ipsilateral gunshot fracture of the lower limb.

nificantly affect the results of the study; refusal to sign the patient's informed consent.

Patients were divided into 2 study groups: main group (84 patients) - patients, who received medical care in the Medical Military clinical Centers and rehabilitation centers of Ministry of Defense of Ukraine in the period from 2016 to 2021. In the patients of this group all elements of the restorative treatment system were used (damage-control orthopedics surgery (DCO), wound preservation, pneumatic tires, extrafocal apparates (including hinged), ultrasonic cavitation, negative pressure therapy (NPWT), monitoring of bacteriological content of wounds, biochemical indicators of blood, conversion technology with using of regenerative technologies, individual rehabilitation program). Telemedical monitoring of patients with analysis of input data and state dynamics was carried out at each stage. The conversion technology includes the use of a combination of bioglass and PRP in the treatment of bone tissue defects. The rehabilitation program includes the use of technology for remote monitoring of movements (ROM and dosed loading on lower limbs).

The control group (62 patients) - patients who received recovery treatment in the same medical centers from 2014 to 2021. Were used the same surgical technlogies but the difference from the main group were: only autoplasty with cancellous bone tissue was included in the method conversion technology. In restorative treatment, telemedical counseling and the technology of remote monitoring of movements were not used (Table I).

In patients with defects of bone tissue up to 3 cm^3 (according to CT), during the conversion of the fixation method, the defect was replaced with the combined bioglass+PRP. Bioglass: "I-Plant" company, Ukraine, composition - 58% SiO2; 33% CaO; 9% PO, synthesis - sol-gel method, burned at 25 ° -850 ° C, specific surface area about 130 m2 / g. Bioglass was formed into blocks with a diameter of 2 mm and a length of 4 mm. Implantation method: ISO 10993-6: 2015. PRP was prepared according to the following technology: 9 ml of venous blood was collected from an patient in a Vacutest™ tube with Na-heparin, centrifuged on a Micromed 3.01 machine (speed of rotation 2500/min, duration 5 minutes). 1 ml of the medium fraction (plasma+platelet concentrate) was taken from the obtained preparation.

The regulatory and legal basis for the application of telemedicine technologies was the Order of the Ministry of Health of Ukraine (October 19, 2015 No. 681) "On the approval of regulatory documents regarding the use of telemedicine in the field of health care" and "The procedure for the organization of medical care at primary, secondary (specialized), tertiary (highly specialized) levels with the use of telemedicine. Teleconsultations were held with "Kyiv-Irpin-Kharkiv-Vinnytsia-Lviv-Odesa-Bakhmut-Pokrovsk-Mariupol" network. Telemedical consultation was carried out on the basis of a request for telemedical consultation in accordance with the form of primary accounting documentation #001/tm "Request for telemedical consultation", approved by order of the Ministry of Health of Ukraine dated October 19, 2015 No. 681 (Form No. 001/tm).

Teleconsultations at the inpatient stage were always conducted in synchronous mode; at the ambulatory stage, in addition to synchronous - offline. Each patient in the main group had sufficient skills in using a computer and mobile applications, and at the outpatient stage, due to a large number of consultations, transferred data to e-mail/Viber/Telegram applications. The frequency of examinations at the inpatient stage was individual, as patients are under the supervision of medical personnel. At the ambulatory stage, a number of patients conducted telemedical interaction as planned every day, but most of them 1-2 times a week.

At the ambulatory stage, control over the amplitude of movements was carried out using the DrGoniometer™ mobile application, which allows to take a picture of the segment in an active functional position, and the program's navigation makes it impossible to take a photo at an angle that is not "perpendicular" to the limb, because in this case system errors would be formed. Then, using the on-screen protractor of this program, the amplitude of movements was manually determined. (Fig. 1).

Each patient had his own smartphone, on which this

Table I. Summary of input data for patients of the comparison groups

	Main (84 patients)	Control (62 patients)
Age	31,66±8,28	33,14±7,92
Gustillo-Anderson	II – 14,1% IIIa – 69,5% IIIb – 16,4%	II – 12,7% IIIa – 65,4% IIIb – 21,9%
Localisation	thigh – 21,3% shin– 42,7% shoulder– 21,8% forearm– 14,2%	thigh – 22,5% shin – 40,1% shoulder – 20,8% forearm– 16,6%
Bone defect volume (according to CT)	2,82 cm3±0,16	2,54 cm3±0,18
Scheme of treatment	The restorative treatment system, which included combined autoaloplasty (BG+PRP) and postoperative telemedical monitoring	Restorative treatment system
The severity of the injury at the time of admission to the II level of ISS medical care	ISS=19,4 (σ=3,1)	ISS=20,8 (σ=2,9)
The number of injured segments	2,31 (σ=0,34)	2,42 (σ=0,32)

Table II. The result of treatment of patients in the comparison groups 6 months after the conversion of the fixation method.

	Main (BG + PRP + telemedicine) N=84	Control (without BG+PRP + telemedicine) N=62	p
Infectious complications (infiltrate)	7 (8,3 %)	7 (11,3 %)	p=0.549
Slowed consolidation	71 (84,5%)	57 (91,9%)	p=0,178
Contracture	30 (35,6%)	26 (41,9%)	p=0,445

Table III. The result of treatment of patients in the comparison groups 12 months after the conversion of the fixation method.

	Main + bioglass + PRP (+ Motion monitoring technology)	Control (without telemedicine)	p
Osteomyelitis	3 (3,6%)	4 (6,5%)	p=0,421
Pseudoarthrosis	4 (4,8%)	4 (6,5%)	p=0,657
Contracture	18 (21,4%)	22 (35,5%)	p=0,042

Table IV. Load monitoring technology vs without movement monitoring technology (12 months)

	Main (+Motion monitoring technology)	Control (without telemedicine)	p
Construction migration (including screw breakage) – 12 months	5 (6,1%)	6 (9,7%)	p=0,399
Pain syndrome after 2 months (nailing) (mean) Visual Analog scale (VAS)	2.7±1,3	3,4±1,9	p=0,001*

Table V. Dynamics of the increase in the function of large joints (hip, shoulder) of comparison groups in the postoperative period (conversion). (% from normal ROM)

	Main	Control	
2 weeks	23,3±9,3	24,7±8,8	P=0,772
4 weeks	38,4±6,9	34,4±7,1	P=0,438
6 weeks	49,6±6,6	42,5±6,8	P=0,11
8 weeks	58,7±6,3	53,2±6,5	P=0,328
10 weeks	64,6±6,2	59,7±6,4	P=0,254
12 weeks	68,1±5,8	64,5±5,6	P=0,198
16 weeks	73,6±5,4	67,4±5,7	P=0,204
20 weeks	77,8±4,4	69,6±4,8	P=0,039
24 weeks	81,5±4,2	74,3±4,5	P=0,043

Table VI. Dynamics of the increase in the function of the elbow and knee in patients of the comparison groups in the postoperative period (conversion). (% from normal ROM)

	Main	Control	
2 weeks	17,6±6,3	17,3±6,8	P=0,582
4 weeks	26,4±5,6	24,4±6,1	P=0,316
6 weeks	38,9±5,3	34,9±6,3	P=0,214
8 weeks	45,5±5,4	39,3±6,4	P=0,075
10 weeks	52,3±5,8	46,8±5,3	P=0,076
12 weeks	57,2±5,3	51,4±5,7	P=0,048
16 weeks	61,5±6,1	56,7±5,5	P=0,204
20 weeks	64,7±5,9	59,3±5,5	P=0,089
24 weeks	67,3±5,2	62,4±5,4	P=0,033

Table VII. Results of restorative treatment in patients of the comparison groups according to the SF-36 scale

	Main	Control	p
Physical activity	72,70%	67,20%	0,066
Restrictions due to physical Health	63.3%	58,40%	0,06
Limitations due to emotional problems	69,50%	63,40%	0,032
Energy	61,60%	58,40%	0,212
Emotional well-being	71,20%	64,60%	0,024
Social interaction	74,20%	65,30%	0,003
Pain	68,40%	63,50%	0,083
General state of health	56,40%	51,20%	0,024
Changes in health	46,40%	41,60%	0,011

program was installed, and the patient, in the absence of other complaints, sent only photographs of the limbs, and the data were evaluated by a consulting doctor and noted.

Assessment of limb load after surgery was performed using the ComeBack Mobility™ program and smart attachments on crutches (Fig. 2). Strain gauges are installed on the nozzles, which assess the load on the right and left crutches separately. When moving on crutches, there are three points of support - the right crutch, the left crutch and one weight-bearing lower limb (while walking, the other limb is in the air). On the comebackmobility.com portal, patients created an account in which passport data, diagnosis, and body weight were indicated. Taking into account the body weight entered into the program, the application automatically determined the load according to the formula:

$m_{body} - (P_{left}+P_{right})=P_{lower\ extremity}$, where:

m_{body} – patient's body weight

P_{left} - load on the left sensor (kg)

P_{right} - load on the right sensor (kg)

$P_{lower\ extremity}$ – load on the lower limb (kg)

At the same time, empirically (since the literature sources do not actually have data on dosed load regimes with equal clinical data), patients were assigned a load regime calculated from a percentage of the patient's body weight. There is an indication on the crutches that signaled each step with colors. The blue color signaled a correctly performed step, red - a low/high load, while the application on the phone made a voice notification "Load less/stronger". In addition, the program formed a load schedule (total number, number of "correct"/"incorrect" steps). Patients also reported tenderness/absence of pain/signs of inflammation in the segment. In case of intense pain syndrome and elevated body temperature above 38°C, urgent teleconsultation was conducted and the next treatment tactics were determined.

The load was increased no more than once a week. The criteria for increasing the load were:

- pain syndrome (below 6 points according to VAS)
- no signs of inflammation.

Results and discussion. Among the total mass of patients with multiply gunshot fracture (MGP) (146 patients), an analysis of treatment results was carried out using the SF-36 scale, as well as indicators of the presence/absence of complications in the form of osteomyelitis, pseudarthrosis, contractures.

Expert assessment of treatment results was carried out in 2 stages - 6 and 12 months after the injury. All examinations were conducted live or in online teleconsultation mode. Selected complications (contracture/pseudoarthrosis/osteomyelitis) are defined as the main ones that affect the outcome of restorative treatment and the quality of life of a patient with MGP.

The SF-36 scale is a non-specific questionnaire for assessing the quality of life, which is widely used in developed

countries and is universal. This questionnaire contains 36 questions assessing the patient's "expectation" and "perception" of his health. This scale assesses the physical and mental components of health. The volume of active/passive movements in the joints was determined at each examination (including using the Dr.Gonimeter program). ROM was determined using a protractor, the axis of which was set corresponding to the axis of the joint, and the branches - along the axis of the segments forming the joint. Movements were measured using the STFR method.

In-depth statistical analysis was carried out using the licensed statistical package STATA 12.1. Before the collection of primary data, the minimum sample size was estimated at the specified marginal level of statistical error of no more than 5% ($p < 0.05$) and research power of at least 80% (level of error of the second kind $\beta=0.2$).

Descriptive statistics of attributive (qualitative) signs are represented by frequency characteristics of indicators - absolute number of cases and their distribution in percentage (%). Quantitative parameters are characterized by arithmetic mean (M), standard (mean square) deviation (SD). To determine the representativeness of the results and calculate the 95% confidence interval of the mean errors of the indicators (m).

Comparison of attributive features in groups was carried out using the Chi-square ($\chi 2$) test and Fisher›s test at a low frequency of the studied clinical characteristics. For quantitative parameters, the character of the distribution of the primary data was evaluated according to the Shapiro-Wilk test for further selection of the statistical method of comparison (parametric or non-parametric). The T-test (parametric method) and Wilcoxon, Mann-Whitney (U) tests (non-parametric methods) were used for comparison.

The marginal level of statistical error at the $p < 0.05$ level was used to evaluate the results.

The results of treatment in these terms and according to the above indicators are presented in tables II - III.

It was noted that these complications occurred in patients of both comparison groups, but their relative indicators are lower in patients of the main group. At the same time, no statistically significant difference in results (6 months) was found.

One year after the final method of fixation, it was established that the relative indicators were also lower in the patients of the main group, and a statistically significant difference was found in the indicator of the frequency of contracture formation ($p=0.042$), which may indicate the timely establishment of low dynamics in increasing the amplitude of movements and appropriate response (redressation, arthrolysis, tenolysis).

To control the impact of movement monitoring technology, two indicators were chosen: the migration of the structure (in the case of bony plating/nailing, extrafocal osteosynthesys according to Ilizarov (including screws/spindles) during loading in a period of up to 12 months and pain syndrome after 2 months in the case of nailing (table IV).

It was established that the migration of the fixators took place in 5 (6.1%) patients of the main group and in 6 (9.7%) of the control group, however, such a slight difference did not have the corresponding level of significance ($p=0.399$). It should also be noted that the migration of the structure took place simultaneously with infectious complications, which may indicate a low impact of the loading regime. At the same time, the pain syndrome in patients was significantly lower in patients of the main group.

The dynamics of growth of large functions is shown in Tables V-VI. The result is noted up to the 3rd week every 2 weeks, up to 6 months - once a month.

The division of patient data into two subgroups was carried out due to the fact that the rehabilitation and prognostic outcome of treatment of patients with injuries of the elbow and knee joints is more difficult than that of the hip and shoulder joints.

Analyzing the dynamics of the increase in the amplitude of movements in large joints, it was noted that the initial data are the same in both comparison groups, but the general function of the increase is linear, and its indicators are slightly lower in the control group. At the same time, a statistically significant difference in the comparison groups (shoulder, hip) was noted at 20 and 24 weeks of the post-conversion period: at 20 weeks 77.8±4.4 (main) and 69.6±4.8 (control) ($p=0.039$), at 24 weeks 81.5±4.2 and 74.3±4.5 ($p=0.043$). Such results were obtained due to the fact that 26 (31.7%) patients of the main group were recommended to undergo a repeat course of sanatorium-resort treatment during the examinations, 4 (4.8%) of them - 2 courses each. Control points were periods of 2 weeks to 3 months, 4 weeks - in the period of 3-6 months, while the patients of the main group were consulted weekly (daily/1-2 times a week).

The main recognized scale for assessing mental and physical health is the SF-36 (short form-36). The results of the treatment of both groups are compared in Table VII.

The patients of the main and control comparison groups, a statistically significant difference in the results of treatment was noted in many positions (restrictions due to emotional problems, emotional well-being, social interaction, general state of health and changes in health). At the same time, the patients of the main group stated that the high frequency of consultations (contacts with the doctor) was an important disciplinary and psychological factor. In the course of teleconsultations, patients received feedback on any issues related to health, the course of postoperative rehabilitation, etc., and in the long term, such an approach to changes will affect both physical and mental health.

DISCUSSION

Analyzing the results of this study, it was determined that the use of telemedicine technologies and combined plastic of small bone defects 6 months after the conversion of the method relatively reduces the frequency of osteomyelitis, reduces the absolute indicators of the frequency of detection of delayed consolidation and contractures, but without a statistically significant difference. 1 year after the final method of fixation, it was

established that the relative indicators were also lower in the patients of the main group, and a statistically significant difference was found in the indicator of the frequency of contracture formation (p=0.042), which may indicate the timely establishment of low dynamics in increasing the amplitude of movements and appropriate response (redress, arthrolysis, tenolysis). It should be noted that there are very few similar studies where telemedicine is evaluated as a tool not only for speeding up care and reducing costs, but also as a clinical aspect. Eichler et al. [18], in a randomized controlled study, demonstrated equivalent results between 2 types of in-person and virtual rehabilitation by comparing functional outcomes, pain, and quality of life. However, patients with in-person rehabilitation had a higher rate of return to work. Muschol et al. determined [19], that improvements in other functional parameters, as well as in quality of life and pain, were achieved in both groups (with telemedical intervention and without). The higher proportion of working patients in the intervention group (64.6%; P=0.01) versus the control group (46.2%) were noted. Specialist evaluation and side effects: the primary outcome, the sum score of the specialist evaluation, was significantly better at UNN compared to RMC (1.72 vs. 1.82, p = 0.0030). The orthopedic surgeons involved evaluated 98% of teleconsultations as "good" / "very good". In our study, a positive effect of telemedicine was also determined, but the category of patients is significantly different. Analyzing the dynamics of the increase in the amplitude of movements in large joints, it was noted that the initial data are the same in both comparison groups, the general function of the increase is linear, and its indicators were slightly lower in the control group. A statistically significant difference between the comparison groups in terms of the amplitude of movements in the shoulder and hip joints was noted at 20 and 24 weeks after the conversion of the fixation method: at 20 weeks 77.8±4.4 (main) and 69.6±4.8 (control) at p=0.039, at 24 weeks 81.5±4.2 and 74.3±4.5 (p=0.043). Thomas et al. [20], Buvik et al. [21] determined the importance of telemedicine as a tool for pre-operative education in arthroplasty patients. In specific cases, rehabilitation of orthopedic surgery is already being carried out with telerehabilitation in selected cases.. the home environment is safe and patients can engage more in rehabilitation in this situation. Braun B.J. et al. [22] determined that control over the load mode not only reduces the probability of breaking structures, but also "disciplines" patients to follow the recommendations of doctors. However, this category of patients is closed single fractures, and the comparison with multiple gunshot fractures is not valid.

Summarizing the obtained results, we can mark that the proposed mode of loading with telemedical control can be optimal for obtaining a better functional result, increasing patient's activity. The control system for restoring the amplitude of movements alone cannot improve the dynamics, but it makes possible to prevent the formation of persistent contractures in time.

CONCLUSIONS

Implementation of telemedicine technologies and combined plastic surgery of bone defects in the restorative treatment system reduce the frequency of major complications that affect the objective result, make it possible to make timely adjustments to the treatment plan, preventing both acute and chronic adverse factors, affect better physical and mental health indicators during the observation period of 12 months.

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