Wiadomości Lekarskie

Medical Advances

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

Volume LXXVII, Issue 11, NOVEMBER 2024 ISSN 0043-5147 E-ISSN 2719-342X

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ALUNA Publishing

Volume LXXVII, Issue 11, NOVEMBER 2024

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

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

Graphic design / production:
Grzegorz Sztank fajne.work

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

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ORIGINAL ARTICLE

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Clinical significance of postoperative acute pancreatitis of non-resection genesis in abdominal surgery

Tetiana V. Formanchuk[1], Maxym A. Hudz[1], Oleg V. Honcharenko[1], Hryhoriy V. Lapshyn[2]
[1]NATIONAL PIROGOV MEMORIAL MEDICAL UNIVERSITY, VINNYTSYA, UKRAINE
[2]UNIVERSITY CLINIC SCHLESWIG-HOLSTEIN, LÜBECK, GERMANY

ABSTRACT

Aim: To analyse in detail the incidence of POAP after urgent abdominal and endoscopic transpapillary surgical interventions.

Materials and Methods: A retrospective analysis of 52 clinical cases of POAP was done, among which 27 patients developed POAP after urgent abdominal interventions and 25 patients – after endoscopic transpapillary interventions.

Results: The incidence of POAP after urgent surgical interventions consisted 0,3%, after endoscopic transpapillary interventions – 18,25%. In the structure of urgent surgical interventions complicated by POAP, gastric and duodenal surgery – 25% (13) and splenectomy – 9,6% (5) were dominating. There was a significant predominance of men in the group of patients with POAP after abdominal interventions and a significant predominance of women in the group with endoscopic retrograde cholangiopancreatography induced POAP (ERCP-induced POAP). POAP can develop at any age; up to 70 years of age, there was an increase in the incidence of POAP with the age of patients, and after 70 years of age the incidence rate was decreasing. The dominant morphological form of POAP was edematous – 67,3% (35).

Conclusions: The issue of a unified definition of POAP after operations on internal organs of nonresectional genesis is not resolved. The incidence rate of POAP after surgeries on internal organs, which have direct anatomical and functional relationship with the pancreas dominate.

KEY WORDS: acute postoperative pancreatitis, ERCP-induced pancreatitis, incidence rate, gender distribution, age distribution

Wiad Lek. 2024;77(11):2119-2126. doi: 10.36740/WLek/197078 DOI

INTRODUCTION

Postoperative acute pancreatitis (POAP) remains an unresolved issue of general surgery and has long been its peculiar «black box». The absence of clear unified international criteria for the diagnosis of this pathology significantly complicates the collection of reliable statistical data and explains the variability in the frequency of this pathology. The highest incidence of POAP is natural after surgical interventions on the pancreas itself, especially with various types of pancreatic resection, which is associated with direct trauma to the pancreas. According to different authors, the incidence of POAP in pancreatic resections varies from 8,3% to 65,7% [1-3], according to the International Study Group of Pancreatic Surgery (ISGPS), the incidence of post-pancreatectomy acute pancreatitis (PPAP) was 32% [4]. The development of POAP after abdominal surgery is associated with the indirect effect of surgical procedures on the pancreas or other indirect factors of aggression (metabolic, circulatory) and, according to different authors, ranges from 2,5 to 85% [5-7].

In the last decade, significant progress has been made in clearly defining this pathology, diagnostic criteria, and a detailed study of its clinical relevance. However, all this was mainly related to acute postoperative pancreatitis of postresection genesis and, accordingly, relatively new recently described terms were proposed – the so-called «postpancreatectomy acute pancreatitis» (PPAP) or «postoperative acute pancreatitis of the pancreatic remnant» as a new clinical form [2]. Some progress has also been made recently in differentiating the concepts of PPAP and postoperative hyperamylasemia, which has long been identified with PPAP [8, 9].

The search for criteria for the diagnosis of POAP was limited to individual publications. Laboratory criteria included a wide range of tests with different diagnostic values. For some time, the diagnostic triad of leukocytosis, C-reactive protein, and blood amylase was widely used among surgeons to diagnose POAP after abdominal interventions [10]. However, it is known that an increase in the total leukocyte count is not a specific marker of acute pancreatic

inflammation and may occur in the postoperative period as a result of other complications. Connor's 2016 definition of acute pancreatic inflammation based on biochemical parameters included an increase in urinary trypsinogen-2 >50 μg/L or serum amylase/lipase > upper limit of normal between 0-2 days after surgery and was related to acute pancreatic inflammation after resectional surgery. Similarly, the definition proposed in 2022 by the International Study Group on Pancreatic Surgery (ISGPS) was used for resection-related POAP. It was based on biochemical, clinical, and radiological criteria: persistent postoperative serum hyperamylasemia (POH) exceeding the institutional upper limit of normal at least during the first 48 hours after surgery, relevant clinically significant signs, and radiological changes consistent with PPAP.

While the concept of POAP after pancreatic resection has made significant progress in recent years, POAP as a complication of other abdominal surgeries is still undefined. The definition paradigm has not changed much, the diagnostic criteria remain unclear, and there are no protocols for the perioperative management of acute postoperative pancreatitis after general abdominal surgery. Therefore, in our opinion, large-scale reports and further detailed study of this pathology are still needed.

AIM

The aim of the study is to analyze the frequency, etiology, gender and age characteristics of acute postoperative pancreatitis in abdominal surgery department.

MATERIALS AND METHODS

We carried out a retrospective analysis of the treatment of 52 patients with POAP. Among them, POAP developed after abdominal surgery in 27 patients who were hospitalized in the department of surgery and endoscopy, and the intensive care unit of the city clinical emergency hospital from January 2018 to December 2022 and in 25 patients with POAP after duodenoscopic interventions, including endoscopic retrograde cholangiopancreatography (ERCP), who were hospitalized in the department of surgery and endoscopy and the intensive care unit of the Vinnytsia regional Pirogov memorial clinical hospital in 2020. There were 23 (44,2%) men and 29 (55,8%) women.

To make the diagnosis of POAP, we used the criteria proposed by Connor in 2016 based on biochemical parameters for diagnosis: the definition of POAP included an increase in urine trypsinogen-2 >50 μg/L or serum amylase/lipase > upper limit of normal between 0-2 days after surgery [11]. All patients gave informed consent to the processing of their clinical data before enrollment in the study. The ethical standards defined in the "Declaration of Helsinki of the World Medical Association" of 2004 were observed.

During hospitalization, patients underwent a standard set of laboratory tests (general and biochemical blood and urine tests) and instrumental studies (gastroscopy and radiological imaging methods). To monitor the development of the pathological process, these studies were repeated in the dynamics. Patients also underwent ultrasound examination of the abdominal cavity in the postoperative period. If necessary, an additional X-ray examination of the chest and computed tomography of the abdominal cavity and retroperitoneal space were performed.

Statistical processing of the obtained results was performed using SPSS software 24.0. The data were tested for normality of distribution using the W-test (Shapiro-Wilk test). Visualization was carried out using the R program package, version 4.3.3. Kernel density plots were created for data visualization. For a general idea of the distribution of data and their characteristics, arithmetic mean and standard deviations were calculated. Quantitative values were presented in the form of median and interquartile range (25th and 75th percentiles) in the case of non-normal distribution of indicators, as determined by the W-test (Shapiro-Wilk criterion) and in the form of mean (M) ± standard deviation of the mean (σ) in the case of normal distribution (p by W-test > 0,05). Continuous variables were compared using the Kruskal-Wallis sum of ranks test. Categorical variables were expressed as absolute and relative (%) frequency and compared using the χ^2 test or the Fisher's exact test. The significance level was set at 95%, and the p-value was considered statistically significant if it was less than 0,05.

RESULTS

The incidence of POAP after endoscopic interventions was 18,25%, after urgent abdominal surgery – 0,3%. Analyzing the age and gender of patients, in patients with POAP after abdominal surgery, except for the ERCP technique, there was a significant prevalence of men over women – 59,3% and 40,7%, respectively ($p<0,05$). At the same time, in the group of patients with ERCP-induced pancreatitis, there was a significant predominance of women over men – 72% and 28%, respectively ($p<0,05$).

The mean age of patients with POAP was 55,56±14,12 years and did not differ between women and men with POAP of different etiologies, except for ERCP-induced

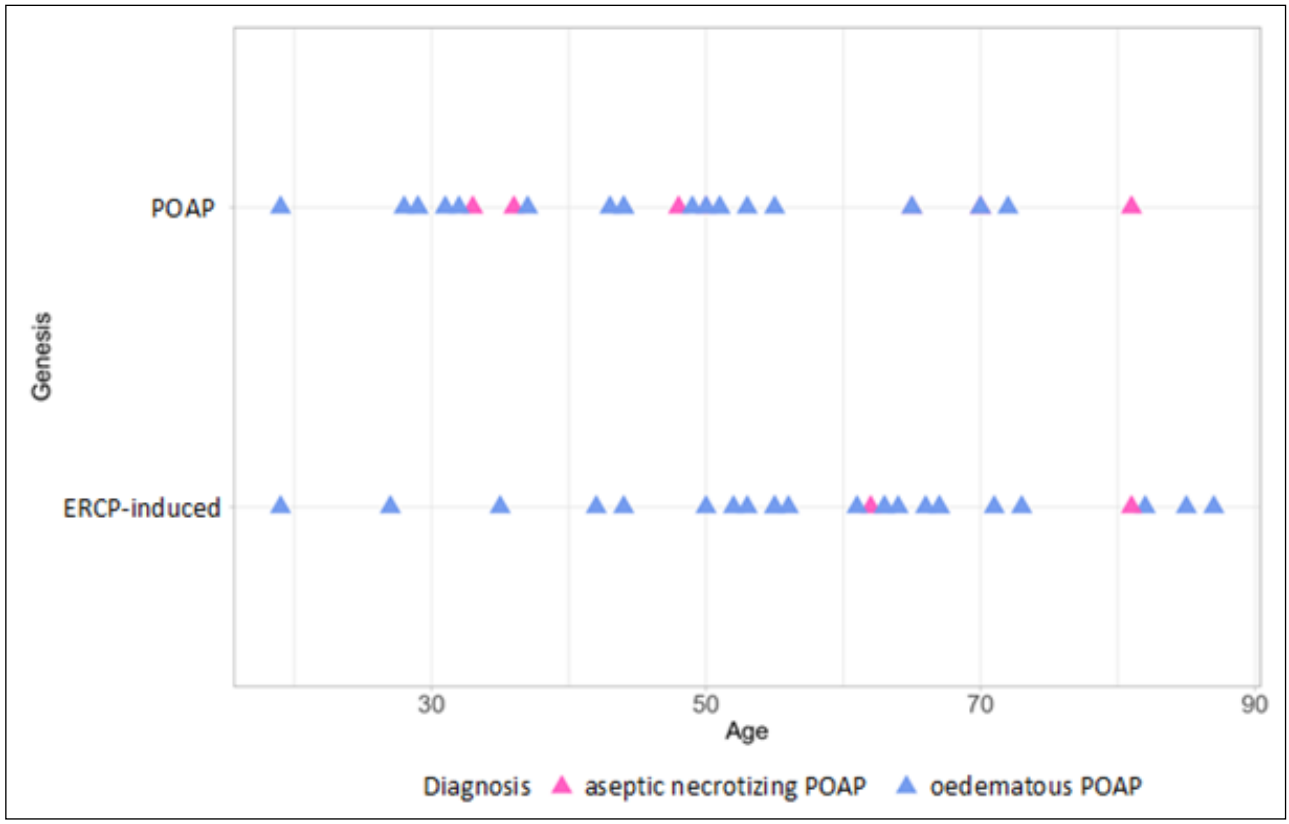

Fig. 1. Distribution of patients with POAP of different genesis by morphological type of inflammation and age.

APPD. Instead, the mean age of men with ERCP-induced pancreatitis significantly exceeded the mean age of women with AP of similar etiology – 67,57±12,08 and 55,94±12,73, respectively (p<0,05) (Table 1).

The development of POAP was characteristic in all age groups, regardless of the etiology and morphologic type of inflammation. The age range of patients did not depend on the etiology and morphologic type of pancreatic inflammation and ranged from 19 to 87 years (Fig. 1).

All surgical interventions that resulted in the development of POAP are conditionally classified as interventions with direct and indirect effects on the pancreas. The high incidence of POAP after operations on internal organs that are anatomically or functionally related to the pancreas is natural. In particular, ERCP-induced POAP was dominant in the structure of POAP development – 48,1% (n=25), which is explained by increased pressure in the ductal system of both the pancreas and extrahepatic bile ducts, trauma of the major duodenal papilla during papillotomy, and its posttraumatic edema. Gastric surgery was the second most common cause of POAP, which consisted one fourth part of all causes of POAP. Intraoperative damage of blood vessels, pancreatic parenchyma, or ducts is often the cause of POAP after these operations, which is especially common in large penetrating ulcers.

Splenectomy was the cause of the development of POAP in 9,6% (n=5) of all patients with POAP. As a rule, this occurs during technically difficult surgical interventions, often accompanied by intraoperative trauma of the tail of the pancreas, especially in patients with polytrauma against the background of intraperitoneal bleeding and anemia, hemorrhagic shock. Surgical interventions on the colon caused the development of POAP in 3,85% (n=2) patients and were not associated with the direct involvement of the pancreas in the pathological process, but they were associated with the peculiarities of mobilization of the tissues surrounding the gland, as well as liver resection.

As for small bowel surgery, it caused the development of POAP in 3,85% (n=2) cases and, in our opinion, developed as a result of the complex effect of indirect factors (metabolic, circulatory disorders, intestinal paresis, etc.). Laparoscopic cholecystectomy is rarely complicated by postoperative pancreatitis, and it was the cause of the development of POAP in 3,85% (n=2) patients. Surgical interventions on the pancreas itself are the most common and pathogenetically sound cause of the development of POAP, when direct trauma to the pancreatic parenchyma, its ducts and peripancreatic tissue, with the release of cytokinase, which activates pancreatic enzymes and a subsequent cascade of biochemical reactions leading to self-digestion of the pancreas.

Table 1. Distribution of patients with POAP by etiology and age

Type of AP	Average age of patients		p
	Men	Women	
POAP (n = 27)	48,5±10,94 (n=16)	48,09±15,92 (n=11)	NS
ERCP-induced AP (n = 25)	67,57±12,08 (n=7)	55,94±12,73 (n=18)	<0,05

Note: NS – non-significant.

Table 2. Distribution of patients by type of surgery that resulted in POAP

№	Surgeries that resulted in POAP (n=52)	Number of patients (n=52)
1.	Surgery on the pancreas	2 (3,85%)
2.	Splenectomy	5 (9,6%)
3.	Surgery on the stomach: Resection of the stomach Suturing of a perforated gastroduodenal ulcer Excision and suturing of a perforated gastroduodenal ulcer	13 (25%) 2 5 6
4.	Laparoscopic cholecystectomy	2 (3,85 %)
5.	Surgeries on the small and large intestine: Operations on the colon (left-sided hemicolectomy, Hartmann's operation + atypical liver resection) Surgery on the small intestine (resection of the intestine, liquidation of the intestinal volvulus)	4 (7,7%) 2 2
6.	Stab wound of internal organs	1 (1,9%)
7.	Endoscopic surgical interventions: ERCP, litho-extraction for gallstone disease ERCP, litho-extraction for postcholecystectomy syndrome ERCP, stenting for malignant tumors of the pancreaticobiliary system	25 (48,1%) 19 3 3

Table 3. Intergroup comparison of patients with different POAP genesis by morphological type and severity of the disease

№	The criterion	POAP (n=27)	ERCP-induced (n=25)	P-value
1.	Morphological type Edematous-interstitial Necrotic aseptic	 19 (70,4%) 8 (29,6%)	 23 (92%) 2 (8%)	0,078*
2.	Severity of the course Mild Moderate Severe	 18 (66,7%) 7 (25,9%) 2 (7,4%)	 17 (68%) 7 (28%) 1 (4%)	NS

Notes: * – the Fisher's exact test; NS – non-significant.

In 2 (3,85%) cases, pancreatic surgery was complicated by POAP. Such a small number of complications can be explained by the fact that the medical institutions where the material was collected are not specialized centers for pancreatic surgery and the total number of surgical interventions on the pancreas is single. Surgical intervention for penetrating stab wounds of the abdominal cavity with damage of the elements of the hepatoduodenal ligament had a direct traumatic genesis of parapancreatic structures and was predictably complicated by the development of POAP (Table 2).

The distribution of patients by the morphological type of POAP was performed similarly to that of AP, based on the criteria of the Atlanta classification 2012. According to them, among all patients with POAP, the edematous-interstitial type of pancreatic inflammation dominated without significant gender preferences and with the involvement of patients of all age groups – 42 (80,8%), the rest of the patients developed necrotizing aseptic POAP – in 10 (19,2%) patients, which was characterized by significant differences in age and gender distribution – in particular, there was a significant increase in the frequency of POAP in women with age, while in men POAP developed in all age groups (Fig. 2).

The issue of classification of POAP by severity is still being actively discussed in the world. We stratified patients according to the severity of the course in accordance with the criteria of the Atlanta 2012 classification. According to it, patients with no complications and no organ disorders had

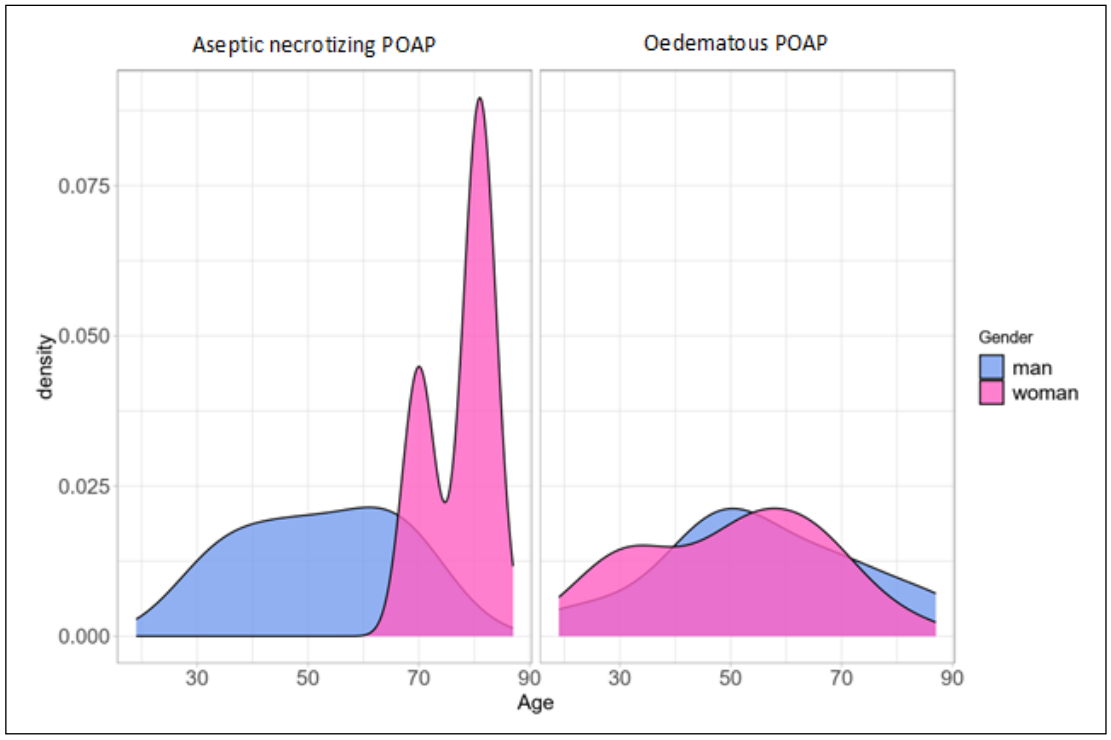

Fig. 2. Distribution of patients by age, gender and morphological type of POAP.

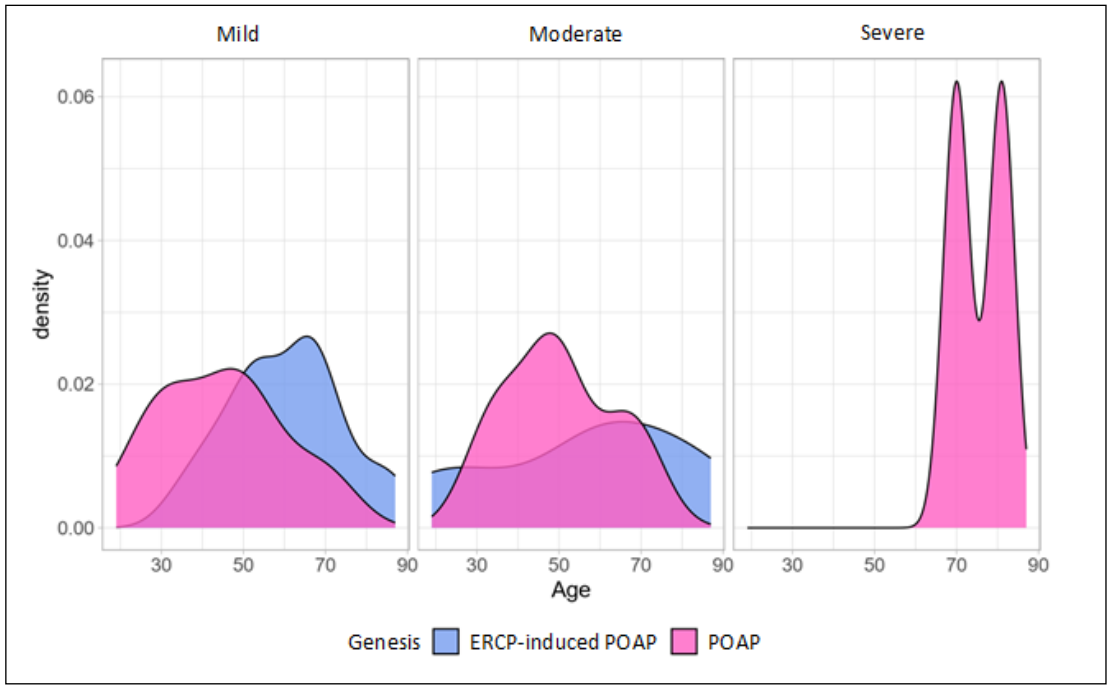

Fig. 3. Distribution of patients by age, genesis and severity of POAP.

mild POAP, moderate POAP was diagnosed in patients with complications and/or transient organ failure. Severe POAP was diagnosed in the presence of persistent organ failure (>48 hours). Thus, the vast majority of patients had a mild course of POAP – 35 (67,3%) cases, 14 (26,9%) had moderate severity of POAP, and 3 (5,8%) patients had severe POAP. Intergroup analysis of various etiological forms of POAP did not reveal a significant difference between groups in morphological forms of the disease with a slight tendency towards reliability and in the severity of the course of the disease (Table 3).

Regarding the age distribution in different etiologic forms of POAP, in both etiologic groups there was a tendency to increase the severity of POAP with age. This can be explained primarily by an increase in the number of comorbidities with age, and with an increase in the number of comorbidities, the severity of POAP (Fig. 3).

DISCUSSION

POAP can develop after any abdominal surgery. The incidence of POAP varies considerably, which, accord-

ing to a recent meta-analysis in 2023, depended on the definition and diagnostic criteria used by the authors [12]. The high incidence of this disease after surgery on organs that have an anatomical and functional relationship with the pancreas is logical, but the development of this pathology is not limited to them and can occur after any intervention on the abdominal organs [13, 14]. In our study, the incidence of POAP after transpapillary endoscopic interventions was 18,25%, while after urgent abdominal surgery, this figure was 0,3%.

In our opinion, such a low incidence of POAP is due to several reasons: insufficient diagnosis due to the lack of a clear definition of the criteria for this pathology; false association of deterioration in the early postoperative period with intra-abdominal complications due to the underlying pathology; a large number of surgical interventions used in the formula for calculating the incidence rate due to hernia repairs, appendectomies, and other operations.

In recent years, significant progress has been made in the study of this pathology, unified criteria for diagnosis and definition of POAP have been proposed, and new views on the etiology have been described. However, in the vast majority of cases, all of this relates to post-resection pancreatitis, in which there is direct trauma of the pancreatic parenchyma and a cascade of complex biochemical reactions is triggered [15-18]. Instead, there are currently no clear unified criteria for diagnosing POAP after urgent and other abdominal interventions, so the issue of POAP requires further comprehensive study.

There are certain gender-specific patterns in the distribution of POAP. As our study showed, the frequency of POAP in men and women differed depending on the etiology. The prevalence of women by 2,5 times over the number of men in the group with ERCP-induced POAP is natural, since women suffer from cholelithiasis 2-3 times more often [19]. In contrast, in the group of patients with POAP not associated with ERCP, there was a predominance of men over women in the ratio of 1,45:1.

Most often, POAP developed after operations on organs that have a direct anatomical and functional connection with the gland due to direct or indirect effects of mechanical or chemical factors: after endoscopic transpapillary interventions – 25 (48,1%), gastric and duodenal operations – 13 (25%), splenectomy – 5 (9,6%).

POAP can develop at any age, regardless of gender. In our study, the age of patients ranged from 19 to 87 years. There was an increase in the incidence of POAP with the age of up to 70 years, after 70 years the incidence of this pathology decreased again, which, in our opinion, was due to the limited scope of surgical interventions in patients of this age group in the setting of concomitant diseases.

The gender and age distribution of patients with POAP in different morphologic forms of AP was quite interesting. In particular, in the edematous form, there was a similar distribution of women and men, as well as the number and age of patients – an increase in the incidence of POAP with age, the highest peak incidence from 50 to 60 years, followed by a gradual decrease. In the necrotic form, there was an increase in the incidence in women over 65 years of age. Previous studies have established that age older than 65 years, together with the diameter of the pancreatic duct, the thickness of the pancreas, neoadjuvant therapy, resection at the level of the body and tail, as well as neuroendocrine histology are independent predictors of acute postoperative pancreatitis [20]. Regarding the severity of POAP, regardless of gender and etiologic distribution, the edematous form of the disease was dominant – 67,3%(35). This echoes the data of other authors, who also noted the predominance of mild and moderate severe forms of acute pancreatitis in patients with postoperative pancreatitis [2].

CONCLUSIONS

The issue of a unified definition of POAP after operations on internal organs of nonresectional genesis is not resolved. In the structure of acute pancreatic complications, operations on internal organs that have a direct anatomical and functional relationship with the pancreas dominate. Further in-depth study of the incidence of nonresectional POAP, its gender and age characteristics, and etiology is needed to develop effective strategies for the prevention of POAP in the future.

PROSPECTS FOR FURTHER RESEARCH

Further studies are needed to expand and provide a better understanding of postoperative pancreatitis and the development of more effective approaches to its prevention and treatment.

Prospects for future research in the field of postoperative pancreatitis of nonresectional genesis may include several areas. An in-depth study of pathogenesis with a detailed investigation of the mechanisms of postoperative pancreatitis, including the role of inflammatory mediators, immune response and the impact of various surgical procedures on pancreatic tissues. This will allow to better understand the processes that cause the development of this complication.

The development and implementation of new preventive strategies is urgent. Further research should be aimed at developing new methods of preventing postoperative pancreatitis, including pharmacological and non-pharmacological approaches. Particular attention should be paid to ways to reduce pancreatic trauma during surgery.

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This article was written within the framework of the research work of the department of surgery No2 with the course "Fundamentals of Dentistry" on the topic "Pathomorphofunctional, pathomorphometric and biochemical parallels of the mechanisms of development of complications of acute surgical pathology: forecasting, diagnosis, individualization of the choice of treatment tactics", 0121U110095, implementation date: 2021 -2025. There were no other sources of funding. All patients undergoing inpatient treatment gave their consent to the processing of their personal data and information obtained during examination and treatment. The collection and analysis of clinical and laboratory material was performed in accordance with all ethical standards defined in the Declaration of Helsinki.

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Tetiana V. Formanchuk
National Pirogov Memorial Medical University
56 Pirogov St., 21018 Vinnytsia, Ukraine
e-mail: mitykt@gmail.com

ORCID AND CONTRIBUTIONSHIP
Tetiana V. Formanchuk: 0000-0002-9565-8213 A C D F
Maksym A. Hudz: 0000-0002-3980-5653 B E
Oleg V. Honcharenko: 0000-0003-0740-9012 B F
Hryhoriy V. Lapshyn: 0000-0002-2030-9748 E F

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

RECEIVED: 10.05.2024
ACCEPTED: 22.10.2024

ORIGINAL ARTICLE

CONTENTS

The effect of cell therapy on the intensity of lipid peroxidation processes in the liver, kidneys and lungs of rats of different ages under conditions of experimental cranio-skeletal trauma

Nataliia V. Izhytska[1], Yurii I. Sushko[1], Arsen A. Hudyma[2], Olha A. Zachepa[3], Iryna V. Antonyshyn[2], Olha V. Denefil[2]

[1]LVIV NATIONAL MEDICAL UNIVERSITY NAMED AFTER DANYLO HALYTSKYI, LVIV, UKRAINE
[2]I. HORBACHEVSKY TERNOPIL NATIONAL MEDICAL UNIVERSITY, MINISTRY OF HEALTH OF UKRAINE, TERNOPIL, UKRAINE
[3]MILITARY MEDICAL CLINICAL CENTER OF THE WESTERN REGION, LVIV, UKRAINE

ABSTRACT

Aim: To determine the effect of cell therapy on the intensity of lipid peroxidation processes in the liver, kidneys and lungs of rats of different ages under conditions of experimental cranio-skeletal trauma (CST).

Materials and Methods: In the experiments, 129 white male Wistar line rats of different age groups were used: immature rats aged 100-120 days and weighing 90-110 g; mature rats aged 6-8 months and weighing 180-200 g; and old rats aged 19-23 months and weighing 300-320 g. In each age group, CST was modeled under thiopental sodium anesthesia. The control rats were only injected with thiopental sodium anesthesia. For the purpose of correction, cryopreserved neuroblast cells (NBC) from Wistar line rats were injected intravenously at a dose of 0,5 ml*106 cells in groups of injured rats of different age groups. Additionally, in separate groups, rat mesenchymal stem cells (MSC) were injected intraperitoneally at a dose of 0,25 ml*105 cells per rat. The animals were taken out of the experiments using anesthesia after 14 days by total heart bleeding. The content of thiobarbituric acid reagents was determined in 10 % extract of liver, kidney and lung homogenate.

Results: In 14 days after the CST was applied in rats of different age groups, a significantly higher content of TBA-active lipid peroxidation products was observed compared to control groups of rats of the corresponding age. Under conditions of NBC monotherapy in experimental groups of different aged rats, a decrease in the content of TBA-active lipid peroxidation products occurred in the liver, kidney and lungs, but the result was statistically significant only in the group of mature rats. The injection of a combination of NBC and MSC for the purpose of correction was accompanied by a significantly greater effect compared to rats without correction. After 14 days of post-traumatic period, the content of TBA-active lipid peroxidation products in the liver and kidneys of different aged rats significantly decreased. In these organs, as well as in the lungs of mature rats, the combination therapy showed a greater antioxidant effect compared to rats without correction and rats with NBC monotherapy. The obtained results shed light on the specificity of the systemic antioxidant effect of combined NBC and MSC cell therapy in rats of different age groups with CST, which should be taken into account in the development of cell transplantation strategies under conditions of severe combined trauma.

Conclusions: Combined transplantation of NBC and MSC during acute period of CST among rats of different age groups is accompanied by a systemic antioxidant effect, which is manifested by a significant decrease in the content of TBA-active lipid peroxidation products in the liver, kidney and lungs, the degree of which is mostly marked among mature rats.

KEY WORDS: cranio-cerebral trauma, skeletal trauma, liver, kidney, lungs, oxidative stress, cell therapy

Wiad Lek. 2024;77(11):2127-2134. doi: 10.36740/WLek/197081 DOI

INTRODUCTION

Combined cranio-cerebral and skeletal trauma affects millions of people worldwide each year and happens to individuals of all ages. Such traumas are accompanied by significant severity and high mortality. All of the survivors are often affected by persistent disorders of the central nervous system and musculoskeletal system. According to the Global Disease Burden study, only the incidence of CCT in 2016 amounted to 27,1 (24,3-30,3) million cases, with a prevalence of 55,5 (53,4-57,6) million [1]. During the acute phase of CCT (up to 2 days), primary neuronal and ganglion damage is noted due to mechanical destruction of nervous and vascular structures with hemorrhages, ischemia, edema, and damage of the blood-brain barrier. In the future, secondary degeneration of nerve structures is noted (2-14 days), which is caused by acute inflammation and ischemia. In its pathogenesis, the formation of reactive

oxygen species, glutamate toxicity, and the release of proapoptotic cytokines play a leading role [2, 3]. All these factors lead to serious neurological dysfunction, localized disorders in the affected bone segment [4] and systemic disorders with the development of traumatic disease (TD) [5].

Despite significant advances in understanding the pathogenesis of cranio-skeletal trauma (CST), there is still no ideal treatment for such an injury. This is due to physiologically low postnatal neurogenesis and a high probability of complications caused by secondary damage to internal organs with the formation of multiorgan dysfunction and deficiency [6, 7].

During treatment strategy for CST after the survivor has recovered from shock, the prevention of secondary brain damage and systemic disorders, as well as stimulation of neurogenesis, which is primarily aimed at reducing neurological deficits, are at the forefront. Under these conditions, therapy involves overcoming the key factors of CST pathogenesis, such as hypoxia, oxidative stress, systemic response of the organism to inflammation, and endogenous intoxication syndrome [5, 8, 9].

According to the data from the literature, cell therapy is a promising pathogenetically based mean of correcting CST. The therapeutic effect of cell transplantation under conditions of CCT has been widely studied over the last 35 years [10]. The main hypotheses of the therapeutic effect are that transplanted cells can differentiate into functional neurons and glial cells, replacing lost tissue and restoring functional networks, or synthesize factors to reduce secondary degeneration of nerve cells and enhance their recovery. In recent years, the systemic effect of cell therapy under conditions of CST has also been confirmed. Thus, intraperitoneal injection of fetal nerve cells under conditions of CST in the acute period, the period of early and late manifestations of TD in the liver, lungs and kidneys contributed to a decrease in the intensity of lipid peroxidation processes, provided protection of the enzyme and glutathione links of antioxidant defense, reduced the manifestations of immune reactions and cytokinogenesis, slowed down microscopic and submicroscopic changes in the studied organs [11, 12].

Pluripotent stem cells have the ability to self-renew and differentiate into other cells. There is more and more evidence in the literature that the functional capacity of stem cells depends on the age. As the organism ages, the ability of stem cells to renew differentiation deteriorates [13]. However, the effect of cell therapy on the course of systemic disorders under conditions of CST depending on age is almost unstudied.

AIM

The aim of the study is to determine the effect of cell therapy on the intensity of lipid peroxidation processes in the liver, kidneys, and lungs among rats of different age groups under experimental CST.

MATERIALS AND METHODS

For the experiments, 129 white male Wistar line rats of three age groups were randomly selected at the vivarium of the I. Horbachevsky Ternopil National Medical University: immature rats aged 100-120 days and weighing 90-110 g; mature rats aged 6-8 months and weighing 180-200 g; and old rats aged 19-23 months and weighing 300-320 g. All rats were maintained on a standard vivarium diet with constant access to water.

In each age group (43 rats in each), under thiopental sodium anesthesia (40 mg·kg^{-1}), CST was modeled according to the method described in work [14]. In immature (young) rats, a dosed mechanical impact was first applied to the left femur with a solid instrument with a wedge-shaped nozzle and an energy of 0,320 J, achieving a closed fracture of the femur. Next, a dosed impact to the skull with an energy of 0,226 J was applied with a dull instrument at a point 3 mm anterior to the interaural line. In mature (adult) rats, a similar impact with an energy of 0,637 J was applied to cause a femur fracture, and a dosed impact to the skull at a point 5 cm anterior to the interaural line with an energy of 0,375 J was applied. In old rats, femoral fracture was achieved by applying a dosed hit to the femur with an energy of 0,796 J, and CCT - by a dosed hit to the skull with an energy of 0,549 J at a point 6 mm anterior to the interaural line.

Impact energy among rats of different age groups caused moderate CCT [5]. In the experiments, no rats with penetrating skull injuries or open femur fractures were used. In the control groups (7 rats each), the animals were only injected with thiopental sodium anesthesia.

For the purpose of correction, cryopreserved neuroblast cells (NBC) of Wistar line rats (ASK Health Medical Diagnostic Center Limited Liability Company, Kharkiv, Ukraine) and rat mesenchymal stem cells (MSC) cultured to the fourth passage, isolated from umbilical cords of Wistar female fetuses at the late stage of gestation, obtained from the cell culture laboratory of the I. Horbachevsky Ternopil National Medical University, Ministry of Health of Ukraine were used in groups of rats of different ages with CST.

After defrosting, the neuroblast cell suspension (NCS) was injected into the tail vein at 12 h after modeling CST in 7 animals of each age group at a dose of 0,5 ml that contained

106 cells. In another experimental group, the injection of neuroblasts and intraperitoneal injection of 0,25 ml of MSC suspension at a dose of $5*10^5$ cells per rat were combined [15]. The effectiveness of cell therapy was evaluated after 14 days. In the comparison groups, physiological solution was injected intravenously and intraperitoneally.

The rats were taken out of the experiments under conditions of anesthesia by total bleeding from the heart. The cooled and blood-washed liver, kidneys, and lungs were homogenized in a Silent Crasher 75000 homogenizer (Germany). The systemic effect of the CST on the rat's body of different ages was evaluated on the basis of the activity of lipid peroxidation (LPO). The content of thiobarbituric acid reagents (TBA-active products of lipid peroxidation) was determined in 10 % of the liver, kidney, and lung homogenate extracts according to the method described in work [16] using a LabAnalyt SP-V1000 spectrophotometer (Granum, China).

The experiments were performed in accordance with the "General Ethical Principles for Experiments on Animals" adopted by the First National Congress on Bioethics (Kyiv, 2001) and agreed with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), as well as the conclusion of the Bioethics Commission of the I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine No. 72 from 06.01.2023.

The obtained digital material was processed in the STATISTICA software package (StatSoft Inc., USA). The median (Me), lower and upper quartiles (LQ, UQ) were determined. For an independent comparison of the degree of indicators deviation among animals of different age groups, the average ratio of individual values of the studied indicators to the average value of the control group was calculated [17]. The significance of differences was assessed by the nonparametric Mann-Whitney criterion.

RESULTS

The research has shown that in 14 days after modeling of CST among rats of different ages, compared to the control, a statistically significantly higher content of TBA-active lipid peroxidation products in the liver was observed (Fig. 1): among immature rats in 2,31 times ($p<0,05$), among mature rats in 2,33 times ($p<0,05$), among old rats in 2,37 times ($p<0,05$). The use of NBC among rats with CST of different age groups compared with rats without correction caused a decrease in the concentration of TBA-active lipid peroxidation products in the liver, but only among mature rats the result was statistically significant. The indicator decreased by 16,8 % ($p<0,05$). Considering all age groups rats, the indicator continued to be significantly higher than in the control ($p<0,05$). Under the influence of the combined injection of NBC and MSC to rats of different ages with CST there was an even greater decrease in the content of TBA-active lipid peroxidation products in the liver. Compared with rats without correction, the indicator decreased by 20,0 % ($p<0,05$) among immature rats, by 31,9 % ($p<0,05$) among mature rats, and by 10,8 % ($p<0,05$) among old rats. Compared with injured rats injected just with NBC, the combined cell therapy caused a statistically significant decrease in the content of TBA-active lipid peroxidation products only in the group of mature rats (by 18,2 %, $p<0,05$). Despite ongoing treatment, the indicator continued to be statistically significantly higher than in the control group ($p<0,05$).

Comparison of the average ratio of the individual values of TBA-active lipid peroxidation products in the liver of different aged rats in 14 days after causing CST to the average value of rats without correction (Table 1) showed that under the conditions of only NBC injection, the studied indicator between rats of different age groups showed no statistically significant difference ($p>0,05$). At the same time, under the conditions of combined cell therapy among mature rats, the indicator was statistically significantly lower than among immature (by 12,5 %, $p<0,05$) and old rats (by 20,4 %, $p<0,05$). Furthermore, the combined cell therapy caused a statistically significant decrease in the value of the index in the groups of immature and mature rats (respectively by 13,0 and 20,4 %, $p<0,05$).

The analysis of the content of TBA-active lipid peroxidation products in the kidney under the influence of CST among rats of different ages revealed (Fig. 2) that after 14 days the indicator was significantly higher than in the control: among immature rats - in 2,41 times ($p<0,05$), among mature rats - in 2,97 times ($p<0,05$), among old rats - in 3,49 times ($p<0,05$). Under the influence of NBC injection, compared to rats without correction, the indicator decreased. The result was statistically significant in the groups of immature and mature rats (respectively, by 10,5 and 15,7 %, $p<0,05$). The combined NBC and MSC injection among rats of different ages caused a further decrease in the content of TBA-active lipid peroxidation products in the kidney. In the group of immature rats, compared with rats without correction, the indicator decreased by 18,8 % ($p<0,05$), in the group of mature rats - by 35,6 % ($p<0,05$), in old rats group - by 8,7 % ($p<0,05$). Compared to rats injected just with NBC, the combined cell therapy was accompanied by a decrease in the content of TBA-active products of lipid peroxidation in the kidney only in the group of mature rats (by 23,6 %, $p<0,05$).

Table 1. The effect of cell therapy on the average ratio of individual values of TBA-active lipid peroxidation products in the liver of different aged rats to the average value of rats without correction at 14 days after cranio-skeletal trauma, Me (LQ;UQ) - median (lower and upper quartiles)

Animal group	NBC	NBC+MSC	p
Immature	0,92 (0,85; 0,95)	0,80 (0,77; 0,84)	<0,05
Mature	0,88 (0,81; 0,91)	0,70 (0,67; 0,74)*	<0,05
Old	0,94 (0,91; 0,95)	0,88 (0,83; 0,96)#	>0,05

Notes. Here and in Tables II, III:
1. * - differences in the group of immature rats are statistically significant (p<0,05).
2. # - differences in the group of mature rats are statistically significant (p<0,05).

Table 2. The effect of cell therapy on the average ratio of individual values of TBA-active lipid peroxidation products in the kidney of different aged rats to the average value of rats without correction at 14 days after cranio-skeletal trauma, Me (LQ;UQ) - median (lower and upper quartiles)

Animal group	NBC	NBC+MSC	p
Immature	0,90 (0,86; 0,95)	0,84 (0,81; 0,89)	>0,05
Mature	0,84 (0,83; 0,90)	0,67 (0,63; 0,68)*	<0,05
Old	0,94 (0,91; 0,95)	0,92 (0,89; 0,96)#	>0,05

Table 3. The effect of cell therapy on the average ratio of individual values of TBA-active products of lipid peroxidation in the lungs of different aged rats to the average value of rats without correction at 14 days after cranio-skeletal trauma, Me (LQ;UQ) - median (lower and upper quartiles)

Animal group	NBC	NBC+MSC	p
Immature	0,94 (0,88; 0,95)	0,90 (0,88; 0,93)	>0,05
Mature	0,86 (0,83; 0,88)	0,77 (0,71; 0,79)*	<0,05
Old	0,96 (0,90; 1,02)#	0,94 (0,89; 0,97)#	>0,05

The research of the cell therapy effect on the average ratio of individual values of TBA-active lipid peroxidation products in the kidney of different age rats to the average value of rats without correction in 14 days after cranio-skeletal injury (Table 2) showed that under the conditions of only NBC injection, the indicator between the experimental groups of rats of different ages did not statistically significantly vary (p>0,05). Under the conditions of combined therapy, the indicator was significantly lower in the group of mature rats compared to immature rats (by 20,2 %, p<0,05) and old rats (by 27,2 %, p<0,05). Under conditions of combined cell therapy compared with NBC monotherapy, the indicator became lower in all experimental groups of rats, but only in the group of mature rats the result was statistically significant - by 20,2 % (p<0,05).

In turn, an increase in the content of TBA-active products of lipid peroxidation was also observed in the lungs of different age rats after 14 days under the influence of CST. Compared to the control group, index of immature rats increased in 2,17 times (p<0,05), of mature rats - in 2,10 times (p<0,05), of old rats - in 2,50 times (p<0,05). Under the influence of NBC injection among rats of different age groups, the indicator decreased, but only in the group of mature rats the result was statistically significant (by 14,5 %, p<0,05). The combined cell therapy did not significantly change the content of TBA-active lipid peroxidation products among immature and old rats compared to the injured rats without correction, but among mature rats it caused a statistically significant decrease in the value of the studied indicator - by 21,0 % (p<0,5). Likewise, in this group, the index became lower in the background of combination therapy compared to rats with NBC monotherapy (by 7,6 %, p<0,05).

The average ratio of individual values of the content of TBA-active lipid peroxidation products in the lungs of different aged rats to the average value of rats without correction 14 days after cranio-skeletal trauma (Table 3) under the influence of NBC was the lowest in the group of mature rats. The differences were statistically significant compared to the group of old rats - by 10,4 % (p<0,05). Under the influence of combined cell therapy, the indicator was also the lowest in the group of mature rats and statistically significantly different from the group of immature rats (by 14,4 %, p<0,05) and old rats (by 18,1 %, p<0,05). In the group of mature rats, combined cell therapy was accompanied by a significant decrease in the value of the studied indicator compared to NBC monotherapy (by 10,4 %, p<0,05).

DISCUSSION

The results obtained indicate that the activation of lipid peroxidation processes in the liver, kidneys and lungs under the influence of CST among rats of different ages is prolonged in time. Even after 14 days of the

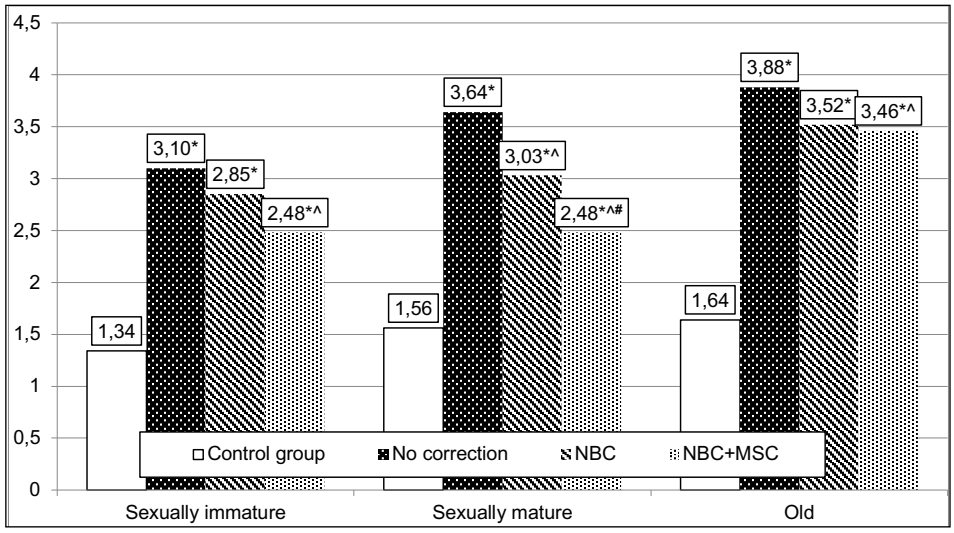

Fig. 1. Effect of cell therapy on the content of TBA-active lipid peroxidation products in the liver at 14 days after cranio-skeletal trauma in rats of different ages.
Note: * - differences in injured rats without correction are statistically significant, $p<0,05$; # - differences in injured rats with NBC correction are statistically significant, $p<0,05$).

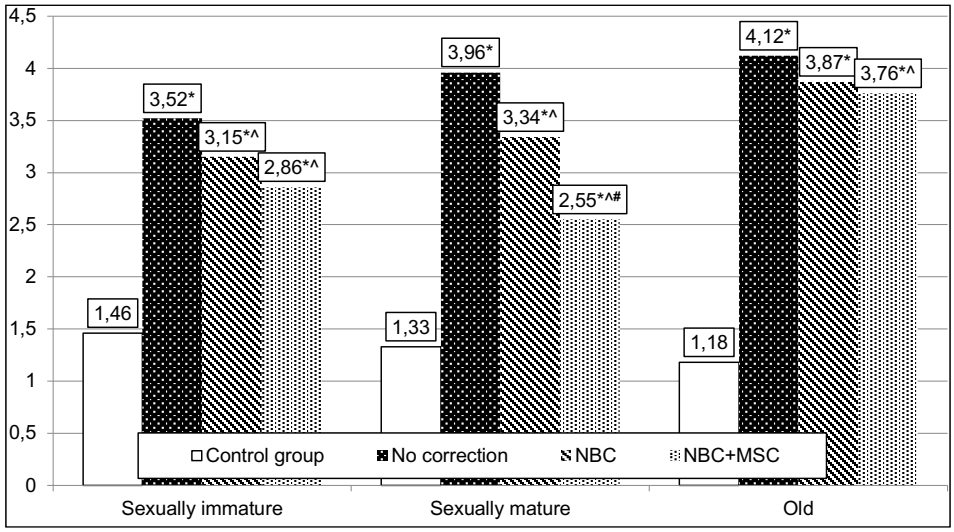

Fig. 2. Effect of cell therapy on the content of TBA-active lipid peroxidation products in the kidney at 14 days after cranio-skeletal trauma in rats of different ages.

post-traumatic period, a significantly higher content of TBA-active lipid peroxidation products was observed in the studied organs compared to the control rats groups of the corresponding age. A prolonged increase in the content of lipid peroxidation products, the source of which is the affected brain and limb tissue, under the influence of CST was also noted by other authors both in the early [17] and late period of TD [18], but their studies were conducted using only mature rats. For the first time we proved the leading role of oxidative stress in the pathogenesis of CST, which, regardless of age, does not subside by day 14 of the experiment.

Under conditions of NBC monotherapy in experimental groups of rats of different ages, a decrease in the content of TBA-active products of lipid peroxidation in internal organs was observed, but in the liver, kidney and lungs the result was statistically significant only in the group of mature rats, and in the kidney of immature rats. Thus, the intravenous injection of allogeneic neuroblasts led to a decrease in the systemic manifestations of CST. Neuroblasts are immature cells of the neuronal lineage that migrate from their places of formation to targeted brain regions, where they turn into neurons and integrate into neuronal networks [19]. As a rule, neurotransplantation is performed locally in certain brain structures, which is accompanied by a decrease in the degeneration of neurons compromised by trauma. Neuroprotective effect is considered to be a key mechanism by which neuroblasts can improve functional outcomes after experimental damage of the central nervous system [20]. This phenomenon is based on the paracrine effect of neuroblasts due to the secretion of trophic factors involved in neuroprotection [21]. In addition, extra mechanisms may be involved in the protection of injured tissues, such as homeostatic support of compromised cells through gap junctions with transplanted progenitor cells [22].

The systemic antioxidant effect detected by us after NBC injection is apparently due to both neuroprotective effects (reduced source of damage and formation of prooxidant factors) and direct antioxidant effects, as shown in some studies [11, 12]. It is remarkable that

the effectiveness of NBC injection was clearly higher in mature rats, somewhat lower in immature rats, and insignificant in old rats. The low systemic therapeutic effect in old rats is consistent with the concept of "stem cell senescence", which implies the inability of pluripotent stem cells to continue to replace body tissues with a sufficient amount of differentiated cells to maintain the original function of this tissue [13].

Among the mechanisms that limit the functional capabilities of stem cells in aging, the authors primarily identify changes in the microenvironment, such as increased levels of reactive oxygen species and proinflammatory cytokines, insufficient antioxidant protection, hormone imbalance, reduced ability of the body to synthesize ATP, etc. In such conditions, stem cells and progenitor cells obviously have a low capacity for development and realization of their functions.

The use of a combination of a NBC and MSC for correction compared to rats without correction is accompanied by a significantly greater effect. After 14 days of the post-traumatic period, the content of TBA-active lipid peroxidation products in the liver and kidneys of rats of different ages significantly decreased. In these organs, as well as in the lungs of mature rats, the combination therapy shows a greater antioxidant effect compared to rats without correction and rats with NBC monotherapy.

As can be seen, the combined effect, which includes 2 cell types with different levels of differentiation and properties, achieves a higher antioxidant effect in internal organs and is greater in mature rats, which is obviously due to the effect of summing the biological effects of both cell types. In this group, the degree of reduction in the content of TBA-active lipid peroxidation products was also significantly higher compared to rats without correction.

According to data from the literature, the MSC is characterized by the suppression of inflammation and the synthesis of biologically active substances that promote neuroprotection and regeneration. Due to the immunomodulatory effect of MSC, a low level of rejection is characteristic [23]. It has been proven that MSC can reduce secondary degeneration due to the secretion of trophic factors such as vascular endothelial growth factor (VEGF), nerve growth factor (NGF), glial cell-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) [24]. At the same time, age creates a negative background for the therapeutic effect of both NBC and MSC. In immature rats on the background of an excess of stem cells and progenitor cells, additional transplantation of NBC and MSC is accompanied by a moderate systemic antioxidant effect. According to the effectiveness of combined cell therapy, rats of different ages with CST can be divided as follows:

Old rats ← Immature rats ← Mature rats

In summary, the results shed light on the specificity of the systemic antioxidant effect of combined NBC and MSC cell therapy among rats of different age groups with CST, which should be taken into account during development of cell transplantation strategies in cases of severe combined trauma.

CONCLUSIONS

Combined transplantation of both NBC and MSC during the acute period of CST among rats of different ages is accompanied by a systemic antioxidant effect, which is manifested by a significant decrease in the content of TBA-active lipid peroxidation products in the liver, kidney and lungs, the degree of which is mostly marked among mature rats.

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The article was written within the framework of a complex research topic of the Educational and Scientific Institute of Modelling and Analysis of Pathological Processes of the I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine "Research of pathogenetic patterns of development of post-traumatic and inflammatory degenerative injuries of organs and systems and their correction" (State Registration No. 0122U000030).

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Arsen A. Hudyma
Maidan Voli 1, 46001 Ternopil, Ukraine
arsgudyma@gmail.com

ORCID AND CONTRIBUTIONSHIP
Nataliia V. Izhytska: 0000-0001-9406-5435 A E F
Yurii I. Sushko: 0000-0002-7601-7904 A B C D E
Arsen A. Hudyma: 0000-0002-1282-2728 A B E F
Olha A. Zachepa: 0009-0009-8680-7043 B C
Iryna V. Antonyshyn: 0000-0002-1252-9177 A D
Olha V. Denefil: 0000-0002-3606-5215 C D

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

RECEIVED: 07.07.2024
ACCEPTED: 24.10.2024

ORIGINAL ARTICLE

CONTENTS

Influence of acute respiratory diseases on hemodynamic in school-age children

Oleksandr H. Buriak, Yurii M. Nechytailo
BUKOVINIAN STATE MEDICAL UNIVERSITY, CHERNIVTSI, UKRAINE

ABSTRACT

Aim: To assess changes of blood pressure and central hemodynamic in children with acute respiratory diseases.

Materials and Methods: A total of 40 children aged 10-12 years with community acquired pneumonia and acute bronchitis in the period of early recovery were enrolled in the study with data comparing with control group of 30 healthy children. The hemodynamic parameters included blood pressure and calculation of systolic and minute blood output volumes.

Results: In children with respiratory diseases a significant decrease in systolic, pulse and average dynamic blood pressure with a slight difference in diastolic pressure were registered. This may indicate the phenomena of diastolic dysfunction due to increase of pulmonary blood flow resistance and deterioration the process of the left heart ventricles filling in diastole. Average dynamic blood pressure was significantly lower in children with respiratory diseases compared with control group: in patients with pneumonia (mean ± standard error of mean) – 76,1±2,1 mm Hg, with bronchitis – 77,1±1,4 mm Hg, in healthy individuals 82,1±1,6 mm Hg ($p<0,05$). The analysis of cardiac output indicators showed in children with respiratory diseases a decrease in stroke volume but in the same time, the indicator of minute blood output volumes was compensated by higher heart rate without significant difference between the groups.

Conclusions: The hemodynamic reduction in children with acute respiratory pathology could be caused by diastolic dysfunction and disorders of autonomic regulation.

KEY WORDS: acute bronchitis, pneumonia, blood pressure, children

Wiad Lek. 2024;77(11):2135-2139. doi: 10.36740/WLek/197128 DOI

INTRODUCTION

Acute inflammatory diseases of respiratory system in children considered worldwide as the most common and serious infectious cause of high morbidity, hospitalization and mortality [1]. Despite modern means of treatment and disease prevention, the number of the respiratory diseases is not decreasing. This pathology has significant seasonality, increases every year in the fall and winter months and is prone to outbreaks [2, 3]. According to multicenter study, only pneumonia is the cause of death of about 16% of children under 5 years of age worldwide and all inflammatory respiratory diseases are leading to a multitude of complications [4]. Between them, many clinical studies have identified an increase in short- and long-term risk of cardiovascular events following acute respiratory infections [5-7]. The mechanism of these complications remains to be fully established. Blood pressure (BP), heart rate and cardiac output, are the principal hemodynamic processes parameters, which could be affected during respiratory diseases. Episodes of diseases often cause increased heart rates to compensate reduced oxygenation, leading to an elevated cardiac workload and potentially heart failure in severe cases [8].

Analysis of the relationship between respiratory diseases and changes in the cardiovascular system has become especially relevant with the emergence of the COVID-19 epidemic [9]. A number of factors determine the severity of hemodynamic changes, including the degree of arterial narrowing and vasoconstriction, increased heart rates, BP, sufficient microcirculation, providing oxygen supplying to the myocardium relative to demands [10]. Acute infections may stimulate platelet activation by bacterial products or directly through pro-inflammatory cytokines [11]. Indirect platelet activation may also result during infection from mechanical stress induced by changes in blood volume and vascular tone. Pneumonia recovering persons do not return to their preinfection health quickly and sometimes experience an increased risk of cardiovascular changes [12]. The mechanisms of this association are not well understood, but a persistent dysregulated inflammatory

response and hemodynamic changes appears to play a central role. Significant alterations in BP, both hypo- and hypertension, may also occur due to the complex interplay between systemic inflammation and vascular dysfunction. For this reason, there is a need to better understanding the inflammatory cross talk between the lungs and the heart during and after pneumonia or bronchitis to develop on preventing measures of respiratory-associated cardiovascular events [13-15].

AIM

Study aimed to assess changes of blood pressure and central hemodynamic in children with acute respiratory diseases.

MATERIALS AND METHODS

In this study 40 children (age 10-12 years) with acute respiratory diseases in period of early recovery – 20 with pneumonia (mean age [mean (M) ± standard error of mean (SEM)] – 11,0±0,2 years) and 20 with bronchitis (mean age – 11,1±0,2 years), were enrolled. Exclusion criteria for both groups included the presence of any kidney or heart disease, which could influence BP parameters. The obtained data were comparing with measurement from control group of 30 healthy children (mean age – 11,2±0,1 years). All participants underwent a comprehensive assessment of medical history, physical examination and evaluation of BP parameters. BP was measured by oscillometric device (Omron HEM-7121, Omron, Japan) with an appropriately sized cuff. The next value of BP were analyzed: systolic BP (SBP), diastolic BP (DBP), pulse BP (PBP), as well as the average dynamic BP (ABP) according to Hickem's formula (ABP = DBP+PBP/3). As the most important indicators of the contractile function of the heart – systolic (stroke) volume (SV) and minute blood output volumes MBV) were calculated by an indirect method according to Star's formula. Systolic volume = 100+0,5*PBP – 0,6×DBP–0,6×A, where "A" is the child's age in years. The minute blood volume calculated by multiplying SV by the heart rate (HR).

Statistical analysis was done using The Statistical Lab (v.3, CeDiS, 2005, freeware). Quantitative data were presented as M ± SEM. Descriptive statistics, normality of the data distribution assessed using the Shapiro-Wilk test and non-parametric tests, such as the Mann-Whitney U test, were used. All p-values were two-tailed and $p<0,05$ was considered statistically significant.

The study was conducted in accordance with the principles of the World Medical Association`s Declaration of Helsinki «Ethical Principles for Medical Research Involving Human Subjects». Informed consent to participate was obtained from all those included in the study (parents of children or their guardians), which emphasizes the absence of invasive interventions. The study protocol was discussed and approved at a meeting of the Biomedical Ethics Committee of Bukovinian State Medical University.

RESULTS

Children from all three groups did not have a significant difference in anthropometric measurements, especially those which could influenced BP – height and body mass index (BMI). Height and weight were in the middle percentile corridors. Resting BP was classified as normotensive and the pulse rate was in main groups nearly the same as in healthy persons (pneumonia patients – 87,6±2,3 bpm, with bronchitis – 88,0±2,8 and healthy – 83,2±1,9, $p>0.05$). But a mean SBP in both groups of patients were significantly lower than in the control group – figure 1 shows a visual representation of the differences in SBP between the all groups. In contrast to this, the mean DBP was at the same level in the main groups (62,3±1,9 mmHg and 62,6±1,3 mmHg) with non-significant difference compared to the control group (65,8±1,4 mmHg) ($p>0,05$).

In children with respiratory diseases, a decrease in pulse BP was registered (Fig. 2), as a result of a lower level of systolic pressure with a slight difference in diastolic pressure. This may indicate the phenomena of diastolic dysfunction due to deterioration of the process of filling the left heart ventricles in diastole. Such a result may indicate an increase in pulmonary blood flow resistance and an increase in BP in the pulmonary vessels [16]. Another causes may be a decrease in myocardium relaxation during diastole, but for this meter an echocardiographic study should be used to detail the mechanism.

In our study the average dynamic pressure, which is considered an integral indicator of BP was calculated. ABP was significantly lower in children with respiratory diseases compared to the control group: in patients with pneumonia – 76,1±2,1 mmHg, with bronchitis – 77,1±1,4 mmHg, in healthy individuals 82,1±1,6 mmHg ($p<0,05$). In the frequency analysis of ABP data in the corresponding groups is noticeable the direction to smaller values in sick children (Fig. 3).

DISCUSSION

The analysis of cardiac output indicators showed in children with respiratory diseases a decrease in stroke volume, which was the smallest in patients with pneumonia

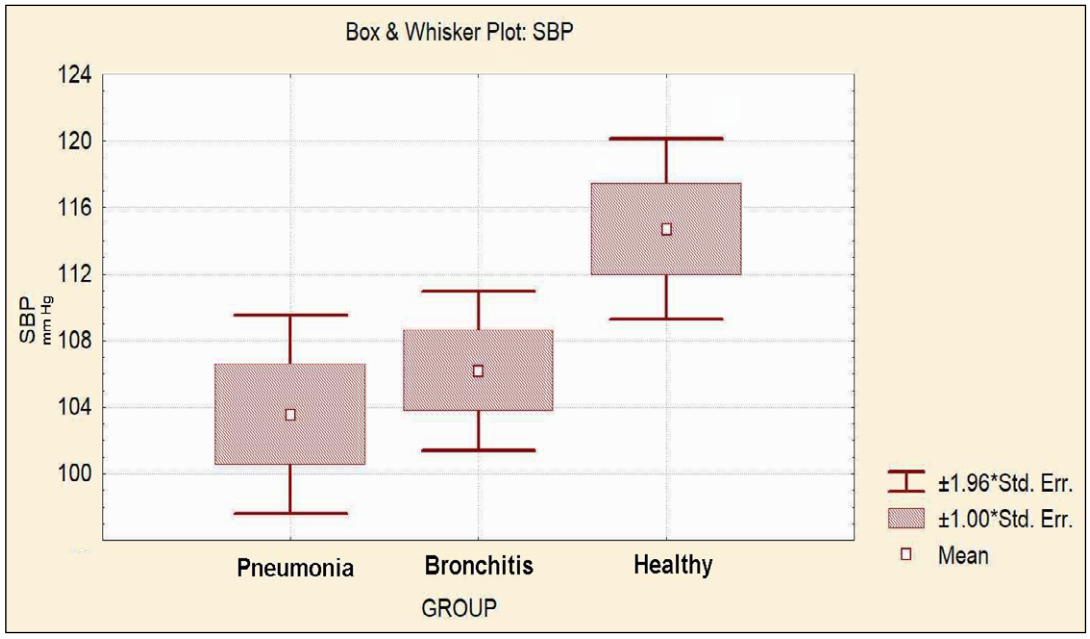

Fig. 1. Comparison of systolic BP between groups.

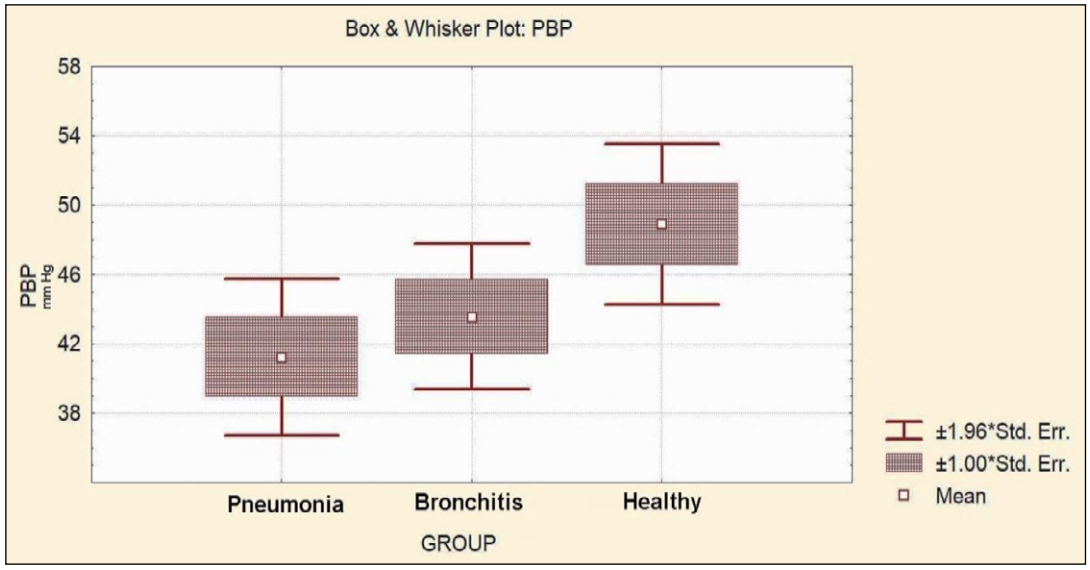

Fig. 2. Comparison of pulse BP between groups.

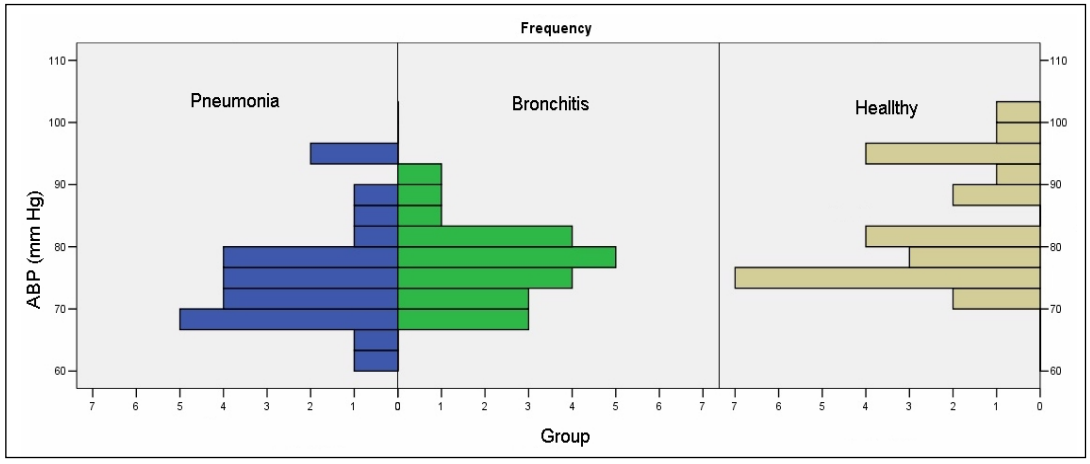

Fig. 3. Comparison of ABP frequency between groups.

(76,5±1,6 ml), when in children with bronchitis it was a little higher (77,6±1,3 ml) but significantly differ with index in healthy persons (81,8±1,4 ml) ($p<0,05$). At the same time, the indicator of MBV was compensated by higher heart rate and was within the range 6,6-6,8 liter per minute without significant difference between the groups. According to investigation of L. Tabacof et al. (2023), the phenomena of SV reduction in respiratory pathology during the recovery period was not related to changes in the myocardium, but to disorders of autonomic regulation – phenomena of dysautonomia [17]. The authors proposed pathophysiologic connections between acute respiratory diseases and dysautonomia because of hypovolemia due to fever, decreased fluid intake, nausea, excessive diaphoresis and prolonged bed rest. These changes are on the basis of increased cardiac sympathetic nervous system outflow, cardiac and extracardiac postganglionic sympathetic neurons activity. In some investigations clinicians show that affected individuals may benefit from effective interventions or oral fluid rehydration and electrolyte repletion as well as pharmacotherapy and autonomic rehabilitation [15]. But by opinion of S. Blitshteyn et al. (2022) such cardiac changes are in fact related to prolonged periods of inactivity, rather than any cardinal pathobiological features of dysautonomia [18]. At the same time, we believe that dysautonomia is the leading pathogenic factor of described hemodynamic changes, but the general mechanisms include the features given by the cited authors. In any case, C. Stotts et al. (2023) indicated on need to better understand the inflammatory cross talk between the lungs and the heart during and after pneumonia and focused on preventing pneumonia-associated cardiovascular events in future. They also discuss opportunities for new clinical strategies to promote inflammatory resolution pathways as a novel therapeutic target to reduce the risk of cardiac events post-pneumonia [15].

CONCLUSIONS

1. In the study, we found that children with acute respiratory diseases in the period of early recover exhibit significantly lower level of systolic pressure and average dynamic BP, with a slight difference in diastolic pressure. This may indicate the phenomena of light diastolic dysfunction.
2. The analysis of cardiac output indicators showed in children with respiratory diseases a decrease in stroke volume which significantly differ with such index in healthy persons. The indicator of MBV was compensated in sick children by higher heart rate and was without significant difference between all groups. The phenomena of SV reduction in children with respiratory pathology could be caused by disorders of autonomic regulation.

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This research was carried out according to the research work of Bukovinian State Medical University for 2022-2026 on the theme of "Chronobiological and adaptation aspects and features of autonomic regulation in pathological conditions in children of different age groups" (state registration number 0122U002245).

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Oleksandr H. Buriak
Bukovinian State Medical University
2 Teatralna Square, 58002 Chernivtsi, Ukraine
e-mail: buriakoleksandr1983@gmail.com

ORCID AND CONTRIBUTIONSHIP
Oleksandr H. Buriak: 0000-0002-6621-7582 A B C D
Yurii M. Nechytailo: 0000-0003-3050-961X E F

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

RECEIVED: 11.06.2024
ACCEPTED: 23.10.2024

ORIGINAL ARTICLE

CONTENTS

Comparison of the effectiveness of regional anesthesia under ultrasound guidance and tumescent anesthesia in the application of modern surgical treatment methods for chronic venous disease of the lower extremities

Volodymyr I. Cherniy, Vitalii S. Myrona
STATE INSTITUTION OF SCIENCE «CENTER OF INNOVATIVE HEALTHCARE TECHNOLOGIES» STATE ADMINISTRATIVE DEPARTMENT, KYIV, UKRAINE

ABSTRACT

Aim: To compare the effectiveness of tumescent anesthesia and regional anesthesia under ultrasound guidance in terms of their impact on nociceptive and stress systems, as well as systemic hemodynamics, in patients with chronic venous disease of the lower extremities, undergoing surgical treatment.

Materials and Methods: Fifty patients (average age 48 ±15 years; 19 [38 %] males and 31 [62 %] females) with chronic venous disease of the lower extremities, who underwent surgical treatment (endovenous laser ablation and miniphlebectomy on one limb), were examined. Twenty-five patients (main group) underwent surgery under femoral nerve block with 150 mg of lidocaine and sciatic nerve block (popliteal fossa) with 150 mg of lidocaine under ultrasound guidance. Another 25 patients (comparison group) underwent surgery under tumescent anesthesia, with the tumescent solution consisting of 400 mg of lidocaine, 50 mg of bupivacaine, and 400 ml of saline solution. We studied the parameters of systemic hemodynamics, heart rate variability, as well as blood glucose and plasma cortisol levels. The pain level was assessed by the use of the Visual Analogue Scale (VAS).

Results: The VAS pain levels during regional anesthesia under ultrasound guidance for endovenous laser ablation and miniphlebectomy were significantly lower than in patients who received tumescent anesthesia.

Conclusions: The regional anesthesia method under ultrasound guidance in patients with chronic venous disease of the lower extremities more effectively reduces surgical stress compared to tumescent anesthesia.

KEY WORDS: endovenous laser ablation, miniphlebectomy, femoral nerve block, sciatic nerve block (popliteal fossa), Visual Analogue Scale, pain

Wiad Lek. 2024;77(11):2140-2147. doi: 10.36740/WLek/197147 DOI

INTRODUCTION

Endovenous laser ablation (EVLA) is one of the modern methods for treating chronic venous diseases of the lower extremities. Instead of ligating or removing the «affected» trunk of the great saphenous vein (GSV), it is heated (coagulated) with a laser from the inside (endovenously) [1, 2]. Most patients report increased skin sensitivity along the treated vein. Some patients experience a painful cord-like sensation along the inner thigh for 5-7 days after the procedure (EVLA). The sensation of tightness and pain during or after the procedure may depend on adherence to the ablation technique and the adequacy of anesthesia [3].

There are several methods of anesthesia used during EVLA, but one of the most common is tumescent anesthesia – a type of local anesthesia. The essence of the method is to anesthetize the area exposed to thermal effects and to form a cushion that surrounds the great saphenous vein. This cushion is created from a balanced solution of local anesthetic adjusted for acid-base composition [4].

The effect of single-dose epidural anesthesia on postoperative pain and hospital stay duration after surgery for chronic venous disease has been studied, comparing it with general anesthesia. Epidural anesthesia provided adequate anesthesia and more effective postoperative analgesia compared to general anesthesia [5]. General anesthesia carries the risk of delayed discharge and prolonged immobilization, which increases the risk of deep vein thrombosis.

The safety and effectiveness of femoral nerve and obturator nerve blocks performed solely under ultrasound guidance were evaluated to reduce or prevent pain during tumescent anesthesia injections for GSV EVLA. Sixty patients in two groups underwent EVLA for GSV insufficiency. All patients received tumescent anesthesia. However, one group received preoperative femoral nerve block (FNB) and obturator nerve block (n. obtura-

torius) under ultrasound guidance. All patients assessed pain levels using the Visual Analogue Scale (VAS) from the start to the end of the laser ablation procedure. No complications from FNB or n. obturatorius block were observed. Pain related to tumescent anesthesia infusion and laser ablation was significantly more intense in the group without FNB and n. obturatorius block. FNB and n. obturatorius block under ultrasound guidance are safe, adequate, and effective options for reducing and/or eliminating intraoperative discomfort associated with tumescent anesthesia injections [6].

EVLA using tumescent anesthesia for treating great saphenous vein insufficiency can be painful and sometimes requires analgesosedation. FNB allows for pain relief in the ablation area (medial surface of the thigh). A double-blind randomized controlled study aimed to determine whether FNB before tumescent anesthesia reduces pain during GSV EVLA combined with miniphlebectomy. The placebo group (control group – 40 patients) received FNB with 10 ml of 0,9% saline before tumescent injection, while the FNB group (intervention group; 40 patients) received 5 ml of lidocaine 5 mg/ml with adrenaline 10 mg/ml combined with 5 ml of 0,9% saline for a total volume of 10 ml for FNB before tumescent anesthesia. It was found that patients undergoing GSV EVLA with miniphlebectomy under tumescent anesthesia experienced mild or moderate pain during tumescent injection. The most painful part of the procedure was the tumescent injection before miniphlebectomy, and FNB significantly reduced this pain [7].

The analgesic effect of specific tumescent anesthetic solutions containing lidocaine, ropivacaine, or a combination of lidocaine and ropivacaine was compared during endovenous radiofrequency ablation for the treatment of chronic venous disease. One hundred forty-nine patients with chronic venous disease of the lower extremities were randomly assigned to three groups: a lidocaine group (Group I), a ropivacaine group (Group II), and a lidocaine + ropivacaine group (Group III). Intraoperative VAS scores in Group II were higher than in Groups I and III, both 8 and 12 hours after the procedure. The use of a tumescent anesthetic solution containing a combination of lidocaine and ropivacaine significantly improved patient comfort [8].

AIM

The aim of the study was to compare the effectiveness of tumescent anesthesia and regional anesthesia under ultrasound guidance in terms of their impact on nociceptive and stress systems, as well as systemic hemodynamics, in patients with chronic venous disease of the lower extremities, undergoing surgical treatment.

MATERIALS AND METHODS

Fifty patients (average age [mean ± standard deviation] 48 ±15 years; 19 [38 %] males and 31 [62 %] females) with chronic venous disease (varicose veins) of the lower extremities, who underwent surgical treatment (EVLA and miniphlebectomy on one limb), were examined. Patients were selected and randomized to receive either regional anesthesia or tumescent anesthesia. Twenty-five patients (Group 1 – main group) were operated under regional anesthesia with ultrasound guidance, while 25 patients (Group 2 – comparison group) were operated under tumescent anesthesia. The exclusion criteria: patients with terminal-stage cancer; patients with decompensated chronic diseases; refusal to sign an informed consent.

The severity of chronic venous disease of the lower extremities was classified using the Clinical-Etiological-Anatomical-Pathophysiological (CEAP) classification, with patients ranging from C2 to C6 according to CEAP.

The protocol for regional anesthesia under ultrasound guidance for EVLA and miniphlebectomy in Group 1 was as follows: premedication with pantoprazole 40 mg intravenous, ondansetron 8 mg intravenous, and dexketoprofen 50 mg intravenous; sedation with propofol 5 mg/kg/hr intravenous, maintenance with humidified oxygen flow adjustment. Symptomatic correction of parameters related to blood pressure, heart rate, SpO_2, and respiratory rate was performed. Intraoperative analgesia included paracetamol («Infulgan») 1000 mg intravenous and fentanyl (0,025-0,05 mg) intravenous (for pain levels of 5 or higher on the VAS); femoral nerve block with 150 mg of lidocaine, and sciatic nerve block (popliteal fossa) with 150 mg of lidocaine. A cushion was formed around the great saphenous vein with saline solution.

The protocol for tumescent anesthesia during EVLA and miniphlebectomy in Group 2 differed in that instead of femoral and sciatic nerve blocks, tumescent anesthesia was performed with a solution containing 400 mg of lidocaine, 50 mg of bupivacaine, and 400 ml of saline. Postoperative analgesia in both groups included intravenous dexketoprofen.

Monitoring of the functional and humoral status of the patient was conducted to assess the adequacy of anesthesia during the perioperative period, taking into account the following indicators: systemic hemodynamics (using a Drager cardiomonitor), heart rate variability (HRV) as a criterion for the balance between nociception and antinociception (normalized low [LFn] and high [HFn] frequency powers, and LF/HF ratio) [10], VAS pain level, with VAS scores studied dynamically over 9 hours. Glycemia was measured using a test strip method (Contour glucometer), and plasma cortisol levels

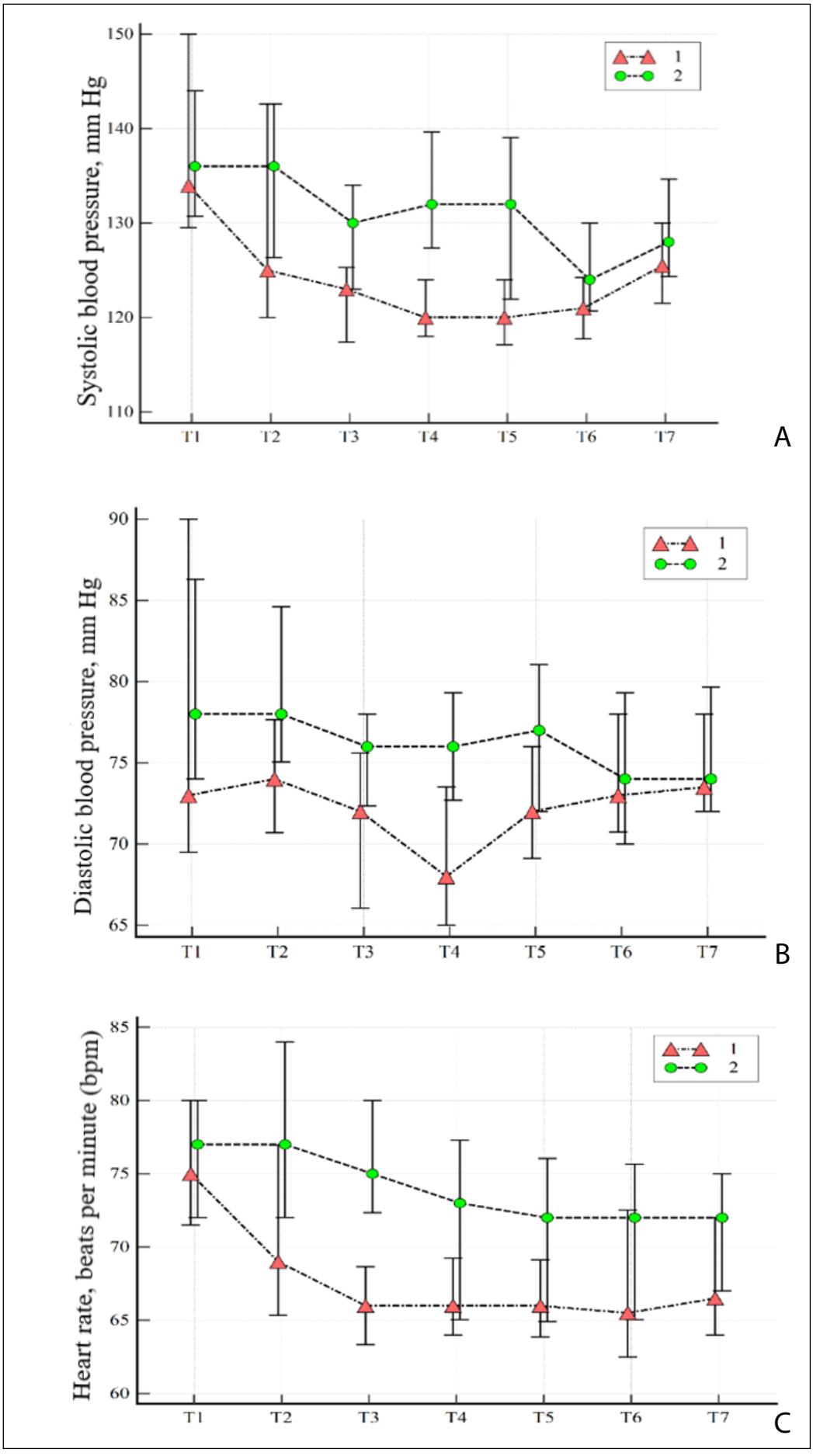

Fig. 1. Hemodynamic indicators (A: systolic blood pressure; B: diastolic blood pressure; C: heart rate) during tumescent anesthesia and regional anesthesia under ultrasound guidance for EVLA and miniphlebectomy. Data presented as Me and 95% CI. Observation periods: T1 – before the surgery; T2 – 15 minutes after the start of the surgery; T3 – 45 minutes after the start of the surgery; T4 – 60 minutes after the start of the surgery; T5 – 90 minutes after the start of the surgery; T6 – end of the surgery; T7 – 60 minutes after the completion of the surgery.

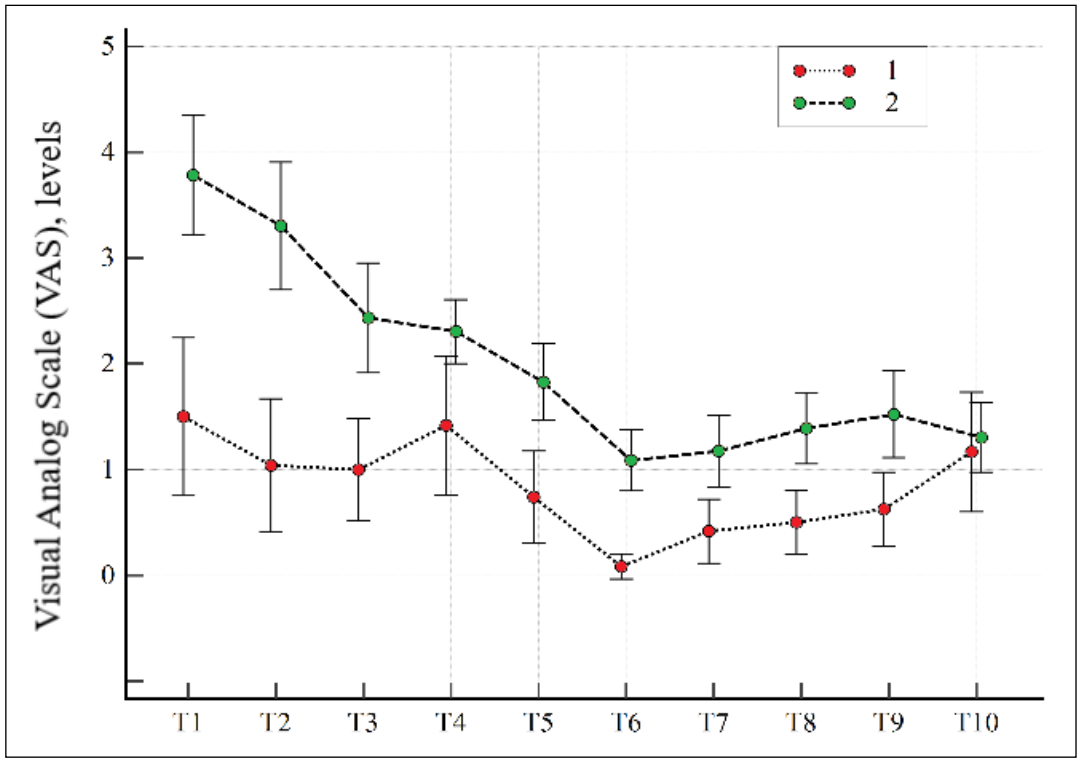

Fig. 2. The VAS pain levels during tumescent and regional anesthesia under ultrasound guidance for endovenous laser ablation and miniphlebectomy. Data presented as M and 95% CI. Observation periods: T1 – 15 minutes after the start of the surgery; T2 – 45 minutes after the start of the surgery; T3 – 60 minutes after the start of the surgery; T4 – 90 minutes after the start of the surgery; T5 – end of the surgery; T6 – 1 hour after the surgery; T7 – 3 hours after the surgery; T8 – 6 hours after the surgery; T9 – 9 hours after the surgery.

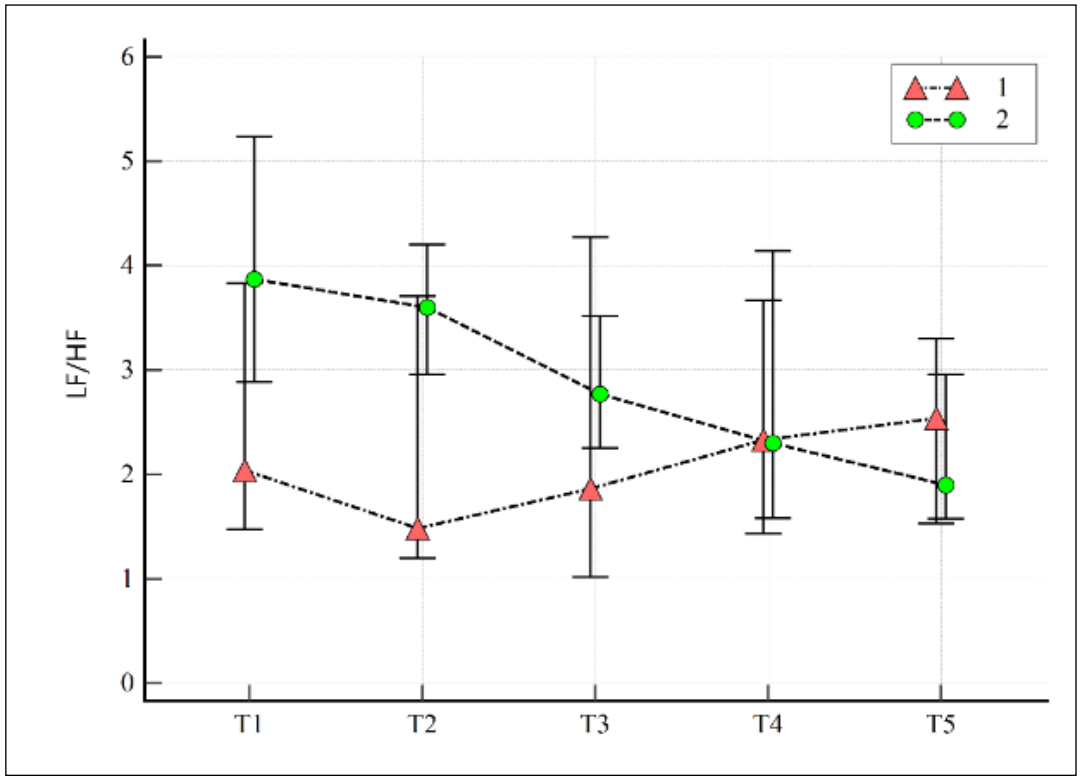

Fig. 3. The LF/HF ratio during tumescent and regional anesthesia under ultrasound guidance for EVLA and miniphlebectomy. Data presented as Me and 95% CI. Observation periods: T1 – before the operation; T2 – 15 minutes after the start of the operation; T3 – 45 minutes after the start of the operation; T4 – 60 minutes after the start of the operation; T5 – 90 minutes after the start of the operation.

were assessed by an immunochemiluminescent method [11]. The dosage of opioids used for analgesia and the number of hospital bed days were also recorded.

Statistical analysis of the data was conducted using the Statistica 10.0 (StatSoft Inc., USA) and EZR v. 1.67 software [12]. Quantitative parameters were presented as the mean (M) ± standard deviation, M with 95 % confidence interval (CI), median (Me) with interquartile range (IQR), and Me with 95 CI. The Student`s T-test was used for the comparison of the studied groups in case of normal distribution of quantitative data. The Mann-Whitney U-test was used, when the quantitative data distribution deviated from normal. Qualitative parameters were presented as absolute and relative (%) frequency. We used the Fisher`s exact test to compare the qualitative (categorical) parameters between the

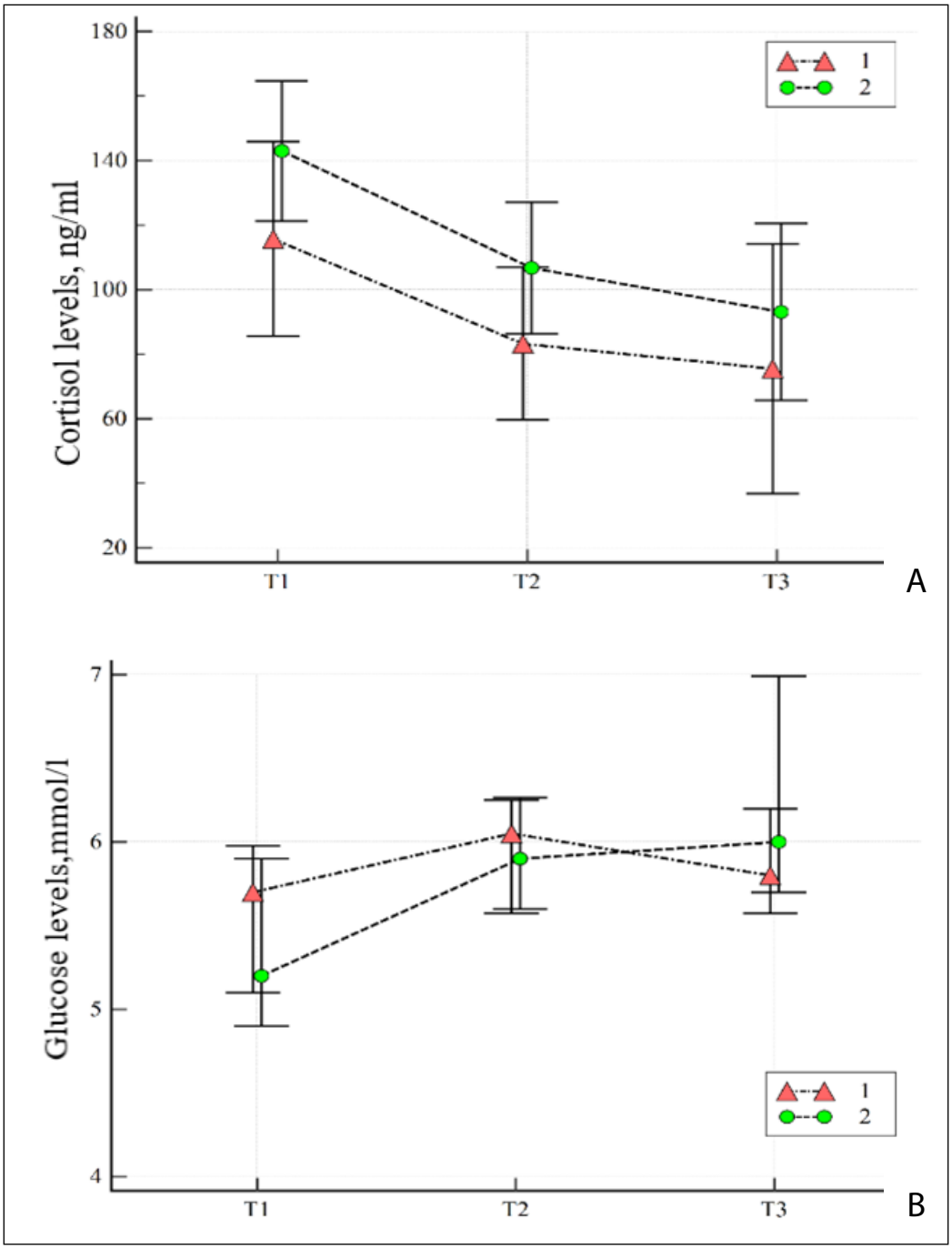

Fig. 4. Levels of cortisol (A [M and 95 CI]) and glucose (B [Me and 95% CI]) in the blood during tumescent and regional anesthesia under ultrasound guidance during endovenous laser ablation and mini-phlebectomy. T1 – start of the operation (incision); T2 – 60 minutes after the start of the operation; T3 – 1 hour after the operation.

studied groups. A p-value less than 0,05 was considered statistically significant [12, 13].

RESULTS

The studied groups were comparable by average age ([Me (IQR)] group 1: 47 [35-63] years; group 2: 44 [35-57] years [p=0,717]), gender (males/females: group 1 – 10/15; group 2 – 9/16 [p=1,000]), as well as the severity of chronic venous disease of lower extremities.

Systemic hemodynamic indicators during tumescent anesthesia and regional anesthesia under ultrasound guidance for EVLA and miniphlebectomy did not significantly differ from age-related norms throughout the entire perioperative observation period. However, a clear trend was observed towards increased systolic (Fig. 1A) and diastolic blood pressure (Figure 1B), and heart rate (Figure 1C) in patients of Group 2. Sixty minutes after the start of the procedure, systolic blood pressure and diastolic blood pressure were 10% ($p < 0,007$) and 11,7% ($p < 0,039$) higher in Group 2, respectively (Figures 1A and 1B, respectively). Ninety minutes after the start of the procedure, these indicators remained 10% ($p < 0,006$) and 11,7% ($p < 0,034$) higher in Group 2, respectively (Fig. 1A and 1B, respectively).

The VAS pain levels during tumescent and regional

Table 1. Average dose of propofol and fentanyl used in the study groups, Me (IQR)

Indicator	Group 1 n=25	Group 2 n=25	p
Propofol, mg	200 (0 – 300)	300 (225 – 400)	0,016
Fentanyl, mg	0 (0 – 0)	0 (0 – 175)	0,044

anesthesia under ultrasound guidance for EVLA and miniphlebectomy differed significantly between the groups throughout the entire perioperative observation period. In Group 1, the VAS pain level was significantly lower compared to patients in Group 2 ($p < 0,001$) – see Fig. 2.

The study of the balance between nociception and antinociception (LF/HF) during tumescent and regional anesthesia under ultrasound guidance for endovenous laser ablation and miniphlebectomy showed a significant decrease in the LF/HF ratio 15 minutes after the start of the surgery in patients of Group 1, with a reduction of 58,6% compared to patients in Group 2 (see Fig. 3).

Cortisol levels in the blood during tumescent and regional anesthesia under ultrasound guidance for EVLA and miniphlebectomy did not differ significantly between groups throughout the perioperative observation. However, a clear tendency towards increased cortisol levels was observed in patients in Group 2 (Fig. 4A). Blood glucose levels also did not differ significantly between the groups (Fig. 4B).

Comparative evaluation of the average doses of propofol and fentanyl used is presented in Table 1. The average doses of propofol and fentanyl used in Group 2 were higher. In Group 1, fentanyl was administered to 4 patients (6 ampoules – 0,6 mg). In Group 2, fentanyl was administered to 10 patients (18 ampoules – 1,8 mg).

The number of bed days for patients with varicose disease in the postoperative period did not differ significantly between the study groups.

DISCUSSION

Activation of the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal system may result from inadequate overall analgesia and be accompanied by clinical effects of SNS hyperactivity, such as arterial hypertension, tachycardia, centralization of blood circulation, tissue perfusion disturbances, increased levels of adrenocorticotropic hormone (ACTH) and cortisol in plasma, and hyperglycemia [3].

Higher invasiveness of surgery elicits a higher degree of surgical stress responses including neuroendocrine-metabolic and inflammatory-immune responses, which are associated with the occurrence of major postoperative complications [13].

Cortisol, along with glucose, is traditionally classified as a stress hormone, with its concentration rising proportionally to the severity of surgical trauma [3].

Surgical interventions inevitably involve tissue trauma, which in turn is accompanied by pain and nociceptive impulses from the surgical site to the central nervous system via the spinothalamic and spinoreticular tracts, leading to what is known as surgical stress. Surgical tissue damage is associated with the release of biologically active substances from the damaged tissues, such as serotonin, histamine, bradykinin, and pro-inflammatory cytokines [14].

Inadequate anesthesia can result in elevated levels of catecholamines in the blood, as well as increased activity of the hypothalamic-pituitary-adrenal and renin-angiotensin-aldosterone systems, leading to increased production and release of ACTH, endorphins, antidiuretic hormone, thyroxine, and cortisol. This causes a stress-induced reconfiguration of the circulatory system, manifested by increased total peripheral vascular resistance, arterial hypertension (primarily due to endothelin-1 production), tachycardia, and a pro-inflammatory shift in vascular endothelial metabolism, which can trigger disseminated intravascular coagulation and the fibrinolytic system [3, 14].

Our comparison of tumescent anesthesia protocols and regional anesthesia methods under ultrasound guidance in patients with chronic venous disease undergoing EVLA and miniphlebectomy indicates that these methods can be the preferred choice. However, the study of the impact of different anesthetic regimens on nociceptive and stress systems, as well as systemic hemodynamics in patients with chronic venous disease, demonstrated that regional anesthesia under ultrasound guidance provides more adequate protection against surgical stress compared to tumescent anesthesia. Pain levels, measured using the VAS scale, were significantly lower during regional anesthesia under ultrasound guidance than in patients receiving tumescent anesthesia.

CONCLUSIONS

1. The regional anesthesia method under ultrasound guidance is more effective in mitigating surgical

stress in patients with chronic venous disease compared to tumescent anesthesia.
2. Since the blockade directly targets the nerve, regional anesthesia under ultrasound guidance uses a smaller amount of local anesthetic than tumescent anesthesia, thereby reducing the risk of toxic effects.
3. The regional anesthesia method under ultrasound guidance in patients with chronic venous disease required a smaller dose of propofol and fentanyl. The number of hospital days for patients with chronic venous disease in the postoperative period did not significantly differ between the groups.

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The study adhered to the basic principles of the Council of Europe Convention on Human Rights and Biomedicine, World Medical Association Declaration of Helsinki on the ethical principles for medical research involving human subjects, and current Ukrainian regulations. The study protocol was approved by the local ethics committee. All patients signed the proposed informed consent to participate in the study.

The study was performed as a fragment of the complex scientific project of the Scientific Department of Minimally Invasive Surgery (State Institution of Science «Center of innovative healthcare technologies» State Administrative Department) «Optimization of surgical treatment of patients under a multimodal program of rapid recovery based on the improvement of operative interventions, in particular with the use of nanobiosensor technologies and their anesthetic support» (state registration number 0122U000233; term: 2022-2024).

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Volodymyr I. Cherniy
State Institution of Science
«Center of innovative healthcare technologies»
State Administrative Department
5 Verkhnia St., 01014 Kyiv, Ukraine
e-mail: vladimircherniy3@gmail.com

ORCID AND CONTRIBUTIONSHIP
Volodymyr I. Cherniy: 0000-0002-9885-9248 A E F
Vitalii S. Myrona: 0009-0000-5942-9344 B C D

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

RECEIVED: 05.07.2024
ACCEPTED: 28.10.2024

ORIGINAL ARTICLE

CONTENTS

Comparison of the experience and perception of artificial intelligence among practicing doctors and medical students

Oksana O. Drevitska[1], Lidiia V. Butska[1], Ostap O. Drevytskyi[2], Valentyn O. Ryzhak[3], Hanna B. Varina[4], Olha V. Kovalova[4], Inna V. Medvid[4]

[1]EDUCATIONAL AND SCIENTIFIC CENTER «INSTITUTE OF BIOLOGY AND MEDICINE» OF TARAS SHEVCHENKO NATIONAL UNIVERSITY OF KYIV, KYIV, UKRAINE

[2]CENTER FOR RESEARCH AND EDUCATION OF TRANSLATIONAL BIOLOGY AND MEDICINE, KYIV, UKRAINE

[3]OPTIMUM MEDIA UKRAINE, KYIV, UKRAINE

[4]BOGDAN KHMELNITSKY MELITOPOL STATE PEDAGOGICAL UNIVERSITY, ZAPORIZHZHIA, UKRAINE

ABSTRACT

Aim: To analyze and compare the experiences and perceptions of artificial intelligence (AI) among practicing doctors and medical students.

Materials and Methods: A survey was conducted among 30 doctors and 30 fifth-year master's students enrolled in the "Medicine" program. Participants were asked about their experiences with AI, their perceptions of AI's impact on their education and practice, and their views on the benefits and drawbacks of AI in the medical field. The data were analyzed to compare the responses between the two groups.

Results: Among the respondents, 8 doctors (26,67%) and 4 students (13,33%) had not used AI in their practice or studies. The analysis was conducted on the remaining 22 doctors and 26 students. The study found that students generally rated the effectiveness of AI higher than physicians did, particularly in areas such as enhancing work and educational experiences. Both groups used AI primarily for information retrieval, with students showing a slightly greater openness to expanding AI's role in education and practice. Despite recognizing the advantages of AI, both groups expressed concerns regarding its accuracy and reliability.

Conclusions: The study indicates that while AI, particularly ChatGPT, is increasingly being adopted in medical education and practice, there is still a level of caution and skepticism among both students and professionals. Further research is needed to optimize the integration of AI in medical curricula and address the ethical implications of its use.

KEY WORDS: Artificial Intelligence, Medical Education, ChatGPT, Clinical Practice

Wiad Lek. 2024;77(11):2148-2153. doi: 10.36740/WLek/197083 DOI

INTRODUCTION

The rapid advancement of artificial intelligence (AI) has profoundly impacted various sectors, with healthcare standing at the forefront of this technological evolution. AI's integration into medical practice and education has brought about significant changes, offering new tools for diagnosis, treatment planning, and the management of patient data. These advancements have the potential to enhance the efficiency and accuracy of healthcare delivery, thereby improving patient outcomes.

AI has been shown to significantly improve diagnostic accuracy, enhance decision-making processes, and streamline clinical workflows. For instance, AI can analyze complex medical data with greater speed and accuracy than traditional methods, offering a new level of support to healthcare professionals [1]. Furthermore, AI's ability to personalize treatment plans and predict patient outcomes is transforming patient care by allowing for more targeted and effective interventions [2].

In the context of medical education, AI is increasingly being recognized for its potential to revolutionize how medical students and professionals acquire knowledge and skills. AI-driven educational tools, such as virtual simulations and adaptive learning platforms, provide students with personalized learning experiences tailored to their individual needs and progress [3]. These tools also enable students to engage in realistic clinical scenarios, improving their problem-solving abilities and readiness for real-world practice. However, despite these advantages, there are ongoing discussions about the challenges associated with AI integration, including concerns about the potential depersonalization of care and the need for rigorous validation of AI tools to ensure their reliability and safety in clinical settings [4].

These advancements have sparked interest in comparing the adoption and perception of AI between practicing doctors and medical students, as their experiences and evaluations of AI can provide valuable insights into the future direction of AI in healthcare. Understanding these perspectives is crucial for developing strategies that optimize AI's use in both educational and practical contexts.

AIM

The objective of this study is to analyze and compare the experiences and perceptions of artificial intelligence (AI) among practicing doctors and medical students. The tasks of the research: to analyze and compare the trends in the frequency of AI usage between practicing doctors and medical students; to analyze and compare the evaluations of the effectiveness, as well as the positive and negative aspects of the use of AI by doctors and medical students; to assess the prospects of AI usage in the educational process and practical activities of doctors and medical students.

MATERIALS AND METHODS

Research Method: Data collection was carried out using a questionnaire that was initially generated by ChatGPT in response to a relevant inquiry. The questionnaire was designed to explore the experience, perception, and evaluation of AI's effectiveness in educational processes and clinical practice. After the initial creation, the questions and their sequence were refined to ensure clarity and ease of analysis using Microsoft Excel.

Special Google Form was then created to facilitate the survey among the selected participants. The participants included students who have experience working in healthcare settings as mid-level practitioners and who are currently pursuing a master's degree in medicine, as well as doctors already working in medical institutions. The anonymous survey gathered data on their experience, attitudes, and evaluation of AI usage in both educational and professional contexts.

QUESTIONNAIRE

1. Have you had experience using artificial intelligence in your medical practice and continuing education? Response Options: Yes/No.
2. What AI programs have you used? Response: Open-ended.
3. In your opinion, has the use of AI improved the effectiveness of your work and continued education? Response Options: Significantly improved – 5 points; Improved – 4 points; Unchanged – 3 points; Worsened – 2 points; Significantly worsened – 1 point.
4. Do you believe AI has enhanced your understanding of complex medical topics? Response Options: Yes, significantly better – 5 points; Yes, better – 4 points; No impact on understanding – 3 points; Worsened – 2 points; Significantly worsened – 1 point.
5. How do you assess the overall impact of AI on your medical work and continuous education? Response Options: Very positive – 5 points; Positive – 4 points; Neutral – 3 points; Negative – 2 points; Very negative – 1 point.
6. Would you recommend expanding the use of AI in the educational process and medical practice? Response Options: Yes, definitely – 5 points; Yes, but with certain conditions – 4 points; Depends on the subjects – 3 points; Not sure – 2 points; Absolutely not – 1 point.
7. In which aspects of education and practice have you utilized AI? (Multiple selections allowed): Response Options: For information search; In educational simulations; For solving clinical tasks; For personalizing educational material; Other (please specify).
8. What benefits of using AI would you highlight? (Multiple selections allowed): Response Options: Time-saving; Greater personalization of education and practice; Improved material retention; Better accessibility of information; Other (please specify).
9. What disadvantages of using AI did you note? Response: Open-ended.

The collected data were analyzed using the statistical tools available in Microsoft Excel, which facilitated both quantitative and qualitative assessments of the participants' responses. This approach enabled a comprehensive evaluation of the differences in AI adoption, perception, and effectiveness between the two groups. Quantitative data were presented as mean ± standard error of the mean. Qualitative data were presented as absolute and relative (%) frequency. To ensure a robust statistical comparison between the groups, we used the Mann-Whitney U-test (for quantitative parameters), and the Chi-square (χ^2) or Fisher`s exact test (for qualitative data). A 2-tailed **p**-value **<0**,05 was considered statistically significant.

The authors take full responsibility for ensuring compliance with Articles 43 and 45 of the Law of Ukraine "Fundamentals of Health Legislation of Ukraine", the Declaration of Helsinki's "Recommendations for Physicians Involved in Biomedical Research Involving Human Subjects" (1964, revised in 1983), Directive 2001/20/EC of the European Parliament and the Council of the EU of April 4, 2001 (as amended). This version makes it clear that the research not only doesn't contradict but actively adheres to bioethical principles.

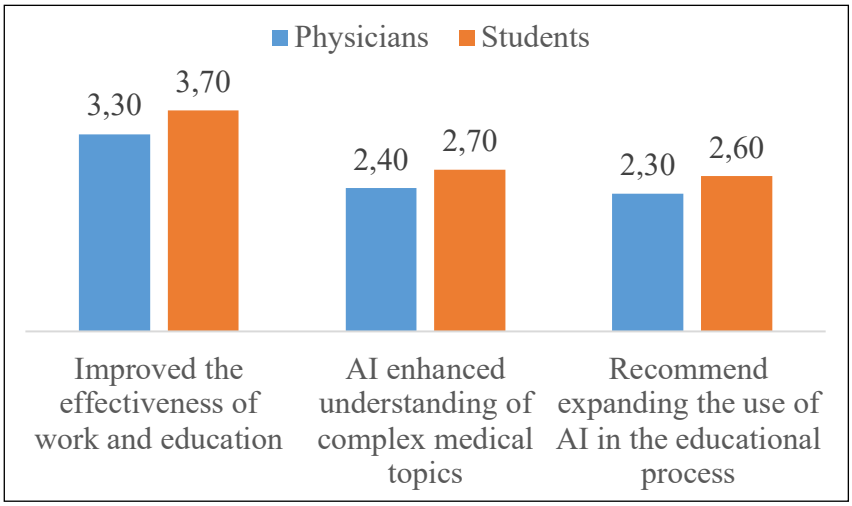

Fig. 1. Indicators (on a 5-point scale) of perception of AI by general practitioners and students.

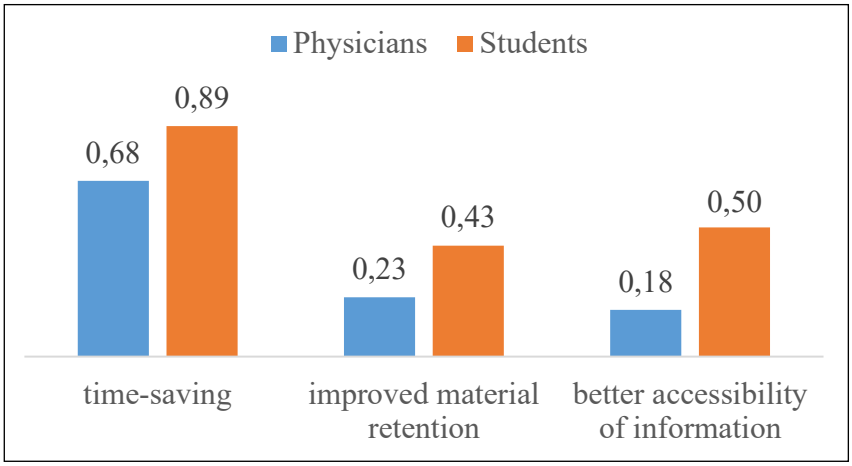

Fig. 2. The differences between doctors and students based on the responses to the questions (expressed as fractions of '1.0', where '1.0' represents all respondents in the corresponding group). * – $p<0,05$ by the Fisher`s exact test.

RESULTS

Based on the survey conducted among 30 doctors and 30 fifth-year master's students of the speciality "Medicine", it was found that 8 doctors (26,67%) and 4 students (13,33%; $p > 0,05$) had not yet used AI in their practice or studies. Therefore, the analysis of attitudes towards AI was conducted using the responses from 22 doctors and 26 students.

Among these respondents, all had experience using the ChatGPT program. Additionally, 8 students (30,77%) and one doctor (4,5%; $p < 0,05$) indicated that they had also used other platforms, including Gamma, Canva.

In a recent survey, the average score for the question, "In your opinion, has the use of AI improved the effectiveness of your work and continued education?" was 3,3±1,0 among physicians and 3,7±1,1 among students ($p > 0,05$). Both groups evaluated the impact of AI on a 5-point scale, with students showing a marginally more positive perception of AI's role in enhancing their work and educational experiences.

In response to the question, "Do you believe AI has enhanced your understanding of complex medical topics?" the average score among physicians was 2,4±0,9, while students rated it at 2,7±0,8 ($p > 0,05$). Although students again rated AI's impact slightly higher than physicians, both groups generally expressed a low to moderate belief in AI's ability to enhance their understanding of complex medical topics.

For the question, "How do you assess the overall impact of AI on your medical work and continuous education?" physicians rated it at 3,6±0,9, while students gave a rating of 3,5±1,0. Both groups generally rated the impact above the average level, with no statistically significant difference between them ($p > 0,05$).

To the question: "Would you recommend expanding the use of AI in the educational process and medical practice?" the average response score of doctors was 2,3±0,9, and of students – 2,6±0,8. So, we observe a somewhat greater openness and willingness to share information for friends among students, but the difference is probably not significant ($p > 0,05$). Fig. 1 shows the results of the survey on the above-mentioned questions.

In response to the question, "In which aspects of education and practice have you utilized AI?" physicians and students reported varying uses. Among the 22 physician respondents, 13 (59,1%) used AI for information retrieval, 7 (31,8%) for educational simu-

lations, 7 (31,8%) for solving clinical tasks, 5 (22,7%) for personalizing educational materials, and 1 (4,5%) for administrative purposes. Among the 26 fifth-year graduate students with medical experience, 15 (57,7%) utilized AI for information retrieval, 8 (30,8%) for educational simulations, 9 (34,6%) for solving clinical tasks, and 8 (30,8%) for personalizing educational materials. These data suggest that both physicians and students predominantly use AI for information retrieval, with similar patterns of utilization across other educational and clinical tasks.

In response to the question, "What benefits of using AI would you highlight?" respondents identified several key advantages. Among physicians, 15 (68,2%) highlighted time-saving, 5 (22,7%) noted greater personalization of education and practice, 5 (22,7%) reported improved material retention, and 4 (18,2%) cited better accessibility of information. Among students, 23 (88,5%) emphasized time-saving, 7 (26,9%) pointed to greater personalization, 11 (43,2%) mentioned improved material retention, and 13 **(50**,0%) noted better accessibility of information. time-saving. Fig. 2 shows the differences between doctors and students according to the questions indicated.

As the results show, there is a tendency for students to pay more attention to saving time, conserving resources, and the availability of information compared to doctors. These differences likely stem from the distinct roles and needs of students versus practicing physicians. For students, AI serves as a crucial tool for managing learning and enhancing their educational experience, whereas physicians may view AI as a complement to their existing expertise and workflows.

In response to the question, "What disadvantages of using AI did you note?" 8 out of 22 doctors (36,4%) and 7 out of 26 students (26,9%) mentioned "incorrect or inaccurate answers," showing no significant difference between the groups. Among the doctors' comments, phrases such as "lack of medical knowledge," "insufficient awareness of medical concepts," "non-regulation at the legislative level," "AI fantasizes", "random errors," "unpredictable results", and "AI lacks practical knowledge and skills" were noted. Students, on the other hand, highlighted concerns like "results need to be checked with specialists," "technical shortcomings and possible misinformation," and "requires detailed explanations in questions".

As we observe, both doctors and students have expressed concerns about the accuracy and reliability of AI, although their specific critiques differ. Doctors focused more on the limitations of artificial intelligence in medical knowledge and the unpredictability of its results, while students were more concerned about the need for peer review and the possibility of technical errors. So, although AI has its shortcomings, it remains a valuable and effective tool in education and clinical problem-solving. Its use requires caution and a critical approach to fully harness its potential while minimizing risks.

DISCUSSION

The application of AI, particularly ChatGPT, in the medical field covers a broad spectrum, ranging from identifying promising topics for education and research to supporting future professionals in clinical and laboratory diagnostics. However, these applications come with several limitations and ethical concerns, particularly regarding reliability and plagiarism [5-7].

ChatGPT can serve as a mediator in idea generation, helping to select a topic and initiate research projects for students and scientists. It has been found that abstracts generated by ChatGPT demonstrated originality in the works created [8]. Several articles have reported the use of ChatGPT for writing scientific literature. One study demonstrated that ChatGPT is capable of producing formal research papers. The authors noted that the language used by AI is grammatically correct and pleasant to read [9]. Doctors and students, in our research, mostly used ChatGPT.

AI can reduce the time, energy, and resources spent on experiments that are more likely to yield unproductive results by analyzing vast amounts of available literature beyond the experience of a single individual. It has the capability to successfully remember previous interactions and user feedback, thereby offering opportunities for continuous improvement [10, 11]. Reduction of time for routine work most attracted doctors and students – in our research.

AI can serve as an important tool for idea generation, supporting education and research, as well as assessing students' clinical skills. However, there are certain limitations and drawbacks to AI. For instance, the accuracy of the text generated by the ChatGPT program largely depends on the quality and nature of the training data. It is important to emphasize that AI is currently unable to fully replace humans, as it lacks a sufficient level of understanding and specialized knowledge in the medical field [12, 13]. Doctors and students in the research process also noted the presence of limitations and shortcomings in the use of AI.

Considering these factors, further research and the integration of AI ethics into medical curricula are important steps to ensure the effective and ethical use of AI in medicine. Given the broad availability and potential of AI in medical education, its use among students

and medical education professionals is expected to increase further.

CONCLUSIONS

The study involving 30 doctors and 30 fifth-year medical students revealed that 26,67% of doctors and 13,33% of students had not yet integrated AI into their professional or academic activities.

The findings indicate some trend: students are more inclined to view AI as a valuable tool for enhancing their educational experience compared to doctors. This is reflected in the slightly higher average scores students gave when asked about AI's impact on their studies and work. Students were more likely to mention benefits such as tips for integrating AI into education and practice, the availability of information, and the time-saving advantages of AI. Despite this, both groups shared a moderate belief in AI's potential to improve their understanding of complex medical topics. When evaluating the overall impact of AI on their work and education, both doctors and students rated it above average, with no significant difference between the two groups.

Although participants acknowledged the benefits of AI, concerns about its accuracy and reliability persisted. However, these concerns differed between the groups. Doctors were more skeptical of AI's limitations in medical knowledge and the unpredictability of its outcomes, while students emphasized the need for peer review and the potential risks of technical errors.

In conclusion, while AI tools like ChatGPT are recognized as valuable resources in medical education and practice, their adoption is accompanied by caution due to inherent limitations and the necessity of critical, careful use. The study underscores the need for further research and the integration of AI ethics into medical curricula to ensure its responsible and effective application in the field.

PROSPECTS FOR FURTHER RESEARCH

Future studies should include a larger and more diverse sample size across different regions and medical specialties to enhance the generalizability of the findings. This would help identify specific trends and challenges unique to various subgroups within the medical community. Studying the effectiveness of incorporating AI training and ethics into medical curricula could inform educational strategies to better prepare future medical professionals for the evolving technological landscape.

Collaborative studies involving experts from fields such as computer science, ethics, and education could foster a more comprehensive understanding of how to optimize AI tools for medical applications. By addressing these areas, future research can contribute to the responsible and effective integration of AI in medical education and practice, ultimately enhancing both learning experiences and patient care outcomes.

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We would like to express our gratitude to the medical students and doctors who participated in this survey. Their valuable insights will contribute to future research and support the integration of AI-related topics into medical education.

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Oksana O. Drevitska
Taras Shevchenko National University of Kyiv
2, Hlushkova Ave., 03127 Kyiv, Ukraine
e-mail: drevitska@ukr.net

ORCID AND CONTRIBUTIONSHIP
Oksana O. Drevitska: 0000-0002-1551-9329 A B C D
Lidiia V. Butska: 0000-0002-7928-0177 D E F
Ostap O. Drevytskyi: 0009-0009-3711-9033 B E
Valentyn O. Ryzhak: 0009-0008-0157-2461 E F
Hanna B. Varina: 0000-0002-0087-4264 A B E
Olha V. Kovalova: 0000-0001-5061-6506 C E
Inna V. Medvid: 0009-0005-4875-3217 B D

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

RECEIVED: 17.06.2024
ACCEPTED: 21.10.2024

ORIGINAL ARTICLE

CONTENTS

Diagnostic value of laboratory markers of enteric dysfunction in preterm infants

Oleksii S. Godovanets
BUKOVINIAN STATE MEDICAL UNIVERSITY, CHERNIVTSI, UKRAINE

ABSTRACT

Aim: To study the peculiarities of food tolerance disorders in premature infants, taking into account the risk factors of gestational age and maternal labor, the peculiarities of the course of perinatal pathology, in order to determine pathogenetically sound clinical and laboratory criteria.

Materials and Methods: A comprehensive clinical and laboratory evaluation was performed on 67 preterm infants of gestational age 32 to 33/6 weeks with severe food tolerance disorders in perinatal pathology. The comparison group consisted of 31 newborns with gestational age of 34 to 37 weeks. In addition to standard laboratory tests, coprofiltrate parameters (albumin, alpha-1-antitrypsin, elastase, PMN elastase, calprotectin) were examined.

Results: The clinical signs of food intolerance included regurgitation and stasis (86,57%), intestinal paresis with delayed meconium and transitional stool (70,15%), flatulence (47,76%), bile or blood impurities in the stool (43,28%). The presence of pain syndromes in conjunction with eating dysfunction was accompanied by episodes of apnea, bradycardia, and decreased blood saturation. The laboratory markers of intestinal dysfunction were the following: increased levels of calprotectin > 390,15 μg/g; albumin > 37,25 μg/g; α-1-antitrypsin > 452,67 μg/g; decreased levels of PMN elastase <95,49 ng/g. The representativeness of these clinical and paraclinical parameters was investigated, which gave grounds to recommend their use in clinical practice in neonatology.

Conclusions: The implementation of recommendations for the use of laboratory markers of food intolerance, along with clinical signs, will increase the effectiveness of diagnostic measures in perinatal pathology in preterm infants.

KEY WORDS: newborn; digestive system; food tolerance disorders; laboratory diagnostics

Wiad Lek. 2024;77(11):2154-2160. doi: 10.36740/WLek/197086 DOI

INTRODUCTION

Annually, up to 16% of children are born prematurely worldwide. Preterm birth is regarded as a principal contributor to neonatal morbidity and mortality. As a consequence of morphological and functional immaturity at the time of birth, children are at elevated risk of developing complications during the neonatal period and of developing long-term consequences of perinatal pathology in later years of life. This category of children is at a high risk of developing physical and neuropsychological disorders, as well as somatic diseases, which have the potential to significantly impair their quality of life in the future [1, 2].

The clinical criteria for disorders of the functional state of the digestive system in diseases of the newborn period are a topic of active discussion in contemporary scientific literature. It is observed that the pathogenetic mechanisms of gastrointestinal dysfunction remain insufficiently studied, which precludes the formulation of general clinical and laboratory criteria for food intolerance [3]. The formation of diseases of the gastrointestinal system in later years of life is caused by low body weight and immaturity of the body of children at birth in the context of unfavorable perinatal risk factors. In light of the above, it is imperative that scientists and practitioners collaborate to conduct clinical trials with the aim of determining pathogenetically based clinical and laboratory criteria for food tolerance disorders in preterm infants.

AIM

The aim of this study is to examine the characteristics of food intolerance disorders in preterm infants, with particular attention to the potential risk factors associated with the gestational period and maternal labor. Additionally, the study aims to elucidate the specific clinical and laboratory criteria that can be used to diagnose and understand the pathogenesis of these disorders.

MATERIALS AND METHODS

A comprehensive clinical and laboratory examination conducted on 67 premature infants with a gestational

age of 32 to 33/6 weeks at birth who exhibited clinical signs indicative of severe perinatal pathology (the main study group). The comparison group consisted of 31 newborns with a gestational age of 34 to 36/6 weeks.

Inclusion criteria: gestational age at birth between 32 and 33/6 weeks, severe course of neonatal diseases, clinical signs of food tolerance disorders, and informed consent of parents to participate in the study.

The exclusion criteria included gestational age at birth of less than 32 weeks or greater than 37 weeks, the presence of congenital malformations, and a lack of informed parental consent to participate in the clinical trial. The data from the pregnant women's exchange cards, birth histories, and newborn developmental histories were subjected to analysis.

The degree of prematurity was determined by considering the gestational age at birth and anthropometric parameters, including weight, height, head circumference, and chest circumference. The degree of correspondence between the child's morphological and functional maturity and their gestational age at birth was evaluated using percentile tables and the Ballard scale. The list of diseases was determined in accordance with the International Classification of Diseases, 10th Revision. The adaptation of children at birth was assessed using the Apgar score and the Score for Neonatal Acute Physiology (SNAPPE II) scale, which was employed to monitor the infants' progress over time [4-6]. To confirm the diagnosis of multiorgan failure, the Neonatal Multiple Organ Dysfunction Score (NEOMOD, 2001) was employed [7, 8]. The clinical assessment of the functional state of the gastrointestinal system in newborns was conducted in accordance with established methodologies, taking into consideration the age-specific characteristics of the neonatal period. Additional laboratory examinations of newborns included a complete blood count, a biochemical blood count and coprofiltrate parameters, namely: Albumin, Alpha-1-Antitrypsin, Fecal Elastase, PMN Elastase and Fecal Calprotectin. The studies were conducted at the German-Ukrainian Laboratory "BUKINTERMED" (Chernivtsi, Ukraine) utilizing the capabilities of the Cottbus Public Laboratory for Medicine, Microbiology and Infectious Epidemiology (Accreditation Certificate D-ML-19676-01-00 according to DIN EN ISO 15189:2014, valid until November 25, 2019). To perform the laboratory tests of the coprofiltrate, an enzyme-linked immunosorbent assay (ELISA) was employed, utilizing reagents from Immundiagnostic AG (Germany).

The research was conducted in accordance with the guidelines set forth in the GCP (1996), the Council of Europe Convention on Human Rights and Biomedicine (1997), the World Medical Association Declaration of Helsinki on the Ethical Principles for Research Involving Human Subjects (1964-2008), and Order of the Ministry of Health of Ukraine No. 690 of 23.09.2009 (as amended by Order of the Ministry of Health of Ukraine No. 523 of 12.07.2012). Approval was granted by the Bioethics Committee of Bukovinian State Medical University (Protocol No. 2, February 9, 2015).

Statistical processing of the data was performed using Statistica (StatSoft Inc., version 13.0, USA) and MedCalc software (version 16.1). Statistical values were calculated by analyzing the standard deviation (SD), standard error (m), arithmetic mean of the sample (M), using the Shapiro-Wilk test (normal distribution with sample size greater than 30; $p<0,05$) and the Kolgomorov-Smirnov test. Quantitative indicators in samples with normal distribution were evaluated using Student's t-test. Results were considered statistically significant at $p<0,05$. The analysis of ROC curves (Receiver Operating Characteristic Curve), AUC (Area Under ROC), as well as sensitivity (SN) and specificity (SP) (with 95 % confidence interval [CI]) was performed using MedCalc software (version 16.1).

RESULTS

The main group of the study consisted of 67 children born at a gestational age of 32 to 33/6 weeks, including 25 girls (37,31%) and 42 boys (62,69%). The mean birth weight of the children was 1548,84±141,48 g, the mean body length was 34,92±1,23 cm, the mean head circumference was 31,41±1,93 cm, and the mean trunk circumference was 26,90±2,08 cm.

Anthropometric parameters of newborns in the comparison group at 34-36/6 weeks of gestation were as follows: body weight – 2245,0 ± 39,45 cm; body length – 44,9 ± 0,07 cm; head circumference – 31,5 ± 0,25 cm; trunk circumference – 28,9 ± 0,23 cm.

The analysis of somatic, obstetric, and gynecological pathology in the mothers of the main group revealed a significant association between repeated pregnancy and childbirth and an elevated risk of preterm birth. Among the cases, 70,15% involved a history of multiple pregnancies and deliveries, with 68,66% of these instances resulting in planned cesarean sections and 17,91% necessitating emergency cesarean sections. During the gestational period, 50,75% of the women exhibited indications of threatened abortion at various stages, along with placental dysfunction (31,34%) and gestosis (28,36%). In 42,30% of cases, premature rupture of the membranes was observed, and in 31,34% of cases, pathology of placental and umbilical cord attachment was prevalence of somatic pathology in the main group was higher than that observed identified.

Table 1. A comparative analysis of coprofiltrate biomarkers in preterm infants (PI) belonging to the main and control groups (M±m)

Index	Main group (n=67)	Control group (n=31)
Calprotectin, μg/g	409,46±19,31*	64,74±3,11
Albumin, μg/g	40,26±3,01*	11,28±0,90
Alpha-1-Antitrypsin, μg/g	478,44±25,77*	241,15±18,90
Elastase, μg/g	270,68±18,04*	246,98±16,79
PMN Elastase, ng/g	90,45±5,04*	257,53±17,30

Note: * — significant difference between comparison groups, p<0,0001.

Table 2. Analysis of ROC curves of coprofiltrate indices in newborns of comparison groups

Index	AUC	Standard error	p	95 % CI	Sensitivity (SN) % (95 % CI)	Specificity (SP) % (95 % CI)
Calprotectin, μg/g	0,977	0,0216	<0,0001	0,930 – 0,996	98,55 (92,2 - 100)	97,83 (88,5 - 99,9)
Albumin, μg/g	0,859	0,0391	<0,0001	0,781 – 0,917	80,60 (69,1 - 89,2)	97,83 (88,5 - 99,9)
Alpha-1-Antitrypsin, μg/g	0,841	0,0392	<0,0001	0,761 – 0,903	71,64 (59,3 - 82,0)	84,78 (71,1 - 93,7)
Elastase, μg/g	0,538	0,0568	0,5010	0,439-0,635	52,24 (39,7 - 64,6)	77,50 (61,5 - 89,2)
PMN Elastase, ng/g	0,805	0,0482	<0,0001	0,720 – 0,874	83,58 (72,5 - 91,5)	89,13 (76,4 - 96,4)

Somatic pathology in mothers of the main group was presented more often, compared to the control group, with diseases of the cardiovascular system (59,70%), endocrine system (43,28%), and urinary system (53,73%) being more frequently diagnosed. Additionally, 11,39% of cases involved gastrointestinal pathology.

The severity of the condition observed in the children in the main group was attributed to hypoxic damage occurring in the context of morphological and functional immaturity of the body. Specifically, 18 newborns (26,47%) were diagnosed with acute asphyxia. All children exhibited hypoxic-ischemic central nervous system damage, with 67 cases (100%) presenting with either an excitation syndrome (43,28%) or a depression syndrome (56,72%). Additionally, five children (7,46%) were diagnosed with hydrocephalus syndrome. Antenatal fetal damage was identified in 29 children (43,28%). Some newborns exhibited indications of hemorrhagic lesions within the central nervous system. In particular, the results of neurosonography revealed that 48 children (71,64%) had subependymal hemorrhages of the I-II degree, while 23 children (34,33%) had intraventricular hemorrhages. Cerebral edema was identified in 17 children (25,37%). Respiratory distress syndrome was observed in 65 children (97,01%) from birth, necessitating long-term respiratory support. In 58 cases (86,57%), the newborns were diagnosed with multiple organ failure syndrome. An analysis of the mother's pregnancy and childbirth history revealed that all newborns in this group were at risk of intrauterine infection.

It has been observed that almost all newborns in the main group exhibited indications of impaired tolerance to enteral nutrition, as evidenced by the presence of clinical manifestations of disease. Specifically, 58 children (86,57%) exhibited regurgitation and stasis; 32 children (47,76%) displayed signs of flatulence; 47 children (70,15%) demonstrated intestinal paresis with delayed meconium and transitional stool; and the presence of bile or blood impurities in the stool was identified in 29 cases (43,28%). The presence of pain syndromes in conjunction with eating dysfunction was accompanied by episodes of apnea, bradycardia, and decreased blood saturation. This is a notable finding in terms of the importance of timely diagnosis and stabilization of the gastrointestinal system to prevent the development of these clinical complications.

In order to ascertain additional laboratory criteria for confirming these disorders, as well as to elucidate their potential pathophysiological mechanisms of development, along with the recommended diagnostic methods, some fecal biomarkers were identified, in particular: Alpha-1-Antitrypsin, Elastase, PMN Elastase, Albumin, Calprotectin were analyzed. The analysis of the obtained indicators was carried out with consideration of the results of the examination of the stated comparison groups.

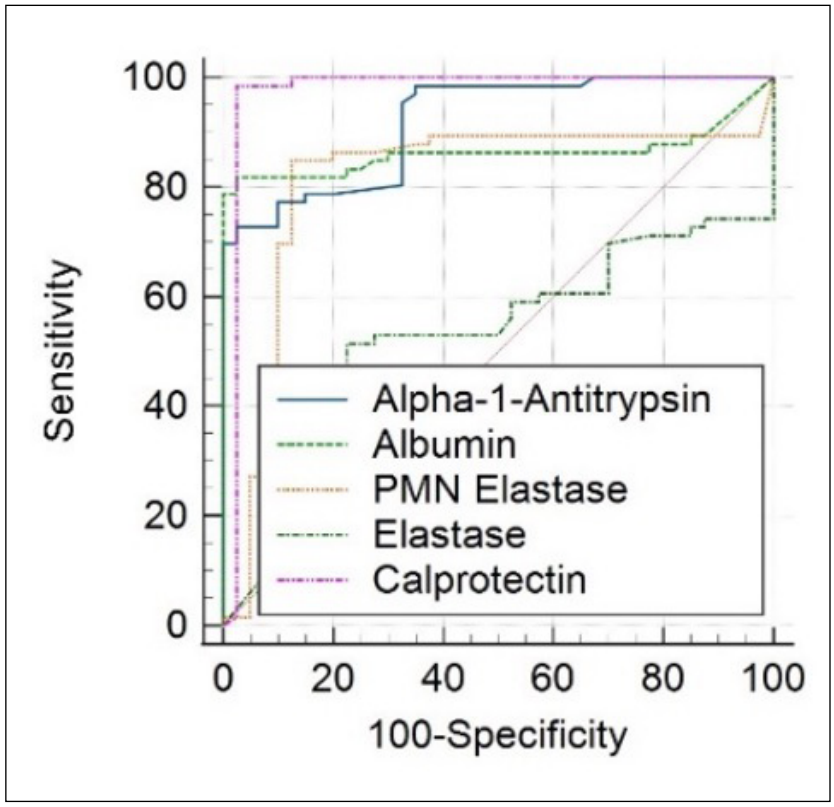

Fig. 1. Analysis of ROC curves of coprofiltrate indicators in the PI of the comparison groups.

The data on the mean values of coprofiltrate parameters are presented in Table 1.

The analysis of the results of the study of coprofiltrate in newborns with clinical signs of digestive dysfunction revealed notable alterations in the observed indices when compared to the control group. In particular, a significant increase in Calprotectin was observed – up to 409,46±19,31 μg/g, Albumin – up to 40,26±3,01 μg/g, Alpha-1-Antitrypsin – up to 478,44±25,77 μg/g and Elastase – up to 270,68±18,04 μg/g. Conversely, there was a notable decline in the level of PMN Elastase – 90,45±5,04 ng/g.

In light of the notable divergence of the utilized indicators from the control values and their pivotal role in elucidating the pathophysiological mechanisms of enteric dysfunction, a ROC analysis was conducted to ascertain their sensitivity and specificity. The results are presented in Fig. 1 and Table 2 for reference.

vwIt is important to note that the ROC analysis has identified calprotectin, albumin, PMN Elastase, and Alpha-1-Antitrypsin as the most significant indicators in terms of sensitivity and specificity.

In conclusion, the panel of biomarkers of gastrointestinal dysfunction, along with the analysis of ROC curves and their operational characteristics in preterm infants with severe forms of perinatal pathology, indicate that the following laboratory parameters of coprofiltrate can be recommended as additional criteria for food intolerance, in addition to clinical signs:

- Calprotectin increase at a threshold > 390,15 μg/g (AUC 0,977 [95% CI 0,930-0,996]; p<0,0001; SN 98,55% [95 % CI 92,2-100%]; SP 97,83% [95% CI 88,5 - 99,9%]);
- increased Albumin level at a threshold > 37,25 μg/g (AUC 0,859 [95% CI 0,781-0,917]; p <0,0001; SN 80,60 % [95% CI 69,1 - 89,2]; SP 97,83% [95% CI 88,5 - 99,9%]);
- increased Alpha-1-Antitrypsin level at a threshold of > 452,67 μg/g (AUC 0,841 [95% CI 0,761 – 0,903]; p <0,0001; SN 71,64% [95% CI 59,3 - 82,0 %]; SP 84,78% [95% CI 71,1 - 93,7 %]);
- decrease in the level of PMN Elastase at a threshold < 95,49 ng/g (AUC 0,805 [95 % CI [0,720 – 0,874]; p<0,0001; SN 83,58% [95% CI 72,5 - 91,5 %]; SP 89,13% [95% CI 76,4 - 96,4 %]).

DISCUSSION

A review of contemporary scientific literature reveals a growing interest among researchers in different countries in establishing laboratory criteria for diagnosing food intolerance in newborns, with a particular focus on preterm infants [9-13]. The extant data suggest the pursuit of scientifically grounded criteria that can elucidate the primary pathophysiological mechanisms of digestive system dysfunction and enhance diagnostic methodologies, with the ultimate objective of devising efficacious therapeutic strategies.

In light of the findings of the study, it can be confirmed that the presence of inflammation of the intes-

tinal mucosa is a consequence of the negative effects of hypoxia on the body of a preterm infant, as evidenced by the significant increase in calprotectin levels in the coprofiltrate. Calprotectin is released from activated neutrophils in response to tissue injury and enters the feces, reflecting the transepithelial migration of granulocytes to the intestinal lumen. Consequently, it is regarded as a marker of integrin inflammation, which enables the differentiation between functional and inflammatory diseases [14].

The elevated albumin concentration is indicative of enhanced intestinal wall permeability and suggests a disruption in the parietal absorption processes. A breach in the integrity of the intestinal barrier results in the leakage and loss of proteins, which is attributed to the inflammatory exudation of the altered epithelium [2]. This also results in an increased translocation of opportunistic pathogens and endotoxins from the intestinal lumen into the general bloodstream. [15]

It is noteworthy that there has been an increase in the level of Alpha-1-Antitrypsin, which is an acute phase protein and a primary inhibitor of serine proteases. This protein is actively secreted under conditions of inflammation, which may explain the observed decrease in the proteolytic activity of PMN Elastase, as documented in our study. Alpha-1-Antitrypsin is primarily synthesized by the liver endoplasmic reticulum, as well as by polymorphonuclear neutrophils, alveolar macrophages, monocytes, enterocytes, and Paneth cells. Alpha-1-Antitrypsin is regarded as the primary antiprotease capable of neutralizing surplus proteases, inhibiting chemotaxis, and preventing neutrophil adhesion. In terms of its mechanism of action, it has been demonstrated to inhibit the release of proinflammatory cytokines and to absorb reactive oxygen species, thereby playing an important role in the activation of the body's anti-inflammatory response [16, 17].

Our data indicate a notable decrease in PMN Elastase in the coprofiltrate of preterm infants exhibiting signs of food intolerance disorders. This protease is secreted in an active form from granulocytes, where it regulates inflammatory and immune responses. It acts together with reactive oxygen species to promote the breakdown of microorganisms [18-20]. The observed decline in the index level may be indicative of an inadequacy in the regulatory function of this crucial mediator in the development of anti-inflammatory mechanisms in hypoxic inflammation. According to some literature data, this may be indicative of the simultaneous presence of cholestasis [16, 19].

The analysis of laboratory parameters revealed that the newborns in the main group exhibited slightly elevated levels of Elastase in comparison to the control group. Elastase is a pancreatic-specific serine endopeptidase that catalyzes the hydrolysis of natural elastin and the digestion of peptides into alanine, glycine, and serine residues. As evidenced in the literature, an increase in the activity of Elastase may manifest at an earlier stage than other enzymes, even at the subclinical stage of inflammation [19, 20]. This may also be indicative of hypoxic inflammation in preterm infants. It is important to note, however, that the results of the ROC analysis indicate that this indicator does not have a significant value as a diagnostic criterion for food intolerance. It is our recommendation that further research be conducted to elucidate the functional significance of Elastase in the context of enteric function in preterm infants. This should be done through the implementation of experimental studies.

In light of the aforementioned evidence, the following synthesis can be made: the intolerance to enteral nutrition observed in preterm infants with perinatal pathology is caused by disorders of the functional state of the digestive system. The aetiology of these disorders is multifactorial. The underlying pathophysiology of these disorders is hypoxic inflammation occurring in the context of morphological and functional immaturity of the body at birth. In addition to the traditional clinical signs, some laboratory criteria are presented that elucidate the mechanisms of food intolerance development in hypoxic inflammation and can be utilized in neonatological practice, as evidenced by their satisfactory sensitivity and specificity.

CONCLUSIONS

1. The basis of functional disorders of the gastrointestinal system in premature infants is hypoxic inflammation against the background of morphological and functional immaturity of the body.
2. The list of clinical signs of food intolerance in newborns includes: regurgitation and stasis (86,57%), intestinal paresis with delayed meconium and transitional stool (70,15%), flatulence (47,76%), bile or blood impurities in the stool (43,28%). The pain syndrome in the context of nutritional dysfunction is accompanied by episodes of apnea, bradycardia and decreased blood saturation, which is important for preventing the development of complications of the clinical course of diseases.
3. The recommended laboratory markers of intestinal dysfunction in premature infants are increased levels of calprotectin > 390,15 μg/g, albumin > 37,25 μg/g, Alpha-1-Antitrypsin > 452,67 μg/g, and decreased level of PMN elastase < 95,49 ng/g.

4. Implementation of recommendations for the use of laboratory markers of food intolerance, along with clinical signs, will increase the effectiveness of diagnostic measures for perinatal pathology in preterm infants.

PROSPECTS FOR FURTHER RESEARCH

Prospects for further research are the study of other significant criteria for laboratory diagnosis of food intolerance in perinatal pathology of newborns, in order to clarify the methods of possible therapeutic correction.

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The study was conducted at the neonatal intensive care unit of the City Clinical Maternity Hospital No. 2 between 2014 and 2015 as part of the research activities of the Department of Pediatrics, Neonatology and Perinatal Medicine of Bukovinian State Medical University (Chernivtsi, Ukraine): the research work "Improving the areas of prognosis, diagnosis and treatment of perinatal pathology in newborns and young children, optimization of schemes of catamnestic observation and rehabilitation" (State registration number 0115U002768, term of execution 01.2015-12.2019); the research work "Chronobiological and adaptation aspects and features of vegetative regulation in pathological conditions in children of different age groups" (State registration number 0122U002245, term of execution 01.2020-12.2024).

CONFLICT OF INTEREST
The Author declare no conflict of interest

CORRESPONDING AUTHOR
Oleksii S. Godovanets
Bukovinian State Medical University
2 Teatralna Sq, 58002 Chernivtsy, Ukraine
e-mail: godovanec.oleksij@bsmu.edu.ua

ORCID AND CONTRIBUTIONSHIP
Oleksii S. Godovanets: 0000-0003-1474-7642 A B C D E F

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

RECEIVED: 09.06.2024
ACCEPTED: 21.10.2024

ORIGINAL ARTICLE

CONTENTS

Outcomes of implementing updated curricula for enhancing digital competence in undergraduate and post-diploma nursing education

Svitlana V. Gordiichuk[1], Maryna V. Kiriachok[1], Serhii M. Hryshchuk[1,2], Lina A. Sikoraka[1], Hlib D. Aleksandrenko[3], Daria I. Sokolovska[3], Oleksandr V. Zvinchuk[3]

[1]ZHYTOMYR MEDICAL INSTITUTE OF ZHYTOMYR REGIONAL COUNCIL, ZHYTOMYR, UKRAINE
[2]ZHYTOMYR IVAN FRANKO STATE UNIVERSITY, ZHYTOMYR, UKRAINE
[3]USAID HEALTH REFORM SUPPORT PROJECT, KYIV, UKRAINE

ABSTRACT

Aim: To assess the effectiveness of updated curricula in developing digital competence and enhancing the use of information and communication technologies in healthcare at the undergraduate and post-diploma levels of nursing education.

Materials and Methods: The updated 'Digigram for Healthcare Professionals' test tool, consisting of 75 questions, was utilized to assess the digital competence of 90 students and 136 practicing nurses before and after training in the Health Informatics curricula, updated through the USAID Health Reform project grant program at Zhytomyr Medical Institute in Ukraine during the first half of 2024. Additionally, the satisfaction of both groups with the curricula content was evaluated.

Results: Students' digital competence increased by 71% following the updated curricula training. The proportion of students with digital competence levels A (Proficient) and B (Above Basic) rose by 38%, from 27% to 65%. Among nurses, digital competence improved by 10%, with the proportion of those at levels A and B increasing by 14%, from 57% to 71%. Additionally, 93% of students and 94% of nurses expressed satisfaction with the content of the new curricula.

Conclusions: The effectiveness of the updated Health Informatics curricula in developing digital competence and promoting the use of information and communication technologies in healthcare for nursing students at undergraduate and post-diploma educational institutions has been demonstrated.

KEY WORDS: curriculum, nurses, digital health, capacity building

Wiad Lek. 2024;77(11):2161-2167. doi: 10.36740/WLek/197087 DOI

INTRODUCTION

In today's world, information and communication technologies (ICT) have become an integral part of the healthcare industry. The World Health Organization (WHO), in its Global Strategy for Digital Health 2020-2025, emphasizes the critical importance of fostering digital competence among healthcare professionals [1]. This objective is also reflected in the national digital health strategies of various countries. It is particularly essential for nurses, who constitute a vital segment of the healthcare workforce and require the necessary knowledge, skills, motivation, and support to effectively integrate ICT into their practice [2]. Insufficient digital competence can result in medical errors, compromised quality of healthcare services, and adverse patient outcomes [3]. Thus, continuous education and professional development for nurses, tailored to their levels of expertise and aimed at identifying barriers and enablers of digital competence development, are crucial for ensuring high-quality healthcare delivery [4, 5]. This need is especially relevant for Ukraine, where the rapid expansion of eHealth initiatives and the electronic healthcare system (EHS) have increased the demand for improved digital competence among the healthcare workforce. The Digital Competence Framework for Healthcare Professionals (hereinafter – the DigComp Framework for HCPs), approved by the Ministry of Health of Ukraine in October 2023, outlines the requirements for digital competence and ICT utilization in healthcare [6].

An assessment conducted by the USAID Health Reform Support (USAID HRS) project in 2021 revealed that only 44% of healthcare workers possessed above-basic digital literacy, 35% had basic skills, and 21% were at the beginner level. Furthermore, only 37% of respondents had received digital literacy training in the last three years [7].

The low level of digital literacy among healthcare professionals in Ukraine indicates the limited involvement

of the medical education sector in the digital transformation of healthcare system. This issue stems from outdated curricula for digital competence development across all stages of medical education — undergraduate, graduate, postgraduate, and continuing professional development [8]. In response, the Government of Ukraine has adopted a number of legislative measures aimed at enhancing the level of digital competence of the healthcare workforce [9-11].

Developing digital competence among nurses has become a critical priority in the modern digital era, as it is essential for ensuring high-quality medical services and driving the successful digital transformation of the healthcare system.

AIM

The aim of this study was to assess the effectiveness of updated curricula in developing digital competence and enhancing the use of ICT in healthcare at the undergraduate and post-diploma levels of nursing education.

MATERIALS AND METHODS

To achieve the research objective, digital competence levels were assessed in accordance with the DigComp Framework for HCPs [6], which comprises five domains (Fig. 1).

The study employed an updated pilot version of the "Digigram for Healthcare Professionals" (hereinafter – Digigram) [12], which was modified in accordance with the DigComp Framework for HCPs and consisted of 75 questions. Digital competence levels were evaluated based on the number of correct responses: A – Proficient (60-75 points); B – Above Basic (45-59 points), C – Basic (30-44 points); D – Low (15-29 points); F – No Skills (0-14 points).

Guided by the DigComp Framework for HCPs and addressing the needs of Ukraine's modern healthcare system, the staff of Zhytomyr Medical Institute (hereinafter – ZhMI) working alongside with external experts on digital health education, developed new curricula for 'Basics of Health Informatics' aimed at both nursing students and practicing nurses [13]. During the first half of 2024 the curricula engaged 101 second-year undergraduate students from ZhMI enrolled in the updated Health Informatics programs, as well as 171 practicing nurses who attended continuing professional development courses at ZhMI. The students' training under the updated program spanned 15 weeks, comprising a total of 90 hours: 20 lecture hours, 22 hours of practical sessions, and 48 hours of individual work. Classes were delivered in a hybrid format (both in-person and online) by Health Informatics instructors.

For practicing nurses, an intensive 6-hour advanced training course on the use of ICT in healthcare was organized, with 2 hours dedicated to lectures, and 4 hours to practical exercises. The training was conducted online by the faculty from the Department of Health Informatics and Postgraduate Education at ZhMI.

To assess the effectiveness of these curricula, the number of correct answers for each domain of the DigComp Framework for HCPs was recorded before and after training, and the statistical significance of the differences between the results was evaluated.

Additionally, a survey was conducted to assess satisfaction with the list of topics and the relevance of the information provided both prior to the modified curricula implementation and after the completed training. Satisfaction with the updated curricula was measured using an anonymous online questionnaire, employing a 5-point Likert scale.

The results were statistically analyzed using the D'Agostino-Pearson test to assess the normality of the sample distribution. The median (Me), 95% confidence interval was determined for each quantitative parameter. Qualitative data were presented as a percentage of the trait and 95% confidence interval. For normally distributed data, the Student's paired T-test was applied to compare the means of two samples (before and after training). For non-normally distributed data, the Wilcoxon W-test was used to compare the central tendencies of paired samples. The McNemar's test with the continuity correction was used to calculate the p-value in a binary comparison of the level of digital competencies and satisfaction of curricula. Statistical analysis was conducted using MedStat v5.2 software. A p-value <0,05 was considered as statistically significant.

The study adhered to the ethical standards and principles of the Helsinki Declaration. Ethical approval was obtained from the Zhytomyr Medical Institute Bioethics Committee (Protocol No. 4 June 12, 2024).

RESULTS

The analysis of learning outcomes was based on data from participants who completed both pre- and post-training surveys: 90 students (89,1% of those engaged with the updated curricula) and 136 nurses (79,5% of those engaged). The mean age of students was 17±0,4 years, with a male-to-female ratio of 0,37. Among practicing nurses, the mean age was 39±1,8 years, with a male-to-female ratio 0,09.

Table 1 presents a comparative analysis of digital competence levels among nursing students, before and after training, categorized by the domains of the DigComp Framework for HCPs.

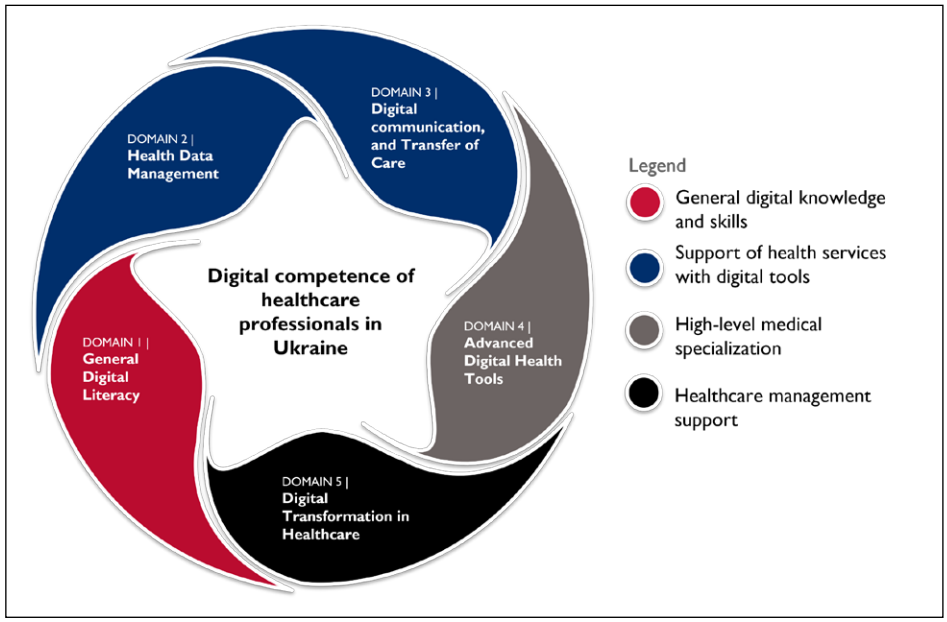

Fig. 1. Domains of the DigComp Framework for HCPs for Healthcare Professionals.

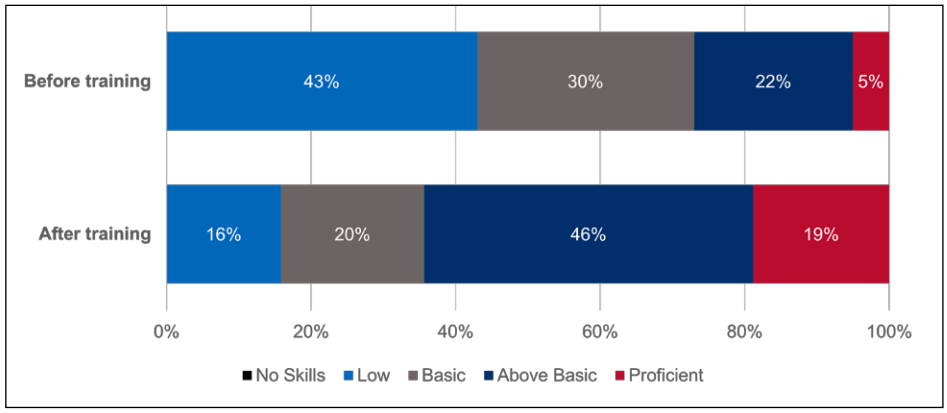

Fig. 2. Proportion of Students by Levels of Digital Competence Before and After Training (N=90).

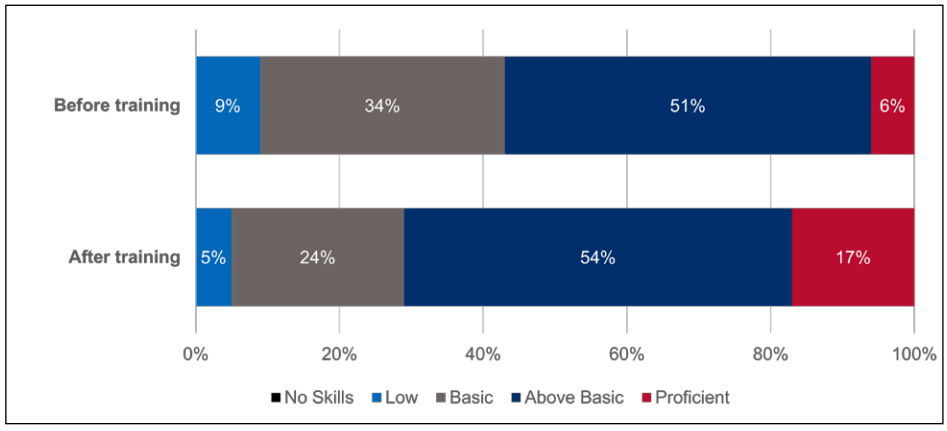

Fig. 3. Proportion of Nurses by Digital Competence Levels Before and After Training (N=136).

The results demonstrated a high level of digital competence in Domain 1, which covers general digital literacy. Additionally, the updated training curricula led to a 30% increase in the number of correct responses within Domain 1. For the other domains of the DigComp Framework for HCPs, the number of correct responses increased by 67% to 100% following the training.

Before the training, the number of correct answers across all areas of the DigComp Framework for HCPs was 31 ± 1,8 (95% CI: 29-37), representing 42% of the total 75 possible answers. After the training, the proportion of correct answers increased to 71%, with the number of correct answers rising to 54 ± 1,8 (95% CI: 49-56).

Regarding the distribution of respondents by their levels of digital competence (Fig. 2), it was observed that before the training, 27% of respondents achieved levels A (Proficient) and B (Above Basic). Following the

Table 1. Assessment Results of Students' Digital Competence Before and After Training of the Updated Curriculum (N=90)

DigComp Framework for HCPs domains	Number of correct answers (max – 15)		% increase
	Before training	After training	
Domain 1	10±0,4 (95% CI: 9-11)	13±0,4 (95% CI: 12-14)	30*
Domain 2	6±0,3 (95% CI: 5-7)	10±0,4 (95% CI: 9-11)	67*
Domain 3	6±0,4 (95% CI: 5-7)	10±0,4 (95% CI: 9-11)	67*
Domain 4	5±0,4 (95% CI: 4-6)	9±0,5 (95% CI: 8-10)	80*
Domain 5	5±0,4 (95% CI: 4-9)	10±0,5 (95% CI: 8-11)	100*
Total	31±1,8 (95% CI: 29-37)	54±1,8 (95% CI: 49-56)	71*

Note: *p<0,001.

Table 2. Assessment Results of nurses' Digital Competence Before and After Training with the Updated Curriculum (N=136)

DigComp Framework for HCPs domains	Number of correct answers (max – 15)		% increase
	Before training	After training	
Domain 1	11±0,3 (95% CI: 10-12)	13±0,3 (95% CI: 12-14)	18*
Domain 2	8±0,2 (95% CI: 7-9)	9±0,2 (95% CI: 8-10)	13*
Domain 3	9±0,3 (95% CI: 8-10)	10±0,3 (95% CI: 9-11)	11*
Domain 4	8±0,3 (95% CI: 7-9)	9±0,3 (95% CI: 8-10)	13*
Domain 5	9±0,3 (95% CI: 8-10)	10±0,3 (95% CI: 9-11)	11*
Total	46±1,1 (95% CI: 44-49)	51±1,2 (95% CI: 49-52)	10*

Note: *p<0,05.

training, this proportion increased to 65%, reflecting 38% improvement (p <0,001).

Table 2 presents a comparative analysis of the digital competence levels among the surveyed nurses before and after training, across the domains of the DigComp Framework for HCPs.

Before engaging with the updated program, nurses averaged 46 correct answers out of 75, representing 61% of the total, which is higher than the 42% average achieved by undergraduate students. Additionally, the updated program led to a notable increase in the number of correct answers across all areas by 10%, based on the 6 hours of training provided.

Regarding the distribution of nurses by their levels of digital competence (Fig. 3), it was observed that before the training, 57% of respondents were at levels A (Proficient) and B (Above Basic). After the training, this proportion rose to 71%, representing a 14% increase (p=0,004).

The final phase of the study involved comparing subjective assessments of satisfaction with the previous and the updated curricula among 57 students and 96 nurses (56,1% and correspondingly 56,4% of those engaged with the updated curricula). For students, satisfaction rose from 63% (95% CI: 50%-75%) with the previous curriculum to 93% (95% CI: 85%-98%) with the updated curriculum (p<0,001). Likewise, for nurses, satisfaction increased from 60% (95% CI: 50%-70%) with the previous curriculum to 94% (95% CI: 88%-98%) with the updated curriculum (p<0,001).

DISCUSSION

The study allowed for the assessment of digital competence levels among students and practicing nurses before and after their participation in the updated Health Informatics curricula The findings revealed that the revised curricula significantly enhanced digital competence at both the undergraduate and post-diploma levels.

In particular, the number of correct answers increased by 71% (from 32 to 54 out of 75 possible) following the students' engagement with the updated curricula. The most significant improvements were observed in the domains of "Digital Transformation in Healthcare" (100%), "Advanced Digital Health Tools" (80%), "Health Data Management" and "Digital Communication, and Transfer of Care" (67%). The proportion of students demonstrating Proficiency and Above Basic skills increased by 38% (from 27% to 65%). The relatively modest increase in the domain of "General Digital Literacy" is likely to the fact that students are from a generation already familiar with active ICT development.

Following the implementation of the updated training program, the number of correct answers provided by nurses increased by 10% (from 46 to 51 out of 75 possible). The proportion of nurses demonstrating Proficiency and Above Basic skills increased by 14% (from 57% to 71%). Compared to students, nurses initially achieved higher results, which can be attributed to their existing practical experience. As they are already employed in the healthcare system and frequently use

EHS and other digital tools, this practical exposure contributes to their enhanced digital competence.

Overall, previous research has demonstrated the importance of a digital literacy curriculum, showing that it can significantly enhance nurses' knowledge and skills, as well as improve their self-efficacy.

For example, a study by A. Kuek and S. Hakkennes [14] demonstrated that a digital literacy curriculum significantly enhanced nurses' knowledge and skills in using information systems before the introduction of an electronic health record.

In the research by J. Kang and E. Suh [15] the effectiveness of smartphone applications designed for the care of patients with chronic diseases was developed and assessed. Their study found that these applications significantly improved nurses' knowledge and self-efficacy in managing hypertension and diabetes. This underscores the potential of integrating digital technologies into educational programs as an effective means of enhancing the competencies of future nurses.

We argue that our results underscore the urgent need for continuous professional development to enhance nurses' digital competence. Previous research by U. Kinnunen et al. [16] has also highlighted the necessity of expanding educational programs to include training on digital devices and assisting patients with digital services, emphasizing that this need is especially critical for older nurses.

A systematic review by E. Kulju et al. [17] demonstrated the effectiveness of educational interventions in enhancing the digital competence of health professionals. The review indicated that interventions are most successful when they incorporate diverse learning modalities, such as theoretical knowledge, practical exercises, and interactive training. Furthermore, the authors highlight the importance of organizational support and personalized guidance throughout the learning process. These findings support the implementation of updated curricula that integrate multiple methods and learning approaches.

C. Buchanan et al. [18] research highlights the shared responsibility of nurses in shaping decisions regarding the integration of artificial intelligence into the healthcare system. This illustrates the importance of not only technical skills but also ethical and communication competence among nurses in the context of the digital transformation in healthcare.

A study by A. Tubaishat and L. Habiballah [19] revealed that Jordanian nursing students demonstrated a moderate level of eHealth literacy. While they were aware of online health resources and knew how to use them, they showed a lack of ability to critically evaluate the quality of these resources. The authors highlight the need to incorporate eHealth competence into nursing curricula. These findings are consistent with the goals of our educational programs, which aim to develop not only technical proficiency but also critical and analytical abilities.

The incorporation of innovative digital competence curricula that integrate diverse teaching modalities and address contemporary healthcare needs – while encompassing technical ethical, communication, and research skills-enhances nurses' preparedness to effectively utilize ICT in their professional practice.

LIMITATIONS AND DIRECTIONS FOR FURTHER RESEARCH

Several limitations should be noted in this study:

1. The study was conducted exclusively at ZhMI, which means that the results cannot be generalized beyond the specific context of this study.
2. Digital competence was assessed immediately following the training program; future studies should evaluate the long-term impact of such training.
3. The evaluation of respondents' satisfaction is inherently subjective and may be influenced by bias.

Further research should expand to include the assessment of the digital competence among healthcare professionals in various regions of Ukraine and investigate how digital skills affect the efficiency of professional activities and the quality of medical services.

CONCLUSIONS

The study results demonstrate the effectiveness of implementing updated Health Informatics curricula in developing digital competence among nurses at both undergraduate and post-diploma levels. By enhancing nurses' digital skills, these curricula support their ability to navigate the national eHealth landscape, utilize electronic healthcare system and other digital tools in their practice, and ultimately improve the quality of medical services and contribute to the successful digital transformation of the healthcare industry.

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This study is made possible by the support of the American people through the United States Agency for International Development (USAID). The contents of this article are the sole responsibility of the authors and Deloitte consulting, LLP and do not necessarily reflect the views of USAID, United States Government, official policy. The study was conducted under contract number 72012118C00001 and USAID Health Reform Support Project Grant Program G-2309-02391 to develop digital competence of healthcare professionals and medical students.
The authors would like to express their gratitude to Anna Tokar, Monitoring and Evaluation Lead at the USAID Health Reform Support Project, for her valuable insights and edits to the article.

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Serhii M. Hryshchuk
Zhytomyr Medical Institute of Zhytomyr Regional Council

46/15 Velyka Berdychivska St., 10008 Zhytomyr, Ukraine
e-mail: zamlkzt@gmail.com

ORCID AND CONTRIBUTORSHIP

Svitlana V. Gordiichuk: 0000-0003-4609-7613 A E F
Marina V. Kiriachok: 0000-0002-0654-9542 A E F
Serhii M. Hryshchuk: 0000-0002-5553-8110 A B C D F
Lina A. Sikoraka: 0000-0002-4785-1086 B C
Hlib D. Aleksandrenko: 0009-0007-2980-8213 A B D F
Daria I. Sokolovska: 0009-0009-3628-8687 B E
Oleksandr V. Zvinchuk: 0000-0001-7163-1932 A E F

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

RECEIVED: 09.06.2024
ACCEPTED: 24.10.2024

ORIGINAL ARTICLE

CONTENTS

The problem of resilience of families with the consequences of war psychotrauma

Nina V. Koliadenko[1,2], Mykola M. Shopsha[2], Olena L. Shopsha[2], Nadiia O. Bachurina[2], Khrystyna S. Zhivago[1], Oksana O. Drevitska[3], Jelena Kolesnikova[4]

[1]SIGMUND FREUD UNIVERSITY OF UKRAINE, KYIV, UKRAINE
[2]INTERREGIONAL ACADEMY OF PERSONNEL MANAGEMENT, KYIV, UKRAINE
[3]TARAS SHEVCHENKO KYIV NATIONAL UNIVERSITY, KYIV, UKRAINE
[4]RIGA STRADINS UNIVERSITY, RIGA, LATVIA

ABSTRACT

Aim: To determine the characteristics of family resilience to the consequences of war psychotrauma.

Materials and Methods: The experimental group included 23 young families (46 people, average age 28,20 ± 1,33 years) forcibly resettled from the zone of active hostilities; the control group consisted of 25 young families (50 people, average age 28,16 ± 1,72 years) from Kyiv, who did not change their place of residence and did not witness active hostilities. The following psychodiagnostic methods were used questionnaires: "Diagnosis of personality behavior in a conflict situation"; "Communication in the family"; Marriage satisfaction test-questionnaire; Connor-Davidson-10 resilience scale.

Results: In families evacuated from the zone of active hostilities a decrease in the vitality and adaptation potential of the family, a violation of its basic functions were revealed, however, there is a significant potential for recovery.

Conclusions: The hypothesis that the situation of a full-scale war has a negative effect on stability, resilience, family relations and family functions in modern Ukraine has been confirmed.

KEY WORDS: family, war psychotrauma, resilience, adaptation, psychological correction

Wiad Lek. 2024;77(11):2168-2173. doi: 10.36740/WLek/197088 DOI

INTRODUCTION

The family in Ukraine is defined as a small social group that is of great importance in the life of every person, ensuring their socialization and satisfaction of needs, providing help and support in difficult life situations [1]. The war gives rise to a number of complex problems that negatively affect the life of the family and its stability [2, 3]. Among the problems faced by the family during the war are the disruption of family education in refugee families, the complication of relations between family members, the growth of adolescent deviations, family violence, etc. [4, 5]. At the same time, the harmony of the social and family environment determines the stability of the morale and fighting spirit of servicemen [6, 7].

Despite the fact that many scientists are currently paying attention to the issue of the influence of the war situation on the institution of the family in Ukraine [8], it remains insufficiently developed. Therefore, the problem of the stability of families with the consequences of military psychotrauma is an actual topic of research.

AIM

The purpose of the study is to determine the characteristics of family resilience to the consequences of war psychotrauma.

MATERIALS AND METHODS

The research methodology was based on a systematic and holistic understanding of a person as a biopsychosocial being and on an empirical approach to the study of family relationships [1].

THE SAMPLE

To participate in the study, an experimental group was formed in a randomized manner, which included 23 young families (46 people, average age 28,20 ± 1,33 years) of forcibly displaced persons who had experience of being in a zone of active hostilities. As a control group, 25 randomly selected young families (50 people, average

age 28,16 ± 1,72 years) from Kyiv, who did not change their place of residence since the beginning of the war, did not witness active hostilities and did not participate in them, were studied. Both groups were comparable in age, family structure (couples with 1-2 children), level of education (unfinished higher education), financial well-being (average level of material well-being).

METHODS OF EMPIRICAL RESEARCH

The following psychodiagnostic methods were used in the work [1, 7]:

- Questionnaire "Diagnosis of personality behavior in a conflict situation" (developer O.S. Kocharyan [1]), which contains 8 scales: I – non-constructive attitude of partners towards marriage; II – depressive moods; III – protective mechanisms; IV – defensive mechanisms; V – presence of aggression; VI – somatization of anxiety; VII – fixation on psychotrauma; VIII scale – control. A value of less than 30 points corresponds to low indicators, 30-70 points to average; 30-40 points – average indicators with a tendency to low; 60-70 points – average indicators with a tendency to high; more than 70 points – high indicators;

- Questionnaire "Communication in the family" (developers: Alyoshina Yu.E., Gozman L.Ya., Dubovska O.M. [1]) – contains 6 scales: 1 scale – the level of trusting communication in the family; II scale – level of mutual understanding between spouses; III scale – the degree of similarity of views of spouses; IV scale – presence of "family language"; V scale – ease of communication between spouses; VI scale – psychotherapeutic nature of family communication;

- Marriage satisfaction questionnaire (developers V.V. Stolin, T.L. Romanova, G.P. Butenko [1]) – consists of 24 questions and is intended for express diagnosis of the level of conflict in a married couple (0-16 points – absolutely unhealthy relationship; 17-22 points – rather unfavorable relationship; 29-32 points – quite safe relationship);

- Connor-Davidson-10 resilience scale – an express questionnaire consisting of 10 statements, the answers to which are evaluated on a 5-point Likert scale (from 0 – "completely false" – to 4 – "true in almost all cases"). The range of points from 0 to 15 indicates a low level of resilience, 16-20 points – a level of resilience below the average, 21-25 points – an average level of resilience, 26-30 points – a level of resilience above than the average, 31-40 points – a high level of stability [7]. The reliability of this questionnaire for assessing viability in wartime conditions was proven by a study of 1,257 respondents from all regions of Ukraine conducted by O.M. Kokun et al., in June-July 2022 [7].

ETHICAL ISSUES

During the study, all regulatory requirements for bioethics specified in the Helsinki Declaration on domestic regulatory documents were strictly observed. The consent of the ethics committee was obtained for the study. Respondents of both research groups gave written informed consent to participate in the experiment. In the process of the research, confidentiality of personal data of its participants and tolerant attitude towards all respondents was ensured. Anonymity of the obtained research results was achieved by special encryption of questionnaire forms.

STATISTICAL PROCESSING

Accumulation, sorting and visualization of research information was carried out in the Microsoft Excel program. The IBM SPSS program (Statistical Package for the Social Science) v. 27 (2022) was used for statistical analysis of the received empirical data. Current statistical calculations were carried out using the Social Science Statistics online calculator. Quantitative indicators were indicated as mean ± standard deviation. Qualitative parameters were presented as absolute and relative (%) frequency. The Student's t-test was used to compare the quantitative indicators between two independent samples. At the same time, the size of the effect was estimated using Cohen's d test, in which the effect size r was considered significant at a value of more than 0,5. To determine the relationship between categorical variables, the χ^2 (chi-square) test was used, which was calculated using 4-field tables. Correlation analysis was performed using the Pearson correlation coefficient, when the relationship between indicators was considered close at the value of the correlation coefficient $r_{xy} \geq 0,7$. A 2-tailed $p<0,05$ was considered statistically significant.

RESULTS

Table 1 shows the comparative results of the research of the respondents of the experimental and control groups using the "Diagnostics of personality behavior in a conflict situation" method.

As can be seen from this table, the indicators of the behavior of family members of the experimental group in a conflict situation are high, while in the control group they are mostly lower, being within the range of average values with a tendency to high. Increased aggressiveness, anger and psycho-emotional rigidity were found in the family members of forced migrants, which led to the use of non-constructive approaches to solving problems. Marital autonomy is also more

Table 1. Results of diagnosis of family behavior of respondents of experimental (A) and control (B) groups in a conflict situation

Scale	GPA		Student's t-test	p	Cohen's d	r
	A	B				
I	80,6±8,4	70,2±9,2	2,64	0,01	1,18	0,51
II	76,4±5,1	63,1±6,3	5,18	<0,001	2,32	0,76
III	59,3±10,2	64,5±5,9	-1,40	0,10	-0,62	-0,30
IV	71,2±8,6	68,6±4,2	0,86	0,21	0,38	0,19
V	82,4±5,2	72,0±9,8	3,29	0,01	1,33	0,55
VI	78,5±6,9	74,3±4,5	1,61	0,07	0,72	0,33
VII	86,2±2,4	76,8±3,6	6,86	<0,001	-225,73	-1,00

Notes: GPA – average score, A – experimental group, B – control group; I – non-constructive attitude of partners towards marriage; II – depressive moods; III – protective mechanisms; IV – defensive mechanisms; V – presence of aggression; VI – somatization of anxiety; VII – fixation on psychotrauma.

Table 2. The results of the study of the peculiarities of communication in the family of respondents of the experimental (A) and control (B) groups

Scale	GPA		Student's t-test	p	Cohen's d	r
	A	B				
I	3,2±0,1	2,9±0,2	3,05	0,008	1,90	0,69
II	3,5±0,1	3,1±0,1	8,41	<0,001	4,00	0,89
III	3,6±0,2	3,4±0,1	2,53	0,02	1,26	0,53
IV	3,3±0,2	3,5±0,1	-2,58	0,02	-1,26	-0,53
V	2,7±0,1	2,6±0,2	1,37	0,10	0,63	0,30
VI	2,1±0,2	2,2±0,3	-0,75	0,24	-0,39	-0,19

Notes: GPA – average score, A – experimental group, B – control group; I – the level of trusting communication in the family; II scale – level of mutual understanding between spouses; III scale – the degree of similarity of views of spouses; IV scale – presence of "family language"; V scale – ease of communication between spouses; VI scale – psychotherapeutic nature of family communication.

Table 3. The results of the study of marital satisfaction and stability of the respondents of the experimental (A) and control (B) groups

Indicator	GPA		Student's t-test	p	Cohen's d	r
	A	B				
Average marriage satisfaction score	28,7±2,6	28,9±2,5	-0,16	0,44	-0,08	-0,04
Indicator of resilience	20,5±4,2	24,8±3,6	-2,46	0,02	-1,10	-0,48

Notes: GPA – average score, A – experimental group, B – control group.

pronounced in the experimental group compared to the control group, which may indicate an increased risk of marital breakdown during the war.

The average depression index of the respondents of the experimental group exceeded 70 points, which indicated its high level. In the respondents of the experimental group, such protective mechanisms as rationalization, isolation, and intellectualization prevailed, while in the control group, prevention of awareness of psychotraumatic information due to its displacement, regression, and denial of existing problems was more often observed. An increase in somatization anxiety reaches a high level, which requires attention, as it may indicate the presence of hidden (somatized) depression.

An addition to the method of researching family attitudes is the method of researching the style of family communication (Y. Ye. Aloshina, L. Ya. Gozman, O. M. Dubovska [1]), the results of which are shown in Table 2. It was established that in the families of temporarily displaced persons, the nature of communication in the family is more trusting, the views of the spouses are more similar to each other, and there is more mutual understanding in the relationship.

However, both in the experimental and in the control research group, the process of communication in the family is equally complex and does not have a psychotherapeutic nature. This indicates the depletion of adaptive resources as a result of military trauma, which prevents the family from performing protective and supportive functions. In the next Table 3 shows the average total score of marital satisfaction and the result of calculating the average viability index according to the short version of the Connor-Davidson-10 resilience scale of the studied respondents of the experimental

and control groups, with appropriate statistical processing. In both groups of respondents, the index of family well-being ranges from a transitional to a fairly prosperous category. Such a result can be explained by the fact that the identified negative characteristics of marital relations are directly related to the psychotrauma of the war, so they are temporary and have a significant potential for recovery.

However, the uncertainty and unpredictability of the future creates a significant risk of aggravation of family problems, especially in the families of evacuees, which requires appropriate psychocorrective and psychoprophylactic measures.

The family's ability to cope with complex psychotraumatic wartime situations was assessed using the short version of the Connor-Davidson Resilience Scale-10 [7]. As can be seen from this table, the indicator of stability of the respondents of the control group is in the range of average values and above the average, while in the experimental group this indicator falls into the range of average and lower values. This indicates that the level of viability of the families of temporarily displaced persons decreases as a result of the psychological trauma experienced during the war, which requires providing them with psychological support and psychocorrective assistance. In both studied groups, a direct and close connection between the vitality of the individual and the characteristics of family relationships was found (r_{xy}=0,854; n=96; p<0,00001). At the same time, in the experimental group, negative emotional states (depression, aggression) were most associated with the level of vitality, and in the control group, with protective defense mechanisms, fixation on psychotrauma, and somatization of anxious experiences.

To determine to what extent the disruption of family functions and family viability depend on the strength of the psychologically traumatic war situation, the chi-square test was calculated. Conflict relationships, violations of educational, communicative, protective and other functions of the family, which caused a decrease in vitality, were found in 38 subjects out of 46 respondents of the experimental group and in 29 people out of 50 respondents of the control group. As a result of statistical calculations, it was established that the chi-square criterion is equal to 6,8818 (p=0,01), which indicates the statistical significance of the obtained result. The Yates adjusted chi-square statistic is 5,7641 (p=0,02). Thus, it can be argued that the strength of the psycho-traumatic situation is a statistically significant risk factor for impairment of viability, so families evacuated from the war zone need constant psychological support, psychocorrective and psychotherapeutic interventions.

DISCUSSION

War is a new experience, the process of acquiring which exhausts adaptation mechanisms, increasing the risk of psychosomatic and psychopathological disorders [13, 14]. The family's reaction to an extreme psycho-traumatic situation, which is a war in Ukraine, can be manifested in the concentration of family members on personal emotions, their loss of the meaning of life and the manifestation of an indifferent attitude towards their own children, which makes it possible to characterize such families as unfavorable [6, 9, 10]. In such families, there is no mutual understanding and mutual support, instead there are manifestations of affectivity, dissatisfaction, irritation, obsessive anxiety, accumulation of aggressiveness, self-esteem decreases, a depressive state occurs [2, 4].

However, according to the sources of scientific literature, the war as a crisis event has an ambiguous effect on family relationships [15]. On the one hand, the fear of loss leads to chronic psychological traumatization and alienation between family members of resettlers [11, 12, 15]. On the other hand, in a difficult situation, it is the family that integrates and becomes a source of support and protection, countering difficulties and preventing negative consequences [1, 4, 5], awareness of the value of family relationships and mutual support of family members is growing [8, 11].

The results of the conducted empirical research – an ascertaining experiment aimed at identifying the impact of war on the stability of families with the consequences of war psychotrauma – confirm the data of the scientific literature about the negative impact of a war situation on the family relations and functions [4, 6, 15]. The respondents of the experimental group had a high level of unconstructive marital attitudes, the presence of symptoms of depression, emotions of aggression and anger, signs of somatization of anxiety, with a predominance of protective mechanisms of rationalization, intellectualization, isolation, displacement, and denial. The indicator of spouse autonomy is more pronounced in the experimental group, which is associated with the transformation of the structure and functions of the family due to the state of war, a decrease in the ability to provide family support due to the exhaustion of the family's adaptive resources, and indicates an increase in the risk of marriage breakdown during the war.

At the same time, it was established that in families of immigrants, the nature of communication in the family is more trusting, and the marriage satisfaction index indicates relative well-being. That is, the identified negative characteristics of marital relations are temporary and have a significant potential for recovery.

LIMITATIONS

The randomization procedure, contributing to the minimization of the risk of systematic error, ensured the relevance of the sample and made it possible to extrapolate the data obtained during the study of the sample population to the general population of young Ukrainian families who experienced the psychological trauma of the war. At the same time, the research procedure did not include a separate study of families evacuated abroad, distant families, and groups of military personnel. Such studies are planned to be carried out in the future.

CONCLUSIONS

The work is devoted to the current problem of researching the stability of the modern Ukrainian family under martial law. The peculiarities of the transformation of family relations as a result of experiencing a situation of full-scale war are determined. The hypothesis that the situation of a war negatively affects family relationships in modern Ukraine is confirmed, and approaches to their psychological correction are scientifically substantiated.

It was found that in young families evacuated from the zone of active hostilities, there is a violation of adaptation resources, which is manifested by increased conflict, non-constructive relationships, symptoms of depression and an aggressive communication style. At the same time, the family becomes unable to perform its main functions, such as protective and educational, and the marriage itself is under threat of destruction. At the same time, it was established that, despite the negative manifestations, spouses who suffered from war trauma retain common interests and values, have a significant potential for restoring the structure and functions of the family, which can be used as a basis for the development and implementation of appropriate psychocorrective measures.

PROSPECT FOR FURTHER RESEARCH

Prospective research is planned to be focused on the study of the viability of remote families and families of military personnel.

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This work (article) was carried out within the framework of the Scientific and Research Work theme «Transformational processes of personality development and formation in crisis situations: modern psychosocial dimensions». Funding sources: 7706 - free of charge (agreement on scientific and technical cooperation, etc.). Implementation stages: beginning November 2022, ending November 2025. Name of the stage: Social and psychological support of the individual in overcoming his crisis states as a result of personal transformations in modern conditions. State registration number: 0122U201363.

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Nina V. Koliadenko
Sigmund Freud University of Ukraine
7 Klovsky Uzviz St., 01021 Kyiv, Ukraine
e-mail: nina-k@ukr.net

ORCID AND CONTRIBUTIONSHIP
Nina V. Koliadenko 0000-0003-2099-0163 A D F
Mykola M. Shopsha 0000-0002-4361-039X A C
Olena L. Shopsha 0000-0002-2192-8911 B E
Nadiia O. Bachurina 0000-0003-3827-3269 B
Khrystyna S. Zhyvaho 0000-0002-4857-1924 C E
Oksana O. Drevitska 0000-0002-1551-9329 B
Jelena Kolesnikova 0000-0002-3280-5044 C F

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

RECEIVED: 05.07.2024
ACCEPTED: 19.10.2024

ORIGINAL ARTICLE

CONTENTS

Impact of the Covid-19 pandemic and state of war on the availability and quality of mental health services

Viktoriia V. Korolenko[1], Bogdan S. Bozhuk[2], Volodymyr V. Korolenko[2]
[1] BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE
[2] KROK UNIVERSITY, KYIV, UKRAINE

ABSTRACT

Aim: To investigate the relationship between the COVID-19 pandemic and the conditions of state of war in Ukraine on the availability and quality of services in the field of mental health and to improve further research in this direction.

Materials and Methods: Bibliographic, epidemiological, sociological, statistical methods were used in the research. The sociological survey covered 315 respondents in Kyiv region and 206 respondents in Kyiv. The quality and availability of mental health care for patients in the context of the COVID-19 pandemic and the ongoing state of war were assessed on a scale from 1 to 7 points. The correlational analysis was performed by the use of Spearman's, Kendall's, gamma rank correlation coefficients.

Results: Respondents in the Kyiv region, evaluating the quality of psychiatric care for patients in the conditions of the COVID-19 pandemic, gave an average rating of 4,0±1,2 points, accessibility – 4,2±1,2 points, and in the city of Kyiv 4,2±1,3 and 4,2±1,3 points. In the conditions of state of war, the average assessment of changes in the quality of psychiatric care in the Kyiv region was 4,6±1,4 points, and availability was 4,9±1,4 points, while in Kyiv it was 4,8±1,5 and 4,8±1,4 points. Correlational analysis proved the presence of a strong direct correlation between assessments of changes in the quality of psychiatric care for patients in the context of the COVID-19 pandemic and during state of war.

Conclusions: Preparing for pandemics and public health crises is critical to ensuring access to mental health care and requires a comprehensive approach.

KEY WORDS: COVID-19, pandemics, mental health, availability and quality of care, state of war

Wiad Lek. 2024;77(11):2174-2179. doi: 10.36740/WLek/197091 DOI

INTRODUCTION

Mental health is important in the fight against the COVID-19 pandemic and in the post-pandemic period. However, the field of mental health often remains underinvested and underappreciated compared to others. Typically, countries spend only 2% of their health budgets on mental health, and international aid in this area is less than 1% of total medical aid. This is a significant challenge, especially given the comorbidity of mental health and diseases such as HIV/AIDS, tuberculosis, and COVID-19 [1].

Crises cause significant stress in society, increasing the risk of mental health problems in the short and long term. Studies have shown that epidemics have a negative impact on mental health. High levels of stress in people affected by COVID-19 have also been confirmed in national surveys [1].

Before the pandemic, access to quality psychiatric care was limited, especially in humanitarian and conflict situations. The COVID-19 pandemic has affected service delivery around the world and made the situation even worse [1].

Despite differences in the level of economic development, social and political structures, and health systems as well as mental health services around the world, emergency measures were implemented that focused on infection control, continuity of care, and ease of access. Some new approaches that have been developed appear to be effective, but they may still carry risks. Therefore, the priority remains the preservation of existing services and the introduction of new, cost-effective practices [2].

A better understanding of the potential impact of changes in essential services, including mental health services, on morbidity and mortality is needed to weigh the benefits and risks of different mitigation strategies. There is an urgent need for documentation and research on what works in different settings at different stages of the pandemic. More effective real-time monitoring of changes in service provision and utilization at the

national and facility level is also needed, as pandemics can change their dynamics [3].

A systematic review of the impact of the COVID-19 pandemic on the use of health services overall found a 37% reduction in the total volume of services, with significant variation and a greater reduction among people with less severe illnesses [4].

The problem of providing psychological support in Ukraine during the COVID-19 pandemic and during the war has become an important challenge for the health care system. Stressful factors that have disrupted normal life have a negative impact on the mental health of Ukrainians, causing anxiety, panic attacks, depression and post-traumatic stress disorder. The relevance of psychological help is increasing due to the need to preserve mental health in crisis conditions. The support of mental health professionals is key to protecting the health of Ukrainians, which is important for the future [5].

AIM

To investigate the relationship between the COVID-19 pandemic and the conditions of state of war in Ukraine on the availability and quality of services in the field of mental health and to improve further research in this direction.

MATERIALS AND METHODS

Bibliographic, epidemiological, sociological, statistical methods were used in the research. Ukrainian and foreign scientific literature on the research topic was used. The sociological survey covered 315 respondents in the Kyiv region, of whom 69,84% (n=220) were educators, 16,51% (n=52) were representatives of the social sphere, 13,02% (n=41) were specialists in health care institutions, 0,63% (n=2) were representatives of the non-state sector; and 206 respondents in Kyiv, of which 52,4% (n=108) were educators, 29,6% (n=61) were medical workers, 17,5% (n=36) were representatives of the social sphere, and 0,5% (n=1) were representatives of the non-governmental sector.

The results were obtained using a questionnaire developed by the authors as part of the framework of PsyCare-Kyiv Region project of the Ukrainian Association of Doctors-Psychologists regarding the state of the mental health system of Kyiv city and the Kyiv region, carried out with support of the Ukrainian-Swiss project "Mental health for Ukraine" (MH4U). The questionnaire included quantitative and qualitative assessments. The quality and availability of mental health care for patients in the context of the COVID-19 pandemic and state of war was assessed on a scale from 1 to 7 points. The level of assistance in the field of mental health was evaluated on a scale from 1 to 10 points. The general background of mental health in the community was evaluated by the following categories: calm; more calm that anxious; anxious in certain population groups; more anxious than calm; anxious. The following categories were proposed for the evaluation of the level of assistance in the field of mental health: the lack of mental health specialists; insufficient funding; insufficient technical base; low salaries of specialists; lack of an established traffic system consumer of services; the assistance is provided at a high level and by qualified specialists.

Statistical processing and analysis of research materials was carried out using biostatistical analysis methods implemented in Microsoft Excel 2016 and BioStat software v. 7.3 packages (AnalystSoft Inc., USA). The mean value and standard error of the mean were calculated for quantitative variables. Qualitative data were presented as absolute and relative frequency (sample proportion ± standard error of the sample proportion). The correlation analysis was performed by calculating the Spearman's rank correlation coefficient (Rho), Kendall's rank correlation coefficient (Tau) and rank gamma correlation coefficient.

The sociological research is anonymous, did not involve the collection of confidential information and personal data (in the sense of the Law of Ukraine "On the Protection of Personal Data" – "information or a set of information about a natural person who is identified or can be specifically identified") [6].

RESULTS

Respondents in the Kyiv region, assessing on a scale from 1 to 7 points whether the quality of psychiatric care for patients has undergone changes in the conditions of the COVID-19 pandemic, gave an average rating of 4,0±1,2 points, the availability of this care in the same conditions – in 4,2±1,2 points. In the conditions of state of war, the respondents of the Kyiv region gave an average score of 4.6±1,4 points for the quality of psychiatric care, and 4,9±1,4 points for accessibility.

The general background of mental health in the community was rated by 37,5±2,7% as anxious in certain population groups in certain population groups, another 15,2±2,0% as more worrying than calm, and 3,8±1,1% – as anxious. At the same time, 34,9±2,7% defined the state as more calm than anxious, and another 8,6±1,6% – as calm (Fig. 1).

Evaluating the level of assistance in the field of mental health in their community on a scale from

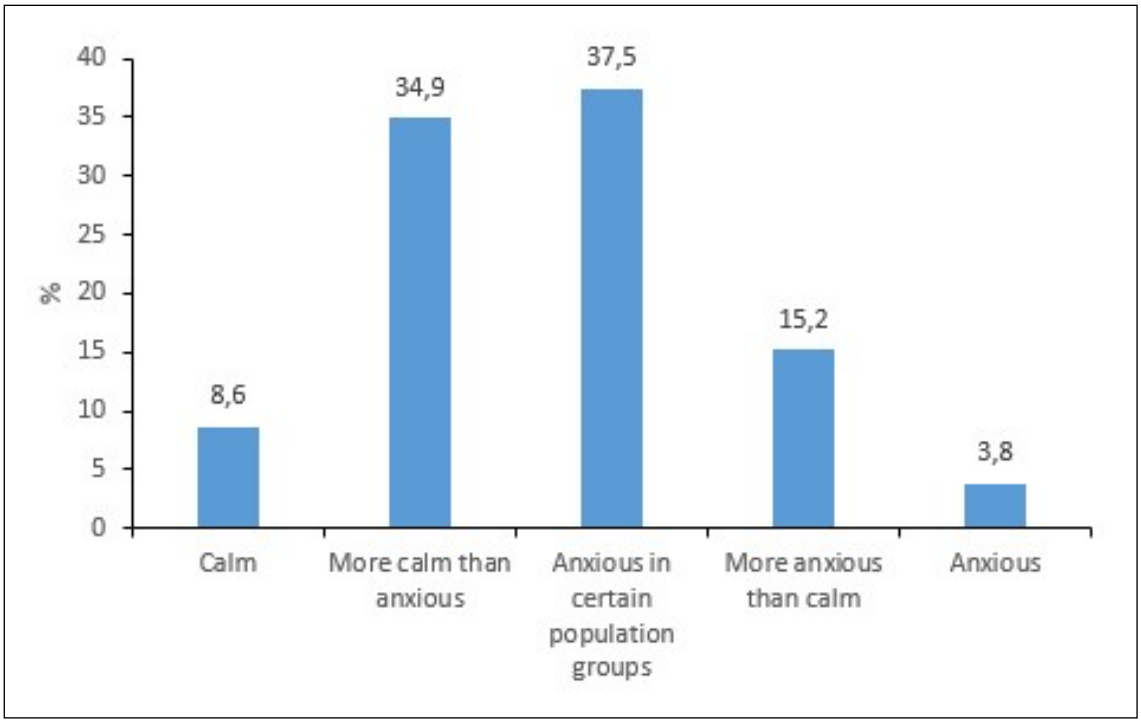

Fig. 1. General background of mental health in the community, Kyiv region.

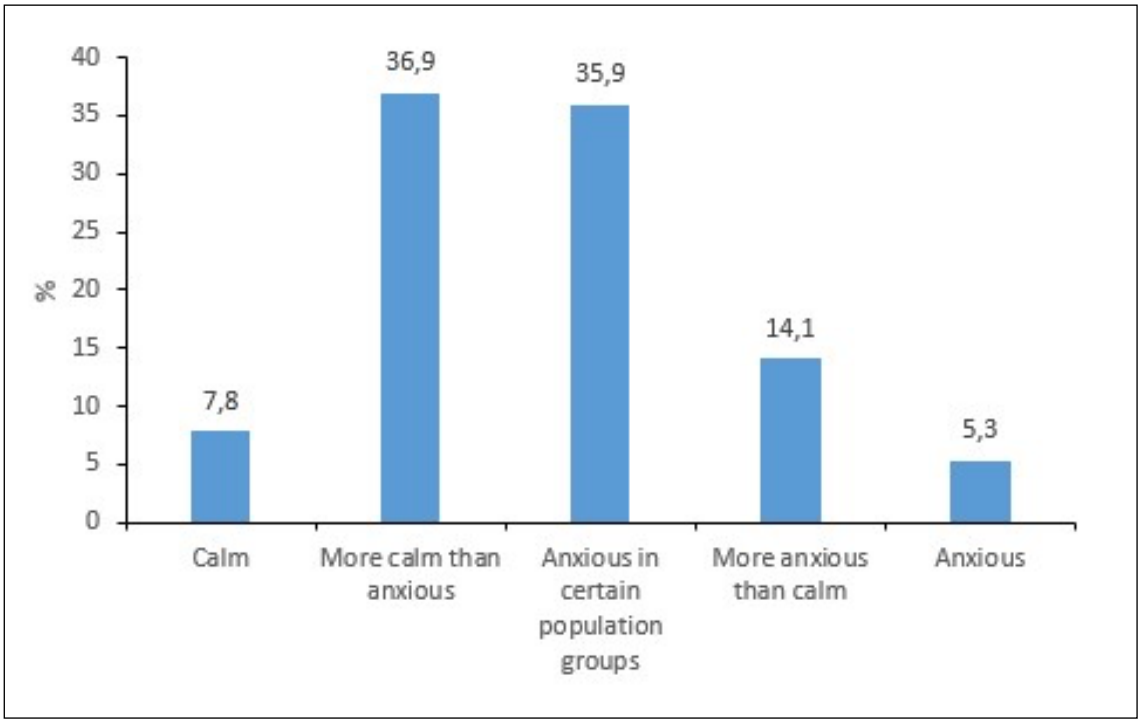

Fig. 2. General background of mental health in the community, Kyiv.

1 to 10 points, the respondents gave an average answer of 5,1±2,1 points. The respondents based their assessment on the lack of mental health specialists (60,3±2,8%), insufficient funding (36,8±2,7%), insufficient technical base (33±2,6%), low salaries of specialists (27,9±2,5%), lack of an established traffic system consumer of services (13,7±1,9 %). According to 11,7±1,8% of respondents, assistance is provided at a high level and by qualified specialists.

Respondents in Kyiv, evaluating changes in the quality of psychiatric care for patients in the conditions of the COVID-19 pandemic on a scale from 1 to 7 points, gave an average rating of 4,2 ± 1,3 points, and changes in availability in the same conditions – 4,2 ±1,3 points. In

the conditions of state of war, the average assessment of changes in the quality of psychiatric care was 4,8±1,5 points, accessibility – 4,8±1,4 points.

The general background of mental health in the community was assessed by 35,9±3,3% as anxious in certain population groups, another 14,1±2,4% as more worrying than calm, and 5,3±1,6% – how anxious. At the same time, 36,9±3,4% defined the state as calmer than anxious, and another 7,8±1,9 % – as calm (Fig. 2).

Evaluating the level of assistance in the field of mental health in their community on a scale from 1 to 10 points, the respondents gave an average answer of 5,8 ± 2,0 points. The respondents based their assessment on the lack of mental health specialists (51,9±3,5%), low salaries of specialists (50,5±3,5%), insufficient funding (49,5±3,5%), insufficient technical base (33±3,3%), service users do not know where to turn for qualified help (32±3,3%), the lack of an established system for the movement of service users (20,9±2,8%). According to 23,3±2,9%, assistance is provided at a high level and by qualified specialists.

Correlational analysis of the data obtained during the survey of healthcare professionals showed the presence of a strong direct correlation between assessments of changes in the quality of psychiatric care for patients in the conditions of the COVID-19 pandemic and during the state of war (Kyiv region: Rho =0,611 (n=41; p<0,05), Tau =0,558 (n=41; p<0,05), Gamma=0,669 (n=41; p<0,05); Kyiv city: Rho =0,584 (n=61; p<0,05), Tau =0,522(n=61; p<0,05), Gamma=0,644 (n=61; p<0,05) and between estimates of changes in the availability of psychiatric care to patients in the conditions of the COVID-19 pandemic and during the state of war (Kyiv region: Rho = 0,596 (n=41; p<0,05), Tau=0,564 (n=41; p<0,05), Gamma=0,670 (n=41; p<0,05); Kyiv city: Rho = 0,602 (n=61; p<0,05), Tau = 0,539 (n=61; p<0,05), Gamma=0,657 (n=61; p<0,05).

DISCUSSION

The disproportionality between the need for mental health care and the provision of appropriate services has always existed, but with the onset of the COVID-19 pandemic, these problems have intensified [7, 8].

During the COVID-19 pandemic, barriers to access to services, limited funding and economic hardship have become more visible. The lack of qualified doctors, fragmented provision of medical care, imperfect policy in the field of mental health, as well as lack of education and awareness about mental illnesses are also acutely felt [9].

The global COVID-19 pandemic has disrupted mental health services worldwide, particularly in many low- and middle-income countries, and concerns about the psychosocial impact of COVID-19 have prompted major funding agencies and governments to seek ways to address this impact [10].

Studies examining the impact of the pandemic on mental health services show a mixed picture: noticeable changes in work, insufficient staffing, equipment, standards, etc [11].

In recent years, a full-scale war had the greatest impact on all aspects of the country's life in Ukraine, which became the most difficult test in the history of its independence. This has led to an increase in the need for medical services, while at the same time reducing the system's ability to provide them, especially in active combat zones [12].

A nationwide study of Ukrainian psychiatric institutions during the Russian invasion in 2022 highlights the significant damage suffered by the structure of mental health care in Ukraine, in particular, hospitalizations decreased by 23,5%, 9,1% of the total number of medical workers was relocated, and 0,5% were injured in all institutions [13].

The prolonged stressful situation of the war negatively affects the psychological and psychophysical health of the population of Ukraine, manifesting itself in emotional and psychosomatic disorders, especially in temporarily displaced persons. Chronic stress worsens general well-being, reduces activity and mood, creating a closed circle of problems. A close relationship between the level of anxiety, vegetative disorders and psychosomatic symptoms was revealed. The risk of developing these disorders does not differ between displaced persons and those who remain [14].

A blended approach that takes into account the needs and digital skills of patients and includes both face-to-face and online methods can improve mental health outcomes in the long term. The need to increase confidence in the use of digital devices, as well as training and experience with all modalities, was identified as a key priority for both clinicians and service users [15].

Emphasis should be placed on creating clearer processes in collaboration with service users for their intended use, incorporating existing guidance and evidence, and exploring barriers [16].

CONCLUSIONS

Challenges related to the organization of mental health became evident during the COVID-19 pandemic and were exacerbated by wartime conditions.

More than half of the respondents in the Kyiv region and the city of Kyiv assessed the general background of mental health as varying levels of anxiety. At the same time, the level

of assistance in the field of mental health was assessed by the majority of respondents as not exceeding the average.

At the same time, the correlational analysis proved the existence of a strong direct relationship between estimates of changes in the quality of mental health care for patients in the conditions of the COVID-19 pandemic and during state of war, and between estimates of changes in the availability of such care for patients in the conditions of the COVID-19 pandemic and during the state of war.

Preparing for pandemics and public health crises is critical to ensuring access to mental health care. Under such circumstances, there is an urgent need for a comprehensive approach to the system of mental health care. To effectively solve these issues, a coordinated interaction between the state, medical institutions and public organizations is needed to guarantee the availability and high quality of psychiatric care for all who need it.

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The article was performed within the framework of the research "Comparative analysis of time series of infectious morbidity and mortality in the structure of general morbidity and mortality of the population of Ukraine and other countries of the European Region in 1990-2023", (2022-2024, number of state registration 0122U000633).
Sociological research was performed as a part of subproject "PsyCare Kyiv Region" (project "Mental health for Ukraine", 01.07.2023-15.12.2023, Swiss Agency for Development and Cooperation (SDC), GFA Consulting Group GmbH, certificate No 281).

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Viktoriia V. Korolenko
Bogomolets National Medical University
34 Beresteiskyi Ave., 03680 Kyiv, Ukraine
e-mail: viki.epi.2019@gmail.com

ORCID AND CONTRIBUTIONSHIP
Viktoriia V. Korolenko: 0000-0002-5483-4620 A B C D
Bogdan S. Bozhuk: 000-0002-8089-2840 A B D E F
Volodymyr V. Korolenko: 0000-0002-9735-0896 D E F

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

RECEIVED: 02.07.2024
ACCEPTED: 14.10.2024

ORIGINAL ARTICLE

CONTENTS

Problems of harmonization of the criminal legislation of the certain continental law countries to ensure the protection of the circulation of medicinal products

Anzhela B. Berzina[1], Olena H. Frolova[2], Andriy M. Orlean[3], Yuriy V. Onishchyk[4], Olga M. Golovko[5], Iryna Y. Khmil[1]
[1]BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE
[2]INTERREGIONAL ACADEMY OF PERSONNEL MANAGEMENT, KYIV, UKRAINE
[3]NATIONAL ACADEMY OF INTERNAL AFFAIRS, KYIV, UKRAINE
[4]KYIV NATIONAL UNIVERSITY OF TECHNOLOGY AND DESIGN, KYIV, UKRAINE
[5]IGOR SIKORSKY KYIV POLYTECHNIC INSTITUTE, KYIV, UKRAINE

ABSTRACT

Aim: To find out the problems of the harmonization of the criminal legislation of the certain continental law countries to ensure the protection of the circulation of medicinal products.

Materials and Methods: This study is based on the analysis of the international legal acts, in particular, the Directives of the EU, as well as Medicrime Convention, national acts of criminal legislation of the certain continental law countries (Germany, Austria, Switzerland, Ukraine, etc.), national judicial practice, data on the number of criminal proceedings in the courts of Ukraine, criminal and medical law legal doctrine (38 normative legal acts and 15 court judgments), data of the Office of the Prosecutor General of Ukraine. Dialectical, hermeneutic, comparative, analytical, synthetic and system analysis research methods were used.

Results: The problems of harmonizing the criminal legislation of the certain continental law countries to ensure the protection of the circulation of medicinal products depend on: a) the level of legal regulation of the field of health care at the national level; b) availability of effective mechanisms for implementing the provisions of international and regional standards.

Conclusions: The formation of a model of criminal law protection of the circulation of medicinal products in continental law countries depends on harmonization with the basic international legal and regional standards and their implementation at the national level.

KEY WORDS: circulation, medicinal products, pharmaceutical activity, international standards, harmonization

Wiad Lek. 2024;77(11):2180-2185. doi: 10.36740/WLek/197092 DOI

INTRODUCTION

The growth of global demand for medicinal products necessitates the existence of effective mechanisms for the protection of relations with the circulation of medicinal products. The circulation of medicinal products is an important element of the health care system both at the international and national levels. Medicinal products must meet the criteria of safety and effectiveness, there must be proper guarantees of the quality of such products, because they are primarily aimed at preserving human life and health.

The pharmaceutical markets of continental European countries have strict legal regulation. It is important to have effective norms of the criminal legislation, which ensure the protection of the circulation of medicinal products at the national level. In the vast majority of these norms have a blanket character. Not only the current international and regional regulatory legislation in the field of circulation of medicinal products, but also the blanket criminal law norms undergo periodic fundamental changes. The reason for this lies primarily in the change in the requirements of international and regional legal acts related to pharmaceutical activity, as well as in the fulfillment of those international obligations assumed by the participating countries of these international legal acts.

AIM

The aim of the article is to find out the problems of the harmonization of the criminal legislation of the certain continental law countries to ensure the protection of the circulation of medicinal products.

MATERIALS AND METHODS

This article is based on the analysis of the international legal acts, in particular, the Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use, as well as Medicrime Convention, national acts of criminal legislation of continental law countries (Germany, Austria, Switzerland, Ukraine, etc.), national judicial practice, statistics on the number of criminal proceedings in the courts of Ukraine, criminal and medical law legal doctrine (51 normative legal acts and 15 court judgments), statistical data of the Office of the Prosecutor General of Ukraine. Dialectical, hermeneutic, comparative, analytical, synthetic and system analysis research methods were used.

RESULTS

It is proposed to consider the specifics of the harmonization of the criminal legislation of the certain continental law countries to ensure the protection of the circulation of medicinal products and the problem of its implementation with reference to the regulatory acts of the EU and international Conventions. Such legal guidelines establish the basis of harmonization and implementation of relevant norms in national legislation. Among them stand out: Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use (hereinafter – Directive 2001/83/EC) [1] and Council of Europe Convention on the counterfeiting of medical products and similar crimes involving threats to public health (CETS No. 211) (hereinafter – Medicrime Convention) [2], etc. Determining the specifics of the criminal law protection of the circulation of medicinal products of the certain continental law countries, we will single out the specifics of restrictions on the use of medicinal products, which are established at the legal level.

The problems of harmonizing the criminal legislation of the certain continental law countries to ensure the protection of the circulation of medicinal products depend on: a) the level of legal regulation of the field of health care at the national level; b) availability of effective mechanisms for implementing the provisions of international and regional standards. The model to follow here is additional acts of criminal legislation of such European countries as: a) German (Medicinal Products Act of 12 December 2005 [3]), b) Austria (Federal Law of Austria on the Manufacturing and Circulation of Medicinal Products of 02 March 1983) [4]), c) Switzerland (Federal Act of Switzerland on Medicinal Products and Medical Devices (of 15 December 2000) [5], d) France (French Public Health Code [6] and additional acts that regulate the criminal law protection of the circulation of medicinal products).

The pharmaceutical legislation of the certain continental law countries should be based on the norms of EU directives and regulations. However, the implementation of these norms is often a problem in legal regulation at the national level. Let us consider the example of Ukraine, which in 2014 signed the Association Agreement between the European Union and its Member States, of the one part, and Ukraine, of the other part. Harmonizing the Medicrime Convention with national legislation, Ukraine has amended the Criminal Code of with Article 321-1 "Falsification of medicinal products or circulation of falsified medicinal products". In this regard, a statistical analysis of the indicators of how the criminalization of such an act as falsification of medicinal products or circulation of falsified medicinal products in the framework of harmonization with European standards affected the implementation of law in Ukraine was carried out (Table 1).

The analysis of the collected statistical data gives reason to say that for the period from 2019 to the first half of 2024, 123 criminal offenses defined in Art. 321-1 of the Criminal Code of Ukraine were registered, of which only 9 were sent to court with an indictment. That is, only 7.3% of criminal proceedings are sent to court with an indictment. As a result, despite the criminalization of the act provided for in Art. 321-1 of the Criminal Code of Ukraine, which took place as a result of the harmonization of Ukrainian legislation with international standards, there are already problems at the national level, which consist, among other things, in the degree of severity of criminal punishment and its actual application. This is confirmed by the processed statistical data of Supreme Court in Ukraine regarding the number of persons convicted of criminal offenses under Art. 321-1 of the Criminal Code of Ukraine (Fig. 1).

Statistically the number of such persons is extremely small (4 in 2019, 3 in 2020, and 1 each in 2022 and 2023. In 2021, not a single person in Ukraine was convicted of the crime provided for in Art. 321-1 of the Criminal Code of Ukraine).

One of the directions of harmonization in Ukraine is the criminalization of acts related to the circulation of medicinal products for veterinary use (in the sense of this concept formulated in the Medicrime Convention). At the national level, the Law of Ukraine "On Veterinary Medicine" [7] of 4 February 2021 does not contain the concept of "Veterinary medicinal products" (the concept of "Veterinary product" is used instead). According to item 15 p. 1 Art. 1 of this Law, a veterinary medicinal product is "any substance or combination of substances with the declared

Table 1. General information on the number of registered criminal offenses and the results of their pre-trial investigation under Article 321-1 of the Criminal Code of Ukraine "Falsification of medicinal products or circulation of falsified medicinal products"

Year	The number of criminal offenses in the reporting period	Criminal offenses in which persons have been served with a notice of suspicion	Criminal offenses for which proceedings have been sent to court	Including with the indictment	Criminal offenses in which proceedings have been closed	Criminal offenses in which a decision has not been made at the end of the reporting period (on termination or suspension)
2019	29	2	0	2	6	27
2020	22	3	2	2	8	19
2021	21	2	0	2	2	21
2022	17	0	0	0	5	17
2023	26	5	2	2	2	24
January-August 2024	8	1	1	1	0	7

property of treating or preventing *animal* diseases…" [8]. In the current Law of Ukraine "On Medicinal Products" of 4 April 1996 [9] and the Law "On Medicinal Products" of 28 July 2022 [10], which has not yet been implemented, the meaning of the concept of "medicinal products" and the concept of "circulation of medicinal products" associated with the appointment of medicinal products to ensure the corresponding functions in the *human* body. This raises the question of the need for criminal protection of medicinal products for veterinary use and their circulation. The current Ukrainian regulatory legislation on pharmaceutical activity does not include such a component as medicinal products for veterinary use and their circulation, since medicinal products for veterinary use are not able to ensure the appropriate "state" of individual and public health of a person, as required by the Law of Ukraine "Fundamentals of the Legislation of Ukraine on health care" of 19 November 1992 [11].

However, if we take into account the international legal standards of the Medicrime Convention regarding the equal legal "regime" of medicinal products for "*human and veterinary use*" (p. "b" of Art. 4), then the regime of criminal legal protection of medicinal products for veterinary use (veterinary medicinal products) and their circulation should not differ from the "regime" of criminal legal protection of medicinal products and their circulation in the sense of item 2 p. 1 Art. 2 of the Law of Ukraine "On Medicinal Products" of 4 April 1996 and item 39 p. 1 of Art. 2 of the Law of Ukraine "On Medicinal Products" of 28 July 2022. Ensuring such compliance with the stated standards of the Medicrime Convention requires the introduction of appropriate changes to the current Ukrainian regulatory legislation in the field of circulation of medicinal products [8].

Another problem for Ukraine is the definition in Chapter II "Substantive criminal law" of the Medicrime Convention of standards regarding the types of criminal offenses related to the counterfeiting of medical products and similar crimes that threaten health care. According to the Art. 5 of the Medicrime Convention, the standard for national legislation is the adoption of the first type of criminal offense: 1) intentional manufacturing of counterfeit medical products, active substances, excipients, parts, materials and accessories (p. 1); 2) any falsification of medicinal products and, as appropriate, medical devices, active substances and excipients (p. 2). According to p. 3 of Art. 5 of this Convention: «Each State or the European Union may, at the time of signature or when depositing its instrument of ratification, acceptance or approval, by a declaration addressed to the Secretary General of the Council of Europe, declare that it reserves the right not to apply, or to apply only in specific cases or conditions, paragraph 1, as regards excipients, parts and materials, and paragraph 2, as regards excipients» [2]. However, the Law of Ukraine "On the Ratification of the Convention of the Council of Europe on the counterfeiting of medical products and similar crimes involving threats to public health" of 7 June 2012 [12] does not contain any reservations regarding the application and implementation in national legislation of the norms of p. 1, 2 Art. 5 of the Medicrime Convention [13]. This means that the current Ukrainian criminal legislation must meet the requirements of paragraphs 1, 2 Art. 5 of the Medicrime Convention. Countries that have ratified the Medicrime Convention and harmonized their legislation are defined in Figure 2.

DISCUSSION

The effort to create a common market for medicinal products of continental law countries led to the emergence of a unique system of pharmacy regulation. The uniqueness of this system is that it has developed a single EU market for medicinal products through a

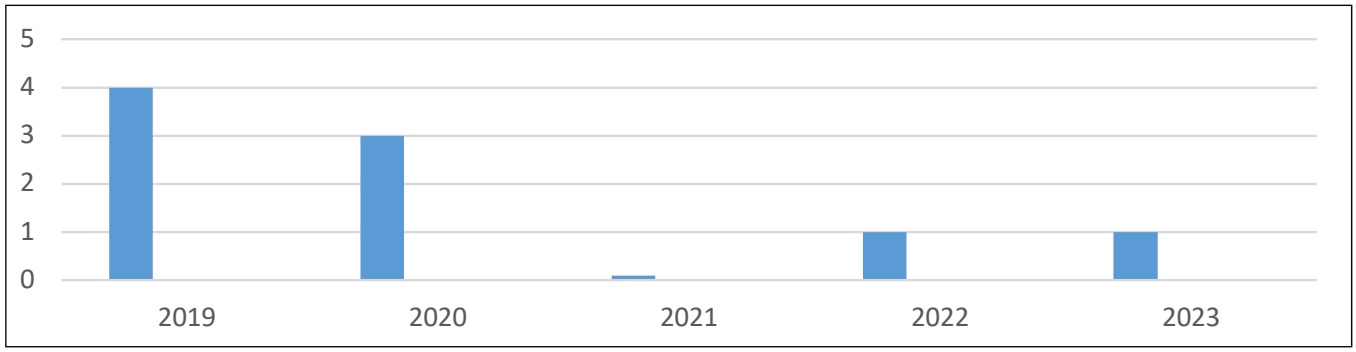

Fig. 1. The number of persons convicted of criminal offenses provided for in Art. 321-1 of Criminal Code of Ukraine «Falsification of medical products or circulation of falsified medical products».

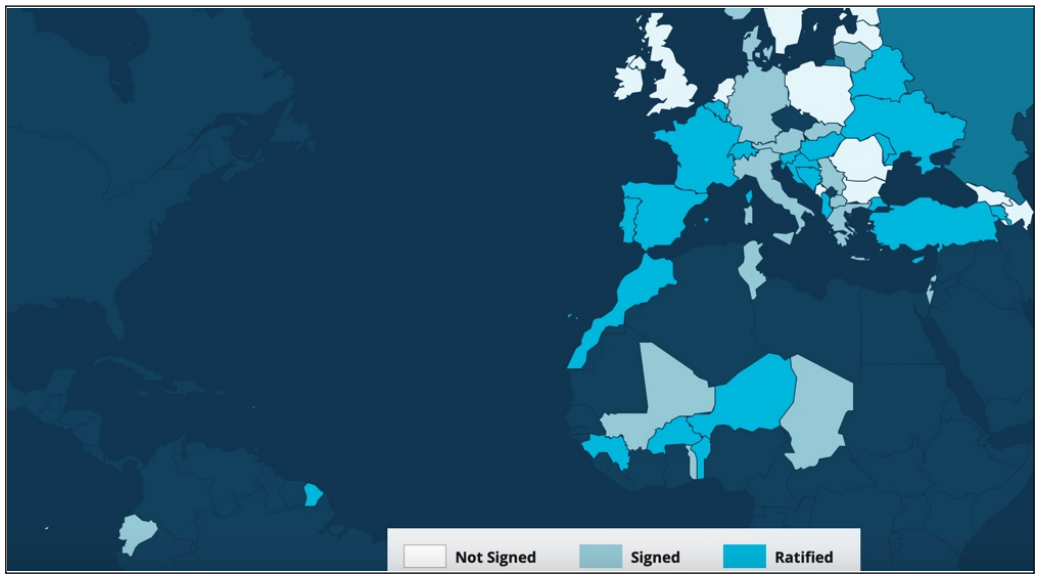

Fig. 2. Countries that have ratified the Medicrime Convention and harmonized their legislation [14].

standardized system of legislation and a harmonized system of procedures for the manufacture and circulation of medicinal products.

Today, the EU regulates pharmaceutical activity between member states and establishes standards that ensure a high level of public health protection and the quality, safety and effectiveness of medicinal products, and member states implement these norms into their national legislation in order to develop their own internal market, and as well as the promotion of innovation [15].

Acts of the main and additional legislation of Switzerland, Germany and Austria provide the largest amount of criminal protection of pharmaceutical activity. Its "blocks" can be certain legislative guidelines for the improvement of the criminal legislation of Ukraine and the construction of its own model system of norms that provide criminal legal protection of pharmaceutical activity [8].

Harmonizing the sphere of circulation of medicinal products to European standards, Ukraine used the main norms of international legal acts as sources of "secondary EU law" preparing the draft of Law of Ukraine "On medicinal products", which was adopted as a law on July 28, 2022. Including those, attention is focused on the norms regarding the features of legal protection of the circulation of medicinal products. After all, at the time of the preparation of this draft law and its adoption, there was "a need ... to review the principles of regulation in the field of circulation of medicinal products, to increase the level of quality, efficiency and safety of medicinal products, as well as their availability" [16]. The harmonization of norms of EU regional standards had a direct impact on provision, including criminal law protection of relevant fragments of pharmaceutical activity.

The scientific discussion is largely focused on the issue of incomplete fulfillment by Ukraine of international legal obligations undertaken during the implementation of harmonization measures [17], in particular after the ratification of the Medicrime Convention [13, 18, 19]. Improving the criminal law mechanism to combat the falsification of medical products at the European regional level requires intensifying of The Medicrime Convention ratification process and full implementation of rules on criminal liability for falsifying medical products into national criminal legislation [20].

CONCLUSIONS

1. In order to properly ensure the criminal legal protection of the circulation of medicinal products, it is necessary to establish which negative, unfavorable actions committed by people are so dangerous for society that the legislator can recognize them as criminal offenses, as well as to determine the punishment that may be appointed by the court on behalf of the state for committing such acts. In the certain continental law countries, such determinations are made on the basis of norms of criminal legislation.
2. The formation of a model of criminal law protection of the circulation of medicinal products in continental law countries depends on harmonization with the basic international legal and regional standards and their implementation at the national level. Such standards determine the main components of the circulation of medicinal products and the connection of such components with the mechanism of state regulation. Moreover, some of the elements of such a mechanism are distinguished as independent and operate under the appropriate legal regime (for example, the "pharmacovigilance system", which is used to collect information about the risks of medicinal products for the health of patients or the population).
3. The term "falsification" in the sense of the Medicrime Convention is used in the official text of this Convention in p. 2 of Art. 5 "Manufacturing counterfeits", when it comes to the need to recognize as a criminal offense the falsification of medicinal products, medical devices, active substances and excipients. Taking into account that in the content of p. 2 of Art. 5 of the Medicrime Convention refers to the "manufacturing of counterfeit medical products, active substances, excipients, parts, materials and accessories", then at the level of the Medicrime Convention, the concepts of "falsification" and "manufacturing of counterfeits" should cover the same actions. This approach of the Medicrime Convention should be taken into account as an international legal reference for the further correlation of the mentioned concepts, which are used in the content of the compositions of specific types of criminal offenses provided for by the Criminal Code of Ukraine.

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The study was performed as a fragment of the complex scientific project of the Kyiv National University of Technology and Design « Legal support for the realization of the rights, freedoms and legitimate interests of subjects of legal relations in the field of public administration» (state registration number 0122U201691; term: 2023-2027).

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Anzhela B. Berzina
Bogomolets National Medical University,
13 Taras Shevchenko Ave., 01601 Kyiv, Ukraine
e-mail: anzhela.kasumova@gmail.com

ORCID AND CONTRIBUTIONSHIP
Anzhela B. Berzina: 0000-0002-9885-309X A B D F
Olena H. Frolova: 0009-0007-5427-7581 A D F
Andriy M. Orlean: 0000-0002-7439-5311 B D E
Yuriy V. Onishchyk: 0000-0003-3355-3392 D E F
Olga M. Golovko: 0000-0001-8963-6598 A B C
Iryna Y. Khmil: 0000-0002-0157-0289 B C

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

RECEIVED: 10.07.2024
ACCEPTED: 25.10.2024

ORIGINAL ARTICLE

CONTENTS

Potential and real dangers of the chemical warfare agents use during the full-scale invasion into Ukraine

Nataliia D. Kozak, Oleh V. Rudynskyi, Andriy V. Verba, Alla A. Asaulenko, Dmytro O. Kozak
UKRAINIAN MILITARY MEDICAL ACADEMY, KYIV, UKRAINE

ABSTRACT

Aim: To analyze modern threats of the use of chemical warfare agents on the line of contact during the full-scale invasion into Ukraine.

Materials and Methods: The study was carried out with the use of theoretical methods (information retrieval, analytical, descriptive, generalization, system analysis and information synthesis) and the construction of trends with the identification of the coefficient of determination.

Results: Most of damage cases of the military men of the Armed Forces of Ukraine were caused by the enemy's use of such groups of chemical warfare agents with an irritating effect as lacrimators and sternites, more specifically K-51 grenades with chloropicrin (PS) and 2-chlorobenzalmalononitrile (CS), RGR grenade with 2-chlorobenzalmalononitrile (CS) and RG-Vo (862-3-23) grenades with chloroacetophenone (CN).

Conclusions: 2698 cases of damage by chemical warfare agents of irritant action were detected among the military men of the Armed Forces of Ukraine, and every month during 2024, more and more injuries were detected among the military men of the Armed Forces of Ukraine (715 cases in May 2024 compared to 444 cases in April 2024), which indicates that a stable upward trend towards a wider trend is emerging deliberate use of chemical warfare agents, the coefficient of determination of R2 of which was high and amounted to 0,9577, and which continues to grow steadily.

KEY WORDS: chemical warfare agents, military men, Armed Forces of Ukraine, full-scale invasion

Wiad Lek. 2024;77(11):2186-2192. doi: 10.36740/WLek/197094 DOI

INTRODUCTION

Chemical warfare agents (CWA) with asphyxiating and irritating effects, such as chemical weapons, were first used during the First World War. In total, from April 1915 to November 1918 inclusive, more than 50 German gas attacks were carried out. During the same period, in opposition to the German troops, 150 British and 20 French gas launches were carried out. On May 15, 1916, French troops resorted to the use of artillery with a mixture of phosgene with tin tetrachloride and arsenic trichloride, chloropicrin, as well as a mixture of hydrocyanic acid with arsenic trichloride, tin tetrachloride in chloroform (a form of vensenite). Mustard gas was first used as a cutaneous blister chemical agent by Germany on July 12, 1917 near Ypres (Belgium) [1].

Chemical warfare agents were regularly used in the 1980s during the Iran-Iraq war. According to various estimates, 5% of Iran's total losses, which amounted to approximately 45 000 people, can be classified as chemical weapons. Libya was suspected of involving chemicals during the invasion of Chad in 1986.

On January 16, 2015, during the anti-terrorist operation in eastern Ukraine, illegal armed groups used tear gas and chemical grenades against the defenders of the Donetsk airport.

The use of chemical weapons by the Syrian Armed Forces on April 4, 2017, when an air strike was carried out on the city of Khan Sheikhoun (Idlib province, Syria) caused a significant resonance in the world. The ammunition contained a nerve agent (most likely sarin). About 100 people died (including 20 children and 17 women), and more than 300 people were poisoned of varying degrees of severity [2].

According to the most common toxicological classification, according to the nature of their effect on the human body, CWA are divided into nerve agents (sarin, soman, VX gases, etc.), cutaneous abscesses (mustard gas, nitrogenous mustard gas, lewisite, etc.), general poisonous (hydrocyanic acid, chlorocyanin), asphyxiating (phosgene, diphosgene, etc.), irritant (chloroacetophenone (CN), 2-chlorobenzalmalononitrile (CS), dibenzoxazepine (CR), adamsite (DM)), psychotomimetic (psychochemical) (Bi-Z (BZ), lysergic acid diethylamide) [3].

AIM

Analysis of modern threats of the use of chemical warfare agents on the line of contact during the full-scale invasion into Ukraine.

MATERIALS AND METHODS

The study was carried out with the use of theoretical methods (information retrieval, analytical, descriptive, generalization, system analysis and information synthesis) and the construction of trends with the estimation of the determination coefficient.

The study was carried out in accordance with the principles of bioethics and legislative norms and requirements for biomedical research, namely: the Helsinki Declaration (2000), the Constitution of Ukraine (1996), the Fundamentals of the Legislation of Ukraine on Health Care (1992), as well as the Law of Ukraine "On Information" (1992) (as amended as of 21.03.2023).

RESULTS

As of the beginning of large-scale aggression against Ukraine on February 24, 2022 until January 2024, according to the General Staff of the Armed Forces of Ukraine, more than 626 cases of the use of ammunition with toxic chemicals by the invaders were recorded [4]. In addition, such cases are becoming more and more frequent. Thus, during January 2024, the defense forces of Ukraine recorded 229 cases [5], and during February 2024 – 250 cases of the use of poisonous substances by the invaders, of which 244 cases of the use of aerosol grenades containing irritating CWA, that is, the vast majority of them [6]. During March 2024, 371 cases of the use of chemical munitions were recorded [7]. During April 2024, 444 cases were recorded. And already in May 2024, 715 cases of the use of ammunition containing dangerous chemicals were documented, which was 271 cases more than in April. From the above data, it follows that there is a clear trend towards an increase in the number of cases of the use of CWA. After calculating the trend and estimating the determination coefficient, it was found that the trend is reliable, since the coefficient of determination R^2 is in the range from 0,8 to 1,0 and is 0,9577, which is a very high value (Fig. 1). In total, from February 2022 to May 24, 2024, 2698 cases of the use of toxic chemicals by the enemy were recorded in Ukraine [8]. During this period, 1385 cases of appeals of servicemen of the Armed Forces of Ukraine to medical institutions with symptoms of chemical damage of varying degrees of severity were recorded, of which only in May – 21 cases [8].

From December 2022 to May 2024, there were repeatedly used chemical grenades K-51, RGR and RG-VO chemical grenades (862-3-23) against the positions of our troops (Fig. 2) in the areas of deployment of units of troops (forces) of the Armed Forces of Ukraine near

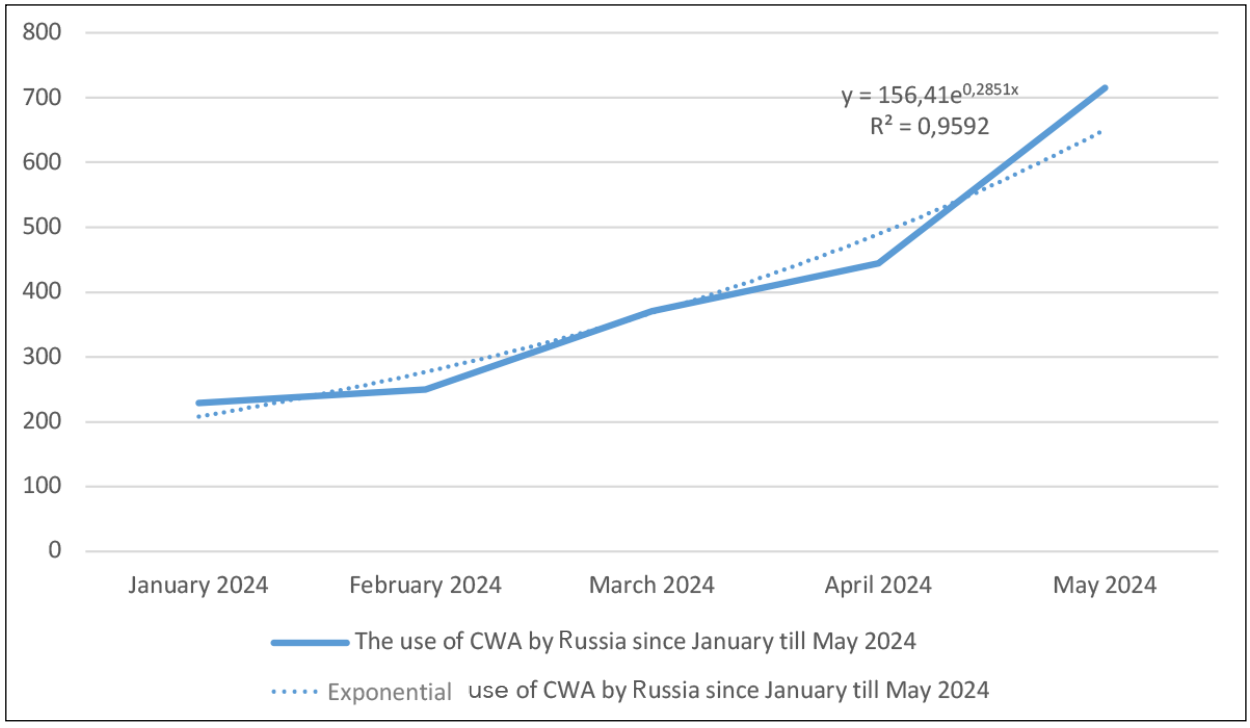

Fig. 1. The trend of the use of chemical warfare agents against military men of the Armed Forces of Ukraine for the period January-May 2024, line, trend equation and coefficient of determination.

Fig. 2. Grenades K-51 (A), RGR (B) and RG-Vo (C).

Fig. 3. Erythematous spots on the surface of the skin of affected military men.

and in settlements, including Mariupol, Klishchiivka, Kurdyumivka, Avdiivka, Novokalynove, Lastochkyne, Pervomaiske, Maryinka [9].

The body of the K-51 grenade is made of hard plastic, the bottom is metal. After inflammation, a reaction occurs, as a result of which the pressure inside the case increases and the bottom is squeezed out, spraying the chemical. As for the content of K-51 grenades, there are two versions of them: garnets containing chloropicrin and 2-chlorobenzalmalononitrile (CS), as well as indoors the effect of the grenade extends to an area of up to 400 square meters. [9].

An irritant hand grenade (RGR) is a 60 mm aerosol hand grenade containing chlorobenzalmalononitrile (CS) [10].

It is reported that in December 2023, the enemy's use of a new type of special RG-VO gas grenades containing CWA chloroacetophenone (CN) was detected for the first time. The substance chloroacetophenone (CN) is prohibited for use as a chemical warfare agent in accordance with the Geneva Protocol, approved by the UN General Assembly in 1969, based on the results of its use during the Vietnam War [11].

Thus, most of the cases of damage to the military men of the Armed Forces of Ukraine were caused by the enemy's use of irritating weapons such as lacrimators and sternites.

Delivery to the contact line and drops of K-51 and RG-VO chemical grenades were carried out mainly by enemy unmanned aerial vehicles.

Conventionally, gas grenades K-51, RGR and RG-Vo are non-lethal means. At the same time, chloroacetophenone (CN) at a concentration of 0,85 mg/l and exposure for 10 minutes leads to the development of toxic pulmonary edema with fatal outcome.

The use of chemical grenades by the enemy, which leads to the release of CWA, temporarily deprives military men of the opportunity to return fire, forces them to leave shelter and move to open "smoke-free" areas of the terrain, which makes them easy targets for enemy lethal weapons. After contact with gas, a person begins to have watery eyes, shortness of breath,

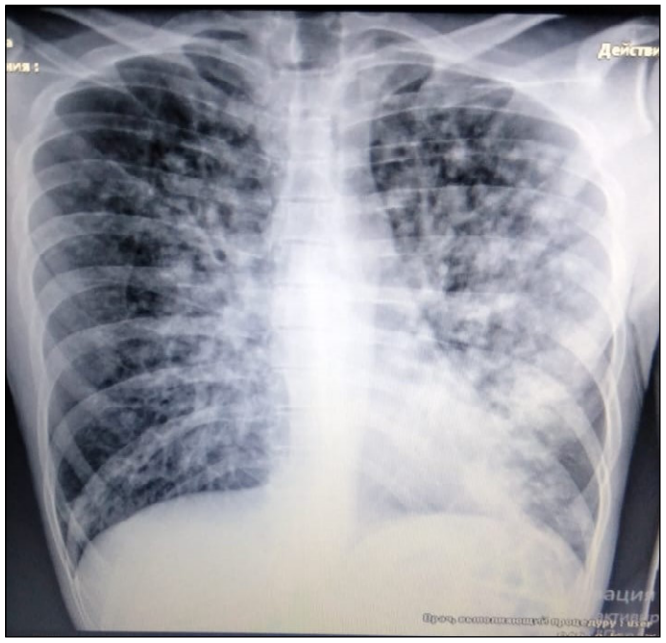

Fig. 4. Patient Sh., 46 y.o. Subtotal pulmonitis, pulmonary failure II.

Fig. 5. Patient R., 31 y.o. Total pulmonitis, pulmonary edema, pulmonary failure III.

cough, sinuses fill with mucus, vomiting is possible, etc. The effect intensifies with every minute a person stays in the toxic cloud.

The clinical picture of sternite lesions in military personnel was differentiated by three degrees of severity of the lesion. In the case of mild damage, the following manifestations were observed: slight irritation of the mucous membrane of the upper respiratory tract, moderate cough, burning pain in the nasal passages, pharynx, symptoms of tracheal irritation, pain behind the sternum, feeling of tightness in the chest, hyperemia of the mucous membranes. During the moderate degree of damage, severe symptoms of irritation of the mucous membranes, involvement of the middle parts of the respiratory tract in the process, headache, earache, excessive rhinorrhea, dry cough, sneezing, salivation, nausea, vomiting were observed. With a severe degree of damage, there were such symptoms as damage to all parts of the respiratory tract, unbearable pain behind the sternum, reflex violation of the mechanics and rhythm of breathing, cardiac disorders (bradycardia, increased blood pressure, slowing and pathological rhythms of breathing, up to its stop – apnea).

The clinical picture of lesions with lacrimators was manifested in all patients by sharp irritation of the conjunctiva and cornea of the eyes, 5-10 seconds after the lesion, severe pain and heartburn in the eyes, lacrimation, photophobia and headache began. Also, lacrimator injuries were classified according to three degrees of lesion severity. With a mild degree of damage, the following manifestations were observed: slight burning pain in the eyes, moderate lacrimation, slight conjunctival hyperemia, and no irritation of the respiratory tract. During the moderate degree of damage, conjunctivitis and spasm of the eyelids, photophobia, excessive lacrimation, pain in the orbits were sharply expressed. During the severe degree of damage, symptoms of general toxicity increased, heartburn in the throat, rhinorrhea and wet cough, severe headaches, dizziness, nausea, vomiting, and general weakness appeared. As a rule, the effect of irritating warfare agents was short-lived. When exiting the "wave of poisonous smoke", the phenomena of irritation in the first 15-20 minutes persisted or even increased, and then gradually decreased over the course of 1-2 hours and disappeared altogether.

The clinical picture in CS lesions was manifested by severe inflammation of the conjunctiva, eye pain, excessive lacrimation, and blepharospasm. Affected military men complained of a burning sore throat, severe cough, respiratory contamination, tightness, tenderness, and then chest pain. Breathing became arrhythmic, the victims became anxious, irritable, intimidated, prone to panic, itching of the skin, burning pains, erythematous spots on the surface of the skin, there could be chemical burns of the skin of the second degree. The action of CN caused severe irritation of the nerve endings of the mucous membranes of the eyes, skin, mucous membranes of the mouth and nose, intense blepharospasm; eye pain with lacrimation (temporary blindness may be observed), difficulty breathing; redness of the skin, burning pain

of the affected area. Affected military men also report heartburn and pain in the mouth and throat. Short-term reflex bradycardia was observed.

When CS came into contact with the skin in military personnel, skin reactions were noted: mainly erythema of the skin of the face and hands with an edematous component, cases of allergic contact sensitization with erythematous spots and vesicular skin rashes were also noted (Fig. 3).

It should be noted that high concentrations of CS and/or CN can cause serious respiratory symptoms such as reactive airway dysfunction syndrome (compromised lung syndrome). Affected military men experienced cough, chest pain, sputum secretion, and in some cases, hemoptysis, difficulty breathing, and nasal discharge, sometimes lasting weeks after the injury. X-ray examination of the lungs revealed the following radiological signs: infiltration of lung tissue, consolidated rounded foci (destruction cannot be ruled out), decreased pneumatization due to infiltrative changes, etc. X-ray of the chest organs of the affected military men is shown in Fig. 4 and Fig. 5.

DISCUSSION

The cardiovascular response of the patients affected by CS and CN was characterized, as a rule, by a change in heart rhythm and an increase in systolic and diastolic blood pressure. In addition to the direct impact of CWA, significant neuropsychic overload of frontline fighters during hostilities can cause not only a significant increase in the incidence of arterial hypertension, but also lead to a significant "rejuvenation" of this disease in the future [12].

The basic level of medical care includes limiting contact with the CWA as soon as possible (putting on a gas mask, leaving the affected area), using a smoke mixture (ficillin) (an ampoule of the drug in a braid is opened and placed under the mask of the gas mask). The smoke mixture has the following composition: chloroform – 40,0; ethyl alcohol – 40,0; ether – 20,0; ammonia – 5 drops. Available in ampoules in a braid of 1,0 [13]. Outside the lesion, rinse the mouth, nose, throat, rinse the eyes and exposed skin with clean water, saline, or 2% sodium bicarbonate solution outside the lesion. The main thing in providing assistance to victims is self-help and mutual aid, as well as the provision of first aid by combat medics of the units, which requires careful training of personnel [14].

As a rule, with mild and moderate injuries, military personnel are not subject to evacuation outside the military area. Most of the victims can be returned to combat capability in a short time – within 1-2 days. At the same time, in case of severe lesions of the eyes and respiratory tract, evacuation is carried out first [15].

When providing first aid, the gas mask is removed, the smoke mixture is reused (if necessary), in case of severe pain, analgesics are administered, the eyes, oral cavity, and skin are washed with plenty of warm running water (with the exception of capsaicin lesions), saline, 2% sodium bicarbonate solution.

The first level of medical care (first aid) includes the administration of metamizole sodium 50% – 2,0 ml, ketorolac 30 mg – 1,0 ml, butorphanol tartrate 0,2% – 1,0 ml intramuscularly; rinsing the eyes, mouth, skin with 2% sodium bicarbonate solution, dripping with 2% novocaine, 1% atropine or 0,5% dicaine for eye pain, applying antibacterial ointment behind the eyelid, gastric lavage and prescribing antacids (if necessary).

All victims of irritants, in the absence of other signs of damage, are classified as lightly affected and, as a rule, do not need to be evacuated to the next stage.

The second level of medical care (qualified medical care) involves complete sanitization with a change of uniforms, pathogenetic and symptomatic therapy. Treatment of bronchospasm, acute respiratory distress syndrome and pneumonia is carried out according to general principles.

The third level of medical care (specialized medical care) consists in the continuation of previous measures, prevention and treatment of complications. Bronchospasm, pneumonia, and, in severe cases, pulmonary edema and acute distress syndrome are treated [16].

CONCLUSIONS

1. As a result of the full-scale armed aggression against Ukraine, 2698 cases of damage by chemical warfare agents of irritant action were detected among the military men of the Armed Forces of Ukraine, and every month during 2024, more and more injuries were detected among the military men of the Armed Forces of Ukraine (715 cases in May 2024 compared to 444 cases in April 2024), which indicates that a stable upward trend towards a wider trend is emerging deliberate use of chemical warfare agents, the coefficient of determination of R^2 of which was high and amounted to 0,9577, and which continues to grow steadily.
2. Injuries to military men of the Armed Forces of Ukraine by chemical warfare agents of irritating effect, in most cases, were of mild and moderate severity. Medical care was provided, in most cases, on the spot, as a rule, they did not need to be evacuated to higher stages of medical evacuation.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Nataliia D. Kozak
Ukrainian Military Medical Academy
45/1, Kniaziv Ostrozkykh St., 01015 Kyiv, Ukraine
e-mail: kozaknd@ukr.net

ORCID AND CONTRIBUTIONSHIP
Nataliia D. Kozak: 0000-0001-9407-9634 A E F
Oleh V. Rudynskyi: 0009-0006-8391-0561 B C D E
Andriy V. Verba: 0000-0002-9661-3084 A B E
Alla A. Asaulenko: 0009-0004-2580-4782 B E
Dmytro O. Kozak: 0000-0001-7007-4687 C D E

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

RECEIVED: 29.07.2024
ACCEPTED: 27.10.2024

ORIGINAL ARTICLE

CONTENTS

A nickel implant induces cell death through autophagy in the connective tissue capsule in an experimental model

Hlib O. Lazarenko[1], Serhii I. Savosko[2], Svitlana M. Shamalo[2]

[1]STATE INSTITUTION OF SCIENCE «CENTER OF INNOVATIVE HEALTHCARE TECHNOLOGIES» STATE ADMINISTRATIVE DEPARTMENT, KYIV, UKRAINE

[2]BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

ABSTRACT

Aim: To identify cellular autophagy markers around nickel-containing implant as evidence of metal hypersensitivity reactions in an animal model.

Materials and Methods: Rats were sensitized to nickel using a modified model involving the administration of $NiSO_4$ with adjuvants. Subsequently, nickel plate implants (Ni content at 98.9%) were placed subfascially in the rats. Five months after implantation, the capsule morphology and autophagy were examined through the immunohistochemical detection of Beclin1 and GRP78. Implants tissue capsules without previous $NiSO_4$ exposition were considered as control.

Results: A high immunoreactions to GRP78 were observed in the implant capsule wall, with Beclin1-positive cells primarily noted at the interface with the implant. GRP78 and Beclin1 were significantly higher (p=0.01) expressed in cases with adjuvants, serving as a model for provoking an acute tissue response to implant.

Conclusions: In addition to inflammation and necrosis, cell death in the connective tissue capsule wall occurs through autophagy. Autophagy, mediated by Beclin1, is prominent at the implant interface and is closely associated with GRP78, which is strongly expressed throughout the capsule thickness, indicating significant oxidative stress in the cells surrounding the nickel plate implant.

KEY WORDS: adverse reaction to metal implant, nickel, capsule, autophagy, cell death

Wiad Lek. 2024;77(11):2193-2198. doi: 10.36740/WLek/197148 DOI

INTRODUCTION

The choice of specific metals or alloys depends on their intended use, but it is important to note that high concentrations of metals can disrupt normal physiological functions. This, in turn, can lead to the development of both local and systemic hypersensitivity reactions.

Hypersensitivity reactions (HSR) are one of the most complex issues in orthopedic and trauma practice, leading to extensive discussions among specialists. These reactions are a function of the adaptive immune system and, according to Gell and Coombs classification, are divided into four types: Type I, Type II (antibody-mediated), Type III, and Type IV (cell-mediated, delayed). Most hypersensitivity reactions associated with orthopedic implants are classified as Type IV, which are delayed-type hypersensitivity reactions [1].

The first documented case of a hypersensitivity reaction to metal (HRM) caused by an orthopedic implant was published in 1966 by Foussereau and Laugier [2]. They described a case of eczematous dermatitis, which sparked significant interest in the medical community. Following this publication, many similar cases have been reported, highlighting the relevance of the issue.

The spectrum of local and systemic reactions associated with metal implants is now collectively referred to as "adverse reaction to metal debris" (ARMD). These include elevated metal ion levels in blood or serum, metallosis (accumulation of metal debris in periprosthetic tissues), adverse local tissue reactions (ALTR), pseudotumors, and osteolysis.

Currently, there is no unified view on the causes of such reactions, highlighting the need for further investigation into the molecular mechanisms of tissue damage around orthopedic implants. It is known that implanted metal components undergo some degree of corrosion, leading to the release of metal ions into the surrounding tissues [3, 4].

These ions can have harmful effects on the structure of bone tissue and bone marrow [5]. Cadosch et al. described the growth and differentiation of osteoclast

precursor cells on the surfaces of stainless steel, titanium, and aluminum implants. They demonstrated that mature osteoclasts can induce corrosion of metal surfaces, leading to the release of metal ions [6-8]. However, metal corrosion can result from mechanisms beyond this, including processes such as crevice corrosion and fretting.

It is important to note that dynamic components of implants often contain particles that are primarily localized in periprosthetic tissues. The accumulation of metal particles in these tissues can lead to sustained antigen concentrations, which in turn may activate a local immune response and potentially cause implant rejection [5].

The inflammatory response of the body is directly related to the amount of metal particles present. These particles have a significant pro-inflammatory effect, activating osteoclasts through macrophage engulfment. Metal ions bind to proteins recognized by antigen-presenting cells [9]. These processes lead to osteolytic lesions around implants. The activity of osteoclasts in periprosthetic areas triggers the activation of their precursors and the release of cytokines, which promote their differentiation and activation [6, 10, 11].

Given the increasing frequency of joint replacement and the use of orthopedic implants, it is expected that the number of complications related to implant reactions will also rise [12-15]. This underscores the need for in-depth study of the molecular mechanisms of cellular damage around orthopedic implants. Such research will help to better understand cellular reactions to metals that may lead to rejection or adverse processes, such as allergic and inflammatory reactions. Understanding these mechanisms can aid in the development of diagnostic and prognostic tests for patients with metal implants.

As a continuation of our previous study [16], which demonstrated capsule growth around a nickel-containing implant and the occurrence of necrosis, this work focuses on cellular mechanisms of cell death, particularly autophagy.

Autophagy is a specific cellular response to damage, such as hypoxia and oxidative stress, aimed at preserving cell survival by removing damaged organelles [17]. However, it remains unclear whether autophagy is involved in regulating cellular reactions around a nickel-containing implant.

AIM

To investigate the cellular response associated with autophagy to a nickel-containing implant in an experimental model of metal hypersensitivity reactions.

MATERIALS AND METHODS

The study was conducted on 25 female Wistar rats, weighing 150-165 grams, aged 3 months at the start of the experiment. The animals were housed in the vivarium of the O. V. Palladin Institute of Biochemistry, NAS of Ukraine. The rats were randomly divided into 3 groups: Group 1 – Intact Group (IT), 6 rats that did not undergo any surgery; Group 2 – Control Group (K), 6 rats received incomplete Freund's adjuvant before implantation of a nickel-containing implant. They were pretreated with Freund's adjuvant with $NiSO_4$, followed by implantation of the nickel-containing implant; Group 3 – Experimental Group (E), 13 rats were pretreated with Freund's adjuvant with $NiSO_4$, followed by implantation of the nickel-containing implant. The immune response associated with nickel was modeled using a modified method described previously [18].

For this, the rats were intraperitoneally (i.p.) injected with 50 µl of NiSO4 solution (CAS 10101-97-0, Sigma-Aldrich, USA) at a concentration of 10 µmol/L in incomplete Freund's adjuvant (Sigma-Aldrich, USA). After 2 and 4 weeks, the rats received NiSO4 through intradermal (i.d.) injections of 50 µl (2 µmol/ml $NiSO_4$ in complete Freund's adjuvant, Sigma-Aldrich, USA) using 28G1/2 needles to restore the immune response.

The implantation of the nickel implant was performed after 6 weeks after last exposition of $NiSO_4$ under aseptic and antiseptic conditions, with general anesthesia using sodium thiopental (Kyivmedpreparat, Ukraine) at a dose of 50 mg/kg body weight, administered intraperitoneally.

The implants for the study were provided by LLC «ORTOSYNTEZ.» All samples were pre-screened by spectroscopy to assess the local elemental composition of the material, with Ni content at 98.9%. The rats underwent a dorsal incision along the vertebral line, and a subfascial pocket was created in the interscapular space to place the nickel-containing implant, measuring 6.0 x 4.0 x 1.0 mm. After the procedure, the wound was closed in layers with Prolene 3-0 sutures (Ethicon Inc, Johnson & Johnson, USA) and treated with the antiseptic Povidone-iodine (Betadine, Hungary). To prevent bacterial infections, the animals were administered the antibiotic Ceftriaxone (Arterium, Ukraine) at a dose of 20 mg/kg body weight, intraperitoneally. The animals were placed in individual cages (60x40x50 cm), and observations were conducted over a period of 5 months. No infections or other postoperative complications were detected.

Five months after implantation, the rats were euthanized using a lethal dose of sodium thiopental (i.p. 150-200 mg/kg) (Kyivmedpreparat, Ukraine). For histological analysis, tissue samples from the capsule around the implant were fixed in 10% neutral formalin. The samples were embedded in paraffin (Leica

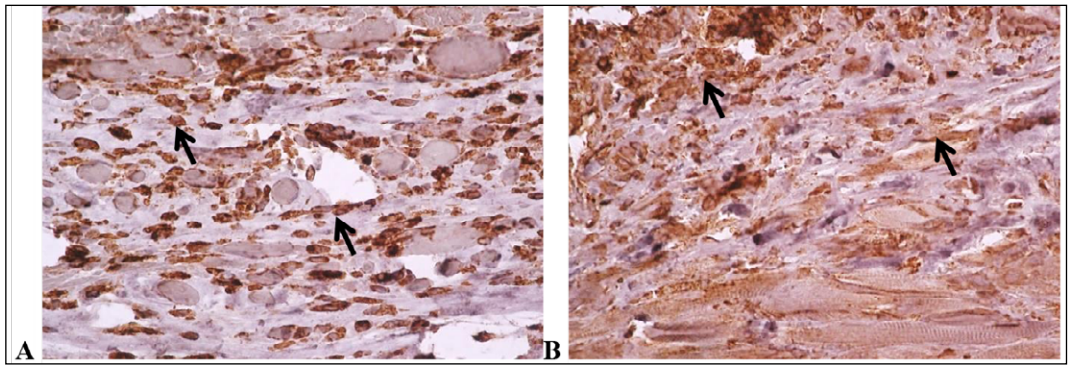

Fig. 1. Cells with GRP78-positive immunoreactivity along the outer contour of the capsule surrounding the implant in the control (A) and experimental (B) groups. Note: ← cells with positive reaction to GRP78. Immunohistochemical staining for GRP78. Magnification x400.

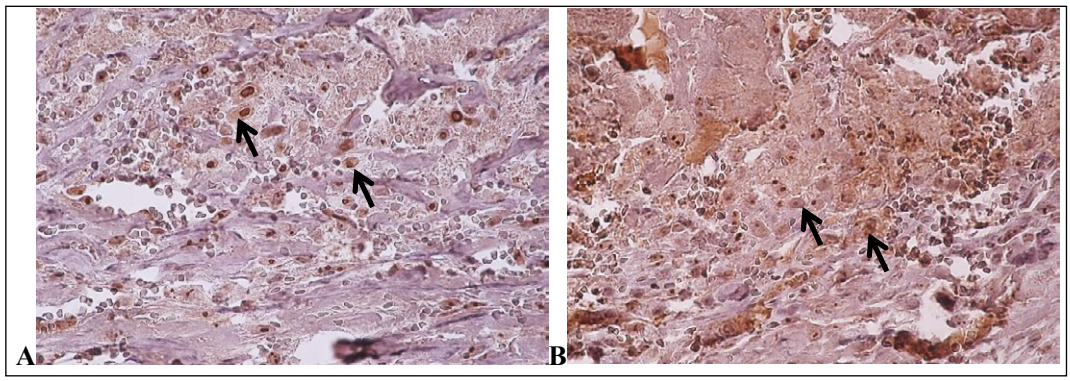

Fig. 2. Cells with Beclin1-positive immunoreactivity in the capsule wall around the implant in the control (A) and experimental (B) groups. Inner layer of the capsule. Note: ← cells with positive reaction to Beclin1. Immunohistochemical staining for Beclin1. Magnification x400.

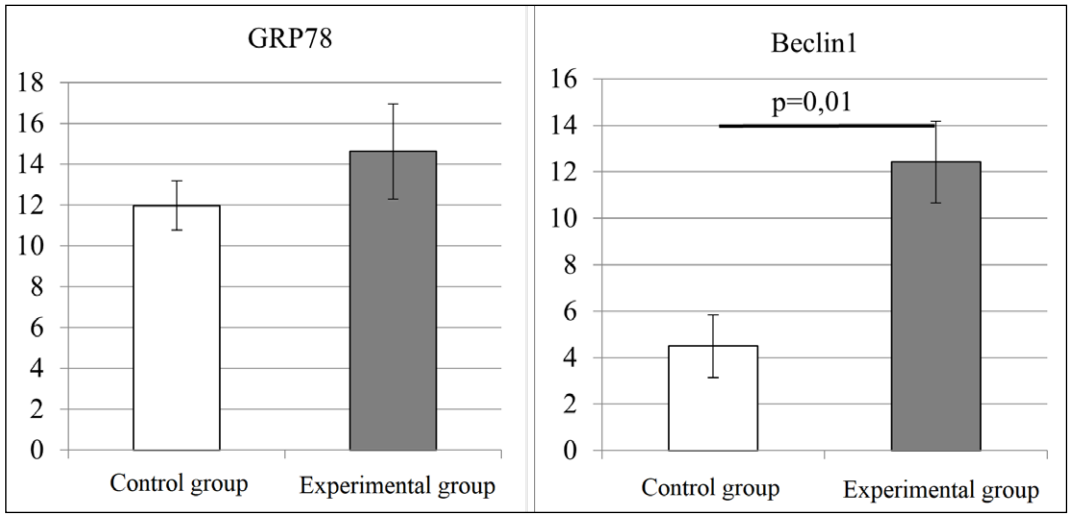

Fig. 3. Quantitative evaluation results of the specific area of immunopositive reaction to GRP78 and Beclin1.

Surgipath Paraplast Regular, Leica, USA). Sections of 4 µm thickness were prepared from paraffin blocks and mounted on Superfrost Plus slides (Thermo Scientific, Gerhard Menzel GmbH, Germany). Antigen retrieval and staining reactions were carried out according to the manufacturer's protocol for antibodies.

Primary antibodies against GRP78 (Invitrogen, PA5-34941, USA) and Beclin-1 (Invitrogen, PA5-20171, USA) were used. The primary antibodies were diluted at a ratio of 1:200. Visualization of the reaction product was performed using a detection system based on diaminobenzidine (EnVision FLEX; Dako, Glostrup, Denmark). The incubation time with primary antibodies was 60 minutes at 24°C, and the incubation time with secondary antibodies was 20 minutes at 24°C. Hematoxylin Gill I was used for staining cell nuclei.

Microscopic examination of the samples was performed using an Olympus BX51 microscope (Olympus, Japan), and microphotographs were obtained with a digital camera Olympus C3040ZOOM. Quantitative assessment of the immunohistochemical reactions was conducted using a densitometric method with ImageJ software.

Statistical data analysis was performed using the Mann-Whitney U test with Origin v.9.0 software. The data were presented as mean (M) ± standard error or mean (m). Differences between groups were considered statistically significant at $p < 0,05$.

RESULTS

Around the implants, a connective tissue capsule with a pronounced inflammatory infiltrate and necrotic material

was observed. On average, 35-45% of the capsule wall was composed of collagen fibers. Microscopically, the wall of the capsule in both the control and experimental groups appeared similar, though a significant cellular infiltrate with hemorrhagic infiltration was noted in the control group. Both groups exhibited multiple accumulations of hemosiderin, macrophages, and cellular detritus.

Immunohistochemical analysis assessed the intensity of GRP78 and Beclin1 synthesis by cells in the capsule wall surrounding the implants. This analysis allowed for the identification of some cellular responses in the capsule wall that could explain processes associated with cell death. GRP78 is associated with endoplasmic reticulum stress, while Beclin1 is linked to autophagy.

The immunohistochemical study revealed that the intensity of GRP78 staining indicated active cytoplasmic immune reactions in the cells of the capsule wall (Fig. 1). These cells were located among muscle fibers, around blood vessels, and between the fibrous elements of the capsule. Based solely on the positive reaction to GRP78, it is not possible to definitively determine the type or population of the cells. However, the majority of cells in the capsule wall exhibited active expression of GRP78. We observed a numerical, but non-significant increase in the density of GRP78-positive cells and specific area of immunoreaction (11,9±1,20 vs 14,6±2,33; p=0,15 via the Mann-Whitney U test). These results indicate that the intensity of processes in the capsule wall, hypothetically associated with cell damage involving the endoplasmic reticulum, was greater in the experimental group or did not differ between comparison groups.

The density of cells with positive immunoreactivity to Beclin1 depended on the distance from the capsule cavity. Thus, their density increased along the inner contour of the capsule and decreased towards the middle thickness of the capsule wall. Cells with positive immunoreactivity to Beclin1 were not detected outside the capsule (Fig. 2). These results indicate a distal dependence of autophagy development in the capsule cells around the implants. It can be inferred that cell death by autophagy occurred along the implant contour. According to densitometric analysis, the specific area of immunopositive reaction was significantly higher (4,49±1,34 vs 12,42±1,75; p=0,01 via the Mann-Whitney U test) in the experimental group compared to the control group (Figure 3). This suggests that autophagy occurs in the cells at the capsule's boundary with the implant, which is the molecular basis of their death. It appears that autophagy processes were significantly less pronounced regarding cellular reactions associated with GRP78, had a distal dependence on the implant, and were potentially more intense in the experimental group.

DISCUSSION

The most common tissue reactions to a metal implant are inflammation and necrosis in the peri-implant tissue. Cellular responses such as proliferation, migration, and cell death occur through various mechanisms. Oxidative stress and peroxidation provide some insight into the cause-and-effect relationship in the development of cell necrosis. However, it turns out that cell death around the implant is a more complex phenomenon and can occur through atypical mechanisms, including cell autophagy. In this experiment, necrotized cells and cellular debris were found around the Ni-containing implant, directly at the interface between the formed connective tissue capsule and the implant. Importantly, among this mass of damaged cells, cells with pronounced Beclin1 expression were detected, which can be considered an immunohistochemical marker of autophagy initiation in these cells. Autophagy is known to be a self-degradation process in eukaryotic cells, involving specific rearrangements of the endoplasmic reticulum to capture and degrade cellular organelles [19]. Reactive changes in cells, which may be related to such responses, include GRP78 expression. This protein responds to oxidative stress and is associated with the endoplasmic reticulum. Studies show that reactive oxygen species (ROS) play a critical role in the initiation of autophagy [20]. Excessive activation of autophagy by oxidative stress can lead to the execution of cell death mechanisms. Increasingly, research indicates that various heavy metals, including Ni, can induce autophagy [20, 21].

There are reports that nickel (Ni) induces mitochondrial damage and mitophagy [22]. In this study, the pronounced reaction of cells expressing GRP78 in the capsule surrounding the implant indicates that the Ni-containing implant induces oxidative stress in peri-implant tissues. It became clear that autophagy occurs on the inner side of the capsule, while cells with a strong immunoreaction for GRP78 were observed throughout almost the entire thickness of the capsule. These results suggest that a certain critical level of oxidative stress around the implant triggers autophagy, mediated by Beclin1. The main conclusion is that autophagy, along with apoptosis and necrosis, and independently of these processes, can regulate the intensity of cell death around the implant with bioactive properties. While necrosis is considered an unregulated process resulting from critical changes and damage to cells, apoptosis and autophagy are regulated processes. Both are triggered by various pathways and conditions and both control cell survival or death. There is clearly a close connection between autophagy and apoptosis, which requires further in-depth study. In this study, we only state the fact of detecting autophagy and its topographical proximity to the Ni-containing implant, which further confirms the bioactivity and toxicity of Ni.

The limitation of this study lies in the restricted methods for detecting autophagy and other mechanisms of cell death. It is important to investigate the connection between autophagy and immune responses to establish a reliable link with reactions similar to allergies and metal hypersensitivity. Therefore, further research is needed to study the pathways and mechanisms of cell death associated with inflammation around Ni-containing implants.

CONCLUSIONS

Undesirable tissue reactions and complications to the Ni-containing implant include inflammation and cell death in the connective tissue capsule surrounding the implant. Autophagy is one of the pathways of cell death in the capsule wall, mediated by Beclin1 and closely linked to GRP78. The latter is a reliable marker of cellular response to damage, where autophagy represents contact-dependent cell death in relation to the implant.

PROSPECTS FOR FURTHER RESEARCH

As a potential approach, one of the likely ways to prevent the initiation of cell death, particularly delayed autophagy, could be coating the implant with a material that prevents cells from coming into contact with nickel or other bioactive metals of the implant.

COMPLIANCE WITH ETHICAL REQUIREMENTS

The experimental studies were conducted in compliance with the requirements for humane treatment of experimental animals in accordance with the Law of Ukraine «On the Protection of Animals from Cruelty» (No. 3447-IV dated February 21, 2006), adhering to the requirements of the European Parliament and Council (2010), and the European Convention «For the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes» (Strasbourg, March 18, 1986).

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The study performed as a fragment of the complex scientific projects of the Scientific Department of Minimally Invasive Surgery (State Institution of Science «Center of innovative healthcare technologies» State Administrative Department) «Surgical treatment optimization based on multimodal fast recovery program using nanobiosencoric technologies and anaesthesiological providing» (state registration number 0122U000233; term: 2022-2024), and the Department of Histology and Embryology of the Bogomolets National Medical University «To study the tissue reactions of various departments of the nervous system and internal organs to the defeat of various genesis, and their modulation. Pedagogical aspects of teaching histology», (state registration number 0120U102691; term: 2023-2025).

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Hlib O. Lazarenko
State Institution of Science
«Center of innovative healthcare technologies»
State Administrative Department
5 Verkhnia St., 01014 Kyiv, Ukraine
e-mail: lazarenkog@gmail.com

ORCID AND CONTRIBUTIONSHIP
Hlib O. Lazarenko: 0000-0001-8585-5355 A C D E F
Serhii I. Savosko: 0000-0001-5145-2195 A B D F
Svatlana M. Shamalo: 0000-0003-1809-1476 C D

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

RECEIVED: 08.07.2024
ACCEPTED: 18.10.2024

ORIGINAL ARTICLE

CONTENTS

Kallikrein-kinin system pathogenetic importance in experimental benign prostatic hyperplasia

Igor A. Lurin[1], Igor P. Khomenko[1], Serhii V. Lul'ko[2], Oleh A. Kashtelyan[2], Ivan V. Savytskyi[2], Serhii V. Tertyshnyi[3], Rooslan S. Vastyanov[3]

[1] NATIONAL ACADEMY OF MEDICAL SCIENCES OF UKRAINE, KYIV, UKRAINE

[2] INTERNATIONAL EUROPEAN UNIVERSITY, KYIV, UKRAINE

[3] ODESA NATIONAL MEDICAL UNIVERSITY, ODESA, UKRAINE

ABSTRACT

Aim: To analyze the mechanisms of regulation of the body's proteolytic systems during inflammation, detection of inflammation markers in blood and prostate secretions in the experimental benign prostatic hyperplasia in rats.

Materials and Methods: The study was conducted on 30 male rats on the model of benign prostatic hyperplasia. Rats were randomized as following: the 1st group (n=6) – intact animals; the 2nd group (n=24) – rats with benign prostatic hyperplasia.

Results: The prostate gland inflammatory damage was also evidenced by the prostate secretion proteolytic potential increase in 9 times as the result of kallikrein activity enhancement on the 21st day of the trial which leads to massive kininogenesis. The analogous kallikrein activity increase (in 3,2 times) we registered in blood serum.

An acute kallikrein activity increase in case of benign prostatic hyperplasia is probably compensated by an increase in the activity of its specific inhibitor – α_2-macroglobulin, the level of which in conditions of investigated pathology increased in 3,25 times on the 21st day of the trial compared to intact rats, and by the proteolytic potential increase resulted in the inhibitory activity of alpha$_1$ proteinase inhibitor content increase in 2,25 times (a protein of the acute stage of inflammation).

Conclusions: An increase in the content and activity of kallikrein-kinin system components in the prostate secretion testifies to its inflammatory damage and the disturbance of the hematoprostatic barrier permeability which can be an important diagnostic criterion.

KEY WORDS: benign prostatic hyperplasia, proteolytic systems, kallikrein-kinin system, pathogenesis

Wiad Lek. 2024;77(11):2199-2203. doi: 10.36740/WLek/197150 DOI

INTRODUCTION

Benign prostatic hyperplasia (BPH), benign prostatic enlargement (BPE) and lower urinary tract symptoms (LUTS) belong to the most frequent diseases in ageing men [1]. Beyond the 6th decade of life, more than 30% of men suffer from moderate to severe LUTS requiring intervention. The pathophysiology of BPH/BPE is still incompletely understood [1]. The dominant role of the androgen system and the androgen receptor is well defined. Androgen receptors are expressed in BPH tissue in which they are activated by the potent androgen dihydrotestosterone. Synthesis of dihydrotestosterone is under control of the 5α-reductase enzyme, activity of which is antagonized by finasteride and dutasteride [2]. More recently, the impact of prostatic inflammation and metabolic parameters particularly for the development of BPE and LUTS has increasingly been recognized [3]. A better understanding of the pathophysiology is a prerequisite for the development of novel, more effective medical treatment options [4, 5]

The activation of proteolysis and one of the most important proteolytic systems of the body – the kallikrein-kinin system (KKS) – becomes of key importance during inflammation [6]. The coordinated action of proteases and their inhibitors is one of the forms of maintaining homeostasis in the body, while a complex and multicomponent sequence of reactions is considered as a universal non-specific response to injury. Kallikrein is a multifunctional proteinase that controls many biological processes, including converting the precursor protein kininogen into bradykinin, a "mediator" of pain and inflammation [6]. In addition, kallikrein causes chemotaxis and aggregation of neutrophils, releases elastase, activates the latent form of neutrophil collagenase. The activity of kallikrein and other proteinases is regulated with the help of special

proteins – inhibitors of serine proteinases, among which the 1-proteinase inhibitor, whose main function is the inactivation of neutrophil elastase, and α_2-macroglobulin, which binds thrombin, plasmin, kallikrein, are present in the largest amount in the blood plasma, elastase and other proteolytic enzymes [1, 5].

There are no research results on the mechanisms of the inflammatory reaction formation in the prostate involving proteolytic systems, as well as protective factors that limit the damage process.

AIM

The aim was to analyze the mechanisms of regulation of the body's proteolytic systems during inflammation, detection of inflammation markers in blood and prostate secretions in the experimental BHP in rats.

MATERIALS AND METHODS

The study was conducted on 30 white, sexually mature male rats kept on a standard vivarium diet. Keeping, processing and manipulation of animals was carried out in accordance with the "General Ethical Principles of Animal Experiments" adopted by the Fifth National Congress on Bioethics (Kyiv, 2013). We used the recommendations of the European Convention on the Protection of Vertebrate Animals for Experimental and Other Scientific Purposes (Strasbourg, 1985), Directive 2010/63/EU of the European Parliament and Council on protecting animals used for scientific purposes, the Law of Ukraine "On protection of animals from cruel treatment" N440-IX of 14 January 2020 and the rules of humane treatment of experimental animals and conditions approved by the Bioethics Commission of the Odesa National Medical University (protocol No. 32D dated 03/17/2016) [7, 8].

Rats were randomized into 2 groups: 1st group (n=6) – intact animals (rats that were on a standard water diet and food); 2nd group (n=24) – rats with BHP. BPH was induced by 30 days sulpiride (i.p., 40 mg/kg) administration. Sulpiride is an antipsychotic agent, the neuroleptic properties of which are associated with antidopaminergic action. With long-term administration (within 30 days), sulpiride induces hypersecretion of prolactin, which causes stimulation of the proliferation of the glandular epithelium in the prostate. In this case, the pathogenesis of acinar hyperplasia is caused by an increase in the activity of 5α-reductase and an increase in the sensitivity of epithelial cells to androgens [9].

The following indicators were investigated both in the blood serum and in the prostate secretion (after its dilution in ratio of 1:9 with physiological solution) to study the role of the body's proteolytic systems in BHP pathogenesis.

The kallikrein activity and the content of prekallikrein after separation from other serine proteinases were determined using ion exchange chromatography by the rate of hydrolysis of N-benzoyl-1-arginine ethyl ether [10]. Alpha$_1$ proteinase inhibitor (α1-PI) and α2-macroglobulin inhibitory activity was studied using a unified enzymatic method [11].

The data obtained were presented as mean (x) and the standard error of the mean (m). For statistical evaluation, biochemical components in blood serum and prostate secretion of rats with benign hyperplasia of prostate were calculated at 4 stages of observation, which were compared with similar indexes in intact rats. For this purpose, the statistical criterion of one-way ANOVA with post-hoc Newmann-Keuls test was used. A $p<0,05$ was considered as statistically significant difference.

RESULTS

Were observed in the blood serum of rats a probable increase in the level of kallikrein and a decrease in the content of its precursor – prekallikrein (Table 1). In the group of control pathology animals, this indicator increased already on the first day on 55,3 % and amounted to 23,6±3,6 nmol/min/ml ($p<0,05$), on the 7th day – on 138,2 % ($p<0,05$) (36,2±4,4 nmol/min/ml), on the 14th day – on 202 % ($p<0,05$) (45,9±5,24 nmol/min/ ml), on the 21st day – on 220,4 % ($p<0,05$) (48,7±6,0 nmol/min/ml) compared to intact animals.

The content of prekallikrein decreased sharply: already on the first day of the trial, its level was 320,3±30,2 nmol/min/ml, which is 10,5% ($p<0,05$) lower than the similar indicator of intact animals. On the 7th day, the prekallikrein level decreased on 11,8% ($p<0,05$) (it was 315,8±28,4 nmol/min/ml); on the 14th day – on 16,7% ($p<0,05$) (298,1±25,3 nmol/min/ml); on the 21st day – on 26,2% ($p<0,05$) (264,2±26,4 nmol/min/ml) compared to the intact group of animals.

The index of inhibitory activity of α1-PI probably increased in the group of animals with simulated BHP pathology. On the first day, its level increased on 21,3% ($p<0,05$) (equal to 30,2±3,0 IU/ml), on the 7th day – on 35,3% ($p<0,05$) (33,7±3,1 IU/ml), on the 14th day – on 70,3% ($p<0,05$) (42,4±3,8 IU/ml), on the 21st day – on 95,6% ($p<0,05$) (48,7±4,4 IU/ml) relative to the similar indicator in the group of intact rats. Studying the level of inhibitory activity of α_2-macroglobulin in the group of control pathology, no probable changes were found compared to intact animals.

It was established during the studying of these indicators in the secretion of the BHP a similar trend in the blood serum of experimental animals. In particular, the level of kallikrein in the group of animals with BHP on the

Table 1. The level of proteolytic enzymes in the blood serum of rats against the background of experimental benign hyperplasia of prostate

Indicator	Intact animals	The investigated indexes in rats with BHP (M±m), n=6			
		1st day	7th day	14th day	21st day
Kallikrein, nmol/min/ml	15,2±3,1	23,6±3,6*	36,2±4,4*	45,9±5,2*	48,7±6,0*
Prekallikrein, nmol/min/ml	357,8±25,8	320,3±30,2*	315,8±28,4*	298,1±25,3*	264,2±26,4*
Inhibitory activity of α_1-PI, IU/ml	24,9±2,7	30,2±3,0*	33,7±3,1*	42,4±3,8*	48,7±4,4*
Inhibitory activity of α^2-macroglobu-line, IU/ml	4,2±0,75	3,6±0,7	3,7±0,7	4,0±0,6	3,9±0,7

Notes: n – the number of animals in the group; * – p<0,05 significant differences of the investigated indexes compared with the same in intact animals.

Table 2. The level of proteolytic enzymes in the secretion of the prostate gland of rats against the background of experimental benign hyperplasia of prostate

Indicator	Intact animals	The investigated indexes in rats with BHP (M±m), n=6			
		1st day	7th day	14th day	21st day
Kallikrein, nmol/min/ml	5,6±0,48	27,2±3,1*	37,6±3,9*	45,3±4,6*	50,8±5,2*
Prekallikrein, nmol/min/ml	273,4±22,3	265,3±25,1	247,1±21,4*	225,3±22,9*	214,2±24,2*
Inhibitory activity of α1-PI, IU/ml	2,4±0,68	3,1±0,72*	3,8±0,85*	4,7±1,2*	5,4±1,1*
Inhibitory activity of α_2-macroglobu-line, IU/ml	0,16±0,02	0,21±0,02	0,32±0,03*	0,46±0,05*	0,52±0,5*

Notes: n – the number of animals in the group; * – p<0,05 significant differences of the investigated indexes compared with the same in intact animals.

first day of the experiment increased by 385,7% (p<0,05) and amounted to 27,2±3,1 nmol/min/ml, on the 7th day – by 571,4% (p<0,05) (37,6±3,9 nmol/min/ml), on the 14th day – by 708,9% (p<0,05) (45,3±4,6 nmol/min/ml) and on the 21st day – by 807,1% (p<0,05) (50,8±5,2 nmol/min/ml) compared to intact animals (Table 2).

In the control group of rats, the level of prekallikrein decreased compared to the level of the intact group of animals: on the first day – by 2,9% (and amounted to 265,3±25,1 nmol/min/ml), on the 7th day – by 9,6% (p<0,05) (247,1±21,4 nmol/min/ml); on the 14th day – by 17,6% (p<0,05) (225,3±22,9 nmol/min/ml); on the 21st day – on 21,7% (p<0,05) (214,2±24,2 nmol/min/ml).

The level of inhibitory activity of α_1-PI probably increased in the group of animals with simulated BHP pathology. On the 1st day its level increased by 29,2% (p<0,05) (equal to 3,1±0,72 μg/ml), on the 7th day – by 58,3% (p<0,05) (3,8±0,85 IU/ml), on the 14th day – by 95,3% (p<0,05) (4,7±1,2 IU/ml), on the 21st day – by 125% (p<0,05) (5,4±1,1 IU/ml) relative to the similar indicator in the group of intact rats.

The inhibitory activity of α_2-macroglobulin in the control group of animals on the first day of observation probably did not differ from intact animals, but on the 7th day its activity increased on 100% (p<0,05) (0,32±0,03 IU/ml), on the 14th day – by 187,5% (p<0,05) (0,46±0,05 IU/ml), on the 21st day – by 225% (p<0,05) (0,52±0,5 IU/ml).

DISCUSSION

The results of the study showed that in experimental BHP there is an increase in the activity of kallikrein in blood serum (p<0,05) compared to a similar indicator in intact animals. The intensity of the inflammatory process in the prostate in CP is indicated by a sharp increase in the inhibitory activity of α_1-PI (p<0,05) – the "protein of the acute phase of inflammation", both in the blood serum and in the secretion of the prostate gland. That is, the activation of the KKS is noted, which leads to the accumulation of bradykinin – one of the main "mediators" of pain and inflammation. As a result of massive kininogenesis, a complex of pathophysiological disorders develops, which are key in the development of the clinical manifestation of BHP: pain, hemodynamic disorders, microcirculation disorders, increased vascular permeability [12, 13].

We have shown that the inhibitory potential of prostate secretion increases dramatically in rats with experimental BHP. α_1-Proteinase inhibitor – protein of the acute phase of the inflammatory process, is the main endogenous regulator of the elastotic activity of the prostate secretion and is secreted during inflammation, thereby reducing the proteolytic activity of leukocyte elastase in the focus of inflammation and thereby preventing excessive tissue damage in the target organs in this pathological process [14].

Thus, α_1-proteinase inhibitor plays an important regulatory role in the anti-inflammatory response in BHP.

It is worth noting that high inhibitory activity of α_2-macroglobulin has been established in the prostate secretion with BHP, which indicates damage to the hematoprostatic barrier and the development of membranopathy. α_2-macroglobulin plays an important role in the regulation of inflammatory processes, as it limits the substrate specificity of most proteolytic

enzymes, turning proteases into peptidases that hydrolyze low-molecular peptides that are mediators of inflammatory processes. In addition, α_2-macroglobulin is the main transporter of regulatory cytokines to cells, participates in signal transmission to the cell and the initiation of a cascade of intracellular reactions that affects the formation of antibodies [14, 15].

CONCLUSIONS

1. The leading link in the pathogenesis of many diseases is a change in the structure and functions of biological membranes – membranopathy, the consequence of which is a violation of hematoprostatic barriers, which is manifested in an increase in the excretion of certain metabolites in the extracellular fluid. In the case of experimental BHP in rats, the appearance in the secretion of the prostate gland of the components of KKS, which are determined in the secretion of the normal prostate in trace amounts, was noted.
2. Inflammatory damage to the prostate was also evidenced by an increase in the proteolytic potential of prostate secretion due to an increase in kallikrein activity on the 21st day by 807,1% ($p<0.05$), which leads to massive kininogenesis. Depletion of the adaptive and compensatory potential of the prostate gland was evidenced by a decrease in prekallikrein by 21,7% ($p<0.05$) compared to the intact group of animals.
3. An acute increase in the activity of kallikrein in BHP is probably compensated by an increase in the activity of its specific inhibitor – α_2-macroglobulin, the level of which in the secretion of the BHP increased on 225% on the 21st day of the experiment compared to intact rats, and the increase in proteolytic potential – by an increase of 125% in the inhibitory activity of α_1-PI, a protein of the acute phase of inflammation.
4. An increase in the content and activity of KKS components in the secretion of the prostate testifies to its inflammatory damage and violation of the permeability of the hematoprostatic barrier, which can be an important diagnostic criterion.

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Source of funding: within the framework of the scientific target program "Mechanisms of epigenetic disorders of the leading links of bioenergetics and nitrogen metabolism in irradiated animals and their descendants" of the Ministry of Health of Ukraine (state registration No. 0121U114601; 2021-2026).

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Rooslan S. Vastyanov
Odesa National Medical University
2 Valikhovsky lane, 65082 Odesa, Ukraine
e-mail: rvastyanov@gmail.com

ORCID AND CONTRIBUTIONSHIP
Igor A. Lurin: 0000-0001-6280-1725 A F
Igor P. Khomenko: 0000-0002-8199-5083 A E F
Serhii V. Lul'ko: 0009-0006-4016-604X B D
Oleh A. Kashtelyan: 0000-0001-9922-4554 C F
Ivan V. Savytskyi: 0000-0002-5841-9993 A B
Serhii V. Tertyshnyi: 0000-0002-4949-5409 D E
Rooslan S. Vastyanov: 0000-0001-8585-2517 C E

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

RECEIVED: 09.07.2024
ACCEPTED: 22.10.2024

ORIGINAL ARTICLE

CONTENTS

Risk factors affecting the success of single-stage laparoscopic choledocholithiasis treatment: a retrospective analysis

Mykhailo V. Maksymenko[1, 2], Roman O. Havryliuk[1, 2]
[1]BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE
[2]CNE «KYIV CITY CLINICAL HOSPITAL OF EMERGENCY CARE», KYIV, UKRAINE

ABSTRACT

Aim: To identify and evaluate the risk factors affecting the success of single-stage laparoscopic treatment for choledocholithiasis.

Materials and Methods: We performed a retrospective analysis of treatment of 139 patients who underwent single-stage laparoscopic CBD exploration and cholecystectomy from 2018 to 2024. Data were analysed by the use of multivariate logistic regression to identify significant predictors of negative outcomes.

Results: Multivariate logistic regression identified advanced age, multiple stones, and preoperative cholangitis as significant negative predictors of procedural success. Age increased the odds of a negative outcome by 20,8% per year (OR 1,208 [95% CI 1,124-1,298]; p<0,001), and each additional stone increased the odds by 21,5% (OR 1,215 [95% CI 1,435-3,127]; p<0,001). Preoperative cholangitis was associated with a 5-fold increase in negative outcomes (OR 5,485 [95% CI 6,397-65,607]; p<0,001). Stone size and altered anatomy were not significantly associated with the procedural success.

Conclusions: Advanced age, multiple stones and preoperative cholangitis significantly impact on the success of single-stage laparoscopic choledocholithiasis treatment, increasing the probability of unsuccessful procedure. Incorporating these predictors into clinical practice can enhance patient care and procedural success. Preoperative imaging and intraoperative techniques are crucial for optimizing outcomes.

KEY WORDS: choledocholithiasis, obstructive jaundice, laparoscopic surgery

Wiad Lek. 2024;77(11):2204-2207. doi: 10.36740/WLek/197095 DOI

INTRODUCTION

Choledocholithiasis to this day remains the most common complication of gallstone disease, accounting for 5-33% of the total incidence [1-4]. This condition carries risks of such complications as cholangitis, pancreatitis, and obstructive jaundice, and requires urgent attention from a multidisciplinary team of specialists. Statistically, the ratio of women to men with calculi of the common bile duct (CBD) is 0,89:1,2, although lithiasis in the gallbladder is more often found in women (1,22:1 compared to men). Pregnancy and parity are considered risk factors for the formation of gallstones. The mean age of patients afflicted with choledocholithiasis is 67 years, which is higher than that of those with gallbladder stones only (56 years). This indicates that the risk of developing gallstone disease depends on such biological factors as gender, age and pregnancy [5, 6].

Nowadays two surgical methods of treatment of choledocholithiasis are successfully employed – single-stage and two-stage.

The two-stage approach includes endoscopic retrograde cholangiopancreatography with papillosphincterotomy combined with laparoscopic cholecystectomy performed as a separate intervention before or after endoscopic intervention. Comprehensive literature evidences the positive results of the use of the two-stage approach, which is primarily related to the more than 50-year development of the use of endoscopic retrograde cholangiopancreatography (ERCP) and the international standardization of endoscopic interventions [4, 7-10].

Single-stage method of treatment of choledocholithiasis involves laparoscopic CBD exploration and lithoextraction with simultaneous cholecystectomy [4, 8, 9]. Despite the obvious advantages, in particular, reducing the duration of inpatient treatment, preserving the sphincter of Oddi, and reducing the overall economic and resource burden, both on the patient and on the medical institution [3, 9], the single-stage technique is not always able to ensure a proper clearance of stones from the common bile duct, influenced by such patient-dependent and intraprocedural factors as age, number and size of stones, presence of cholangitis in the preoperative period, and altered anatomy [11, 12].

AIM

The aim of the study was to identify and evaluate the risk factors affecting the success of single-stage laparoscopic treatment for choledocholithiasis.

MATERIALS AND METHODS

A retrospective analysis of the treatment of 139 patients afflicted with choledocholithiasis, who underwent one-stage laparoscopic CBD exploration with subsequent gallstone extraction and cholecystectomy in the period from 2018 to 2024, was conducted. The mean age of patients was (mean [M] and the standard error of mean [m]) 59,5±1,7 years (17 to 86 years), among them men – 56,9±1,7 years, women – 62,1±1,7 years. In 29 (20,8%) patients, CBD exploration and lithoextraction were performed through the cystic duct, and in the remaining 110 (79,1%) – through the CBD incision. Operative intervention was performed using an Olympus CHF-V choledochofibroscope with a diameter of 5 mm and a Karl Storz with a diameter of 3 mm; lithoextraction was performed through choledochotomy access using Dormia-type baskets (FG-55D, FG-55D Olympus).

Complicated choledocholithiasis was documented in 73 patients (52,5%), namely, such were considered those cases, where there were specific factors that made it difficult to remove calculi of the CBD by the laparoscopic method, specifically, the characteristics of the calculi, their localization, number and size, as well as the presence of altered anatomy and preoperative cholangitis. Large stones (>15 mm) were observed in 18 patients (12,9%), multiple choledocholithiasis (>3 calculi, size >10 mm) was noted in 31 patients (22,3%), an atypical form of the concrement (barrel-shaped) was detected in 2 patients (1,4%). Calculi in the intrahepatic ducts were detected in 2 patients (1,4%). Mirizzi syndrome was noted in 8 patients (5,8%). Stenosis of the esophagus, stomach or duodenum was not detected. Four patients (2,8%) had previously undergone gastrectomy or Billroth II gastric resection. Parapapillary diverticulum was detected in 8 patients (6,2%). Cholangitis in the preoperative periods was detected in 63 patients (45,3%). Vitreous edema of the duodenum was not detected. Various terminal conditions were not documented, significant coagulation disorders were not detected.

All procedures involving human subjects were conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants, with clear explanations provided regarding the nature, purpose, potential risks, and benefits of the study. Data confidentiality and participant privacy were rigorously maintained throughout the study. All personal information was anonymized, and data were stored securely to protect participants' identities.

The data analysis was performed by the use of IBM SPSS Statistics v. 26.0 (Armonk, NY: IBM Corp., USA). To determine the independent factors, impacting on the success of one-stage laparoscopic treatment of choledocholithiasis, we used a multivariate logistic regression analysis. The strength of the association between the studied factors and the studied outcome (the absence of the procedural success) was measured by an odds ratio (OR) with 95 % confidence interval (CI). A p-value <0,05 was considered statistically significant.

RESULTS

The obtained data on the previously defined anamnestic and intraprocedural factors impacting the successful performance of one-stage laparoscopic CBD exploration and lithoextraction (accounted specifically for technical difficulties in performing the procedure, bile ducts clearance rate, presence of post-procedural complications and the need for repeated surgical interventions were taken into account) are highlighted in Table 1.

According to the multivariate logistic regression analysis, the advanced age, multiple stones and preoperative cholangitis appeared to be significant negative predictors of procedural success (Table 2). Namely, the age increased the odds of a negative outcome by 20,8% per 1-year increase (OR 1,208 [95% CI 1,124-1,298]; p<0,001), and each additional stone increased the odds by 21,5% (OR 1,215 [95% CI 1,435-3,127]; p<0,001). Preoperative cholangitis was associated with a 5-fold increase in negative outcomes (OR 5,485 [95% CI 6,397-65,607]; p<0,001). Stone size and altered anatomy were not significantly associated with the procedural success.

DISCUSSION

The abovementioned findings underscore the critical importance of identifying and managing negative predictors in the surgical management of choledocholithiasis. Advanced age, the presence of multiple stones, and preoperative cholangitis are significant factors that can markedly impact procedural outcomes.

Older patients, for instance, may face higher rates of incomplete stone clearance due to a variety of factors associated with aging. Physiological changes that accompany advanced age, such as decreased bile duct motility and compromised liver function, can affect the efficacy of surgical interventions. Additionally, older individuals often have comorbidities that further complicate surgery and healing processes. These factors necessitate a tailored approach to surgical planning and execution, ensuring that age-related challenges are addressed proactively to improve outcomes [2, 9].

Similarly, the presence of larger and multiple stones presents its own set of technical challenges during laparoscopic

Table 1. The logistic regression model characterizing the impact of anammestic and intraprocedural factors on the success of single-stage laparoscopic treatment of choledocholithiasis*

Variable	β–Coefficient	Standard error	p	OR	95% CI
Age	0,189	0,037	<0,001	1,208	1,124–1,298
Number of concrements	0,195	0,206	<0,001	1,215	1,435–3,217
Size of concrements	0,092	0,097	0,342	1,097	0,907–1,326
Preoperative cholangitis	1,702	0,594	<0,001	5,485	6,397–65,607
Altered anatomy	1,779	1,242	0,152	5,926	0,520–67,541

Note: * – the absence of procedural success as the studied outcome ($\chi 2 = 118{,}234$; $df = 5$; $p<0{,}001$).

extraction. The complexity of removing multiple or larger stones can lead to difficulties in achieving complete clearance through laparoscopic methods alone. This situation may require adjunctive procedures such as endoscopic stone extraction techniques or even conversion to open surgery if laparoscopic methods are inadequate. The decision to convert to open surgery is often based on intraoperative findings and the surgeon's judgment, aiming to ensure that all stones are effectively removed and to prevent postoperative complications [2, 11-13].

To mitigate these challenges, several strategies can be employed. Preoperative optimization is crucial and can be achieved through advanced imaging modalities. Techniques such as magnetic resonance cholangiopancreatography (MRCP) and ERCP are instrumental in assessing the stone burden and ductal anatomy with high precision [10, 14-16]. MRCP provides detailed images of the bile ducts and helps in identifying the size, location, and number of stones, as well as any anatomical anomalies. ERCP can facilitate both diagnostic and therapeutic interventions, including stone removal, thereby reducing the complexity of subsequent surgical procedures. These imaging techniques allow for better surgical planning and can improve the overall success rate of the surgery.

Intraoperative techniques also play a critical role in managing choledocholithiasis effectively. Intraoperative cholangiography (IOC) is a valuable tool that can help navigate anatomical variations and ensure complete stone clearance [17, 18]. By providing real-time images of the bile ducts during surgery, IOC allows surgeons to identify any remaining stones and verify that the bile ducts are free of obstruction. This technique enhances the accuracy of the surgery and reduces the risk of postoperative complications.

Moreover, patient counseling and informed consent are essential components of preoperative preparation. It is important to discuss with patients the potential need for staged procedures or conversion to open surgery based on intraoperative findings. By setting realistic expectations and preparing patients for the possibility of additional interventions, surgeons can enhance patient satisfaction and ensure a more informed and cooperative approach to treatment.

CONCLUSIONS

The advanced age, the presence of multiple stones and preoperative cholangitis significantly impact on the success of single-stage laparoscopic choledocholithiasis treatment, increasing the probability of unsuccessful procedure. Understanding and addressing negative predictors are paramount in optimizing the success of single-stage laparoscopic choledocholithiasis treatment. By incorporating these predictors into clinical decision-making and surgical planning, clinicians can improve procedural outcomes, minimize complications, and enhance patient care. Future research should focus on prospective validation of these predictors across diverse patient populations and explore novel techniques or technologies to further refine the management of choledocholithiasis. Finally, an integrating these insights into clinical practice will lead to better outcomes and quality of care for patients undergoing one-stage laparoscopic treatment for choledocholithiasis.

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The study was performed as a fragment of the complex scientific projects of the Department of Surgery with a Course of Emergency and Vascular Surgery (Bogomolets National Medical University) «Combined minimally invasive technologies in the treatment of obstructive diseases of hepatopancreatobiliary zone» (state registration number 0121U108878; term: 2022-2023).

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Roman O. Havryliuk
Bogomolets National Medical University
39/1 Shovkovychna St., 01601 Kyiv, Ukraine
e-mail: romangavryliuk2@gmail.com

ORCID AND CONTRIBUTIONSHIP

Mykhailo V. Maksymenko: 0000-0003-2507-1238 A B E F
Roman O. Havryliuk: 0009-0009-8641-5787 C D

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

RECEIVED: 02.07.2024
ACCEPTED: 20.10.2024

ORIGINAL ARTICLE

CONTENTS

Assessment of society's readiness for the legalization of euthanasia, carried out in the field of palliative medicine

Valentyna G. Nesterenko[1], Olha Yu. Kosilova[2], Iryna V. Redka[3], Roman O. Sukhonosov[1], Oleksandr O. Shevtsov[1], Yana V. Zhuravel[1]

[1]KHARKIV NATIONAL MEDICAL UNIVERSITY, KHARKIV, UKRAINE

[2]STATE INSTITUTION "I. MECHNIKOV INSTITUTE OF MICROBIOLOGY AND IMMUNOLOGY OF THE NATIONAL ACADEMY OF MEDICAL SCIENCES OF UKRAINE", KHARKIV, UKRAINE

[3]V.N. KARAZIN KHARKIV NATIONAL UNIVERSITY, KHARKIV, UKRAINE

ABSTRACT

Aim: To determine the signs of society's readiness to legalize euthanasia for palliative patients by interviewing both the patients themselves and their relatives (caregivers) and specialists who provide medical, psychological, social and spiritual services at the end-of-life.

Materials and Methods: The study was carried out using a sociological method among palliative patients of three regions of Ukraine (Lviv, Kyiv and Kharkiv) on a random sample of 377 people surveyed during 2022–2024. The conducted research confirmed and clarified the previous results with greater reliability.

Results: Among the interviewed palliative patients, 60,0% complained of unbearable chronic pain; 69.4% thought about suicide at least once or tried to commit it. At the same time, only 40.3% thought about the possibility of euthanasia (or looked for ways to perform it) (1,7 times less). The prevalence of opinions about the possibility of euthanasia was higher among relatives (caregivers) of palliative patients (47,4%), and lower among those who provide services (30,1%). Among all respondents, suicide was discussed more often than euthanasia 1,5 times more often. And a broad social dialogue on the legalization of euthanasia is considered possible only by a little more than half of the respondents.

Conclusions: Ukrainian society is not ready for a broad dialogue about the need to legalize euthanasia, which actually means the continuation of the suffering of a significant number of palliative patients who do not receive adequate pain relief and most do not have the financial opportunity to use euthanasia tourism.

KEY WORDS: palliative and hospice care, chronic pain, suicide, questionnaires

Wiad Lek. 2024;77(11):2208-2214. doi: 10.36740/WLek/197096 DOI

INTRODUCTION

The organization of palliative and hospice care in the country and integration of this system into the general health care system, reflect the country's ability to care for vulnerable segments of the population, to be a "social" state, to fulfill the declarative promises of its Constitution and other laws. Within the limits of the right to life, states independently decide what freedoms should be granted to their citizens regarding the disposition of their life, health, and death. The most controversial in all societies are the rights to abortion and euthanasia.

"Euthanasia" (from Greek εu – good, θάνατος – death) as an easy painless death, without torment and suffering is an alternative to dying with unbearable chronic pain caused by an incurable disease in countries such as Sweden, the Netherlands, Belgium, Switzerland, Finland, Germany, Spain, Luxembourg, Chile, Canada, 20 US states. Perception or rejection of ideas about the possibility of legalizing euthanasia in these countries took place with the participation of terminally ill patients, their relatives (family members, guardians, caregivers), people who, in their official duties or volunteer (public) activities, often come into contact with patients who die in pain and ask to take their lives. But even doctors, who are more often faced with difficult patients and their pleas for relief from suffering, are often not ready to perform euthanasia due to religious beliefs and ethical guidelines of medical universities [1]. The vast majority of low-income countries are unable to provide palliative care for patients at a sufficient level, so euthanasia can be offered as an alternative to dying in pain. Refusing to consent to such a voluntary medical procedure should not violate the right of palliative patients to die with dignity in their own country.

The most important argument for speeding up the euthanasia legalization is always the insufficient

Table 1. Distribution by age, gender and religiosity of patients interviewed at the second stage of the study, their caregivers and persons providing services to patients

Questionnaire questions and answers		Interviewees' categories						Total in three categories	
		patients		caregivers		services' providers			
		abs.	%	abs.	%	abs.	%	abs.	%
Sex	male	65	48,5	21	21,7	58	39,7	144	38,2
	female	69	51,5	76	78,3	88	60,3	233	61,8
	total	134	100,0	97*	100,0	146*	100,0	377	100,0
Age	adults	103*	76,9	-	-	-	-	-	-
	children	31	23,1	-	-	-	-	-	-
Religious	yes	35	33,9	37	38,1	82	56,2	154	44,5
	no	68	66,1	60	61,9	64	43,8	192	55,5
	total	103*	100,0*	-	-	-	-	346	100,0

Note: * – The answer, whether the respondent is a believer, was calculated only for adults.

Table 2. Distribution of surveyed patients (adults and children) by main palliative diagnosis

Category of diseases	Diseases of adults (with ICD-10 codes)	Diseases of children (with ICD-10 codes)	Number, persons (%)
- oncological diseases	malignant neoplasms (C00–C97, D00–D48)		35 (26,1)
- neurological diseases	dementia (F00–F03); epilepsy (G40–G47); multiple sclerosis (G35)	severe perinatal conditions (Q00–Q99); infantile cerebral palsy (G80); mental retardation (severe and profound) (F72–F79); Inflammatory diseases of the central nervous system (G00, G03, G04, G06, G08, G09);	57 (42,5)
- cardiovascular diseases	(I00–I99)		9 (6,7)
- infectious diseases	tuberculosis (A15–A19); HIV/AIDS (B20–B24)		11 (8,2)
- endocrine diseases	diabetes (E10–E14)		2 (1,5)
- arthropathy	rheumatoid arthritis (M05–M06)	-	2 (1,5)
- kidney disease	(N00–N15, N20–N23)	-	1 (0,8)
- liver disease	liver fibrosis and cirrhosis (K74)	chronic hepatitis (K73, K75.2, K75.3)	3 (2,2)
- lung disease	COPD (J43–J47)	-	1 (0,8)
- congenital malformations	-	(Q00–Q99)	7 (5,2)
- orphan diseases	-	phenylketonuria (E70.0); cystic fibrosis (E84); mucopolysaccharidoses (E76)	6 (4,5)
Total, persons (%)	103 (76,9)	31 (23,1)	134 (100,0)

Notes: CNS – central nervous system;
COPD – chronic obstructive pulmonary disease;
HIV/AIDS – human immunodeficiency virus/acquired immunodeficiency syndrome;
ICD-10 – International Classification of Diseases of the 10th revision [7].

analgesia of severe patients, which is one of the main needs of palliative patients [2]. The quality of pain relief for the majority of such patients has a strong influence on the ranking of countries according to the Quality of Death Index. In this ranking, most of the countries with the best quality of death are countries with a high level of income. But there is also an exception to this rule: in the "A" list, which includes the best countries, there is Uganda (31st place), which belongs to low-income countries. And, on the contrary, the Czech Republic (66th place) and Portugal (75th place) with high levels of profit are classified as the "worst" countries [3].

The integral assessment of the Quality of Death Index takes into account 20 indicators, combined into 5 cat-

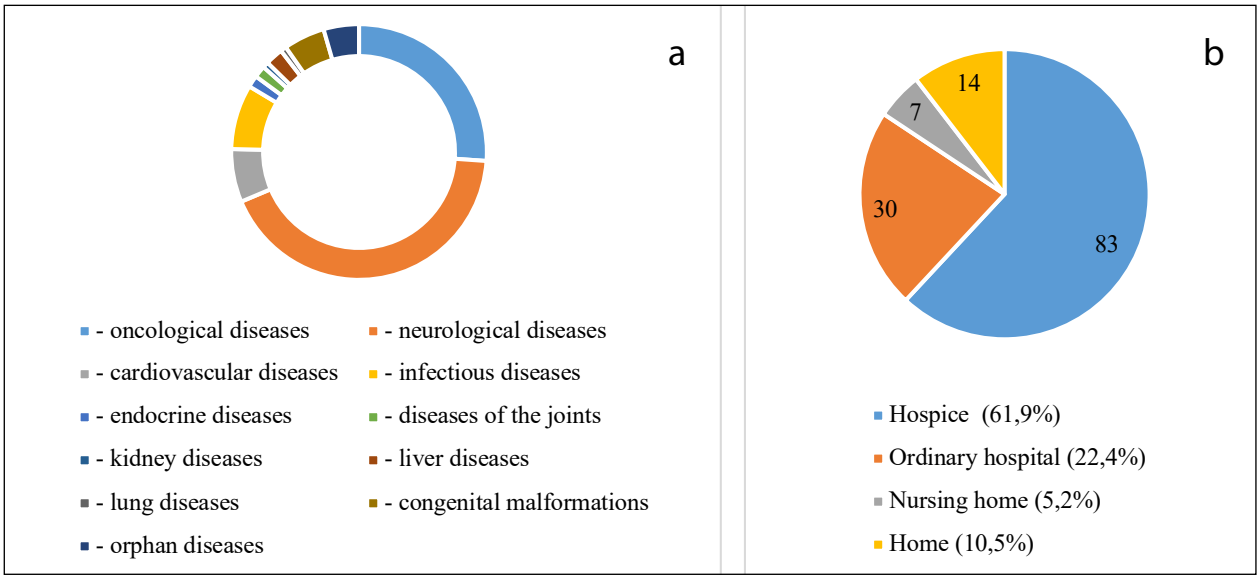

Fig. 1. Distribution of interviewed palliative patients: a) by diagnosis; b) at the place of treatment.

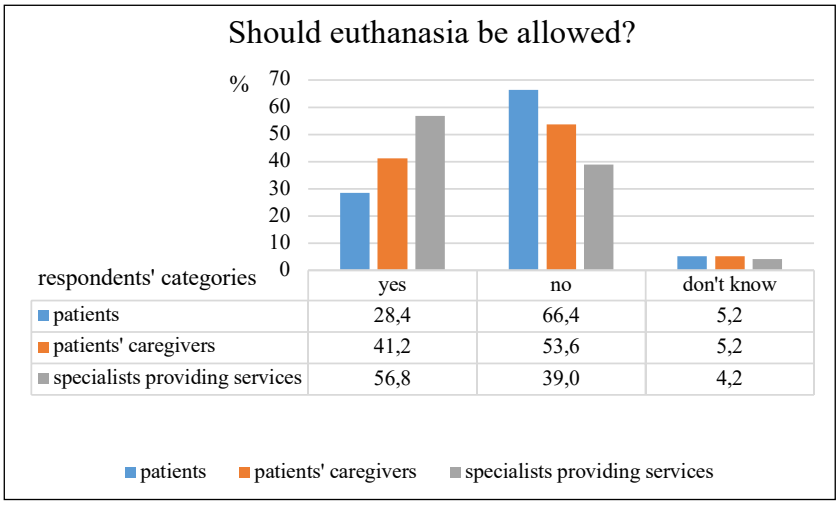

Fig. 2. The result of patients' survey, their caregivers and services' providers, at the second stage of the study regarding the need to legalize euthanasia.

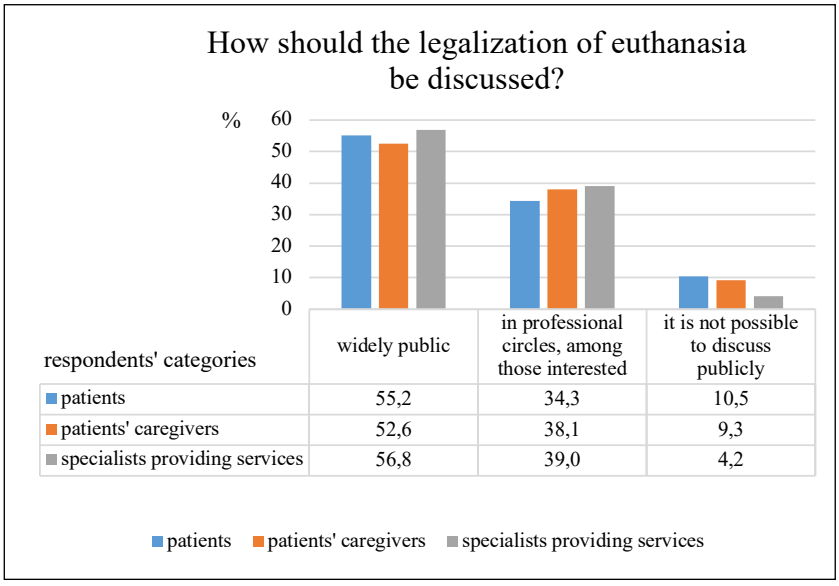

Fig. 3. The result of patients' survey, their caregivers and services' providers, at the second stage of the study regarding the possible format of the public debate on the euthanasia legalization.

Table 3. Findings from a survey of patients, caregivers and patient care providers in the second phase of a study on opinions about end-of-life patients' suffering by suicide or euthanasia

Questionnaire questions and answers		Interviewees' categories						Total in three categories	
		patients		caregivers		services' providers			
		abs.	%	abs.	%	abs.	%	abs.	%
The patient's thoughts about suicide / suicide attempt	yes	93	69,4	34	35,1	88	60,3	215	57,0
	no	20	14,9	63	64,9	58	39,7	141	37,4
	not answer	21	15,7	-	-	-	-	21	5,6
Total		134	100,0	97	100,0	146	100,0	377	100,0
The patient's thoughts about the euthanasia / search for the possibility	yes	54	40,3	46	47,4	44	30,1	144	38,2
	no	66	49,3	51	52,6	102	69,9	219	58,1
	not answer	14	10,4	-	-	-	-	14	3,7

egories. Among the categories, the quality of medical care has the greatest qualitative weight (30%). Three categories (human resources of the system of medical care and social provision; accessibility of medical care for the population; and direct adequacy to the needs of the organization of palliative and hospice care) have an impact on the final assessment in the amount of 20% each. Another 10% depend on community involvement [4]. The last fact shows the insignificant influence of public opinion on palliative medicine as a whole. Therefore, in order to determine the readiness of society to discuss the need to legalize euthanasia, it is necessary to study the opinion of patients, their relatives, and persons who provide services (treatment and support) to such palliative patients and their relatives.

AIM

To determine the signs of society's readiness to legalize euthanasia for palliative patients by interviewing both the patients themselves and their relatives (caregivers) and specialists who provide medical, psychological, social and spiritual services at the end-of-life.

MATERIALS AND METHODS

The research was conducted in 2022–2024 in hospices and palliative care units in three regions of Ukraine, namely Kharkiv (east of the country), Kyiv (center of the country) and Lviv (west of the country) using a sociological method: using a specially developed anonymous questionnaire [5]. 589 people were interviewed, from which 377 were randomly selected (the critical value of the standard normal distribution for the 95% confidence level was approximately 1,96). The responses of 377 adults were taken into account, 31 of whom responded on behalf of their minor child with a palliative diagnosis (parents or guardians, each adult on behalf of one child). The other 346 responses counted were on their own behalf. The respondents were divided into three groups (134 palliative patients, including 103 adults and 31 children, whose answers were obtained through parents; 97 caregivers of palliative patients; 146 specialists who provide medical, psychological, social and spiritual services to palliative patients at the end-of-life, and namely doctors, nurses, social workers, priests, volunteers), which is shown in Table 1.

At the previous stage of the research (2022–2023) [6], 284 people were interviewed, of whom 100 respondents were selected by customization with quota subgroups (10 subgroups of 10 people each). The survey result had a reliability level of $p<0,05$ for the entire group (100 people), but $p>0,05$ for each of the subgroups, which became one of the reasons for continuing the survey and increasing the sample. Separate data from the first and second stages of the study were compared.

At the second, current stage, the groups were enlarged (3 instead of 10). The data obtained from the respondents in the first stage in the city of Vinnytsia were not taken into account in the second stage. The answers of 59.0% of the respondents, included in the groups of the first stage of the study, were also taken into account when calculating the results of the second stage. The study of this stage covered an additional period (2024). Generalization of the diagnoses of palliative patients was carried out according to the same principles in the first and second stages of the study. The diagnoses of the second stage of the study are shown in Table 2 and Fig. 1.a, and the distribution of patients by place of treatment is shown in Fig. 1.b.

The questionnaire data were automatically transferred to the associated Google Forms file. Sample customization and randomization were performed using the "Randomizer & Calculator" application ver.

1.3.0/2023 (Antika Inc., Turkey). Randomized results were reported to Excel 2019 (Microsoft, USA) for final statistical processing. The result of calculations expressed as a percentage was rounded to the nearest tenth.

RESULTS

The most important result of the study is the ratio of answers to questions about thoughts or suicide attempts, thoughts or search for a way of euthanasia, the opinion about the need to legalize euthanasia and the feeling of constant unbearable pain caused by a palliative disease (for patients and their relatives who state complaints of constant pain), which presented in Table 3. Refusal to answer questions about suicidal thoughts or attempted suicide, as well as about possible euthanasia or finding a way to perform it, was accepted only from palliative patients. Their loved ones (caregivers) and persons providing services (treatment and support) to palliative patients were asked to answer these questions with a yes or no. The results of the survey of all categories of respondents about the need for legalization of euthanasia and the possible format of the discussion about such legalization are shown in Fig. 2 and Fig. 3. 75 out of 134 (60,0%) surveyed patients complained of unbearable chronic pain.

Attention is drawn to the greater prevalence of opinions about the possibility of euthanasia among relatives (guardians) of palliative patients than among the patients themselves. At the same time, palliative patients report their thoughts about the suicide of their loved ones only in about half of the cases.

DISCUSSION

It should be noted that our previous studies established the need to expand the list of palliative diseases for adults and children [8], in accordance with the WHO recommendation and the development of the Ukrainian Center for Public Data (2018). An expanded list of diseases will allow not only to treat more patients with serious diseases at the end of their lives as palliative, with appropriate state funding, but also bring palliative patients closer to the possibility of euthanasia instead of suicidal thoughts or attempts.

Today, chronic excruciating pain unfortunately prompts palliative patients more often to thoughts of suicide than to thoughts of euthanasia. The distribution of the interviewees into 3 approximately equal groups by region of the country, gender (48,5% of male to 51,5% of female) allows us to claim that the place of residence and gender had no influence on the average result. An important fact established by the study is that 60% of palliative patients experienced chronic unbearable pain, 69,4% thought about suicide at least once in their life, but only 40,3% of patients thought about performing euthanasia (or sought such an opportunity) (1,7 times less than suicidal thoughts). In our opinion, this indicates a misunderstanding of the procedure by a large part of patients. At the same time, the procedure of suicide tourism is not available for the majority of palliative patients of Ukraine, considering the high cost of euthanasia in Switzerland, which is the closest country to Ukraine, where you can get a similar service. Similarly, the proximity of Mexico, which has legalized euthanasia, is an option for US residents [9].

Seeking medically assisted end of life results in significantly less stigma than suicide attempts, as indicated by Eilers J.J. & Kasten E. (2022) [10]. Carrying out a legal, state-controlled euthanasia procedure entails less risk of lengthy criminal investigations for the loved ones of the deceased. In addition, the development of the euthanasia system, providing patients with an alternative to a dignified end of life in conditions of ineffective treatment, and especially analgesia, increases the assessment of the development of the entire system of palliative and hospice care [11–15].

CONCLUSIONS

The creation of a civilized system of euthanasia for the hopelessly ill with chronic excruciating pain that cannot be treated is an opportunity to realize the right of every person to a dignified death. The existence of such a right for everyone does not deprive believers of the right to refuse not to resort to euthanasia. Ukrainian society is not ready for a broad dialogue about the need to legalize euthanasia, which actually means the continuation of the suffering of a significant number of palliative patients who do not receive adequate pain relief, and most of them do not have the financial opportunity to use euthanasia tourism. The fact that severe palliative patients are more likely to think about suicide than about euthanasia indicates a lack of quality information about the procedure of medically assisted end of life.

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Due to the anonymous nature of the questionnaire, informed consent to participate in the study was not obtained. The general design of the study was approved by the Bioethics Commission of the Kharkiv National Medical University in 2022 (Protocol No.1).

The study was carried out within the scope of the topic "Medical and social justification of the improvement of the system of palliative and hospice care in Ukraine in the context of reforming the health care system", in accordance with the subject of the research work of the Department of Public Health and Health Care Management of the Kharkiv National Medical University 0124U002696 (2024–2026).

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Valentyna G. Nesterenko
Kharkiv National Medical University
4 Nauky Avenue, 61022 Kharkiv, Ukraine
e-mail: vh.nesterenko@knmu.edu.ua

ORCID AND CONTRIBUTIONSHIP

Valentyna G. Nesterenko: 0000-0002-3773-9525 A B C D F
Olha Yu. Kosilova: 0000-0001-6558-0710 E F

Iryna V. Redka: 0000-0002-9620-9452 E F
Roman O. Sukhonosov: 0000-0002-5177-2970 E F
Oleksandr O. Shevtsov: 0000-0003-1459-9141 E F
Yana V. Zhuravel: 0000-0002-2856-5439 E F

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

RECEIVED: 17.06.2024
ACCEPTED: 28.10.2024

ORIGINAL ARTICLE

CONTENTS

Follow-up performance of patients with cutaneous melanoma: recommendations for an effective strategy

Olena O. Oshyvalova[1], Serhii M. Mazurenko[1], Ivan G. Voinov[1,2]

[1]STATE INSTITUTION OF SCIENCE «CENTER OF INNOVATIVE HEALTHCARE TECHNOLOGIES» STATE ADMINISTRATIVE DEPARTMENT, KYIV, UKRAINE
[2]STATE INSTITUTION «NATIONAL SCIENTIFIC CENTER OF SURGERY AND TRANSPLANTATION NAMED AFTER O. O. SHALIMOV TO NATIONAL ACADEMY OF MEDICAL SCIENCES OF UKRAINE», KYIV, UKRAINE

ABSTRACT

Aim: To investigate the incidence of progression and median time for metastasizing, the development of recurrent localization, and to identify localizations with a high tendency to recurrence of melanoma during 10 years of patient follow-up observation.

Materials and Methods: The retrospective data of dynamic observation by dermatovenereologists of SIS «CIHT» SAD of 183 patients with skin melanoma for the period 2014 – first half of 2024.

Results: Among 183 cases of melanoma, 23 cases (12,6%) were detected in stage III and 11 cases (6,0%) in stage IV, so the neglect rate was 18,6%. Melanoma progression was detected in 39 patients (21,4%) after an average of (mean ±m) 5,3±1,8 years of follow-up, including 9 patients (23,1%) with stages I-II melanoma and all patients (30 people – 76,9%) with neglected cases (stages III-IV). In our study, the predominant localization of primary melanoma on the skin of the lower limbs (52,9%) and trunk (29,3%) resulted in further progression of the disease in the first 5-6 years of patients' follow-up.

Conclusions: Our study has supplemented the scientific data on the timing of follow-up of patients with skin melanoma and possible progression factors (tumour thickness, lymph node involvement, localization of the primary tumour), which should be taken into account in the dynamic monitoring of patients in order to detect disease progression timely.

KEY WORDS: skin melanoma, factors for disease progression, dynamic monitoring of patients

Wiad Lek. 2024;77(11):2215-2224. doi: 10.36740/WLek/197099 DOI

INTRODUCTION

The incidence of melanoma continues to increase worldwide, with the highest burden of melanoma occurring in Australian, North American and European populations [1]. The prognosis of primary cutaneous melanoma is largely dependent on the characteristics of the primary tumour, with a one-year survival rate of 98% for thin (<0,8 mm), non-ulcerative primary melanomas and 75% for thick (>4 mm), ulcerative tumours [2].

To make a decision about additional diagnostics and specific anti-cancer treatment, it is necessary to accurately classify the patient's melanoma stage at the time of diagnosis. The guidelines of The American Joint Committee on Cancer (AJCC) for the staging of melanoma are regularly updated based on new evidence, and in the 8th edition (AJCC 8) mitosis was removed as a criterion for staging, the thickness size for thin invasive melanomas was reduced to 0,8 mm, and the measurement of lesion thickness was rounded to the nearest 0,1 mm for all tumour categories [2, 3].

It is known from scientific sources that there is no threshold for the thickness of a primary melanoma tumour at which the process of metastasis cannot be realised. Since the proportion of thin melanomas is very high, today more patients die from T1 tumours than from T4 tumours in absolute numbers [5].

Survival estimates for melanomas in the T2 to T4 tumour category are now based on a cohort of patients with a negative sentinel lymph node (SLN) biopsy. The spread of the disease to the lymph nodes, lungs and central nervous system is now differentiated from 'all other internal organs' to provide better prognostic information for patients and clinicians [3].

Surgical intervention remains the main treatment for local recurrences of melanoma. In patients who are examined at regular time intervals, local recurrences can be detected at a stage at which they can be treated by surgical resection [4].

However, there are differences among clinical guidelines regarding the standard for clear margins of

resection for primary melanoma. Recommendations vary around the world, with some countries, such as the United States and Australia, suggesting 5-10 mm, and others, such as South Africa, suggesting 18 mm for the treatment of malignant lentigo [4].

Factors predisposing to recurrence in patients with T1 or T2 melanoma include positive SLNs, ulceration, thickness, and stage of primary melanoma [5].

In the study by L. von Schuckmann et al. [6], in a cohort of patients with high-risk primary melanoma who received wide excision of the primary tumour with or without SLN, the 2-year survival rate was 95% for T1b tumours and 67% for T4b tumours. Patients who did not undergo SLN had a significantly lower 2-year survival rate compared to patients with melanoma of the same tumour category and a negative-SLN. Patients who did not undergo SLN had a significantly lower 2-year survival rate compared to patients with melanoma of the same tumour category and negative-SLN.

The presence of ulceration significantly decreased survival at all tumour thicknesses. Other factors associated with a worse 2-year prognosis included mitotic rate greater than 3 per 1 mm^2 and the location of the primary tumour on the scalp or neck [6].

Most of the first recurrences occurred in the area of the primary tumour (70,2%). In 57,8% of patients who underwent surgical resection of locoregional recurrence, they remained disease-free for another 2 years, while 31,3% of patients without surgical resection of locoregional recurrence developed further recurrence in a distant location [6].

Usually, a recurrence of melanoma is detected by the patient in 75% of cases. However, in addition to looking for suspicious skin lesions, patients should be aware of other signs that may indicate recurrence: dense subcutaneous nodes, enlarged lymph nodes, or persistent and unexplained systemic signs.

It is known from scientific sources that approximately 5-10% of patients develop a second invasive melanoma, and more than 20% develop a new melanoma in situ some time after the initial diagnosis [7].

Clinical follow-up is important not only to ensure timely diagnosis of tumour recurrence or detection of a new tumour localization, detection of regional lymph node involvement, distant metastasis, but also to provide ongoing patient education, reassurance and psychosocial support [8].

The frequency and duration of clinical follow-up is usually based on the stage of the disease at the time of diagnosis, but should be tailored to the individual needs of the patient, for example, due to the presence of multiple melanocytic or atypical nevi [3].

There is limited evidence on the ideal follow-up schedule for patients with melanoma. However, it is recommended that the frequency and duration of clinical examination should be based on their stage [8]:

T1a stage – annually for at least ten years after the initial diagnosis;

T1b-T2a stages – every six months for two years, then annually for at least ten years after the initial diagnosis;

T2b-T2c and T3a-N3d stages – every four months for two years, every six months for the third year, and then annually until at least ten years after the initial diagnosis;

T4 stage – according to the recommendations for stage III with additional visits as needed [3].

While there are no universal recommendations for follow-up monitoring for primary melanoma survivors, the general consensus is that regular skin and lymph node examinations are necessary for the early detection of disease recurrence or subsequent new primary tumours [3, 9, 10].

With the implementation of targeted immune therapies for the treatment of metastatic melanoma, including possible adjuvant therapy, a detailed understanding of the risk of melanoma recurrence can help clinicians to consult patients with primary tumours with a high risk of metastatic disease [9, 10].

AIM

The aim of our study was to investigate the frequency of progression and development of cutaneous melanoma recurrence, the average time to metastasis and target organ, and to identify localizations with a high propensity for melanoma recurrence within 10 years after the diagnosis of the primary tumour, using data from a retrospective cohort of patients who were observed at a particular healthcare facility.

MATERIALS AND METHODS

The study was conducted at the State Institution of Science «Center of innovative healthcare technologies» State Administrative Department (hereinafter – SIS «CIHT» SAD) and is a fragment of the research work «Medical and social substantiation, development and implementation of a modern model of a system for continuous improvement of the quality of integrated medical care in the work of a multidisciplinary healthcare institution» (state registration number 0122U000232). The retrospective data of dynamic observation by dermatovenereologists of SIS «CIHT» SAD of 183 patients with skin melanoma for the period 2014 – first half of 2024, data on the morphological characteristics of the primary tumour and foci of progression were used. In

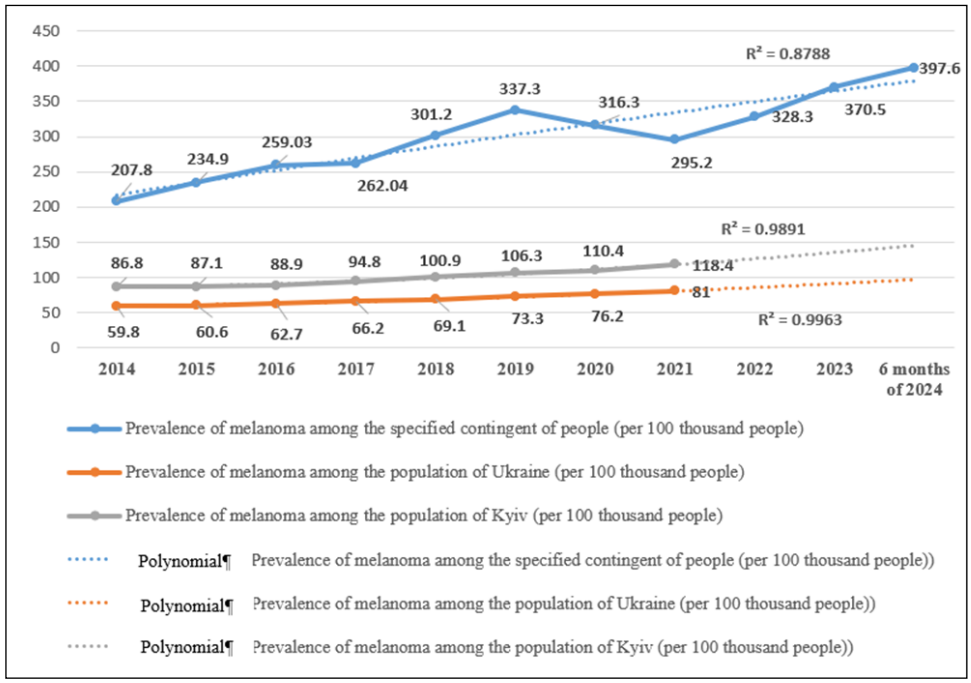

Fig. 1. Prevalence rate of melanoma among the specified contingent of people (per 100 thousand people) for the period 2014 – first half of 2024 compared to the prevalence rate of melanoma among the population of Ukraine (per 100 thousand people) and the population of Kyiv (per 100 thousand people) for the period 2014-2021.

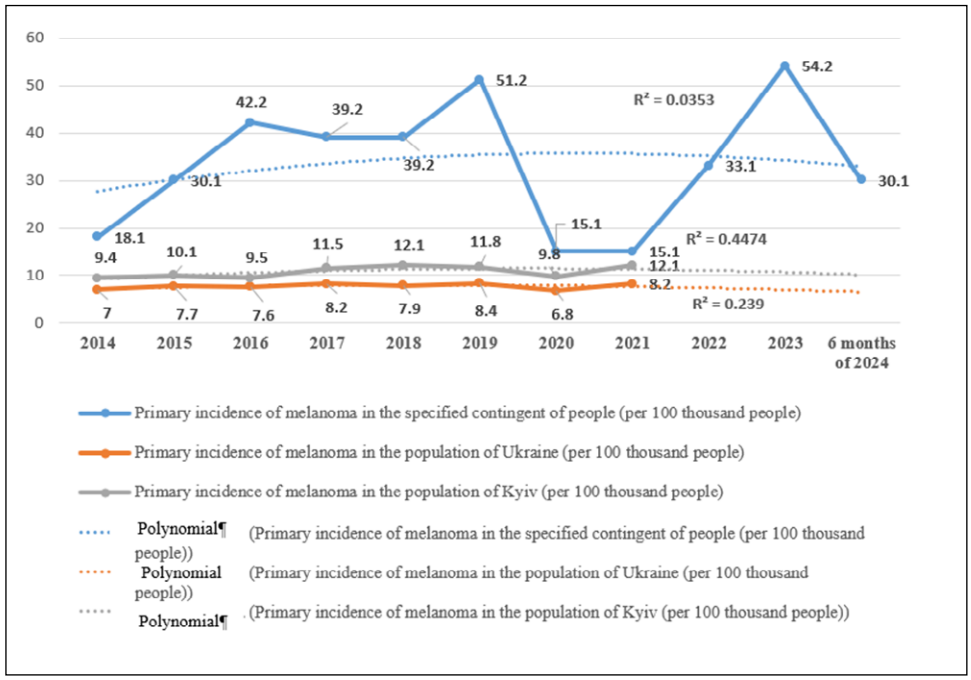

Fig. 2. Primary melanoma incidence rate among the specified contingent of people (per 100 thousand people) for the period 2014 – first half of 2024 compared to the primary melanoma incidence rate among the population of Ukraine (per 100 thousand people) and the population of Kyiv (per 100 thousand people) for the period 2014-2021.

the course of the study, bibliosemantic, general clinical and instrumental, laboratory diagnostic and medical-statistical research methods were used, including Mean – arithmetical average; m – standard deviation error (hereinafter: mean ± m), the coefficient of determination (hereinafter: [R^2]). This research followed the regulations of the World Medical Association Declaration of Helsinki and ethical principles for medical research involving human subjects.

The statistical software Excel and IBM SPSS Statistics v. 23 were chosen for calculations. The package of standard functions contains all necessary database analysis for calculations.

RESULTS

There were 183 patients with skin melanoma under dynamic observation, the average age of which was (hereinafter: mean ± m) 63,9±2,1 years, including 103 (56,3%) men aged 64,1±1,8 years and 80 (43,7%) women aged 62,8±2,3 years. Only 23 % (42 people) were of the working age, with women making up the majority at 13,7% (25 people).

The level of melanoma prevalence among the specified contingent of people for the period 2014-2021 exceeded the level of melanoma prevalence among the population of Ukraine and the population of Kyiv (in 2014 – 2,4 and 3,5 times, respectively; in 2021 – 2,5 and

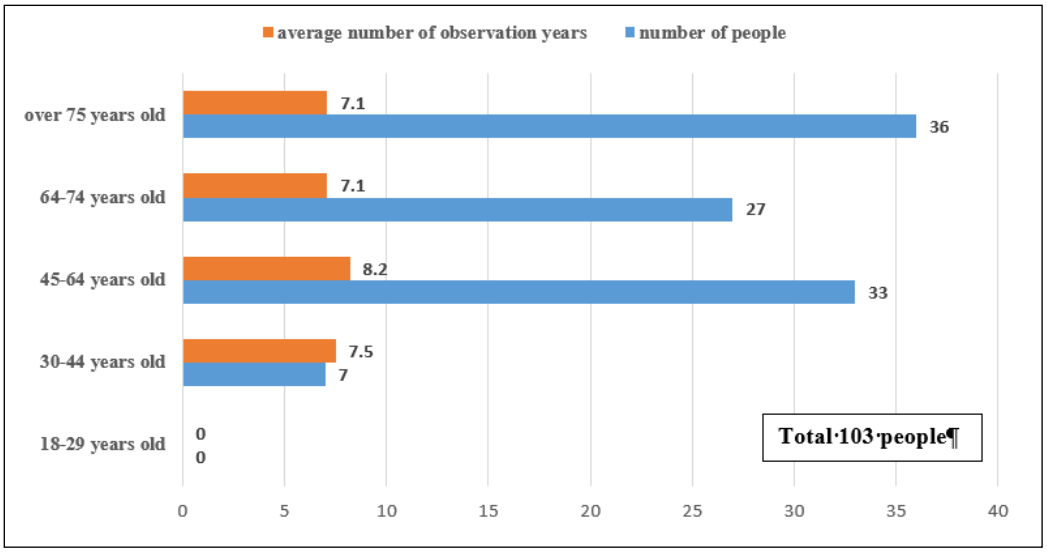

Fig. 3. Duration of observation of male melanoma patients among the specified contingent of people for the period 2014 – first half of 2024.

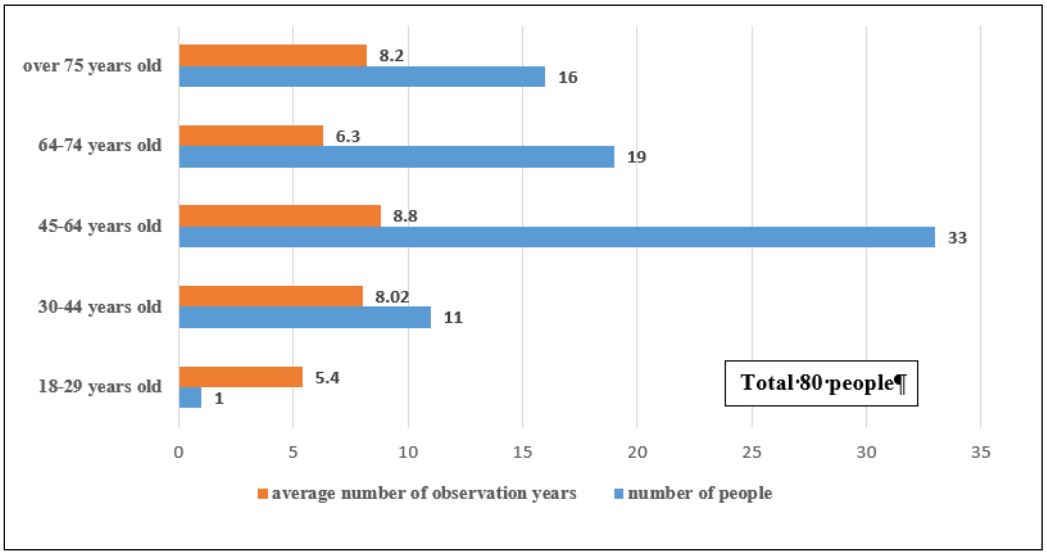

Fig. 4. Duration of observation of women with melanoma among the specified contingent of people for the period 2014 – first half of 2024.

3,6 times, respectively) and had a significant upward trend (the coefficient of determination [R^2] = 0,8788), as well as the prevalence level of melanoma among the population of Ukraine (R^2 = 0,9963) and Kyiv (R^2 = 0,9891) (Fig. 1).

For the period 2014 – first half of 2024, no significant increase in primary melanoma incidence among the specified population was observed (R^2 = 0,0353), which corresponds to the general trend among the population of Ukraine (R^2 = 0,239) and the population of Kyiv (R^2 = 0,4474) (Fig. 2). At the same time, the level of primary incidence of melanoma among the defined population was significantly higher than the corresponding indicator among the population of Ukraine and Kyiv: 2,6 and 1,9 times in 2014, respectively; 6,1 and 4,3 times in 2019, respectively (Fig. 2).

The average observation period for melanoma patients among the specified contingent of people was 8,4±1,7 years, including the average observation period for men – 8,2±1,6 years, women – 8,5±1,9 years.

The largest number of men with melanoma among the specified contingent of patients was registered at the age of 45-64 years (33 patients – 32%) and at the age of 75 years (36 patients – 35%) (Fig. 3). There were no men aged 18-29 years among our patients. The longest mean observation period was in the group of men aged 45-64 years (8,2 ± 1,1 years), the lowest in the groups aged 64-74 years (7,1 ± 1,9 years) and over 75 years (7,1 ± 0,8 years) (Fig. 3).

The largest number of women with melanoma among the specified contingent of people was also registered at the age of 45-64 years (33 people – 41,2%) (Fig. 4). Only one woman aged 18-29 years with a mean observation period of 5,4 years was registered among our patients. The longest mean observation period was also in the group of women aged 45-64 years (8,8 ± 1,2 years) (Fig. 4).

Morphological confirmation occurred in 98,9% of cases, which corresponds to the rate of confirmed melanoma cases among the population of Ukraine

Table 1. Morphological characteristics of the tumour process

Breslow thickness rate				Presence of ulceration, %	Mitotic activity, per 1 mm² (M±m)
invasion rate	average invasion rate, mm (M±m)	Number of cases			
		Abs.	%		
in situ	0	14	7,7	0	0
up to 1 mm	0,67±0,03	72	39,3	0	1,5±0,5
up to 2 mm	1,61±0,02	63	34,4	0	2,6±0,4
up to 3 mm	2,93±0,01	14	7,7	1,7	4,4±0,6
up to 4 mm	3,8±0,01	9	4,9	3,4	8,7±0,3
>4 mm	4,45±0,03	7	3,8	1,7	7,3±0,7

Table 2. Distribution of deaths from melanoma depending on the term of observation after detection of the pathology

Number of years of observation before death from melanoma	Number of patients who died from melanoma	
	Abs.	% of the total number of patients
up to 1 year	2	1,1
up to 5 years	10	5,5
up to 10 years	10	5,5
up to 15 years	4	2,2
up to 20 years	1	1,1
Total	**27**	**15,4**

Table 3. Characteristics of melanoma progression cases

Number of years of observation before metastasizing	Number of progression cases			
	in men		in women	
	Abs.	%	Abs.	%
up to 1 year	5	2,7	4	2,2
up to 5 years	7	3,8	5	2,7
up to 10 years	7	3,8	5	2,7
up to 15 years	2	1,1	2	1,1
up to 20 years	2	1,1	0	0
Total	**23**	**12,52**	**16**	**8,7**

and Kyiv (98,4% and 100%, respectively, in 2022). The morphological characteristics of the tumour process are presented in the Table 1.

Among 183 cases of melanoma, 14 cases (7,7%) were detected at stage 0; 72 cases (39,3%) at stage I; 63 cases (34,4%) at stage II; 23 cases (12,6%) at stage III; and 11 cases (6,0%) at stage IV. The highest level of mitotic activity was detected in stages III and IV – 8,7±0,3 and 7,3±0,7 per 1 mm^2. Ulceration was also observed at stages III and IV of tumour growth – 3,4% and 1,7%, respectively (Table 1).

It was detected during preventive examinations in 56,9% (106 cases) of patients with melanoma. In 183 patients with melanoma, 179 (97,8%) primary tumour localizations were detected, and in 4 cases (2,2%) the primary lesion was not detected (2 lesions in men and women).

In men, the predominant localization of the primary tumour was on the skin of the trunk (59 cases (58,4%), of which: 35 cases (34,6%) on the skin of the back, 16 cases (15,8%) on the skin of the chest, 8 cases (7,9%) on the skin of the abdomen) and on the skin of the upper limb (15 cases (14,8%), of which: 12 cases (11,8%) on the skin of the shoulder, 3 cases (3%) on the skin of the forearm) (Fig. 5).

In women, the predominant localization of the primary tumour was on the skin of the trunk (26 cases (33,3%), including: on the skin of the back – 18 cases (23,1%), chest – 5 cases (6,4%), abdomen – 3 cases [3,9%]) and on the skin of the lower limb (25 cases (32%), including: 8 cases (10,3%) on the thigh, 14 cases (17,9%) on the lower leg, and 3 cases (3,8%) on the skin of the foot (Fig. 5).

Specialized treatment was provided to 100% of primary melanoma patients, including 108 patients (59,0%)

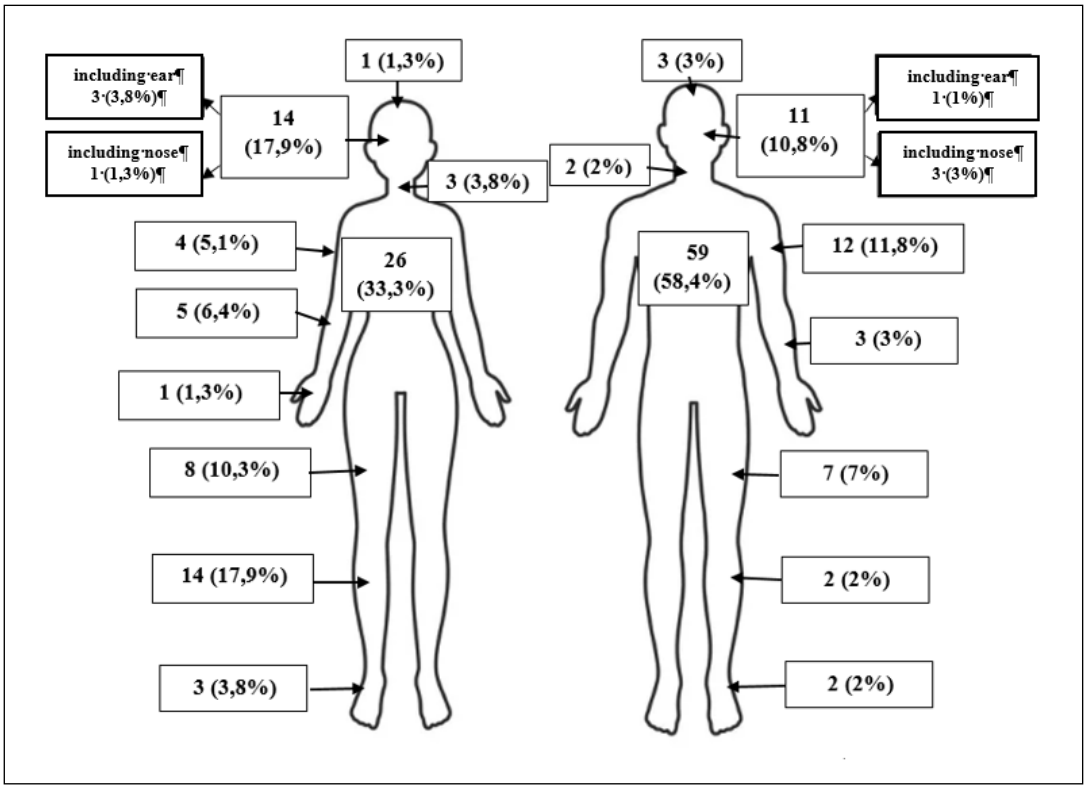

Fig. 5. Distribution of primary melanoma localization on the body skin in men and women under observation.

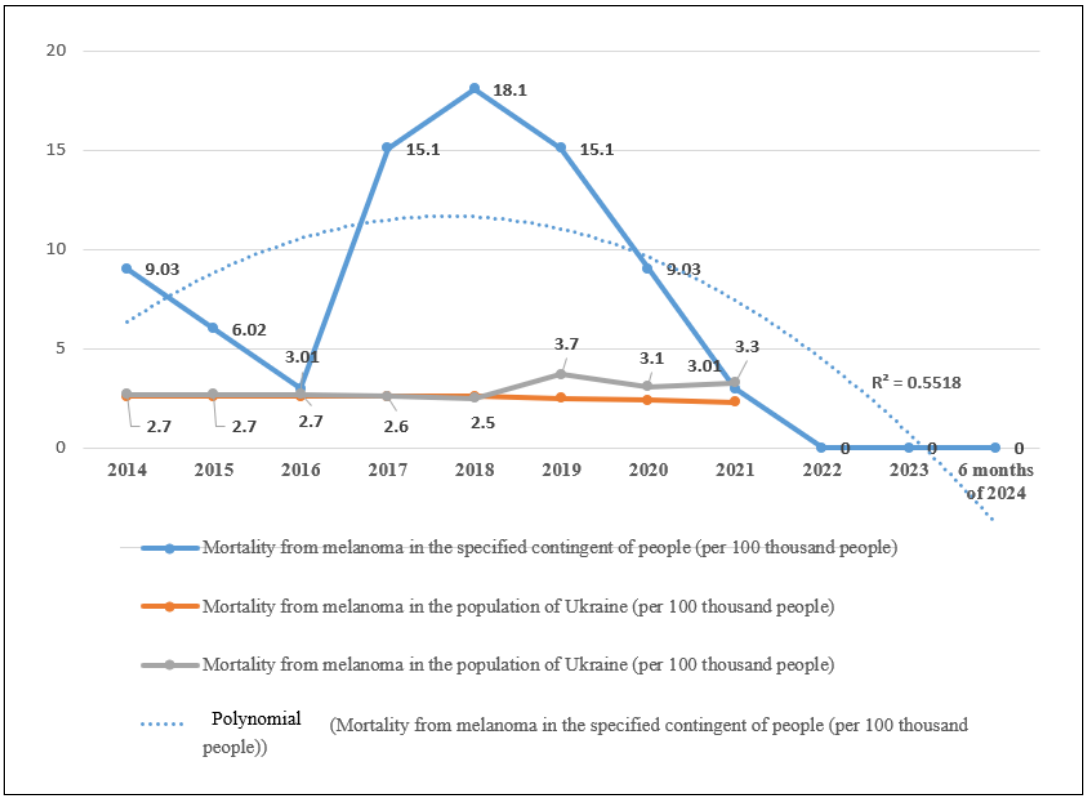

Fig. 6. Mortality rate from melanoma in the specified contingent of people (per 100 thousand people) for the period 2014 – first half of 2024 compared to the mortality rate from melanoma in the population of Ukraine (per 100 thousand people) and the population of Kyiv (per 100 thousand people) for the period 2014-2021.

who received surgical treatment only, and 75 patients (41%) who received combined and complex treatment. According to the National Cancer Registry of Ukraine, in 2021, 90,9% of primary patients were covered by special treatment, including 71,1% with surgical treatment only, and 16,2% with combined and complex treatment.

The mortality rate from melanoma of the specified contingent of people (per 100 thousand people) for the period 2014-2020 exceeds the mortality rate from melanoma of the population of Ukraine (per 100 thousand people) and the population of Kyiv (per 100 thousand people) by 3,3 times in 2014 and 2020, and by 7,2 times in 2018 (Fig. 6). Since 2019, the mortality rate from melanoma in the specified contingent of persons

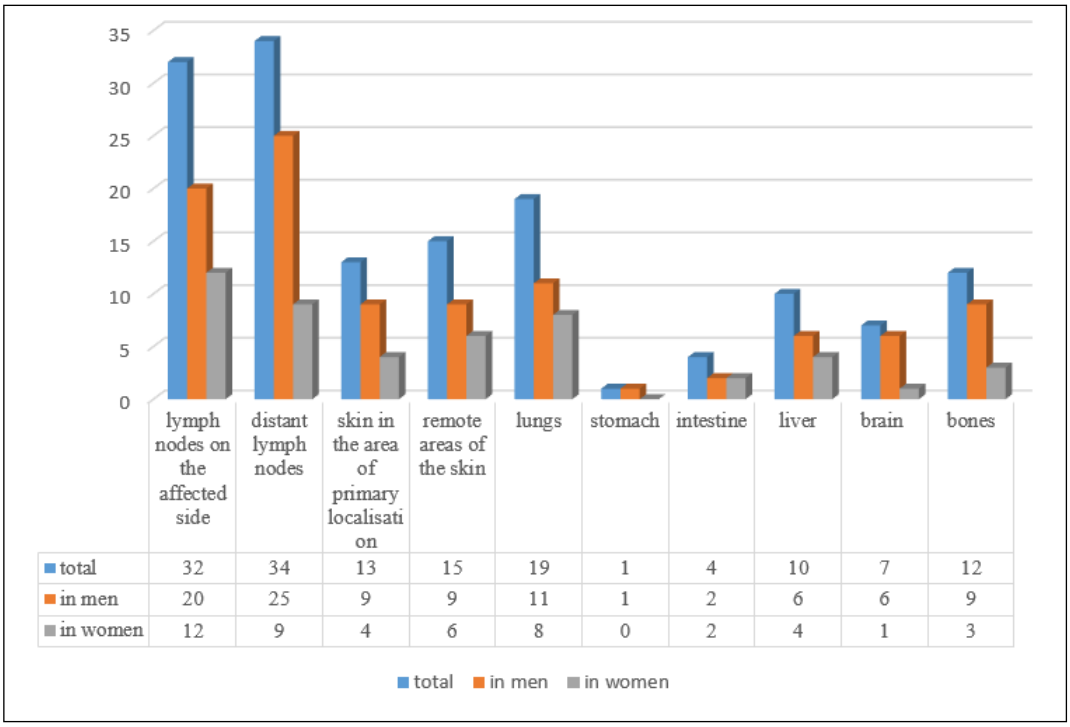

Fig. 7. Distribution of metastasis foci localization in men and women under observation.

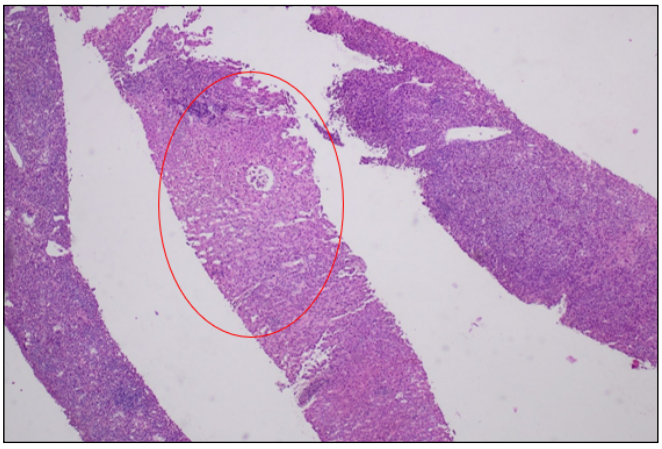

Fig. 8. Trepan biopsy material of a liver mass, hematoxylin-eosin stain. Magnification x40. The biopsy material shows liver tissue (highlighted by a red oval) with preserved histoarchitectonics and signs of fatty degeneration of hepatocytes.

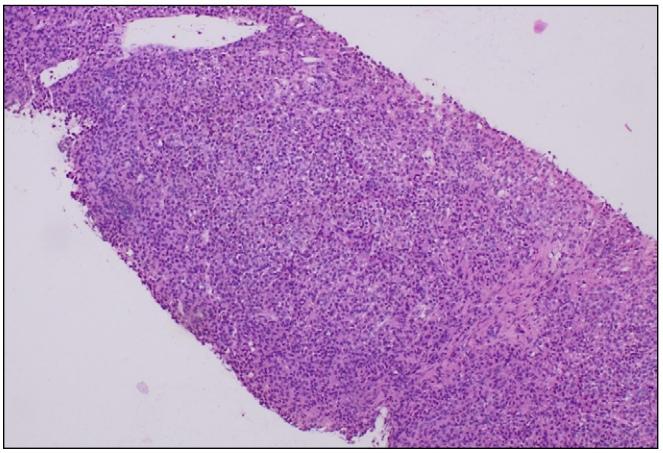

Fig. 9. Trephine biopsy material of the liver mass, hematoxylin-eosin stain. Magnification x100. Tumour tissue: the tumour consists of solid layers of atypical polymorphic epithelioid cells with an increased nuclear-cytoplasmic ratio and 1 nucleus, with foci of fibrosis, moderate mitotic activity. In some places, a small amount of brown pigment is detected.

has significantly tended to decrease ($R^2 = 0,5518$) and in 2021 decreased to the level of the corresponding indicator among the population of Ukraine and Kyiv (Fig. 6).

The distribution of deaths in patients with melanoma depending on the period of observation after the pathology was detected is presented in Table 2.

Among the patients with melanoma who continued follow-up, repeated tumour localizations were detected in 7 cases (3,8%) on average after 6,5±2,1 years of observation with localization on the skin: back and face in 2 cases (28,5% each), 1 case on the scalp, shoulder and forearm (14,3% each) (fig. 7).

We observed the progression of the tumour process in melanoma in 39 cases (21,4%) after an average of 5,3±1,8 years of observation, the average age of patients with metastatic melanoma was 64,1±2,4 years. The characteristics of metastasis cases are presented in Table 3.

The predominant number of cases of progression occurred in the first 5-10 years of follow-up in both men and women, with a predominance of men – 19 cases (10,3%) compared to 14 cases (7,6%) in women (Table 3).

Among men and women, cases of metastasizing of melanoma to distant lymphatic collectors (87,2%) and lymph nodes on the side of the lesion (82,1%), to the lungs (48,7%), skin in the area of the primary

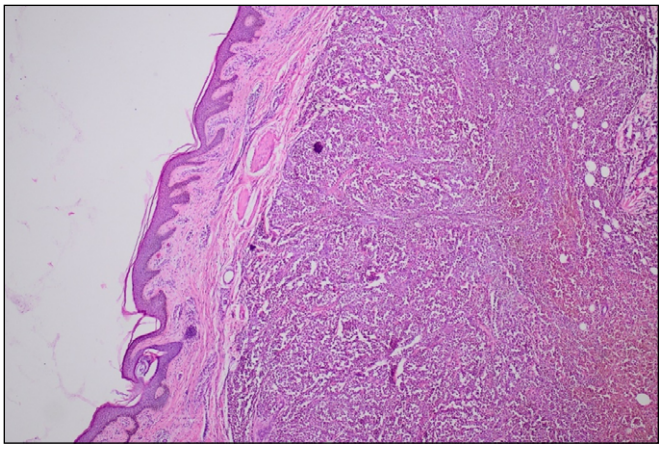

Fig. 10. Material of melanoma recurrence in the scar, haematoxylin-eosin stain. Magnification x40. Skin with fibrosis, slight lymphocytic infiltration. The dermis of the presented fragment shows tumour growth that is not associated with the epidermis. The tumour consists of atypical epithelioid cells with an increased nuclear-cytoplasmic ratio, focally brown pigment is present.

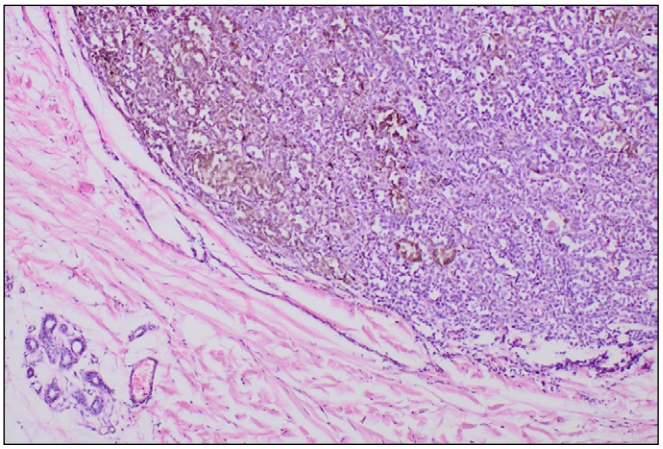

Fig. 11. Material of melanoma metastasis to the skin, hematoxylin-eosin stain. Magnification x100. Tumour growth with the presence of brown pigment is detected in the dermis. The tumour consists of atypical epithelioid cells with an increased nuclear-cytoplasmic ratio and moderate mitotic activity.

tumour (33,3%) and distant skin areas (38,5%), to the liver (25,6%) and bone system (30,8%) predominated.

All cases of tumour progression were diagnosed using ultrasound diagnostics, computed tomography, magnetic resonance imaging of tissues and confirmed morphologically.

For morphological studies was used biopsy material obtained during various types of surgical interventions: excisional biopsy, punch biopsy, trephine biopsy.

Figures 8 and 9 (the photos of the biopsy material) show the morphological picture corresponding to the metastasis of epithelioid cell melanoma to the liver tissue.

Figure 10 shows a morphological picture of melanoma recurrence in the scar after removal of the primary tumour lesion.

Figure 11 shows the metastasis of melanoma to the skin at a distance from the primary focus of the tumour process.

In an additional study of 39 cases of tumour progression, we found that there were 9 cases (23.1%) of progression in patients with stages I-II melanoma and 30 cases (76.9%) in patients with stages III-IV, which corresponds to all the neglected cases of melanoma that were under our observation.

In this group, 18 (46,2%) diagnostic biopsies of sentinel lymph nodes were performed, including 16 (41%) in patients with stage III-IV of the disease and 2 (5,2%) in patients with stage I-II of the disease. Seventeen biopsies (43,6%) were positive, 1 biopsy (2,6%) was negative (stage I of the disease).

The characteristics of primary melanoma localizations that subsequently progressed to the regional lymphatic collectors are presented in Figure 12.

DISCUSSION

The level of prevalence and primary incidence of melanoma among the specified population for the period 2014 – first half of 2024 had an upward trend, as well as among the population of Ukraine and Kyiv [11]. The highest number of melanoma patients was registered in the age group 45-64 years, both among men (33 people – 32%) and women (33 people – 41,2%), which indicates the prevalence of the disease in people of working age.

The average observation period of melanoma patients among the specified contingent of individuals was 8,4 ± 1,7 years, including in the group of men aged 45-64 years (8,2 ± 1,1 years) and in the group of women aged 45-64 years (8,8 ± 1,2 years), which ensures the representativeness of the data obtained in the study.

Among 183 cases of melanoma, 23 cases (12,6%) were detected in stage III and 11 cases (6,0%) in stage IV, so the neglect rate was 18,6%, which is higher than the neglect rate among the population of Ukraine and the population of Kyiv (2022 – 12,1% and 7,4%, respectively) [11].

The predominant localization of primary melanoma in men was the skin of the trunk (59 cases – 58,4%) and upper limb (15 cases – 14,8%), while in women – the skin of the trunk (26 cases – 33,3%) and lower limb (25 cases – 32%). Subsequently, this distribution of primary melanoma localization provided the mechanisms of disease progression through the respective regional lymphatic collectors.

The mortality rate from melanoma among the specified contingent of people exceeded the corresponding rate among the population of Ukraine and Kyiv, but since 2019 has had a significant downward trend and

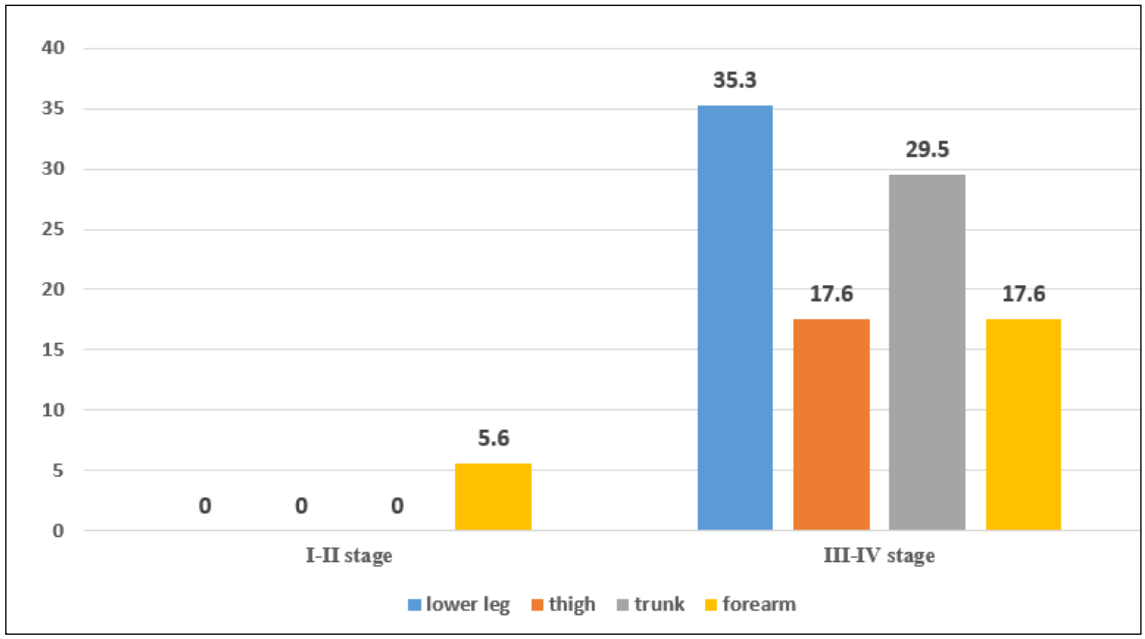

Fig. 12. The proportion (%) of primary melanoma localizations that subsequently metastasized to regional lymphatic collectors of the total number of positive sentinel lymph node biopsies.

in 2021 corresponded to the national level of mortality from melanoma (3,3 per 100 thousand people; 3,01 per 100 thousand people in Kyiv; 3,7 per 100 thousand people in Kyiv; 2,7 per 100 thousand people in Ukraine) [11].

Of the newly diagnosed melanoma patients, 2 people (1,1%) did not live for 1 year. The analogous figure among the population of Ukraine in 2021 was 9,3%, among the population of Kyiv – 7,9% [11]. The five-year and ten-year survival rate of melanoma patients among the defined population was 94,5% in 2021.

Progression of melanoma was detected in 39 patients (21,4%) after an average of 5,3±1,8 years of observation, of which 9 patients (23,1%) had stages I-II melanoma and all patients (30 people – 76,9%) had neglected cases of the disease (stages III-IV). Particularly for the group of patients with neglected cases of melanoma, the average level of primary tumour invasion according to Breslow ranged from 2,93±0,01 mm to 4,45±0,03 mm, with an average level of mitotic activity from 4,4±0,6 per 1 mm^2 to 8,7±0,3 per 1 mm^2, and the occurrence of ulceration in 16,4% of cases.

The abovementioned results confirm the scientific data on the use of such tumour indicators as Breslow tumour thickness, mitotic activity and ulceration, and disease stage to predict the course of the disease [2, 3, 5, 8].

In our study, the most common localization of primary melanoma on the skin of the lower limbs (52.9%) and trunk (29.3%) resulted in further progression of the disease in the first 5-6 years of observation.

CONCLUSIONS

In our study, we have supplemented the scientific data on the timing of patient observation and possible localizations of the primary tumour, which should be taken into account in the dynamic observation of patients with melanoma in order to detect disease progression in a timely manner.

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This research followed the regulations of the World Medical Association Declaration of Helsinki and ethical principles for medical research involving human subjects.
The study was conducted at the State Institution of Science «Center of innovative healthcare technologies» State Administrative Department and was a fragment of the research work «Medical and social substantiation, development and implementation of a modern model of a system for continuous improvement of the quality of integrated medical care in the work of a multidisciplinary healthcare institution» (state registration number 0122U000232; term: 2022- 2024).

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Olena O. Oshyvalova
State Institution of Science
«Center of innovative healthcare technologies»
State Administrative Department
5,Verkhnia St., 01014 Kyiv, Ukraine
e-mail: oshivalovaea@gmail.com

ORCID AND CONTRIBUTIONSHIP
Olena O. Oshyvalova 0000-0002-0395-0269 A D E F
Serhii M. Mazurenko 0009-0000-0756-1351 B C E
Ivan G. Voinov 0000-0003-1118-2359 B C D

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

RECEIVED: 07.07.2024
ACCEPTED: 24.10.2024

ORIGINAL ARTICLE

CONTENTS

The state of formation of a careful attitude towards health in the modern youth

Svitlana P. Palamar, Larysa V. Harashchenko, Olena D. Litichenko, Victorija V. Zhelanova, Liudmyla L. Nezhyva, Dana O. Sopova, Liudmyla O. Pluhatar
BORYS GRINCHENKO KYIV METROPOLITAN UNIVERSITY, KYIV, UKRAINE

ABSTRACT

Aim: The aim of the study was to explore the factors that influence the development of a caring attitude toward health in modern youth as a foundation for an active life stance.

Materials and Methods: Various methods, including analysis of normative and scientific sources, systematic analysis, and empirical research, were employed. An anonymous survey of 114 pedagogical students was conducted to assess how much they value their health. This survey gathered information on health value, motivations for a healthy lifestyle, and self-assessments of physical and emotional well-being.

Results: The findings indicate that 86% of students recognize the importance of maintaining health. While 88% attribute decreased life expectancy to war and diseases, 12% cite harmful habits and a sedentary lifestyle. Positive factors identified by 60% include clean water, quality food, and effective medications. Regarding mental health, 61% consider their state normal, while 39% feel sad and anxious. Only 25% believe they eat properly, and 75% do not follow a healthy diet. Additionally, 88% view physical education as essential for health, and 68% see themselves as active participants in their lives. However, 46% do not grasp the importance of personal responsibility in maintaining their health.

Conclusions: A significant portion of pedagogical students do not prioritize health as a guiding principle. It is essential to structure the educational process to transform societal values into personal achievements, making health a lifelong necessity.

KEY WORDS: health attitude, physical education, motivation, mental health

Wiad Lek. 2024;77(11):2225-2231. doi: 10.36740/WLek/197101 DOI

INTRODUCTION

At the current stage of societal development, there is a negative trend in the declining health of the younger generation. The interest in health is due to the tendency to deteriorate in today's complex socio-economic and environmental conditions [1, 2]. The concept of «values of health» includes the following positions [3-6]: mastering the content of this concept, understanding it as an important characteristic of socio-economic and cultural development of society, as well as the values of human existence; mastering the means and methods of preserving, shaping and strengthening their own health and the health of children; active dissemination of the idea of health care among children, parents, teaching staff; improvement of knowledge, skills and abilities in the field of physical education and rehabilitation.

It is well known that various social, medical, preventive, and sports institutions are involved in the health care of children and youth. However, this does not improve the situation. It is evident that government programs promoting health are not being implemented very effectively. On the other hand, parents' demands for the intellectual development of their children, starting from an early age, are increasing, while they do not pay enough attention to the formation of physical health, endurance, and resilience in their child's body. The problem also lies in the fact that parents pay significant attention to their children's health; however, due to a lack of comprehensive information on healthy lifestyle issues and the absence of personal examples, their health-promoting efforts are not always effective or successful [7].

In light of the above, we believe that the formation and strengthening of children's and youth's health is not only a medical and social issue but also an educational one.

AIM

The aim of the study was to explore the factors that influence the development of a caring attitude toward health in modern youth as a foundation for an active life stance.

MATERIALS AND METHODS

To actualize the didactic aspects of the problem, the methods of analysis of normative and scientific sources, systematic analysis and generalizations, the results of our own empirical research, questionnaire materials, and active modeling of the formation of a caring attitude towards health of modern youth were used.

To achieve the aim of the research, we have conducted experimental task, during complying which we considered a belief that students' acknowledged and value relation to health depends on several factors. They are: knowledge of main one's health factors and children's health, factors that influence health and means of preserving and forming it; worldview rethinking of physical culture and wellness work prioritization in the conditions of preschool education institution; wellness solutions skills; ability to use health preserving and enforcing skills to one's own health and children's health; ability for critical analysis and appropriate application of physical health forming technologies, providing optimal mental health and forming social health of children.

We conducted experiments using empirical method of conducting scientific pedagogical researches, that is, survey method. We chose half-open type survey (except for choosing an answer, the respondents could also express their own point of view).

To process results we used mathematic registration method, which allowed revealing certain qualities in the analyzed phenomena. To improve clarity and convenience of further findings analysis we used graphical method of representing experimental data. We also used methods of hypothesis, systemic analysis and generalizing, which allowed drawing conclusions in our research.

During the research, we based our findings on the belief that the development of a careful attitude toward one's own health, as well as the health of others, depends not only on the awareness and positive perception of health as a value. First and foremost, it depends on the desire and ability to organize one's own activities aimed at realizing this value.The formation of a careful attitude toward one's own health is possible only if the individual is an active participant and a creative subject of their own life. To implement a health-oriented lifestyle, it is essential to be able to make appropriate health decisions on a daily basis. In accordance with the aims and objectives of the research, 114 students from educational majors were surveyed. The students are undergraduate degree seekers majoring in 012 Preschool Education and 013 Primary Education at Borys Grinchenko Kyiv Metropolitan University, studying in their second and third years of full-time education. All respondents are female and are aged 18-19 years.

The questionnaire consisted of three sections. The first section included questions about the structure of factors influencing the personal health of students:

1. How many years do you plan to live?
2. What could prevent you from living to that age, and which of the factors you mentioned can you avoid through your own efforts?
3. Who (or what) will help you prolong your life?

The second section included questions about self-analysis of physical condition:

1. Are you satisfied with your weight?
2. What can you do to change it? (What methods can help you?)
3. How do you feel when you wake up, and why?
4. Do you get tired easily, and what tires you the most?
5. How do you understand the concept of «proper nutrition»? What does it mean to you?
6. Are you eating properly?
7. What is your opinion on vegetarianism and raw food?
8. What do you think about eating food from street vendors? Please comment on why.
9. How often should you wash your hands? Why?

The third section included questions about the motives, incentives, and guidelines for adequate health behavior:

1. In your opinion, are you prepared for the challenges that come with family life?
2. What (or who) will help you cope with these challenges?
3. How much time would you allocate for sports activities during family life?
4. Are you willing to change your routine for the sake of your child?
5. What will help you with this?
6. What can help you maintain productivity and energy throughout the day, week, or at any time?
7. What could prevent your husband from living a long life?
8. Do you believe that heredity affects lifespan? What can help overcome such hereditary factors?
9. Would you consider it essential to actively participate in your children's physical games?

The research was carried out in accordance with the principles of the Declaration of Helsinki (VMA, 1964), and was approved by the Bioethics Committee of the Science Center.

RESULTS

In response to the first question of the first section of the questionnaire, all respondents indicated that they wish to live a long life (100%). Based on the responses to the second question of the questionnaire, we can note that

Table 1. Responses of students to Section I, which included questions about the structure of factors influencing personal health

Question number of the questionnaire	Section I of the questionnaire	Percentage amount
1.	Wish to live a long life.	100%
2.	Causes of shortened lifespan:	
	war and illnesses, harmful habits, other tragedies.	88%
	bad habits, a sedentary lifestyle, accidents.	12%
	Factors for a long life through personal efforts:	
	leading a healthy lifestyle, maintaining an active life, avoiding stress.	68%
3.	Who (or what) will help prolong life:	
	clean water, quality food, effective medicine.	60%
	family, friends, doctors, coaches.	46%

Table 2. Responses of students to Section II, which included questions about the structure of factors influencing personal health

Question number of the questionnaire	Section II of the questionnaire	Percentage amount
1.	Satisfied with own weight. Dissatisfied with own weight.	87% 13%
2.	Methods of weight change: Healthy lifestyle, sports activities, individual sessions with a coach.	88%
3.	Mood with which students wake up:	
	Positive.	61%
	Normal, sad, anxious.	39%
4.	Reasons for a sad mood:	
	War, anxiety, anticipation of distressing news from the front, lack of good news, illness, absence of restful sleep or relaxation, a lot of tasks.	79%
5.	Reasons for fatigue:	
	War, bad news, unpredictable events, stress, lack of a clear study plan, boring tasks during classes, heavy workload.	47%
	Certain people.	1%
	Understanding the essence of proper nutrition.	96%
6.	Do you eat properly?	
	Yes.	25%
	No.	75%
7.	Negative attitude towards vegetarianism and raw food.	75%
8.	Positive attitude towards street food.	75%
9.	Frequent handwashing throughout the day.	100%

the majority of respondents identified war and illness as reasons for a shortened lifespan (88%). In addition, these 88% indicated that harmful habits and other life tragedies could hinder living a long life. 12% of respondents consider harmful habits, a sedentary lifestyle, and accidents as causes of a shortened lifespan. In response to the question about what from the aforementioned can be avoided through personal efforts, a significant number of respondents pointed to the importance of leading a healthy lifestyle, maintaining an active life, and avoiding stress (86%). To the third question of the questionnaire, 68% responded that everything depends on them, while the remaining 46% indicated that doctors, family, friends, loved ones, a coach or instructor, as well as a positive attitude, would help prolong their life. Another 60% of respondents identified clean water, quality food, and effective medicine as factors influencing longevity (Table 1).

Responses to the questions in Section I of the questionnaire indicate that students wish to live long and productive lives. They believe that achieving this depends on their own activity, as well as on their family,

Table 3. Responses of students to Section III, which included questions about the structure of factors influencing personal health

Question number of the questionnaire	Section III of the questionnaire	Percentage amount
1.	Ready for family life. Not ready for family life.	61% 39%
2.	Family will help cope with family pressures.	78%
3.	Time you would spend on sports during family life:	
	5% of your time, 30 minutes, three times a week, it will all depend on the family budget, 2-3 hours a day.	75%
4.	Ready to change your routine for the sake of your future child.	89%
	Not quite ready.	11%
5.	Family will help with childcare.	81%
6.	Factors for maintaining productivity:	
	Rest, coffee, delicious food, friends, family support, morning exercises, healthy sleep.	75%
7.	Factors influencing your husband's lifespan:	
	War, stress.	81%
	Negative habits.	19%
8.	Heredity influences lifespan:	
	Yes.	52%
	No.	48%
9.	Will actively participate in their own children's active games.	83%
	Will not always actively participate in their own children's games.	5%

doctors, coaches, and others. A significant number of students lack personal beliefs about their responsibility for maintaining and developing their health.

Responses from students to the questions in Section II of the questionnaire allowed for certain conclusions regarding their physical condition. When asked if they are satisfied with their weight, the majority of them answered affirmatively (87%). A small portion of respondents (13%) indicated that they are dissatisfied or partially dissatisfied with their weight, mentioning that they need to lose weight or build muscle. The majority of respondents noted that the means of changing their weight include a healthy lifestyle, engaging in sports, and individual training sessions with a coach (88%). In response to the question about the mood with which students wake up and why, the answers were distributed as follows: 61% of respondents indicated that they wake up in a positive mood, while 39% wake up with varying moods (normal, sad, anxious). The majority of respondents (79%) indicated that their mood is influenced by factors such as war, nighttime air raid alerts, explosions, the anticipation of distressing news from the front, the lack of good news, illnesses of family and friends, the absence of restful sleep or relaxation, and a heavy workload, among others. When asked if they get tired easily and what tires them the most, half of the respondents answered no, as they usually feel energetic (53%). The other 47% reported that they tire quite quickly, citing reasons such as studying, monotonous tasks during classes, heavy workloads, as well as war, bad news, unforeseen events, stress, and the lack of a clear work plan, among others. 1% indicated that certain individuals in their environment tire them.

Regarding the question about proper nutrition, students showed consensus in their responses. 96% of respondents indicated that it involves a balanced diet and eating routine. When asked if they eat properly, the majority answered «no» (75%), while only a quarter of those surveyed (25%) believe they do. For the seventh question in Section II of the questionnaire, a significant portion of respondents indicated a negative attitude (75%), 4% stated that they were indifferent and had not thought about the issue, expressing a neutral stance but not seeing it as a prospect for themselves. Additionally, 1% of respondents had a very negative view of vegetarianism and raw food (Table 2).

In response to the eighth question of Section II of the questionnaire, students stated that eating on the go is acceptable because it is convenient, quick, and saves time for more important tasks (75%). However, they noted that such quick meals are not entirely healthy. Regarding the question about the necessity of frequent

handwashing, all respondents (100%) agreed that it is important and mentioned that frequent handwashing has become a daily habit due to the spread of the coronavirus disease.

Responses to the questions in Section II of the questionnaire indicate that students understand that physical health is the foundation of a healthy personality. They also recognize the reasons (both objective and subjective) for the deterioration of their mental (emotional) health.

Responses to the questions in Section III of the questionnaire allowed for the following conclusions. For the first question, a significant portion of respondents answered affirmatively (61%), while the remaining 39% answered negatively. Regarding the second question, a common viewpoint emerged among 78% of respondents, stating that family would help them cope with various challenges.

For the third question, the majority of respondents (75%) indicated that they would allocate time for sports during family life as follows: 5% of their time, 30 minutes, three times a week, depending on the family budget, or 2-3 hours a day. Notably, students recognize the importance of physical exercise, even within family life.

In response to the fourth question, almost unanimously, respondents (89%) stated that they are willing to change their lifestyle for the sake of their child, while 11% said that perhaps not at this time.

For the fifth question, 81% of respondents indicated that the family should be the primary support in caring for a child. In response to the sixth question, nearly 75% of respondents cited rest, coffee, good food, friends, family support, morning exercises, and healthy sleep as essential factors for maintaining well-being.

To the seventh question, a significant portion of respondents identified war and stress as major influences (81%), while 19% pointed to negative habits. For the eighth question, responses were almost evenly split: about half (52%) expressed belief in overcoming negative hereditary factors, while the other half (48%) did not. They noted that self-improvement, the desire to change, self-confidence, consultations with various specialists, and medicine can help combat negative heredity.

Finally, for the ninth question, a large majority (83%) responded that they would actively participate in their children's physical games, while a few noted they would not always do so (5%) (Table 3).

Responses to the questions in Section III of the questionnaire indicate that students understand that family life comes with certain risks and is complex and challenging. As a result, some young people approach the formation of a new family with responsibility. However, a significant portion is not ready for family life. This highlights the issue of family upbringing for the younger generation.

DISCUSSION

The analysis of the questionnaire results indicates that students recognize health as a key factor in leading an active, happy, and effective life. They agree with the axiom that maintaining health is a complex issue that requires hard work both from individuals and society [5, 6]. Students are aware of the objective and subjective reasons for adopting a healthy lifestyle and acknowledge that physical education is an effective means of promoting and strengthening health. They understand the importance of proper nutrition and personal hygiene and recognize that environmental degradation, chronic fatigue, and negative habits contribute to declining health and well-being [8, 9].

However, while students demonstrate knowledge and understanding of these issues, this awareness does not always translate into personal acceptance or conviction. The information they acquire remains somewhat detached from their daily lives and does not motivate them to prioritize their health. This disconnect suggests that contemporary societal challenges necessitate a reevaluation of personal attitudes toward health. Unfortunately, many young individuals lack a strong internal drive to prioritize their well-being [10].

This situation points to a gap in the formation of daily skills for making health-related decisions. Understanding health as a value is different from actively organizing one's activities or educational efforts to actualize that value. As a result, values can sometimes be reduced to mere declarations. Furthermore, students may not fully grasp that a low level of general educational culture significantly impacts an individual's health-oriented mindset.

To address these issues, it is essential to create supportive environments that foster personal responsibility and promote health literacy. By integrating health education into daily routines and providing practical tools for decision-making, we can help students transition from awareness to action, ultimately encouraging a culture of health and well-being.

It is evident that not all students are prepared for family life. While they express a positive attitude toward it and show a willingness to change their lifestyle, rhythm, and routine for the sake of a future child, simply having a positive outlook is not enough.

Moreover, students are not fully convinced that they should be active participants and creative agents in their own lives. They do not entirely recognize the necessity of personal responsibility and the importance

of being a role model for their future children. Only the daily health-oriented behavior of adults serves as a model to emulate, and a healthy lifestyle should be the norm every day. Any gaps in the process of taking responsibility for one's health or in finding effective ways to strengthen it can lead to a loss of health for individuals or even entire generations.

CONCLUSIONS

The results of the questionnaire analysis indicate that a significant portion of respondents is confident that maintaining health and developing health-oriented behaviors depend on the individual. They understand the importance of personal responsibility in building a healthy trajectory. At the same time, another considerable group of respondents believes that the issue of health preservation lies within the family, preschool institutions, schools, and medical facilities. These students think that not everything depends on their own efforts. They feel that family, coaches, and professionals from various fields can assist them in adopting a health-oriented lifestyle.

It is important to note the positive attitude and interest of contemporary students in physical exercise and sports. They consider physical culture to be an effective means of strengthening and promoting health. However, students did not demonstrate a conscious approach to evaluating their own health or the development of health preferences and habits.

We believe that knowledge about a healthy lifestyle is essential for students to engage in active living right now. It is equally important for this system of knowledge and skills to be transformed into a healthy way of life.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Svitlana P. Palamar
Borys Grinchenko Kyiv Metropolitan University
18/2 I. Shamo Blv., 01601 Kyiv, Ukraine
e-mail: s.palamar@kubg.edu.ua

ORCID AND CONTRIBUTIONSHIP
Svitlana P. Palamar: 0000-0001-6123-241X A B C D
Larysa V. Harashchenko: 0000-0002-7847-632X A B C D
Olena D. Litichenko: 0000-0002-7004-3837 A B C D
Victorija V. Zhelanova: 0000-0001-9467-1080 A B D
Liudmyla Nezhyva: 0000-0001-9520-0694 D E F
Dana O. Sopova: 0000-0002-0814-6667 D E F
Liudmyla Pluhatar: 0000-0002-3421-5566 E

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

RECEIVED: 11.07.2024
ACCEPTED: 29.10.2024

ORIGINAL ARTICLE

CONTENTS

Characteristics of therapeutic and diagnostic errors in the management of traumatic brain injury by healthcare professionals: insights from forensic medical examinations on the quality of medical care

Alina O. Pletenetska, Yevhenii A. Varfolomeiev, Ivan S. Demchenko
BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

ABSTRACT

Aim: To identify defects in the diagnosis and treatment of traumatic brain injury (TBI) committed by medical personnel at various stages of providing medical care based on the results of forensic medical examinations.

Materials and Methods: The article based on the study of medical care defects in cases of TBI. The research was conducted according to the data of forensic medical examinations by the SI "Main Bureau of Forensic Medical Examination of the Ministry of Health of Ukraine" from 2012 to 2021. The study of the main clinical defects affecting the subsequent evaluation of TBI by forensic medical experts was performed.

Results: During the analysis of forensic medical conclusions, the defects of medical care were identified in 163 cases, accounting for 85,8±2,5%. A causal relationship between the defect and the adverse outcome was established in 103 (54,2±3,6%) cases. Among the cases with a causal relationship, 72 (37,9±3,5%) directly impacted the outcomes. Among the groups of defects was described such as incorrect assessment of patient condition, misinterpretation of clinical symptoms, failure to perform diagnostic measures, etc.

Conclusions: Defects in treatment were identified on different levels of medical care in a majority of cases (85,8%) and causal relationship between the defects and outcomes was established in 54,2% of cases. The findings indicate that a multifaceted approach is necessary to address the defects in medical care for TBI patients. This includes better diagnostic accuracy, improved treatment protocols, and addressing organizational and administrative issues.

KEY WORDS: forensic medicine, traumatic brain injury, medical care defects

Wiad Lek. 2024;77(11):2232-2238. doi: 10.36740/WLek/197102 DOI

INTRODUCTION

Traumatic brain injury (TBI) presents a substantial clinical challenge, with management requiring precise therapeutic and diagnostic approaches. Despite advances in treatment protocols, errors in both diagnosis and therapy remain prevalent, significantly impacting patient outcomes [1-3]. The complexity of TBI management is highlighted in comprehensive guidelines which emphasize the importance of timely and appropriate interventions [4, 5].

The integration of advanced diagnostic tools, such as quantitative pupillometry, is crucial for improving the accuracy of TBI assessment and management [6]. Effective respiratory management strategies are also essential in preventing secondary brain injury and improving recovery outcomes [7]. However, the inconsistency in the implementation of these guidelines often leads to suboptimal care.

Diagnostic errors in the emergency department significantly impact the clinical course of TBI patients. Misdiagnosis or delayed diagnosis can result in inappropriate or delayed treatments, exacerbating brain injury and leading to poorer outcomes. A systematic review highlights the high prevalence of diagnostic errors at the emergency department, emphasizing the need for improved diagnostic accuracy and clinical decision-making processes [8].

The assessment of the quality of medical care makes it possible to identify widespread errors with the aim of their further prevention [8-11].

Assessment of the correctness of therapeutic and diagnostic measures in the provision of medical assistance to TBI patients is one of the tasks of forensic medical examination in Ukraine.

AIM

The aim of the article is to identify defects in the diagnosis and treatment of TBI committed by medical

Table 1. Quantitative characteristics of commission forensic medical examinations in cases of TBI carried out by the SI for medical affairs for 2012-2021

Years	Total for medical cases, n	In cases of TBI			
		In total			
		N	P, %	m_p, %	95 % CI
2012	119	14	11,8	3,0	5,8
2013	149	18	12,1	2,7	5,2
2014	151	16	10,6	2,5	4,9
2015	145	16	11,0	2,6	5,1
2016	147	20	13,6	2,8	5,5
2017	205	27	13,2	2,4	4,6
2018	153	18	11,8	2,6	5,1
2019	151	20	13,2	2,8	5,4
2020	169	21	12,4	2,5	5,0
2021	166	20	12,0	2,5	5,0
In total	1555	190	12,2	0,8	1,6

personnel at various stages of providing medical care based on the results of forensic medical examinations.

MATERIALS AND METHODS

The article was based on the study of defects in the provision of medical care by doctors in cases of TBI, conducted according to the data of commission forensic medical examinations of medical cases by the State Institution "Main Bureau of Forensic Medical Examination of the Ministry of Health of Ukraine" (SI) from 2012 to 2021 (from the total number of 1555 examinations on the improper provision of medical care by medical workers, we selected 190 that related to TBI) to study the main clinical defects affecting the subsequent evaluation of TBI by forensic medical experts with examples of practical cases.

When characterizing qualitative indicators, the share of variants possessing the studied features (n) was expressed as a percentage (P) of their total number, the error of percentage (m_p), and the 95 % confidence interval (CI). Data processing and analysis were carried out in OpenOffice software packages (Base, Calc, Writer, Draw, Math), GNU Octave, with original documents saved in *.doc, *.xls formats. This software is open source and its use is governed by the GPL (GNU General Public License).

RESULTS

During the statistical analysis of examinations carried out in the forensic medical department, it was found that among all examinations on such medical cases, their percentage in cases of TBI ranged from 10,6 ± 2,5% (in 2014) to 13,2 ± 2,8% (in 2019) (Table 1).

The distribution of all medical case examinations by doctor's specialties showed that the number of neurosurgeons and anesthesiologists accounted for 14 ± 4,9% and 10 ± 4,2%, respectively, of all forensic medical examinations in medical cases. However, in cases of improper provision of medical care for TBI, criminal proceedings followed by the appointment of expert examinations, in addition to neurosurgeons and neurologists, were also opened against medical workers of other specialties: anesthesiologists, surgeons, paramedics of emergency teams, traumatologists and obstetrician-gynecologists (birth trauma of a newborn), that is, those who are more often faced with emergency conditions.

When analyzing the commission forensic medical examinations of TBI in medical cases of the SI from 2012 to 2021 (190 cases), the following findings were noted. Defects were identified in 163 cases, accounting for 85,8±2,5%. A causal relationship between the defect and the adverse outcome was established in 103 (54,2±3,6%) cases. Among the cases with a causal relationship, 72 (37,9±3,5%) had significant defects directly impacting the outcomes.

In the remaining cases, the influence of defects on outcomes was either indirect or the primary factor could not be determined. In addition to the diagnostic defects described in the previous section (incorrect, unjustified diagnosis, incomplete or ignored neurological symptoms characteristic of TBI, absence/incompleteness of trauma history, poorly or undocumented external injuries, simulation, and aggravation of clinical manifestations of TBI, etc.), medical practitioners also committed other defects directly affecting patient treatment and adverse outcomes.

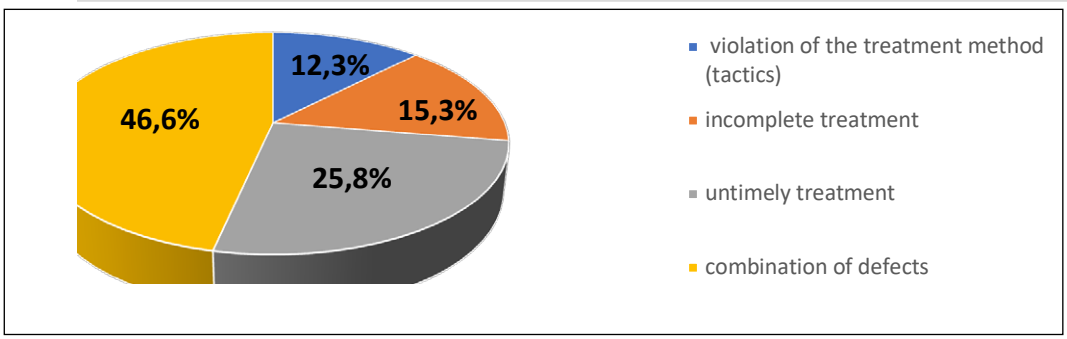

Fig. 1. Percentage distribution of TBI treatment defects based on commission forensic medical examinations in "medical cases" of the SI.

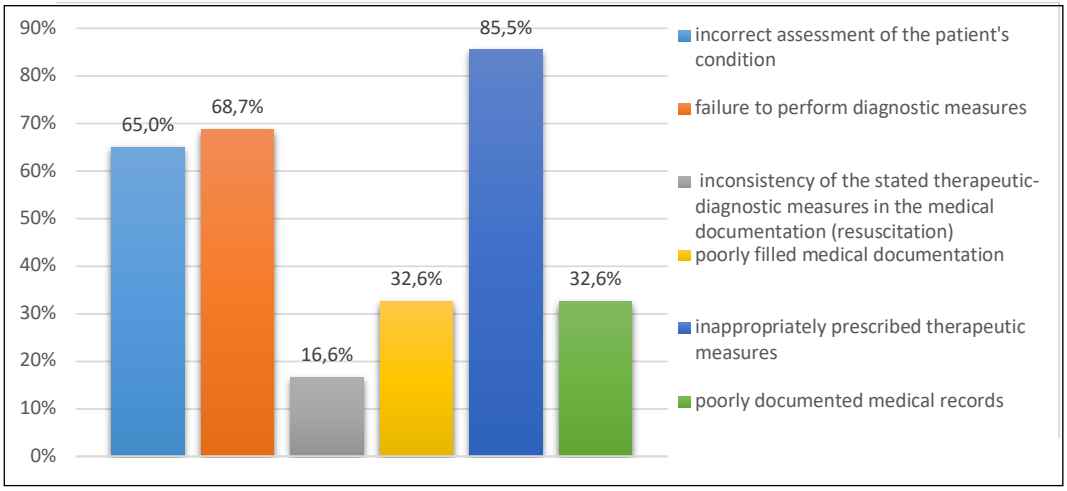

Fig. 2. Percentages of defects affecting forensic medical assessment in victims and the assessment of the quality of medical care for TBI.

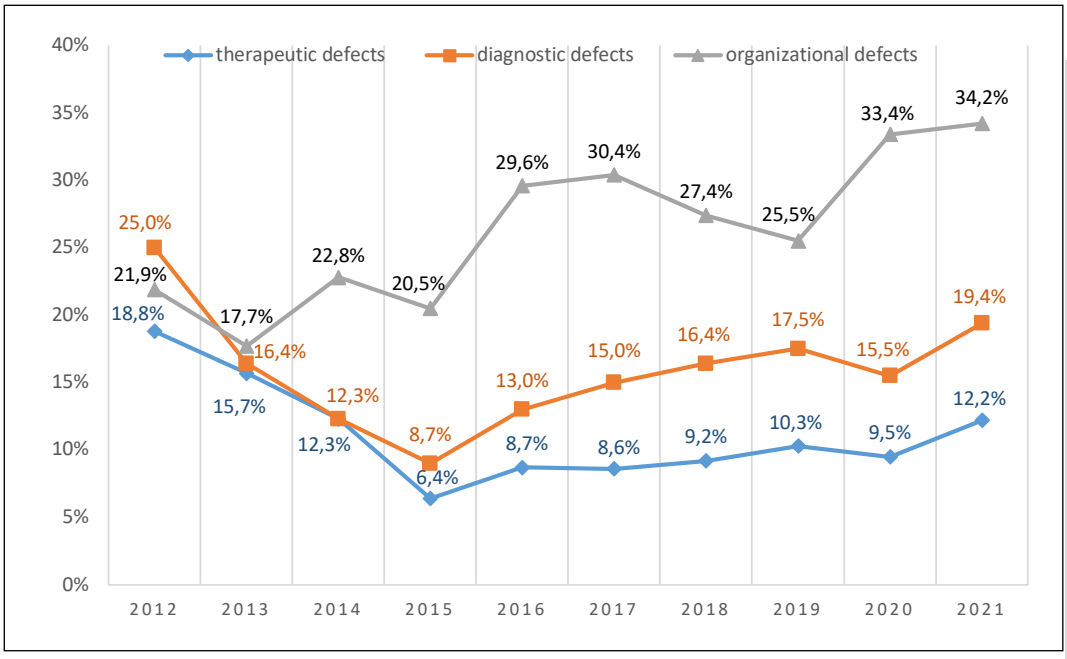

Fig. 3. Dynamics of therapeutic, diagnostic, and organizational defects made by medical practitioners based on forensic medical examination results of the SI from 2012 to 2021.

Incorrect assessment of patient condition was found in 106 cases (65±3,7% of the 163 commission forensic medical examinations where defects in medical care were found). For example, a patient's medical record indicated unconscious admission, anisocoria, bradycardia, and a "lucid interval," yet the condition was assessed as moderate severity. The patient subsequently developed cerebral edema with herniation into the foramen magnum.

Special attention should be given to cases of misinterpretation of clinical symptoms of TBI against a background of alcohol intoxication, leading to incorrect condition assessment and subsequent consequences. These cases accounted for almost a third (31,9±3,7% – 52 cases) of the 163 commission forensic medical examinations where defects in medical care were found.

Failure to perform diagnostic measures was identified in 112 cases (68,7±3,6% of the 163 commission foren-

sic medical examinations where defects in medical care were found). For example, a trauma doctor was convicted under part 2 of Article 139 of the Criminal Code and sentenced to three years in prison. According to the "Expert Conclusion," "...the attending physician did not follow the neurosurgeon's recommendations and did not perform a necessary CT scan of the brain to determine the nature and extent of the TBI," while "...the patient required a full diagnostic examination, including a CT scan of the brain, to clarify the nature of the TBI before prescribing treatment that corresponded to the established clinical form."

Discrepancies between documented and actual therapeutic and diagnostic measures were found in one examination, where a patient with severe TBI, who was unconscious, was given oral medications according to the prescription sheet. In 27 examinations (16,6±2,9% of the 163 commission forensic medical examinations where defects in medical care were found), reanimation measures documented in medical records were not confirmed during subsequent forensic examinations (tracheostomy, conicotomy, venous access, etc.).

Incorrectly prescribed therapeutic measures were evaluated among all 163 (85,5±5,0%) commission forensic medical examinations where defects in medical care were found, considering the method (tactics) of treatment, completeness, and timeliness of treatment. Violations in the method (tactics) of treatment were noted in 20 cases (12,3±2,6%), completeness violations in 25 cases (15,3±2,8%), and untimeliness of treatment in 42 cases (25,8±3,4%), with a combination of defects in 76 cases (46,6±3,9%). (Fig. 1)

In the treatment of TBI, three key aspects must be considered: the method (tactical approach), completeness, and timeliness of medical care [4-15].

1. METHOD (TACTICAL APPROACH)

In TBI treatment, the method is divided into conservative and operative approaches. The choice depends on the severity of the injury, the clinical picture, and the presence or absence of complications such as intracranial hematoma, brain swelling, or tissue compression.

• *Conservative treatment* is used for mild and moderate TBI cases where there are no severe structural brain damages requiring immediate surgery. This includes: medication therapy to stabilize intracranial pressure (e.g., diuretics, corticosteroids), reduce brain swelling, relieve pain (analgesics), and control seizures (antiepileptic drugs); monitoring the patient in the intensive care unit to assess vital functions and track changes in neurological status.

• *Operative treatment* is necessary in cases of severe TBI where there is life-threatening damage such as intracranial hematomas, skull fractures with brain tissue damage, or other complications requiring urgent surgical intervention: craniotomy, decompressive craniectomy, skull fracture.

2. COMPLETENESS OF TREATMENT

Completeness refers to a comprehensive approach that involves all necessary diagnostic and therapeutic procedures to fully assess the injury, determine the patient's condition, and ensure proper management throughout recovery.

• *An incomplete list of diagnostic and therapeutic procedures* includes: computed tomography (CT), Magnetic resonance imaging (MRI), Neuromonitoring, Lumbar puncture.

• In addition to diagnostics, the *therapeutic aspect* includes: medication management, including intracranial pressure control, oxygenation, and hypothermic methods to reduce brain swelling; intensive care, aimed at supporting vital functions like lung ventilation and fluid therapy to maintain stable hemodynamics.

3. TIMELINESS OF TREATMENT

Timeliness is crucial in TBI treatment. Clinical guidelines define therapeutic and surgical "windows" within which certain actions must be taken to achieve the best outcomes:

• Therapeutic windows: For mild and moderate injuries, where a conservative approach is used, it is essential to start medication therapy and stabilize the patient within the first hour after injury ("golden hour"). This includes: ensuring adequate oxygenation (ventilation), controlling intracranial pressure, quickly administering medications to prevent seizures and reduce brain swelling.

• Surgical windows: In severe TBI cases (e.g., subdural hematoma), time is critical for performing surgical correction. According to clinical guidelines, surgery should be done as soon as possible, typically within the first 4–6 hours after the injury, to minimize brain damage caused by prolonged compression or ischemia. Delays can lead to irreversible neurological damage or death.

Timeliness is about ensuring that all necessary actions are carried out within the specific time frames outlined in clinical guidelines. Delayed treatment significantly worsens the prognosis and increases the risk of death or long-term disability.

Detailed examination of commission forensic medical examinations conducted to assess the quality and qualification of medical care provided to TBI patients showed that therapeutic defects were related to diagnostic ones.

Poorly documented medical records included cases with missing entries, errors, illegible writing, etc.

There were 62 such cases, accounting for nearly a third (32,6±3,4%) of the 190 analyzed commission forensic medical examinations in medical cases. (Fig. 2)

Overall, the analysis of the examinations revealed the most common groups of defects made by doctors when providing medical care in TBI cases: therapeutic (general, tactical, and technical) – incorrect choice of methods and means for diagnosis, incorrect, incomplete, or unprofessional assessment of research results, errors in determining indications and contraindications for treatment methods, insufficient examination of the patient, incorrect execution of diagnostic or therapeutic manipulations, which can be generalized as non-compliance with medical care standards and clinical treatment protocols; diagnostic, related to diagnosis according to ICD-10 (The International Classification of Diseases) (by disease and complications; by quality and formulation of diagnoses; by discrepancy between initial and final diagnoses); organizational - administrative (from untimely delivery of the patient to the medical facility and shortcomings in hospital planning, such as inefficient use of premises, to insufficient control over the quality and effectiveness of therapeutic work, including failure to comply with job and regulatory documents regulating the work of doctors of various specialties), and documentation (incorrect and incomplete filling of operation protocols, documents, certificates, extracts from medical histories, sick leave sheets; shortcomings and gaps in filling out outpatient cards, medical histories, operating journals; inadequate registration journals, etc.).

When comparing different types of defects in TBI in percentage terms, it is noticeable that during the specified period, the dynamics of defects showed a wave-like nature, with the number of therapeutic and diagnostic defects decreasing from 18,8% in 2012 to 12,2% in 2021 and from 25% in 2012 to 19% in 2021, respectively. (Fig. 3)

The lowest number of therapeutic and diagnostic defects was noted by forensic commissions in 2015 (6,4% and 9%, respectively). Meanwhile, the number of organizational defects significantly increased from 21,9% in 2012 to 34,2% in 2021.

DISCUSSION

The management of TBI patients with co-existing conditions, such as alcohol intoxication, was particularly challenging, leading to misinterpretation of clinical symptoms and improper treatment. This complexity is supported by the guidelines discussed by Hawryluk et al., which emphasize the need for tailored therapeutic strategies in complex TBI cases [5]. Our study identified a high prevalence of diagnostic errors, consistent with the findings of Newman-Toker et al., who conducted a systematic review on diagnostic errors in the emergency department [12]. The failure to perform necessary diagnostic measures, as noted in our study, underscores the importance of adherence to established guidelines, such as those outlined by Carney et al. and IHL/GC/GR/JS/JW/JP, for the management of severe TBI [4, 13]. Implementing standardized diagnostic protocols, including timely imaging studies, can help mitigate diagnostic errors and improve patient care. Therapeutic errors, including incorrect assessment of patient conditions and inadequacies in treatment, were prevalent in our study. These findings resonate with the guidelines provided by Abdelmalik et al., emphasizing the need for comprehensive therapeutic interventions tailored to individual patient needs [14]. Additionally, the recommendations of Hawryluk et al. for decompressive craniectomy in severe TBI cases highlight the importance of evidence-based therapeutic strategies in improving outcomes [5].

Organizational issues, including delayed patient transfer and insufficient hospital planning, were significant contributors to poor outcomes. Incomplete and poorly documented medical records were found in 32.6% of cases, highlighting systemic issues in medical record-keeping. These organizational shortcomings are echoed in the guidelines for the management of severe brain injuries, which stress the importance of efficient hospital processes and meticulous documentation [15].

Organizational issues, such as delayed patient transfer and insufficient hospital planning, contributed to poor outcomes in our study. These findings are consistent with the observations of Hautz et al., who emphasized the impact of diagnostic errors on mortality and hospital length of stay, further underscoring the importance of efficient organizational protocols in TBI management [2]. Improved documentation practices, as advocated by Newman-Toker et al., are essential for ensuring accurate recording of patient information and facilitating continuity of care [12].

The study's findings underscore the critical impact of diagnostic and therapeutic errors on TBI outcomes. The high incidence of defects and their direct correlation with adverse outcomes highlight the need for improved adherence to established guidelines. Implementing comprehensive diagnostic and therapeutic protocols, such as those proposed by Capizzi et al. and Carney et al., can enhance the quality of TBI management [4, 6].

Furthermore, the integration of advanced diagnostic tools and timely therapeutic interventions, as recommended by Asehnoune et al., can help mitigate these

errors [7]. Organizational improvements, including better hospital planning and efficient patient transfer protocols, are also essential for enhancing patient care. Ensuring thorough and accurate documentation, as emphasized in various guidelines, is crucial for maintaining the quality and continuity of care.

In conclusion, addressing the diagnostic, therapeutic, organizational, and documentation defects identified in this study requires a multifaceted approach. By aligning medical practices with established guidelines and adopting best practices from the literature, healthcare providers can significantly improve the outcomes for TBI patients.

CONCLUSIONS

The analysis of commission forensic medical examinations of TBI in medical cases of the SI from 2012 to 2021 has revealed several critical findings:

1. Defects in medical care were identified in 85,8% of cases, with 54,2% of these defects directly contributing to adverse patient outcomes, highlighting significant gaps in diagnostic and treatment processes.
2. Misinterpretation of symptoms, especially in cases of alcohol intoxication, and the failure to perform necessary diagnostic measures were common issues, underscoring the need for better training and adherence to protocols.
3. While therapeutic and diagnostic defects decreased over time, the rise in organizational defects (34,2% in 2021) indicates growing systemic problems.

In conclusion, the study underscores the critical need for systemic improvements in the management of traumatic brain injury, from diagnosis to treatment and documentation, to enhance patient outcomes and reduce the prevalence of medical errors. Continued efforts in training, adherence to clinical guidelines, and administrative reforms are essential to achieving these goals.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Alina O. Pletenetska
Bogomolets National Medical University, Kyiv, Ukraine
5 Mechnikov St., 01133 Kyiv, Ukraine
e-mail: fantasyalinka@gmail.com

ORCID AND CONTRIBUTIONSHIP
Alina O. Pletenetska: 0000-0002-7029-3377 A C D E F
Yevhenii A. Varfolomeiev: 0000-0003-0539-9682 B
Ivan S. Demchenko: 0000-0001-8721-2775 D

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

RECEIVED: 30.06.2024
ACCEPTED: 28.10.2024

ORIGINAL ARTICLE

CONTENTS

Periodontitis upon hypothyroidism of young people

Oksana M. Repetska, Zoryana B. Popovych, Iryna Z. Chubiy, Mykola M. Rozhko, Ivan V. Paliichuk
IVANO-FRANKIVSK NATIONAL MEDICAL UNIVERSITY, IVANO-FRANKIVSK, UKRAINE

ABSTRACT

Aim: To study the periodontal tissues and to establish the Magnesium content in oral liquid and capillary blood of young people with hypothyroidism from areas with Iodine deficiency.

Materials and Methods: In order to study the prevalence of periodontal disorders there were 330 people aged from 25 to 44 years from areas with natural Iodine deficiency enrolled in this examination. Primary hypothyroidism was diagnosed in 195 people (they made up the group I), and 135 people did not have any accompanying endocrine pathology (group II). The most prevalent were 30–34-year-olds – 38,46% in the group I and 39,26% in the group II. Patients aged 25-29 years were the least: 27,18% and 28,89% in the I and II groups. Biochemical analysis of oral fluid and capillary blood was performed to investigate the Magnesium content.

Results: According to the results of the dental examination, it was established that 85,13% of young people with hypothyroidism were also affected by periodontal disorders. On the other hand, periodontal disorders were observed in 69,63% of people of the same age range without endocrine pathology ($p<0,01$). Chronic generalized periodontitis of I and II degree dominated in the spectrum of periodontal disorders. The concentration of Magnesium in the blood serum of individuals with hypothyroidism was lower than in those without hypothyroidism.

Conclusions: The obtained data indicate a negative effect of primary hypothyroidism on the development of periodontal tissue diseases.

KEY WORDS: periodontitis, hypothyroidism, biochemical research

Wiad Lek. 2024;77(11):2239-2244. doi: 10.36740/WLek/197103 DOI

INTRODUCTION

The etiology and pathogenesis of periodontal diseases are multifactorial and are influenced by a number of exogenous and endogenous determinants and triggers [1, 2]. The main common predictors of periodontal tissue disorders include somatic pathology such as endocrine diseases, blood and cardiovascular pathology, diseases of the gastrointestinal tract, viral illnesses etc. [2-10]. It has been scientifically proven that the state and interaction of the main regulatory systems (immune, endocrine, nervous) determines the body's homeostasis and its response to any pathogenic influence. The progression of the pathological process in periodontal tissues to a certain extent depends on the state of the endocrine system [6, 7, 10]. The occurrence and rapid progression of periodontitis are substantiated by the association with diseases of the thyroid gland in particular [4, 7, 8]. Diseases of the thyroid gland contribute to the development and rapid progression of periodontitis [9-11].

A large number of professional sources also trace the relationship between somatic pathology and periodontal diseases, which occur simultaneously, mutually aggravating each other [6, 11, 12].

Hypothyroidism, for example, is one of the endocrine pathologies that significantly affects development and severity of periodontal disorders. This disease often occurs in those from regions with natural Iodine deficiency. A large part of European countries has a more or less pronounced natural Iodine deficiency. The latter is most common in the western regions of Ukraine and in the Chernihiv region. From Iodine deficiency suffers about 70% of the population due to its insufficient content in the soils remote from the sea, especially in the mountain and foothills areas [12]. Epidemiological study [13] of children from the regions with iodine deficiency (Ivano-Frankivsk region) confirmed the presence of endemic goiter in 54% of individuals. Moreover, the high rate caries was found in 50% of patients with endemic goiter as well as 31% of affected children were diagnosed also with systemic and focal hypoplasia. At the same time, 23% of the examined had a combined pathology – caries and hypoplasia at the same time.

There is a decrease of the pH from 6,4 to 5,0 in the oral liquid of children with thyropathology compared to healthy ones according to researchers [10].

Insufficient Iodine intake leads to the development of consistent adaptive processes that are able to maintain

the normal synthesis of thyroid hormones. However, if the deficiency of this trace element persists for a long time, adaptation mechanisms are disrupted, followed by a decreased synthesis of thyroid hormones. The main biological value of Iodine is determined by the fact that this element is essential for the thyroxine and triiodothyronine synthesis and secretion. Thyroxine and triiodothyronine regulate the tissue and cell metabolism, influence body growth and development. Thyroid hormones control all aspects of cell division and growth, the state of the immune, cardiovascular systems, and liver function. Iodine deficiency violates biochemical processes that control metabolism and production of thyroid hormones. Disturbances of hormone synthesis associated with Iodine deficiency are the reasons for a number of diseases and pathological conditions.

Illnesses of the thyroid gland are the second most common among endocrine disorders. Problems of the thyroid status based on constant Iodine deficiency in children impede mineral, protein, carbohydrate, vitamin and other types of metabolism, which affects the homeostasis of the oral cavity on the whole. Experimental studies directly confirm the key role of thyroid hormones in the regulation of bone metabolism. Since thyroid hormone receptors are present in human bone, it has been suggested that they may act directly on bone cells through specific nuclear receptors or indirectly through increased secretion of growth hormone and insulin-like growth factor [6]. Pathologies of the thyroid gland belong to systemic diseases accompanied by hormonal changes of the body and influence vital processes in many organs, and are considered as determinants in the development of comorbid conditions [6, 7, 8, 10].

Hypothyroidism is one of the most common diseases of the endocrine system caused by insufficient production of thyroid hormones [8]. Primary hypothyroidism is the most common. The overall prevalence of primary manifest hypothyroidism in the population is about 0,2-2%, subclinical – 7-10% among women and 2-3% – among men [9].

In case of hypothyroidism and periodontitis there are some biochemical changes regarding bone structure, especially in micro mineral content. Although there are many studies concerning Calcium and Phosphorus, much less information is about the role of Magnesium.

Magnesium, however, is an important trace element. The content of Magnesium in the human body ranks fourth after Calcium, Potassium and Sodium. This element is present in cartilage and bone tissue at the initial stages of bone formation, and its amount is dynamically regulated by the intensity of osteogenesis. About 60% of Magnesium is stored in bones, where Magnesium is a part of the mineral bone matrix together with Calcium and Phosphorus. Magnesium affects the processes of bone remodeling, stimulating the activity of osteoblasts and increase osteogenesis.

Recently, Magnesium has been recognized as being an important factor for the physiological bone assessment. Therefore, we decided to study this trace element in our research.

AIM

The aim of the research was to study the periodontal tissues and to establish the content of Magnesium in the oral liquid and capillary blood in people (25-44 years old) from an area with Iodine deficiency and are diagnosed with hypothyroidism.

MATERIALS AND METHODS

Ivano-Frankivsk National Medical University performed a clinical examination of patients and research in order to study the periodontal tissues in young people (25-44 years old) with hypothyroidism caused by Iodine deficiency and to determine changes in the content of Magnesium. Clinical studies were conducted in accordance with the legislation of Ukraine and the principles of the Helsinki Declaration on Human Rights.

Examinations of patients with chronic generalized periodontitis were carried out at the Postgraduate Dentistry Department. Laboratory determination of Magnesium content was carried out at the «Center of Bioelementology» of the Ivano-Frankivsk National Medical University. Each patient provided a written consent for the examination.

Clinical examination of patients was conducted according to unified clinical protocols. The diagnosis of «hypothyroidism» was established by an endocrinologist based on clinical and laboratory data according to a unified clinical protocol.

There were 330 residents of Ivano-Frankivsk of ages between 25 to 44 years (according to the World Health Organization age classification) enrolled in this study. Primary hypothyroidism was diagnosed in 195 people (they made up the group I). The group II consisted of 135 people without endocrine pathology. In both observation groups were mostly people aged 30-34 years: 38,46% in the group I and 39,26% in the group II. Patients aged 25-29 years were the least: 27,18% and 28,89% in the group I and group II respectively.

Blood and saliva samples were collected at the same time, in the morning, before breakfast for biochemical analysis. First, saliva was collected. Mixed saliva (5 ml) was obtained by spitting without stimulation into an Eppendorf test tube prior tooth brushing (to prevent

Table 1. Prevalence of periodontitis in observation groups depending on age

Age groups (year)	Group I			Group II		
	Number of examined (n)	With periodontitis (N)	%	Number of examined (n)	With periodontitis (N)	%
25 – 29	53	39	73,58	39	19	48,72*
30 – 34	75	64	85,33	53	41	77,36
35 – 44	67	63	94,03	43	34	79,07*
All together	195	166	85,13	135	94	69,63**

Note: * – $p<0,05$ is a significant difference in the frequency between the I and II groups;
** – $p<0,01$ is a significant difference in the frequency between the I and II groups.

trauma to the gums and getting into the blood sample), after mandatory oral rinsing with distilled water. The obtained saliva was centrifuged at 3000 rpm for 15 minutes. The separated serum and the supernatant fraction of saliva were placed in standardized plastic disposable Eppendorf microtubes of 1,5 ml. Determination of biochemical indicators was usually carried out on the day of sampling.

Blood for research was taken from the gingival papilla as follows: the oral cavity was rinsed with warm water, the dental arch was covered with cotton pads, the gums were applied with an antiseptic and dried. A 3,5 mm deep papilla puncture was performed most often around the 34, 33, 43, 44 teeth by a sterile scarifier for blood sampling. Vaseline was used to prevent the blood from spreading. Heparin (30 units per 1,0 ml of blood) was used as an anticoagulant. Estimation of the Magnesium content in blood serum and oral fluid was carried out by a kit for the determination of trace elements in biological fluids («Simko» research and production company, Ukraine).

As chronic generalized periodontitis of the I and II degrees of severity was diagnosed the most among others periodontal disorders, biochemical studies were carried out in those affected. The first group included 50 patients with chronic generalized periodontitis diagnosed with primary hypothyroidism: 25 patients with chronic generalized periodontitis of the I degree of severity and 25 patients with chronic generalized periodontitis of the II degree of severity. The group II consisted of 50 patients with chronic generalized periodontitis without endocrine pathology (25 people with chronic generalized periodontitis of the I degree and 25 patients with chronic generalized periodontitis of the II degree).

The statistical processing of the obtained data was carried out by the use of MedCalc v. 23.0.5 (MedCalc Software Ltd) and EZR v. 1.68. Continuous variables were presented as mean ± the standard error of mean, and were compared in the studied groups by the Welch`s unpaired T-test. Qualitative variables were presented as absolute and relative (%) frequency, and were compared in the studied groups by the Fisher`s exact test. A 2-tailed $p<0,05$ was considered as statistically significant.

RESULTS

The results of the examination demonstrated that 85,13% (166 of 195) of young patients diagnosed with hypothyroidism were also affected by periodontal disorders. On the other hand, periodontal disorders were observed in 69,63% (94 of 135) of young people of the same age, but without endocrine pathology, which was 1,2 times lower ($p<0,01$). Chronic generalized periodontitis of I and II degree dominated in the spectrum of periodontal disorders.

Periodontal disease was detected in 73,58% of the 25-29-year-olds diagnosed with hypothyroidism, which was 1,5 times more than in patients of the same age without endocrine pathology (48,72%; $p<0,05$) (Table 1). In the age range of 30-34 years, the percentage of periodontal tissue diseases in patients with hypothyroidism was 85,33%, being numerically but non-significantly higher than in the II group (77,36%).

In patients aged between 35 to 44 years diagnosed with primary hypothyroidism, periodontitis was observed in 94,03% of all examined. However, this indicator was 1,2 times lower (79,07%; $p<0,05$) in this age category of patients without endocrine pathology. Thus, a significantly higher prevalence and intensity of periodontal tissue diseases was found in patients with hypothyroidism, compared to individuals without endocrine pathology in general, as well as in age groups «25-29» and «35-44» years (Table 1).

Furthermore, biochemical studies regarding indicators of mineral metabolism also showed that there were changes of Magnesium content in young people with chronic generalized periodontitis upon primary hypothyroidism.

The indicators of Magnesium in the blood serum and oral liquid were at the lower limit of the norm, and with

Table 2. Indicators of Magnesium content in the capillary blood of the gums and in the oral liquid of the examined patients

Indicator	I group (n=50)		II group (n=50)	
	Chronic generalized I degree periodontitis (N=25)	Chronic generalized II degree periodontitis (N=25)	Chronic generalized I degree periodontitis (N=25)	Chronic generalized II degree periodontitis (N=25)
Mg concentration in blood serum (mmol/l)	0,68±0,06	0,55±0,05*	0,84±0,09	0,82±0,09
Mg concentration in oral liquid (mmol/l)	0,46±0,03**	0,34±0,02***/#	0,72±0,07	0,69±0,06

Notes: Mg – Magnesium; * p<0,05 – a significant difference between the groups I (with hypothyroidism) and II (without hypothyroidism) among patients with II degree periodontitis; ** p<0,01 – a significant difference between the groups I and II among patients with I degree periodontitis; *** p<0,001 – a significant difference between the groups I and II among patients with II degree periodontitis; # p<0,01 – a significant difference between the groups of I and II degree periodontitis among patients with hypothyroidism (group I)

the progression of the process, they exceeded the limits of the normative values. Normally, Magnesium content in the blood serum is 0,65-1,03 mmol/l and in the oral liquid it is 0,41-0,93 mmol/l.

The average estimation of Magnesium content in the blood serum of young people diagnosed with hypothyroidism and affected by chronic generalized periodontitis (0,62±0,05 mmol/l [n=50]) indicated moderate hypomagnesemia, and it was 1,3 times more pronounced than the similar data in people with periodontal disease without hypothyroidism (0,83±0,09 mmol/l [n=50]; p<0,05).

The average value of Magnesium content in the oral liquid of patients affected by chronic generalized periodontitis diagnosed with hypothyroidism was at the lower limit of the norm for this indicator (0,40±0,03 mmol/l [n=50]), and was 1,7 times less than the indicator of people without hypothyroidism (0,70±0,07 mmol/l [n=50]; p<0,01).

Magnesium content in biological fluids of patients affected with chronic generalized periodontitis and diagnosed with thyroid hypofunction is presented in Table 2.

The concentration of Magnesium in the blood serum of people with hypothyroidism, and affected by chronic generalized periodontitis of the degree I (0,68±0,06 mmol/l) was on the border of the lower margin of the norm for this element. With the progression of chronic generalized periodontitis, the amount of Magnesium lowered by 1,2 times to a digital value of 0,55±0,05 mmol/l, which exceeded the norm (numerical, but non-significant difference).

The content of Magnesium in the blood serum did not differ significantly in the groups of the I and II degree periodontitis among patients with or without hypothyroidism (Table 2). At the same time, the Magnesium blood serum level in patients with hypothyroidism and chronic generalized periodontitis of the II degree was significantly lower, as opposed to the corresponding patients without hypothyroidism (0,55±0,05 vs. 0,82±0,09 mmol/l, respectively; p<0,05) (Table 2).

In contrast to the Magnesium concentration in the blood serum, we observed its significantly lower level in oral liquid in patients with chronic generalized periodontitis of the II degree, as compared to less severe disease (I degree) (Table 2). Moreover, patients with hypothyroidism and chronic generalized periodontitis of the I and II degree demonstrated the significantly lower levels of Magnesium in oral liquid, in comparison with the corresponding patients free from hypothyroidism. The content of Magnesium in the oral liquid did not differ significantly in the groups of the I and II degree periodontitis among patients with or without hypothyroidism (Table 2).

DISCUSSION

At the moment, there is no definitive opinion regarding the effect of thyroid hormones on bone metabolism, since the duality of their effects has been proven. Various studies show mixed data concerning metabolic changes in periodontal tissues upon dysfunction of thyroid gland. Bone tissue is a dynamic structure that is constantly renewed and is controlled by numerous systemic and local factors, among which thyroid hormones are of vital importance [8, 10].

Along with Calcium, Magnesium is an essential element for optimal mineral density of the bone. It helps to maintain a normal Calcium level, permanent bone renewal and prevents Calcium loss. With a limited Magnesium supply to the body, the formation of calcium phosphate is inhibited [8].

Researchers [14] revealed an expressive negative impact of thyroid diseases, hypothyroidism in particular, on the development and spread of osteopenic

syndrome (84%). Author [14] noted a slow type of bone remodeling with suppression of both bone formation and bone resorption. The study demonstrated that L-thyroxine replacement therapy in case of hypothyroidism has a protective effect on bone tissue.

According to our gained results, the duration of all phases of bone remodeling increases dramatically by hypothyroidism. This is confirmed by the results of other researchers [9, 14, 15] as well. Osteoblasts influenced by thyroid hormones stimulate the secretion of prostaglandins which increase the activity of osteoclasts.

The results suggest that the intact periodontium was found only in 29 (14,87%) patients in the group I, while the number of patients in the group II with healthy periodontium exceeded by 2 times (n=41 [30,37%]; p<0,01). Accordingly, periodontal disorders were observed in 94,03% of all examined patients affected with primary hypothyroidism aged 35-44 years. In patients without endocrine pathology, however, this indicator was 1,2 times lower (79,07%; p<0,01). It can be assumed that the prevalence of periodontitis was higher in patients diagnosed with hypothyroidism, its progression was faster and more intense compared to the periodontological status of people without endocrine pathology.

CONCLUSIONS

The obtained data indicate a negative effect of primary hypothyroidism on the development of periodontal tissue diseases. Prevalence of periodontal tissue diseases was significantly higher in patients with hypothyroidism as compared to the individuals without it. The study also demonstrated that there was a hypomagnesemia found in young people suffering from chronic generalized periodontitis with hypofunction of the thyroid gland, which worsened with the progression of the pathological process in the periodontium. The average Magnesium content in the oral liquid of patients with chronic generalized periodontitis diagnosed with thyroid hypofunction was at the lower limit of the normative values for this indicator. The detected pathological changes definitely indicate a violation of metabolic processes in periodontal tissues in case of endocrine pathology.

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The study was carried out within the framework of two researches: the research «Clinical effectiveness of complex treatment of diseases of hard dental tissues and periodontium in the population from ecologically bad regions», which was financed from the state budget of Ukraine (state registration number 0118U004144, implementation period 2018-2020); and the research «Complex morpho-functional study and substantiation of the use of modern technologies for the treatment and prevention of dental diseases», which is financed from the state budget of Ukraine (state registration number: 0121U109242, implementation period: 2021 – 2023).

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Zoryana B. Popovych
Ivano-Frankivsk National Medical University
2 Galytska St., 76018 Ivano-Frankivsk, Ukraine
e-mail: zpopovych@ifnmu.edu.ua

ORCID AND CONTRIBUTIONSHIP
Oksana M. Repetska: 0000-0003-2880-9971 A B C
Zoryana B. Popovych: 0000-0003-4472-3748 A D E
Iryna Z. Chubij: 0009-0001-0826-5500 A B C
Mykola M. Rozhko: 0000-0002-6876-2533 E F
Ivan V. Paliichuk: 0000-0002-2375-6367 E F

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

RECEIVED: 11.07.2024
ACCEPTED: 22.10.2024

ORIGINAL ARTICLE

CONTENTS

The role of bone inflammation in the pathogenesis of maxillary sinus cyst formation

Volodymyr O. Shkorbotun[1, 2], Yaroslav S. Nachesa[1], Nataliia I. Levchuk[3]

[1] SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE

[2] STATE INSTITUTION OF SCIENCE «CENTER OF INNOVATIVE HEALTHCARE TECHNOLOGIES» STATE ADMINISTRATIVE DEPARTMENT, KYIV, UKRAINE

[3] STATE INSTITUTION " V.P. KOMISARENKO INSTITUTE OF ENDOCRINOLOGY AND METABOLISM OF THE NATIONAL ACADEMY OF MEDICAL SCIENCES OF UKRAINE", KYIV, UKRAINE

ABSTRACT

Aim: To evaluate the role of maxillary bone inflammation in the formation of MSCs through the comprehensive assessment of histological and radiological research results and the determination of receptor activator of nuclear factor kappa-B (RANKL) in tissue homogenates, nasal secretions, and blood plasma.

Materials and Methods: We included 25 patients aged 20 to 65 with maxillary sinus cysts. We analyzed computed tomography and used enzyme-linked immunosorbent assay with human TNFSF11 (RANKL) in biological samples. Nasal secretion collection and blood sampling were performed before surgery, and tissue samples from the cyst were taken during surgery for further homogenization.

Results: Patients with pseudocysts had deep untreated carious lesions in the upper molars and premolars or adentia on the affected side of the maxillary sinus in 86% of cases, and a higher level of RANKL in the homogenates of removed cysts at 71,7±31,11 pg/μg of protein. In patients with true cysts, deep untreated carious lesions or adentia were not found in our research, and the level of RANKL in the homogenates was lower, at 32,2±5,05 pg/μg of protein ($p<0,001$). We found no statistically significant correlation between the concentration of RANKL in cyst homogenates, blood plasma, and nasal secretions.

Conclusions: Patients with histologically confirmed pseudocysts more frequently exhibited deep carious lesions in the upper molars and premolars than patients with true cysts. Additionally, they demonstrated a significantly higher level of RANKL in the homogenates of removed cysts.

KEY WORDS: diseases of the respiratory system, periodontitis, immunity factors, combined pathology, RANKL, morphology

Wiad Lek. 2024;77(11):2245-2251. doi: 10.36740/WLek/197104 DOI

INTRODUCTION

The primary diagnostic method for maxillary sinus cysts (MSC) is radiological, particularly computed tomography, due to its accuracy and ease of performance [1]. It provides information about the presence of cystic formations, their sizes and locations, and their relationship with the dental row, which is fundamental for surgical preparation and the choice of surgical approach. However, these diagnostic methods do not allow for the assessment of immunochemical processes at the tissue level, which could offer better insights into the pathogenesis of the disease and help refine treatment strategies. Clear recommendations for immediate removal are limited to mucoceles and cysts that exhibit signs of bone tissue destruction or damage to neural and vascular structures [2]. The management strategy for patients with small cysts remains debatable [2, 3]. In this case, using immunoassays as an additional research method may serve as an effective complement to standard diagnostic approaches.

Given the presence of inflammation in cysts, particularly its heightened manifestation in pseudocysts, assessing the involvement of bone tissue in the process can influence treatment strategies [4]. Inflammatory remodeling of bone tissue, especially in carious lesions of upper molars and premolars, can become the primary substrate for MSC inflammation. In such cases, primary treatment should be directed not at the cyst itself but at dental sanitation and the reduction of bone inflammation. Modern immunoassay methods allow the detection of proteins in biological fluids at concentrations as low as 100 picograms per milliliter. This sensitivity enables the detection of proteins, which are markers of internal tissue processes, in external secretions of the body [5]. One of the main markers of bone tissue remodeling, applicable in this case, is RANKL—a ligand of the receptor activator of nuclear factor kappa-B.

RANKL belongs to the tumor necrosis factor superfamily–11 and is the only ligand complementary to the extracellular portion of the RANK receptor. RANKL exists

in two forms: soluble and membrane-bound, with the latter predominating under normal conditions. However, in the presence of pathological changes—either inflammatory or neoplastic—the soluble form emerges [6]. This can occur due to proteolysis of cells or alternative mRNA splicing.

The RANK/RANKL system—the receptor activator of nuclear factor kappa-B and its ligand—participates in many regulatory processes within the human body. The primary function of RANKL is to activate and differentiate osteoclasts responsible for bone tissue resorption [7]. Their role has been demonstrated in the development of both primary malignant tumors from bone and cartilage tissues and the formation of bone metastases from non-bone tumors, particularly in breast cancer [8, 9]. RANK/RANKL activation in postmenopausal osteoporosis enables the use of monoclonal antibodies against RANKL, such as denosumab, for the correction of bone disorders [10]. Furthermore, the role of the RANK/RANKL system in the formation of glandular epithelium in the mammary glands has been proven [11]. In inflammatory processes, RANKL is expressed in B-lymphocytes, T-lymphocytes, epithelial cells, and fibroblasts [6].

Determining the level of RANKL in nasal secretions and directly in cyst tissue will allow for the assessment of local bone microinflammation. A comprehensive assessment of radiological and immunological research methods in MSC cases may simplify the selection of patient management strategies and determine the need for involving dental specialists in the treatment process.

AIM

The aim of the study was to evaluate the role of maxillary bone inflammation in the formation of MSCs through the comprehensive assessment of histological and radiological research results and the determination of RANKL – a protein marker of inflammatory bone tissue remodeling – in tissue homogenates, nasal secretions, and blood plasma.

MATERIALS AND METHODS

Twenty-five patients aged 20 to 65 years (average age [mean (M) ± standard error of the mean (m)] was 31±12,2 years; 14 males and 11 females) with a diagnosis of maxillary sinus cyst (MSC) were included to our study. The diagnosis was established based on general clinical and specialized rhinological examination, including endorhinoscopy and computed tomography (CT) of the head. The examinations were conducted at the Department of Otorhinolaryngology of the Shupyk National Healthcare University of Ukraine at the clinic of Municipal Non-Profit Enterprise «Kyiv City Clinical Hospital No. 9» and the Department of Fundamental and Applied Problems of Endocrinology of the State Institution "V.P. Komisarenko Institute of Endocrinology and Metabolism of the National Academy of Medical Sciences of Ukraine."

Inclusion criteria were patients with cystic formations in the maxillary sinus. Exclusion criteria were patients with CT signs of facial bone resorption unrelated to sinus pathology, a history of skull fractures within the last six months, and a confirmed oncological diagnosis. All patients underwent endoscopic endonasal maxillary sinusotomy with removal of the cystic formation.

Nasal secretion collection and blood sampling were performed before surgery, and tissue samples from the cyst were taken during surgery for further homogenization. The average cyst size was 23,2±1,18 mm. The cysts were located in the alveolar recess of the maxillary sinus.

Nasal secretion collection was carried out according to the method of G. Rasp et al. (1994) [12], modified by M. San Nicolo et al. (2019) [13]. Absorbent cotton ("Hartmann," Germany) in a cone shape, 40 mm in length and approximately 6 mm in diameter, was inserted into the middle nasal meatus under anterior rhinoscopy control for 20 minutes. It was then centrifuged using a filtering mesh at 3000 rpm at +4°C for 15 minutes. The obtained samples were stored at -20°C until immunoassay analysis. Blood sampling was performed with disposable systems, followed by centrifugation at 3000 rpm for 10 minutes to separate the plasma.

The MSC cyst membrane tissue was weighed on electronic scales (SPU 123, "OHAUS Corporation," China), placed in a 2,0 ml plastic tube, and phosphate buffer ("Sigma," USA) was added at a 1:10 ratio. Tissue homogenization was performed by intensive shaking, using metal beads, on a TissueLyser II device ("QIAGEN," Germany). After homogenization, the suspension was centrifuged at 10 000 rpm for 10 minutes at 4°C. The supernatant was collected and frozen at -80°C for further biochemical and immunoassay analysis. Protein concentration in the solution for further calculation of immunoassay results was determined by the Bradford method [14]. This method is based on the change in the light absorption capacity of the Coomassie dye-protein complexes compared to its pure form, allowing for quantitative protein content determination.

For detection, a colorimetric method was used with a sensitivity of <10 pg/ml and a range of 78–5000 pg/ml for human TNFSF11 (RANKL) using an ELISA kit ("Abcam," UK). The ELISA method consisted of the following steps: adding 100 µl of the sample to the appropriate wells and incubating at 37°C for 90 minutes, washing

Table 1. Characteristics of comparison groups

Patient groups	Number of patients (n)	Age, years (M±m)	Gender, n (%)	
			Women	Men
Group I – patients with retention cysts	11	36±4,1	4 (36%)	7 (64%)
Group II – patients with pseudocysts	14	33±2,7	7 (50%)	7 (50%)
Total	25	p=0,181	p=0,250	

Table 2. Condition of the teeth in patients with maxillary sinus cysts based on computed tomography data

Condition of the teeth of the upper jaw	Group I (n=11)	Group II (n=14)	p*
Healthy teeth, n (%)	5 (46)	1 (7)	0,057
Superficial caries (without pulp involvement), n (%)	4 (36)	0**	0,026
Deep caries (with pulp involvement) fully sanitized, n (%)	2 (18)	1 (7)	0,565
Deep caries (with pulp involvement) not sanitized or partially sanitized, n (%)	0***	8 (57)	0,003
Adentia, n (%)	0***	4 (29)	0,105

Notes: * – the Fisher`s exact test; ** – 95% CI [0-13%]; *** – 95% CI [0-16%].

the plate, then adding 100 µl of biotinylated antibody and incubating at 37°C for 60 minutes. The plate was washed three times for 1 minute with 300 µl of 0.01M PBS, followed by the addition of 100 µl of working ABC solution and incubation at 37°C for 30 minutes. The plate was then washed five times with 300 µl of 0.01M PBS for 1–2 minutes each, 90 µl of prepared TMB Color Developing Agent was added, and the plate was incubated at room temperature in the dark for 20 minutes. Finally, 100 µl of TMB stop solution was added, and the results were read at 450 nm within 30 minutes.

All patients were informed about the study's purpose, provided informed consent, and were informed of their right to withdraw at any stage. The study was conducted in compliance with the Helsinki Declaration and Ukrainian legislation.

Statistical analysis was performed by the use of StatPlus software v 8.0.4.0. Quantitative data were presented as M ± m, and being compared by the the Mann-Whitney U test. Qualitative parameters were presented as absolute and relative (%) frequency (with 95% confidence interval [CI] in the certain cases), and being compared by the Pearson's χ^2 test or Fisher's exact test. Correlation analysis was performed by the use of the Pearson correlation coefficient (r). For all tests, a statistically significant difference was considered at $p<0,05$.

RESULTS

All patients, based on the histological examination of the cyst membranes obtained during surgery, were divided into two groups. Group I included patients with retention cysts, characterized by the presence of a three-layered wall—connective tissue septum lined with epithelium on both sides, maintaining the structure of columnar epithelium with possible areas of hyperplasia and metaplasia into the stratified epithelium and possible infiltration by inflammatory cells. Group II consisted of patients with confirmed "false" cysts (pseudocysts), characterized by a two-layered wall that, in certain areas, completely lost the epithelial component and was represented only by connective tissue with significant infiltration by inflammatory cells. The characteristics of the groups are presented in Table 1.

As can be seen from Table 1, the studied groups were comparable by age and sex.

The analysis of the condition of the teeth and the bone system of the upper jaw and the corresponding sinuses was carried out based on the patient's computed tomography scans. The principal criterion for the impact on the maxillary sinus was the involvement of the deep structures of the premolars and molars and the state of their treatment on the affected sinus side.

The data on the distribution of different teeth status in the studied groups are summarized in Table 2. The data presented in Table 2 indicate that patients with histologically confirmed pseudocysts (Group II) significantly more often demonstrated the inadequately treated deep lesions of the upper premolars and first and second molars on the side of the affected maxillary sinus. In general, patients with pseudocysts (Group II) were characterized by the higher frequency of significant pathological teeth changes (including not sanitized or partially sanitized deep caries [with pulp involvement] and adentia), as compared to the Group 1: 86% (95% CI [61-99%]) vs. 0 (95% CI [0-16%]), respectively ($p<0,001$).

To confirm the theory of bone involvement in the inflammatory process during the formation of max-

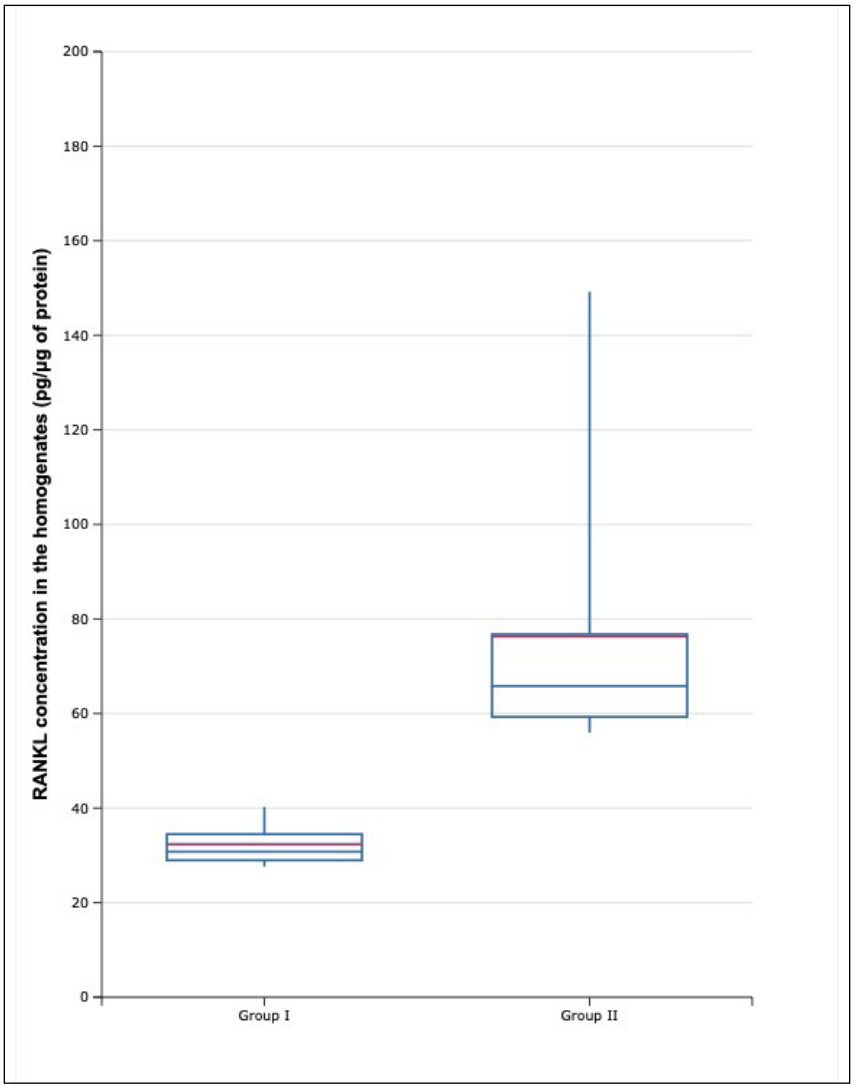

Fig. 1. Comparison of the concentration of the bone inflammation marker protein RANKL in the homogenates of retention cyst (Group I [n=11]) and pseudocysts (Group II [n=14]) of the maxillary sinus (box plot).
*p<0,001.

illary sinus cysts, we performed an enzyme-linked immunosorbent assay (ELISA) on the homogenates of the removed cysts to determine the level of RANKL—a protein marker of bone tissue remodeling (Fig. 1).

Fig. 1 shows that the concentration of RANKL in patients with pseudocysts was significantly higher (71,7±31,11 pg/µg protein) than in patients with cysts (32,2±5,05 pg/µg protein) (p<0,001), supporting the hypothesis of bone inflammation in the formation of this type of cysts.

With respect to the correlation analysis results, we did not observe any statistically significant correlations between the concentration of RANKL in cyst homogenates, blood plasma and nasal secretions (Fig. 2 and Fig.3).

DISCUSSION

The pathogenic impact of caries spreading to the deep structures of the teeth, which are in contact with the roots of the maxillary sinus, involves the inflammatory process of the periodontal tissue and surrounding bone tissue. The pulp, due to its structure as loose connective tissue, unlike the dense enamel, determines the speed and generalization of destruction and inflammation when involved [15]. The group of patients with retention cysts (Group I) mainly had healthy teeth or adequately treated and filled canals. Tooth loss, the terminal stage of the carious process in the tooth, was only found in patients with pseudocysts (Group II).

The involvement of deep tooth structures and bone tissue of the maxillary sinus in the inflammatory process is confirmed by the significant increase in the concentration of RANKL in the homogenates of pseudocysts compared to retention cysts, suggesting a dental cause as primary in the formation of pseudocysts.

Considering the above facts, the deep lesions of the teeth adjacent to the maxillary sinus, and the increase in inflammatory bone tissue remodeling markers in these same patients, we can conclude that the primary substrate for the spread of inflammation is dental pathology, which may influence the management tactics for these patients.

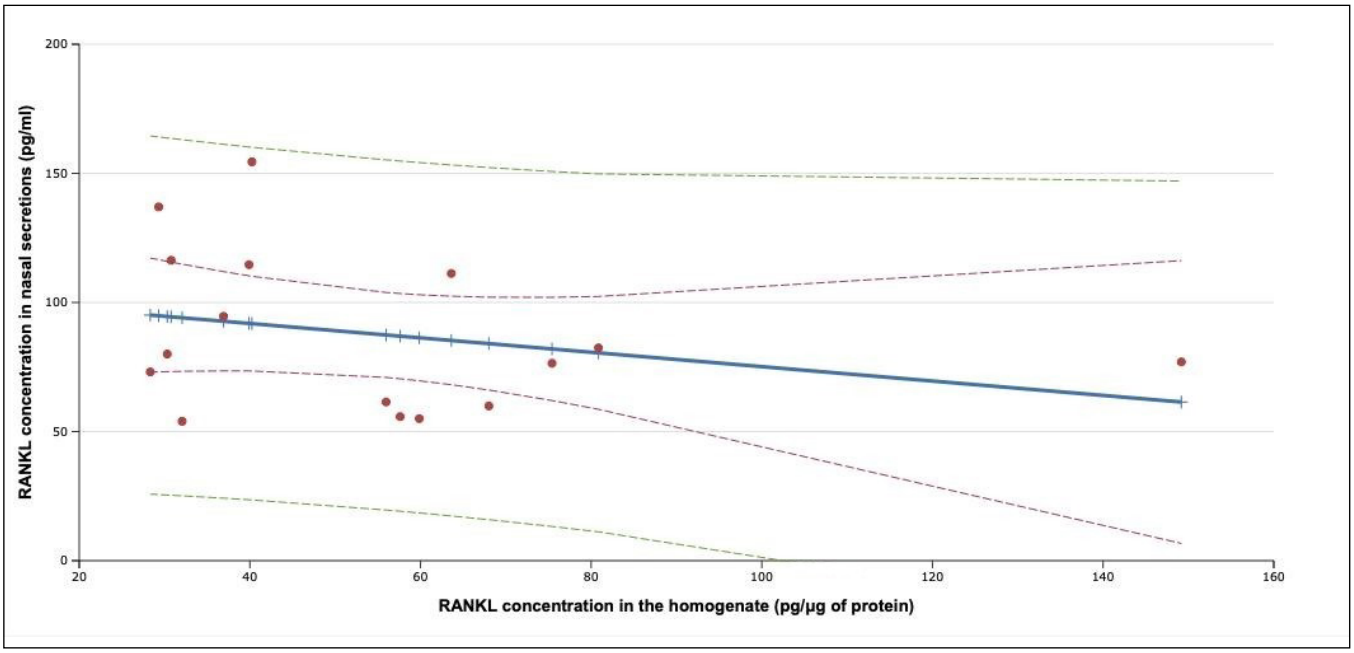

Fig. 2. Scatter plot of the concentration of the maxillary sinus protein marker in tissue homogenates and nasal secretions (r=0,277; p=0,299 [N=16]).

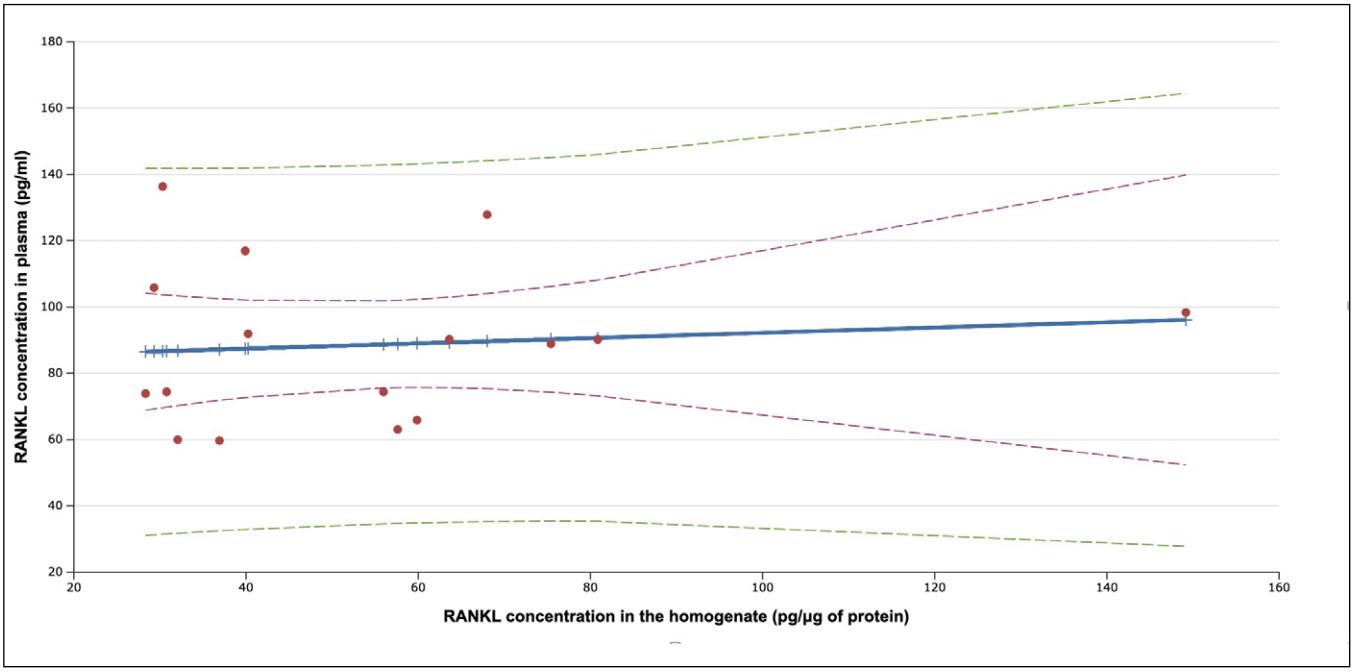

Fig. 3. Scatter plot of the concentration of the bone inflammation marker protein RANKL in patients with maxillary sinus cysts in tissue homogenates and plasma (r=0,103; p=0,706 [N=16]).

The absence of correlation between the concentration of RANKL in cyst homogenates and nasal secretions may be related to the mucus formation mechanism in the nasal cavity. Since the process involves the mucous glands of all paranasal sinuses, the affected maxillary sinus, even if it retains functioning mucous glands, produces relatively little mucus compared to the entire mass of nasal and paranasal sinus mucosa [16]. Moreover, according to the study by M. Tos and C. Mogensen (1979), the largest mass of mucous glands is located on the medial wall of the maxillary sinus [17]. Therefore, the concentration of inflammatory proteins in nasal mucus is not representative of their concentration in the affected tissues.

The concentration of RANKL in blood plasma depends on many factors and may increase in various pathological processes, such as trauma, tumors, autoimmune processes, and degenerative diseases of the musculoskeletal system [18]. However, the impact of a localized process in the upper jaw on the homeostasis of protein markers is insufficient for their correlated fluctuations in plasma.

CONCLUSIONS

1. The unsanitized carious lesions of the deep structures of the upper premolars and molars or adentia on the affected side are intrinsic to patients with histologically confirmed pseudocysts and observed in 86% of cases. In contrast, no such findings were noted in the group with verified retention cysts (p<0,001).
2. Evaluation the concentration of the bone inflammation remodeling marker protein RANKL in the homogenates of cystic formations confirmed higher bone inflammation activity in patients with pseudocysts and deep tissue involvement, that allows identifying the dental cause of pseudocyst formation as primary.
3. No correlation was found between the concentrations of RANKL in cyst homogenates, nasal secretions, and blood plasma, likely due to the localized and minor activity of the process at the organism level.
4. The RANKL test of the maxillary sinus cyst wall can be recommended to determine the value of the odontogenic factor in the development of the cyst.

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The study was conducted as a fragment of the complex scientific projects of the Otolaryngology Department of Shupyk National Healthcare University of Ukraine «Improving methods of diagnosis and treatment of patients with pathology of the nose and paranasal sinuses,» (state registration number 0122U200964; term: 2022-2026).

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Yaroslav S. Nachesa
Shupyk National Healthcare University of Ukraine
9 Dorohozhytska St., 04112 Kyiv, Ukraine
e-mail: doctor.yaroslavnachesa@gmail.com

ORCID AND CONTRIBUTIONSHIP
Volodymyr O. Shkorbotun: 0000-0003-0769-6242 A E F
Yaroslav S. Nachesa: 0000-0003-0512-3029 B C D
Nataliia I. Levchuk: 0000-0003-0482-5176 B C

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

RECEIVED: 04.07.2024
ACCEPTED: 23.10.2024

ORIGINAL ARTICLE

CONTENTS

Low anterior resection syndrome in distal rectal cancer patients and its correction methods

Bogdan V. Sorokin[1], Sergiy O. Zlobenets[1], Vladyslav O. Yakovenko[2], Volodymyr Yu. Pyrogovsky[1], Anna O. Milyanovska[1]

[1]SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE

[2]BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

ABSTRACT

Aim: To evaluate and compare the functional outcomes and quality of life in distal rectal cancer patients after low anterior resection with the formation of a transverse double-fold reservoir (TDFR) and without it.

Materials and Methods: The retrospective study enrolled 80 patients with distal rectal cancer: 40 patients who underwent low anterior resection with the formation of a TDFR, and 40 patients with the formation of a colorectal anastomosis (control group). The follow-up period was 10-60 months, with a median follow-up of 36 months after primary surgery. Functional outcomes were assessed at 3, 6, 12, and 24 months after stoma closure using anal manometry, balloonometry, and questionnaires including LARS Score, Wexner, and FIQL.

Results: The 5-years overall survival rate was comparable in main and control groups (85% and 79%, respectively). Rectal sensitivity threshold, urge to defecate and maximum tolerated volume were higher in the TDFR group at 3, 6, and 12 months post-operatively. Manifestations of low anterior resection syndrome (LARS) were more frequently observed in the control group. Quality of life, as assessed by the FIQL questionnaire, was significantly better in the TDFR group. The internal sphincter function was better preserved in the TDFR group, in contrast to control group.

Conclusions: The TDFR formation in patients with distal rectal cancer after low anterior resection leads to improved anal continence within 12 months post-operatively, reduction of LARS manifestations, and enhancement of quality of life for patients while maintaining oncological radicality.

KEY WORDS: rectal cancer, colonic reservoir, low anterior resection syndrome, transverse double-fold reservoir, videocolonoscopy

Wiad Lek. 2024;77(11):2252-2260. doi: 10.36740/WLek/197105 DOI

INTRODUCTION

Colorectal cancer (CRC) ranks among the top malignancies in terms of incidence rates globally, significantly surpassing most other cancer locations [1, 2]. In recent years, according to statistics from the International Agency for Research on Cancer (IARC), nearly 1 million new cases of this type of cancer are detected each year [2]. Specifically, the incidence of CRC in the European Union is reported at 21,5 per 100 000 population, with a mortality rate of 12,8 per 100 000 population [2, 3]. According to the National Cancer Registry of Ukraine, the incidence rate in 2021 was 18,6 per 100 000 population [4]. In Ukraine, the standardized mortality rate from CRC in 2021 was 10,3 per 100 000 population [4]. In 60-65% of cases, CRC is localized in the distal sections, with more than 30-35% occurring in the lower ampullary region of the rectum [4-6]. Currently, surgical treatment is the primary approach for patients with tumors located in the distal rectum [6, 7]. Radiation and chemotherapy are used as part of a comprehensive treatment strategy, complementing surgical interventions [8, 9]. According to reviews of contemporary literature, several main directions in the surgical treatment of distal rectal cancer can be identified: 1) the application of total mesorectal excision (TME); 2) expanding indications for sphincter-preserving surgeries; 3) developing and implementing surgical interventions aimed at modeling the reservoir function of the rectum [3, 5, 6, 8, 10].

The problem of surgical rehabilitation for patients who have undergone surgery for distal rectal cancer is of particular relevance [10-18]. The introduction of TME with the formation of low anastomoses is a fundamental component of modern rectal surgery. However, the loss of the rectal ampoule leads to worsened functional outcomes of the operation. Typically, patients experience frequent bowel movements (up to six times a day), prolonged incomplete evacuation, imperative false urges to defecate, and varying degrees of anal incontinence [18]. The primary pathogenic element of these disturbances is the loss of reservoir and

evacuatory function of the rectum [8]. In the literature, this symptom complex is referred to as low anterior resection syndrome (LARS) [9]. Various studies [10-12] indicate that the incidence of LARS ranges from 25% to 50%, depending on classification and follow-up duration. In addition to the loss of the rectal reservoir function, other causes leading to LARS include anatomical and functional damage to the anal sphincter, resulting from both direct trauma to the sphincter [13] and nerve damage [14]. Injury to the autonomic nerves results in a decreased recto-anal inhibitory reflex (RAIR) [15]. The presence of RAIR allows receptors in the anal transitional zone to distinguish the nature of the rectal contents (formed stool, gases). A decrease in this reflex is directly related to the function of continence [15]. Thus, not only the loss of the ampulla, but also the integrity of the transition zone of the anal canal are important components in the maintaining of anal incontinence. One of the primary methods for preventing LARS (low anterior resection syndrome) is the modeling of the rectum's reservoir function. Since the emergence of LARS in the mid-1980s up to the present, attempts have been made at surgical correction of this disorder aimed at compensating for the reservoir function of the lost rectal ampulla in combination with the principles of oncological radicalism [1, 16, 17]. Since 1986 R. Park and F. Lasorthes published studies on forming a pelvic colonic reservoir in the shape of the letter J, using the colon based on the prototype of the small intestinal reservoir [14, 17]. Currently, there are several main types of reservoirs and their modifications (Fig. 1) [1].

AIM

The aim of the study was to evaluate and compare the functional outcomes and quality of life in distal rectal cancer patients after low anterior resection with the formation of a transverse double-fold reservoir (TDFR) and without it.

MATERIALS AND METHODS

A retrospective comparative analysis of the surgical treatment outcomes was conducted on 80 patients with distal rectal cancer who underwent surgery between 2018 and 2022. The inclusion criteria for the study were as follows: tumor localization in the lower-middle rectal ampulla, with a distance of 2-3 cm from the dentate line; disease stage T0-4N0-2M0-1; no invasion of the internal sphincter or levator ani muscle; and adenocarcinoma histotype. Patients with tumor localization in the lower rectal ampulla accounted for 42 (52%) cases, while in 38 (48%) cases, the tumor was located in the middle rectal ampulla. Among the operated patients, 54 (67%) were men and 36 (33%) were women, aged between 25 and 74 years. The average age of the patients was (mean [M] and standard deviation [SD]) 62 ± 5,3 years. For adequate study, a standard diagnostic examination complex was used, including pelvic magnetic resonance imaging with contrast, transrectal ultrasound, chest, abdominal, and pelvic computed tomography, videocolonoscopy, rectosigmoidoscopy with biopsy, sphincterometry to assess the function of the rectal sphincter apparatus. Neoadjuvant chemoradiotherapy (CRT) was administered to 68 (85%) patients according to treatment standards: fractionated radiotherapy of 45-50 Gy in 1,8-2,0 Gy fractions with capecitabine modification (850 mg/m^2). Surgery was performed 6-8 weeks after CRT.

The 80 patients were divided into two groups: 1) the main group included 40 patients who underwent low anterior resection with the formation of a reservoir with TDFR; 2) the control group included 40 patients who underwent standard low anterior resection with end-to-end colorectal anastomosis. Among baseline characteristics, the studied groups were compared by age, sex, body mass index (BMI) and CR-POSSUM score (prediction of the expected in-hospital or 30-day mortality).

Low anterior resection of the rectum was performed according to standard principles, with high ligation of the inferior mesenteric artery and D2 lymph node dissection. In all cases, the procedure was accompanied by total mesorectum excision with the formation of a protective stoma (95% – transverse colostomy, 5% – ileostomy). Gastrointestinal continuity was restored using a circular stapling device. In the main group, to correct LARS, low anterior resection was supplemented by the formation of a TDFR using the original technique [19] see Fig. 2.

The method involved a longitudinal incision of the serosa of the colon without opening the lumen, located above the planned anastomosis. The transverse suture line was formed with three guiding sutures and two continuous sutures, creating two transverse folds in the colon. The first fold was positioned 4-5cm above the anastomosis, with the second fold 4-5 cm from the first. The time required to create the reservoir was approximately 15±5 minutes.

All patients underwent proctoreservoirography before the restorative surgery (see Fig. 3). In both groups, the preventive stoma was closed after 3 months, provided there were no inflammatory processes in the rectum and no signs of anastomotic insufficiency. In cases of diversion colitis, the closure of the colostomy (coloplasty) was postponed for 1-2 months, with corresponding

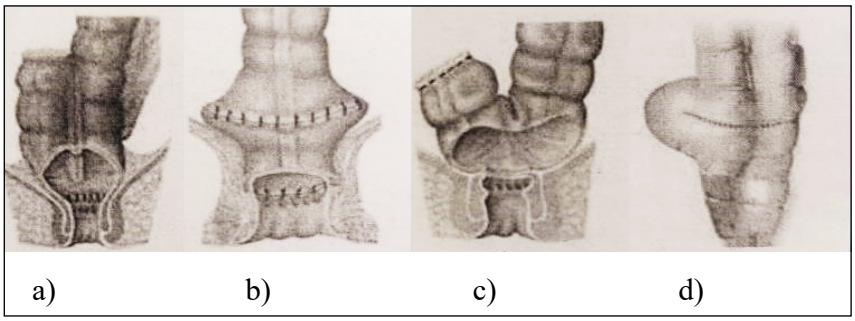

Fig. 1. Main types of colonic reservoirs: a) J-shaped reservoir; b) transverse reservoir; c) side-to-end anastomosis reservoir; d) C-shaped reservoir [1].

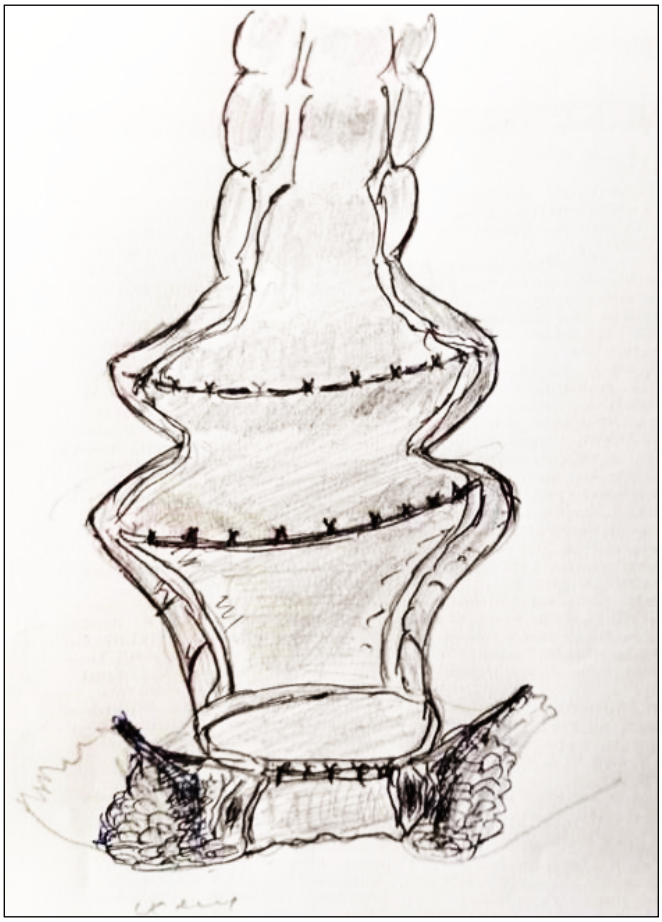

Fig. 2. The original technique of TDFR formation [19].

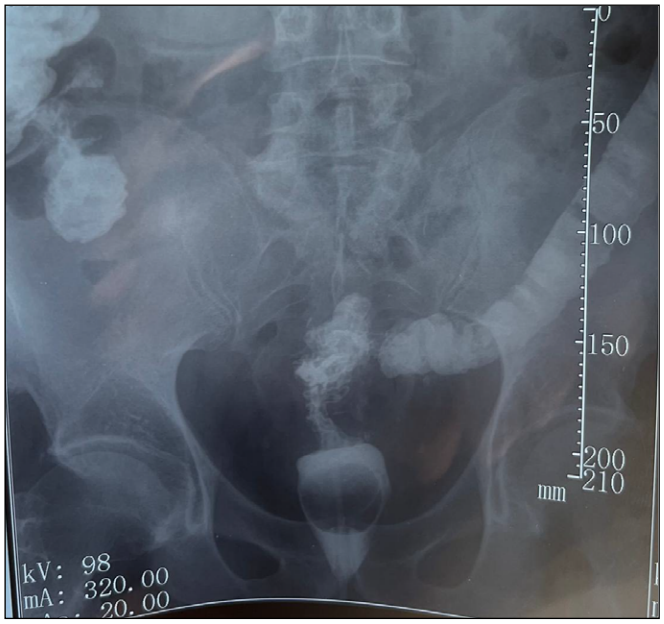

Fig. 3. TDFR on proctography.

anti-inflammatory therapy using 5-aminosalicylic acid medications.

The measurement of pressure in the anal canal was conducted by the use of the THD-Anopress device. To study the volume of the newly created reservoir, balloon manometry was performed.

The functional outcomes of surgical treatment were assessed by the use of the following questionnaires: LARS Score [2, 9, 18] and Cleveland Clinic Continence Score (Wexner score) [3].

The elements of the LARS Score include: gas incontinence, liquid stool incontinence, increased frequency of defecation, frequent tenesmus during defecation, and urgency of defecation. The scoring ranges from 0 to 42 points, where: 0-20 points indicate no symptoms of LARS; 21-29 points indicate moderately expressed LARS; and 30-42 points indicate severely expressed LARS [2, 9, 18]. The Cleveland Clinic Continence Score (Wexner score) evaluates results based on total points (0 points indicate complete control, and 20 points indicate complete anal incontinence) [3].

The quality of life assessment was conducted using the Fecal Incontinence Quality of Life (FIQL) questionnaire [3].

All operated patients were followed up for 10 to 60 months, with a median follow-up of 36 months after the primary surgery.

The statistical analysis was performed by the use of IBM SPSS Statistics v. 23 and MedCalc v. 22.009. The continuous variables were presented as mean and standard deviation. The qualitative parameters were presented as absolute and relative (%) frequency. The comparison of the studied groups was performed by the use of the Mann-Whitney U-test (for continuous variables) and χ^2 test or Fisher`s exact test (for qualitative data). The clinical effect size was assessed by the odds ratio (OR) (with 95 % confidence interval [CI]). The Cox proportional hazards model was used to compare the survival rates in the studied groups

Table 1. The baseline characteristics of the studied groups

Parameters	Main group N=40	Control group N=40	p
Age, years	65,4 ± 12,3	67,3 ± 11,2	0,478
Males, n (%)	24 (60,0)	23 (57,5)	0,820
BMI, kg/m^2	24,3 ± 5,1	25,1 ± 6,2	0,184
CR-POSSUM, points	6,3 ± 1,5	6,2 ± 1,8	0,788
Cases of chemoradiation, n (%)	36 (90,0)	34 (85,0)	0,499

Table 2. The distribution of patients by TNM stages (8th edition, 2017 [20])

TNM stage	Main group N=40	Control group N=40	P
Stage I (pT1N0M0), n (%)	1 (2,5)	2 (5,0)	0,939
Stage II (pT2N0M0), n (%)	4 (10,0)	2 (5,0)	
Stage II (pT3N0M0), n (%)	15 (37,5)	14 (35,0)	
Stage II (pT4N0M0), n (%)	2 (5,0)	2 (5,0)	
Stage III (pT2-4N1-2M0), n (%)	16 (40,0)	17 (42,5)	
Stage IV (pT1-4N0-2M1), n (%)	2 (5,0)	3 (7,5)	

Table 3. The postoperative complications in the studied groups

TNM stage	Main group N=40	Control group N=40	P
Failure of anastomosis, n (%)	2 (5,0)	3 (7,5)	0,488
Anastomosis stricture (after 6 months), n (%)	1 (2,5)	3 (7,5)	
Necrosis of the small intestine, n (%)	0	1 (2,5)	
Without postoperative complications, n (%)	37 (92,5)	33 (82,5)	
Complications in total*, n (%)	3 (7,5)	7 (17,5)	0,311

Note: * – OR 0,38 [95% CI 0,09-1,60]; p=0,188.

Table 4. The overall survival in the studied groups

Study groups	12 months	24 months	36 months	48 months	60 months
Main group (%)	100	96	93	88	85
Control group (%)	100	95	92	87	79

(with the assessment of hazard ratio [HR] with 95 % CI). A 2-tailed p<0,05 was considered statistically significant.

The treatment of patients was carried out in accordance with the Helsinki Declaration of the World Health Organization association, ESMO guidelines and the recommendations of the ethical committee of the medical institution, which required informed consent from the patient for the application of treatment and diagnostic methods.

RESULTS

The studied groups were comparable by the certain baseline characteristics (Table 1), as well as the distribution of patients by TNM stages (Table 2). The timing of colostomy closure ranged from 3 to 4 months in both groups.

The immediate postoperative complications are summarized in Table 3. The postoperative complications occurred in 7,5% of cases in the main group and in 17,5% of cases in the control group, and were managed conservatively (OR 0,38 [95% CI 0,09-1,60]; p=0,188). There were no cases of bladder atony and relaparotomy, as well as no postoperative mortality in either group.

The data on overall and recurrence-free survival are shown in Tables 4 and 5, and in Figure 4. At 5-years follow-up, the overall survival in the main group was 85%, as compared to 79% in the control group (HR 0,74 [95% CI 0,45-2,91]; p = 0,732 (Table 4, Fig. 4). Moreover, the recurrence-free survival in the main group was 70%, while in the control group it was 65% (HR 0,86 [95% CI

Table 5. The recurrence-free survival in the studied groups*

Study groups	12 months	24 months	36 months	48 months	60 months
Main group (%)	100	95	85	78	70
Control group (%)	100	93	85	76	65

Note: * HR 0,86 [95% CI 0,31-2,51); p=0,809.

Table 6. The Indicators of the Wexner scale in the studied groups

Groups	Observation term			
	3 months	6 months	12 months	24 months
Main group, points	11,7±2,5	10,2±1,9	7,3±1,2	6,2±1,1
Control group, points	12,1±2,0	11,1±2,0	8,4±1,3	7,3±1,3
p	0,432	0,042	0,002	0,001

Table 7. The quality of life in patients at 6- and 12-months follow-up after colostomy closure in the studied groups

FIQL scales, points	Main group		Control group		p	
	6 months	12 months	6 months	12 months	6 months	12 months
Lifestyle	2,8 ± 0,6	3,1 ± 0,5	2,5 ±0,6	2,6 ± 0,7	0,028	0,004
Experiencing stress	2,5 ± 0,6	2,7 ± 0,6	2,8 ±0,7	2,9 ± 0,7	0,043	0,174
Depression/ self-esteem	3,0 ± 0,6	3,1 ± 0,5	2,5 ±0,5)	2,6 ± 0,6	0,001	0,001
Shyness	3,2 ± 0,5	3,4 ± 0,6	2,8 ± 0,6	2,9 ± 0,6	0,002	0,004

Table 8. The average pressure in the anal canal at rest in the studied groups, mmHg

Term	Main group	Control group	p
Before closing the stoma	45 ±6	31 ±7	0,001
After closing the stoma			
In 1 month	28 ±6	19 ±6	0,001
In 3 months	34 ±7	24 ±5	0,001
After 6 months	39 ±7	28 ±6	0,001
In 12 months	42 ±7	34 ±6	0,001

Table 9. The volume of the newly created reservoir by the use of balloon manometry in the studied groups

Term after closing the stoma	Maximum portable volume, cm^3		p
	Main group	Control group	
After 1 month	115 ± 18	110 ± 17	0,205
After 3 months	140 ± 12	140 ± 14	0,990
After 6 months	185 ± 14	160 ± 15	<0,001

0,31-2,51); p=0,809 (Table 5). Thus, the studied groups were comparable by the overall and recurrence-free survival.

At 5-years follow-up, the local recurrence rate was 10% (4 cases) in the main group and 15% (6 cases) in the control groups (OR 0,63 [95% CI 0,16-2,43]; p=0,502).

When comparing the degree of LARS manifestations, according to the LARS Score questionnaire, from 3 to 12 months after colostomy closure, the following results were obtained (see Fig. 5).

In patients from the main group, better results were obtained compared to the control group. Specifically, the frequency of defecations was lower in the main group by 12 points after 3 months. This trend persisted at 6 and 12 months, with score differences favoring the main group of 10 and 12 points, respectively. One year after colostomy closure, the advantage of the main group over the control group in score assessment was 2,3 times greater. The differences were statistically significant for all follow-up periods (Fig. 5).

The results of the functional assessment of stool and gas retention (Wexner score) are presented in Table 6.

According to the Cleveland Clinic Continence Score (Wexner score) data, 48% of patients showed no significant impairment of the anal sphincter function. In 42% of patients, there were signs of solid stool incontinence

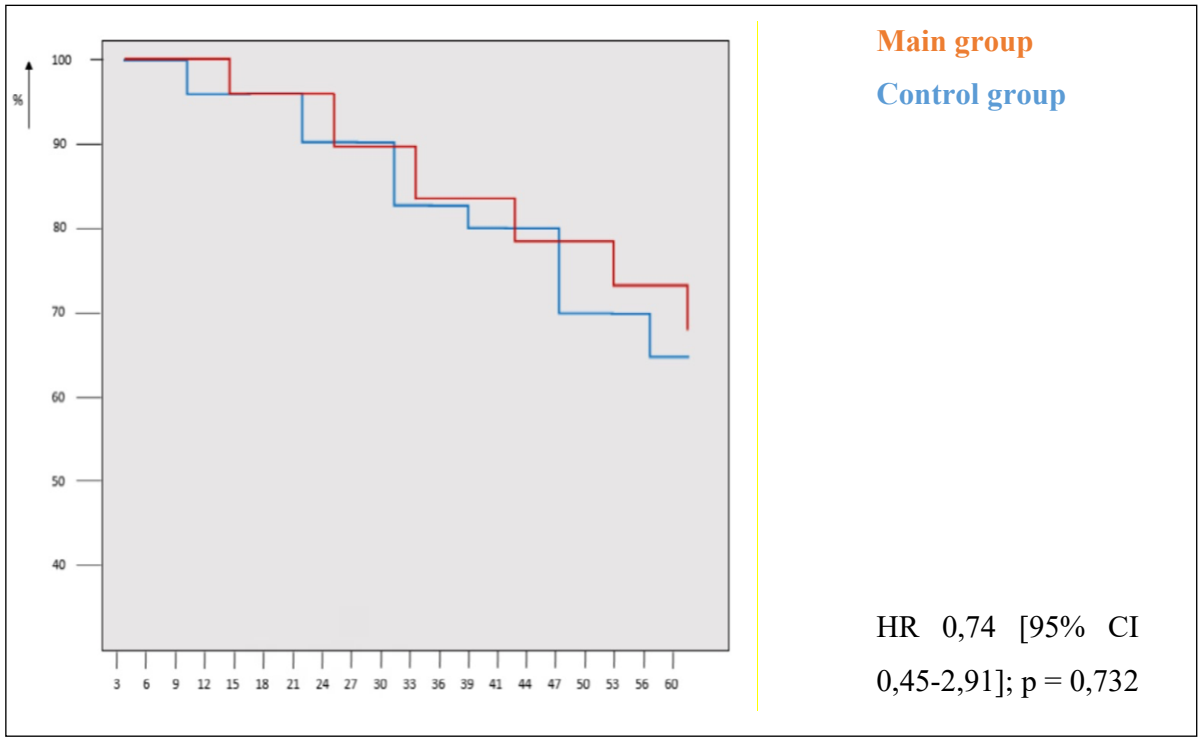

Fig. 4. The overall survival in the studied groups.

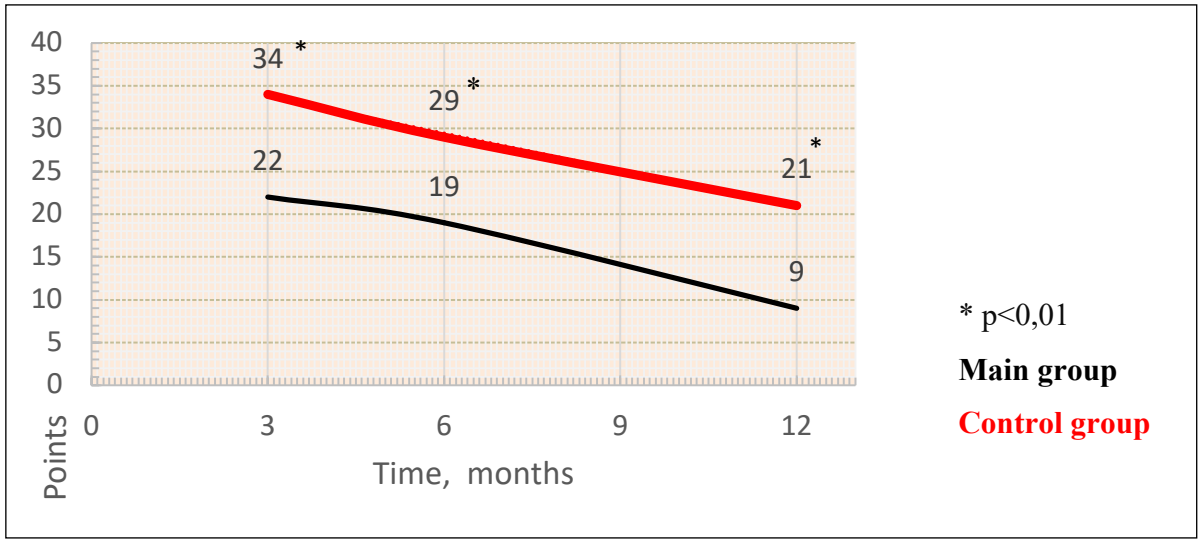

Fig. 5. Functional results according to the LARS Score scale.

and an inability to control bowel movements over the course of a year. During the first year, both groups exhibited gradual improvement in anal sphincter function, with more satisfactory results observed in the main group (Table 6).

The results of the quality of life assessment by the FIQL questionnaire are presented in Table 7.

Based on the obtained results, there is an observed improvement in quality of life in both groups over the period of 6 to 12 months, with a more pronounced dynamics in the main group. In particular, the adaptation period progresses more quickly, with patients being less vulnerable to depression and more psychologically stable (Table 7).

The functional advantages of the reservoir construction are confirmed by the data on specialized examination methods. The results of the measurement of pressure in the anal canal, conducted using the THD-Anopress device, are presented in Table 8. According to the obtained results, the differences between the groups were statistically significant in all follow-up periods.

When observing the patients after stoma closure over various periods, it is noted that the function of the internal sphincter is less impaired in the main group

compared to the control group, particularly evident in the first months following stoma closure. After 3 months, the internal sphincter gradually adapts and increases the pressure for stool retention by 10 mmHg, which is observed again at 6 months (11 mmHg) and at 12 months (8 mmHg) (Table 8).

In patients of the main group, 6 months after colostomy closure, there is an observed increase in the volume of the maximum tolerable urge by 70 cm^3, indicating partial restoration of the reservoir function of the lowered intestine (Table 9).

DISCUSSION

Various models of the rectal ampoule are utilized for the treatment of the LARS. It is known that the introduction of a J-shaped reservoir from the colon has reduced the intensity of LARS, but when assessing the separate outcomes, patients experience problems with the evacuation of intestinal contents due to excessive stretching of the reservoir walls and increased capacity, requiring additional cleansing procedures [10-18]. In 1999, considering the disadvantages of the aforementioned reservoir, K. Z'Graggen et al. developed a reservoir by longitudinal incision and then transverse suturing with a continuous suture [16]. The operation ends with the formation of an end-to-end anastomosis using manual or mechanical sutures. Some advantages of this reservoir include ease of formation, applicability in obese patients, and a short mesentery of the colon, although the volume of the neo-rectum is, in our opinion, not substantial [19]. A. Heriot et al. [21] conducted a meta-analysis that included data from 35 studies involving 2240 patients and found no significant differences in functional indicators between groups with a J-shaped reservoir and a coloplastic reservoir.

To date, several studies compare the functional and physiological results of these reservoirs, showing that coloplastic reservoirs demonstrate better evacuation function in the early postoperative period. In 2003, M. Machado et al. [22] proposed an end-to-end colorectal anastomosis, which, according to the authors, is technically simpler compared to the J-shaped reservoir and is associated with a lower frequency of anastomotic strictures. According to the results of five randomized studies (C. Brown et al. [23]), no significant difference was found in functional indicators between the J-shaped reservoir and the lateral anastomosis. Thus, the pursuit of quality of life, especially after low anterior resections that involve the loss of the rectal ampoule and its reservoir function, remains an important and unresolved issue [21].

Therefore, LARS is one of the negative aspects of surgical intervention on the rectum, and despite the variety of surgical correction methods, the question of choosing a method for modeling the rectal ampoule remains open. In our opinion, the proposed a reservoir with two transverse fold, based on the obtained surgical, oncological, and functional results, is a preferred method for patients after low anterior resections of the rectum. Its advantages include the absence of lumen opening of the colon, simplicity of formation, short formation time, and adequate length of the intestinal extension (neo-rectum).

CONCLUSIONS

1. The formation of TDFR does not increase a number of postoperative complications and does not affect the survival of distal rectal cancer patients after low anterior resection, as compared to patients with the formation of a colorectal anastomosis.
2. The assessment of the quality of life and functional results of distal rectal cancer patients showed a significant difference between groups. At 1-year follow-up, the LARS Score was 2,3 times higher in the TDFR group as compared to the control group. The FIQL scale («lifestyle», «depression/self-esteem» and «shyness» domains) favored the TDFR group at 6- and 12-months follow-up. The assessment of reservoir function by balloonometry indicated an increase in the volume of the maximum tolerable urge by 70 cm^3 in patients of the main group at 6-months follow-up after colostomy closure.

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The work was carried out within the framework of the research topic of the Shupyk National Healthcare University of Ukraine «Optimization diagnostics and treatment of different malignant patients» (state registration № 0117U002467; term: 2017-2026).

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Bogdan V. Sorokin

Shupyk National Healthcare University of Ukraine
9 Dorogozhytska St., 04112 Kyiv, Ukraine
e-mail: viktorovichbogdan50@gmail.com

ORCID AND CONTRIBUTIONSHIP

Bogdan V. Sorokin: 0000-0003-0511-7550 A B C D E F
Sergiy. O. Zlobenets: 0009-0009-7762-0041 B C D
Vladyslav O. Yakovenko: 0000-0002-2419-6971 D E F
Volodymyr Yu. Pyrogovsky: 0000-0002-7453-991X A B E
Anna O. Milyanovska: 0000-0003-4757-8291 B C D

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

RECEIVED: 10.07.2024
ACCEPTED: 27.10.2024

ORIGINAL ARTICLE

CONTENTS

Modern combined approach to the surgical treatment of patients with post-traumatic tympanic membrane perforation due to blast injuries and eustachian tube dysfunction

Anastasiia K. Tarasenko, Ilona A. Srebniak

STATE INSTITUTION «INSTITUTE OF OTOLARYNGOLOGY NAMED AFTER PROF. O.S. KOLOMIYCHENKO OF THE NATIONAL ACADEMY OF MEDICAL SCIENCES OF UKRAINE», KYIV, UKRAINE

ABSTRACT

Aim: To improve the effectiveness of surgical treatment for patients with post-traumatic tympanic membrane perforations and concurrent Eustachian tube dysfunction through simultaneous combined surgical methods.

Materials and Methods: We analyzed clinical and functional outcomes of 35 patients (mean age: 34 ± 10,5 years) with tympanic membrane perforations caused by acoustic and blast injuries. The patients were divided into two groups: the first group (n=17) underwent only tympanoplasty type 1, while the second group (n=18) underwent simultaneous septoplasty, inferior turbinectomy, and tympanoplasty with prolonged middle ear ventilation using a subanular Silverstein tube. Parameters analyzed included time from injury to surgery, perforation size and localization, and audiometric results.

Results: Both groups demonstrated significant hearing improvement, with a reduction in the air-bone gap. The results demonstrate the clinical and functional effectiveness of both simultaneous combined surgical treatment (septoplasty, inferior turbinectomy, and tympanoplasty) and isolated tympanoplasty in military patients with tympanic membrane perforations due to blast wave trauma, acoustic barotrauma, and explosion injuries. After treatment, the findings show significantly reduced bone-air gaps and improved air-conducted sound perception thresholds.

Conclusions: Simultaneous septoplasty, inferior turbinectomy, and tympanoplasty offer a modern and effective approach to treating patients with post-traumatic tympanic membrane perforations and Eustachian tube dysfunction. Early intervention is particularly critical for military patients exposed to blast injuries to ensure faster recovery.

KEY WORDS: tympanic membrane perforation, Eustachian tube dysfunction, acoustic barotrauma, blast injury, tympanoplasty

Wiad Lek. 2024;77(11):2261-2264. doi: 10.36740/WLek/197107 DOI

INTRODUCTION

Acoustic barotrauma is a severe injury to the auditory system caused by exposure to intense sound pressure, particularly relevant in the context of military actions where blast wave trauma is a primary cause of damage [1]. This injury can lead to significant temporary or permanent impairments in hearing, balance, and spatial orientation, affecting both physical and mental health. Blast-related injuries to the auditory system are common among military personnel and civilians exposed to explosions, highlighting the need for effective treatment strategies [2].

Acoustic barotrauma and tympanic membrane perforation are closely related. Sudden sound pressure can rupture the tympanic membrane, causing severe pain, hearing loss, and bleeding. Tympanic membrane perforation is a primary manifestation of acoustic barotrauma, especially in individuals exposed to explosions. Understanding this relationship is crucial for timely diagnosis and the development of effective treatments to prevent complications and improve patient outcomes.

Tympanic membrane perforations vary in location, size, and shape. Damage typically occurs in the pars tensa but can also affect the pars flaccida [3]. Perforations may be central, marginal, or extend to the fibrous annulus, ranging from small to large, covering over 75% of the membrane. Their size and location critically influence the degree of hearing impairment and the likelihood of spontaneous healing. Surgical repair, such as tympanoplasty, is often necessary for larger or marginal perforations.

Several surgical approaches, including myringoplasty and tympanoplasty, are used to restore tympanic membrane integrity. Myringoplasty involves the use of grafts to close the membrane defect through underlay or overlay techniques [4]. Endoscopic methods with

double-layer cartilage grafts have achieved high success rates (90-95%) in closing perforations, regardless of size [4]. Tympanoplasty involves reconstruction of the tympanic membrane and ossicular chain if needed, though complications like recurrent perforation and graft misplacement are common [5, 6].

To ensure surgical success, nasal breathing and Eustachian tube function must be assessed preoperatively, and procedures like septoplasty may be necessary before tympanoplasty [7]. Modern surgical techniques allow for both procedures to be performed during a single hospitalization under one anesthetic intervention, which is especially beneficial in wartime conditions where resources are limited [8].

AIM

The objective of the study is to improve the efficiency of surgical treatment for patients with post-traumatic tympanic membrane perforations and accompanying Eustachian tube dysfunction by using simultaneous combined surgical methods for faster patient recovery.

MATERIALS AND METHODS

A clinical and functional analysis was conducted on 35 male patients, mean age (mean [M] ± standard deviation [SD]) 34 ± 10,5 years, with acquired tympanic membrane perforations following acoustic and blast wave trauma in active combat zones. They were operated on in the Ear Microsurgery and Otoneurosurgery Department of the State Institution «Institute of Otolaryngology named after Prof. O.S. Kolomiychenko of the National Academy of Medical Sciences of Ukraine». The patients were divided into two groups based on the extent of care provided. The first group (n=17) included patients who had previously undergone septoplasty or whose nasal septum condition did not affect breathing and the eustachian tube's ventilation function. In the second group (n=18), septoplasty was performed simultaneously with tympanoplasty during one hospitalization. The inclusion criteria for the first group were: results of the inflation-deflation test (ETF test) confirming grade 1 Eustachian tube function (according to Miller classification), a history of septoplasty or endoscopic data of its absence, and a positive or weakly positive Valsalva maneuver. The inclusion criteria for the second group included the absence of a history of septoplasty, nasal breathing problems, endoscopic confirmation of nasal septum deviation, and Eustachian tube function of grades 3-5 (according to Miller classification) based on the ETF test.

An audiological study of auditory function was carried out on an ITERA audiometer (Denmark) according to the generally accepted scheme using pure tone threshold tonal and speech tests. The results were evaluated before and 6 months after surgery, using the international classification of hearing impairment according to WHO at frequencies of 500 Hz; 1; 2 and 4 kHz.

The study complied with the Helsinki Declaration and followed the protocols for providing highly specialized medical care. The study design was approved by the local bioethical committee. Written informed consent to participate in the current study was obtained from all patients.

The statistical analysis was performed by the use of Statistica v. 14.0 (TIBCO Software Inc., USA). Quantitative variables were expressed as M ± SD, being compared by the use of unpaired Student`s T-test in the studied groups (independent samples). A paired Student`s T-test was used for dependent samples comparisons (before and after treatment within each group). A p-value of less than 0,05 was considered statistically significant.

RESULTS

The studied groups were comparable regarding the average patients` age (group 1: 36 ± 12,2; group 2: 32 ± 8,6 years [p=0,392]) and the perforation size (group 1: 5,7 ± 2,09; group 2: 5,7 ± 2,22 mm [p=0,982]). The localization distribution of perforations between the groups was also comparable, with minor deviations in the anterior and posterior sections. The baseline data in both groups were considered equivalent and balanced, allowing for a valid comparison of treatment outcomes between the groups. During the analysis of clinical results of treatment—healing process (total epidermisation of the tympanic membrane)—no significant differencies were noted in the both groups and complete epidermisation was noted in 6-8 weeks after tympanoplasty. No surgical complications were observed in either group.

The pure tone audiogram data before and after treatment in the studied groups of patients are summarized in Table 1.

Comparison of results between the groups after surgery (independent t-test) shows no statistically significant difference between the groups at frequencies of 500 Hz, 2000 Hz, and 4000 Hz, indicating that combined simultaneous surgical treatment (septoplasty + tympanoplasty) is as effective and safe as performing only tympanoplasty and allowed to achieve hearing gain on the investigated frequencies. Hearing gain with significantly decreased air bone gap at the 1000 kHz

Table 1. The pure tone audiogram data before and after treatment in the studied groups of patients

Frequency (Hz)	Group 1 n = 17			Group 2 n = 18			p_2	p_3
	Before (dB)	After (dB)	p_1	Before (dB)	After (dB)	p_1		
500	27,7 ± 15,03	9,7 ± 5,36	<0,001	30,7 ± 12,81	7,0 ± 4,16	<0,001	0,526	0,111
1000	31,2 ± 16,55	13,4 ± 7,91	<0,001	32,6 ± 13,58	7,7 ± 4,99	<0,001	0,780	0,016
2000	33,9 ± 15,27	15,7 ± 8,56	<0,001	33,9 ± 13,18	13,4 ± 9,51	<0,001	0,990	0,466
4000	48,3 ± 25,78	25,1 ± 19,56	<0,001	44,3 ± 22,89	17,7 ± 15,52	<0,001	0,629	0,219

Notes: p_1 – the significance of difference before and after treatment in the corresponding study group; p_2 – the significance of difference before surgery between the studied groups; p_3 – the significance of difference after surgery between the studied groups.

was noted in both groups. In the second group with simultaneous surgery the hearing gain after surgery significantly increased by almost two times compared to the data in the first group and amounted to (7,7 ± 4,99) dB.

DISCUSSION

In the meta-analysis conducted by S. Son et al. [8], the effectiveness of septoplasty and its influence on improving Eustachian tube function in patients with deviated nasal septum was studied. The authors performed a systematic review and meta-analysis of existing studies to determine whether surgical correction of nasal septal deviation affects Eustachian tube function. The main methods used in the studies included the evaluation of ET function before and after septoplasty using tests such as the Valsalva maneuver, inflation-deflation test (ETF), and audiometry. This meta-analysis showed significant improvement in Eustachian tube function after septoplasty, demonstrating a close relationship between nasal breathing and middle ear ventilation. Our study's results align with the findings of S. Son et al. [8], as the simultaneous combined surgical procedure, including simultaneous septoplasty and tympanoplasty, also demonstrates a positive effect on restoring Eustachian tube function, particularly in post-traumatic tympanic membrane perforation cases. However, there are differences, which the different contexts of the patients studied may explain. In our case, the patients were military personnel who had sustained blast and acoustic injuries within active modern war zone, whereas the meta-analysis examined patients with nasal septal deviation and TM perforation not associated with trauma or any external damages.

In the study conducted by C. Singh et al. [9], the effectiveness of single-stage endoscopic tympanoplasty combined with septoplasty in patients with chronic mucosal otitis media and nasal septal deviation was explored. The approach involved using an autologous septal cartilage graft for TM reconstruction. The results of this study align with our findings regarding the effectiveness of the combined simultaneous surgical approach.

In the study by M. Hassan et al. [10], the effectiveness of single-stage surgical intervention, where septoplasty is performed simultaneously with tympanoplasty, was discussed. The researchers highlighted potential complications, such as negative pressure in the middle ear post-septoplasty, which could worsen tympanoplasty outcomes. Our study shows that the simultaneous combined surgical approach is effective in treating military patients with post-traumatic tympanic membrane perforations without any complications within the study period.

CONCLUSIONS

The results demonstrate the clinical and functional effectiveness of both methods of surgical treatment – simultaneous combined surgical treatment (septoplasty, inferior turbinectomy, and tympanoplasty) and isolated tympanoplasty after previously performed nasal surgeries in military patients with tympanic membrane perforations due to blast wave trauma, acoustic barotrauma, and explosion injuries. After treatment, the findings showed significantly reduced air-bone gaps and improved air-conducted thresholds in the both groups after surgeries (independent t-test) demonstrated no statistically significant difference between the groups at frequencies of 500 Hz, 2000 Hz, and 4000 Hz, indicating that combined simultaneous surgical treatment (septoplasty + tympanoplasty) is as effective and safe as performing only tympanoplasty. In the second group with simultaneous surgery the hearing gain after surgery significantly increased by almost two times at 1000 frequency compared to the data in the first group and amounted to (7,7 ± 4,99) dB.

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The study was conducted as a fragment of complex scientific projects of the National Academy of Medical Sciences (State Institution «Institute of Otolaryngology named after Prof. O.S. Kolomiychenko of the National Academy of Medical Sciences of Ukraine», Kyiv, Ukraine) «Clinical and experimental research of external ear canal architectonic and inner ear labyrinth and their influence to the effectiveness of cochlear vestibular disorders treatment, that occurred due to the blast injuries and labyrinth hydrops» (state registration number 0121U113544; term: 2022-2024).

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Anastasiia K. Tarasenko
Institute of Otolaryngology named after Prof. O.S. Kolomiychenko
of the National Academy of Medical Sciences of Ukraine
3 Zoologichna St., 03680 Kyiv, Ukraine
e-mail: tarasenko.anastasi@gmail.com

ORCID AND CONTRIBUTIONSHIP

Anastasiia K. Tarasenko: 0009-0006-5815-0556 A B C D E F
Ilona A. Srebniak: 0000-0002-2972-9415 B C D E

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

RECEIVED: 12.07.2024
ACCEPTED: 25.10.2024

ORIGINAL ARTICLE

CONTENTS

Early results of transabdominal preperitoneal inguinal hernia repair (TAPP) using invasive and non-invasive methods of mesh fixation

Artem V. Trots, Oleksandr V. Perekhrestenko
STATE SCIENTIFIC INSTITUTION «CENTER FOR INNOVATIVE MEDICAL TECHNOLOGIES OF THE NATIONAL ACADEMY OF SCIENCES OF UKRAINE», KYIV, UKRAINE

ABSTRACT

Aim: To evaluate the early postoperative results of surgical treatment of patients with inguinal hernias after laparoscopic transabdominal preperitoneal inguinal hernia repair (TAPP) using invasive and non-invasive methods of fixation of prosthetic material.

Materials and Methods: The study included 76 patients (average age 61,4±15,3 years; 69 [91 %] males, 7 [9 %] females) with primary uncomplicated unilateral inguinal hernias who underwent TAPP. Patients were divided into two groups: group 1 – 52 patients in whom the AbsorbaTack fixation device was used to secure the mesh; group 2 – 24 patients in whom the LiquiBand FIX8 cyanoacrylate adhesive was used. In both groups, in the early postoperative period, the severity of pain according to Visual Analogue Scale (VAS) and functional activity according to the standard Carolinas Comfort Scale (CCS) were assessed.

Results: The severity of pain syndrome, according to VAS score, in the cyanoacrylate glue group was lower starting from postoperative day 7 (1,4±0,7 vs. 1,8±0,6 in group 1, respectively; p=0,026). On postoperative day 30, the functional activity indicators were better in the cyanoacrylate glue group (mean CCS score was 13,3±4,2 vs. 23,3±7,4 points, respectively; p=0,001). There was no difference in the incidence of early postoperative complications among the groups.

Conclusions: Fixation of the mesh with cyanoacrylate glue during laparoscopic transabdominal preperitoneal inguinal hernia repair is a safe and effective technique that helps to reduce the level of postoperative pain and provides better functional results in patients in the early postoperative period compared to traditional methods of mesh fixation.

KEY WORDS: adhesives, quality of life, pain, functional status

Wiad Lek. 2024;77(11):2265-2270. doi: 10.36740/WLek/197109 DOI

INTRODUCTION

Given the current trends in inguinal hernia surgery regarding the active implementation of minimally invasive surgical techniques and adherence to the principles of enhanced recovery after surgery (ERAS), there has been a gradual shift in emphasis towards functional outcomes of surgical treatment, and not just the reliability of anatomical reconstruction of the abdominal wall [1].

In turn, postoperative pain syndrome is a crucial factor that makes it impossible to achieve the optimal functional outcome of surgical treatment and worsens the associated indicators of physical activity and, accordingly, the quality of life of patients [2, 3].

Among the many options for solving the problem, it was proposed to use non-invasive methods of fixing prosthetic material. Thus, the method of fixing the mesh using cyanoacrylate glue was introduced, that potentially allowed to ensure the reliability of fixation without a statistically significant difference in the incidence of inguinal hernia recurrence and at the same time reduce the level of postoperative pain [4].

However, the vast majority of existing studies focus on long-term outcomes, namely the development of chronic postoperative inguinal pain (CPIP) at rest, losing sight of the patient's functional activity after surgical treatment, which is a crucial indicator in assessing quality of life. This was the key element that we focused on in the context of our work.

AIM

To evaluate the early postoperative results of surgical treatment of patients with inguinal hernias after laparoscopic transabdominal preperitoneal inguinal hernia repair (TAPP) using invasive and non-invasive methods of fixation of prosthetic material.

MATERIALS AND METHODS

Our study was performed in line with the principles of the Declaration of Helsinki. Also, the Local Ethics Committee of State Scientific Institution «Center For Innovative Medical Technologies of the National Academy of Sciences of Ukraine» approved this study. All participants signed an informed consent.

This study included 76 patients (69 [91 %] males, 7 [9 %] females; the age ranged from 20 to 85 years; average age was [mean (M) ± standard deviation (SD)] 61,4±15,3 years) with primary uncomplicated unilateral inguinal hernias who underwent laparoscopic transabdominal preperitoneal inguinal repair (TAPP). The patients included in this study were divided into two groups: group 1 included 52 patients who underwent TAPP using the AbsorbaTack fixation device to fix the prosthetic material; group 2 included 24 patients who underwent TAPP with mesh fixation and sealing of the parietal peritoneum with cyanoacrylate glue LiquiBand FIX8.

The data evaluated (in addition to age and sex) included: intraoperative blood loss (ml), duration of surgery (min), hospital stay (days), incidence of early postoperative complications in the 30-day period after the intervention (number of cases).

Pain syndrome was assessed preoperatively and in the postoperative period using a Visual Analogue Scale (VAS) on postoperative days 0, 1, 2, 3, 7 and 30. The results were interpreted on a 10-point scale from 0 to 10, where 0 points is no pain and 10 points is maximum pain.

The modified Carolinas comfort scale (MCCS) and the standard Carolinas comfort scale (CCS) questionnaire were used to assess the functional activity of patients at the preoperative stage and on postoperative day 30, respectively.

The standard CCS questionnaire consisted of 8 questions, each containing 3 items to separately assess pain at rest and during exercise, the sensation of prosthetic material, and limitation of mobility during exercise (except for the 1st question where only pain and mesh sensation were assessed). Each item in the CCS was scored on a 5-point scale. The lowest score for each item was 0 points, which corresponded to the complete absence of pain, subjective sensation of prosthetic material or restriction of mobility during physical activity, and the highest score was 5 points, which meant the greatest severity of pain, subjective sensation of mesh or restriction of mobility during physical activity. The lowest score for each question was 0 points, the highest was 15 points (except for the 1st question where the maximum score was 10 points). Issues assessed: pain and mesh sensation at rest; pain, mesh sensation and limitation of movement while bending over, sitting down, performing routine daily activities (getting out of bed, taking a bath, getting dressed), coughing, sneezing, deep breathing, walking or standing, going upstairs or downstairs or while doing physical exercises (not related to the main job). The total score was calculated by summing the scores of each of the eight questions and could range from 0 points to 100 points.

The MCCS questionnaire used in the preoperative period differed from the standard CCS questionnaire in the absence of an item on the subjective feeling of the presence of a mesh implant. Thus, the total maximum score that a patient could receive on the MCCS was 75 points.

Patients' scores on the MCCS questionnaire in the preoperative period were converted to percentages of the maximum possible score for the questionnaire. In the CCS questionnaire, each score obtained corresponded to 1% in the conversion.

To facilitate further analysis and the formation of clinical groups according to the level of satisfaction with functional outcomes, the CCS and MCCS scores were stratified according to the severity of symptoms. Accordingly, patient groups were formed: very satisfied (≤5%), generally satisfied (>5% … ≤30%), unsatisfied (>30% … ≤60%), very unsatisfied (>60%).

The technical aspects of the survey were the same for all patients. All patients received the same explanations and detailed descriptions of the specifics of filling out each of the questionnaires used.

TECHNICAL ASPECTS OF TAPP

The traditional horizontal positioning of the patient on the operating table on the back with the arm brought counter-laterally to the side of the hernia defect was used. Prior to the surgical intervention, under visual control, unilateral (surgical side) transversus abdominis plane block (TAP block) with 20.0 ml of 0.25% bupivacaine solution was performed in the projection above the anterior superior iliac crest and below the rib margin [5]. Then, after creation of carboxyperitoneum and placement of trocars, the peritoneum was cut and dissection of preperitoneal space begun with the "critical view of safety" (CVS) achievement in accordance with the recommendations of the European Hernia Society (EHS) [6]. After isolation of the hernia sac, visualization of all critical anatomical structures and hemostasis control, a 15x10 cm Parietene polypropylene mesh prosthesis (Covidien, USA) was inserted into the preperitoneal space through a 10 mm port. The mesh was placed so that it completely overlapped the area of the myopectineal orifice with a margin of 3,0-4,0 cm. The next step was fixation of the mesh with AbsorbaTack 5 mm fixation device (Covidien, USA) or LiquiBand FIX8 cyanoacrylate glue (Advanced Medical Solutions Group, UK) at

6-8 safe points (in Cooper's ligament and 2 cm above the iliopubic tract), taking into account anatomical structures ("triangle of pain", "triangle of doom", inferior epigastric vessels). After the implantation of the prosthetic material, the CO_2 pressure in the abdominal cavity was reduced to 4-5 mm Hg, after which the mesh position was checked to prevent its folding and hemostasis was controlled again. The final stage was the restoration of the integrity of the parietal peritoneum using V-Loc 3-0 sutures (Covidien, USA). In case of cyanoacrylate glue fixation, it was also used to restore the parietal peritoneum. After removal of the ports under visual control of the laparoscope, the skin defect was closed with monofilament sutures on a cutting needle.

STATISTICAL PROCESSING METHODS

Statistical processing of data was carried out using the methods of variation and descriptive statistics using the statistical analysis package SPSS Statistics: An IBM Company, version 23. Before starting the data analysis, all indicators were checked for normality of distribution using the Shapiro-Wilk test and for equality of variances using the Levene's test. The study used descriptive statistics such as M ± SD for quantitative parameters, and absolute and relative frequency (%) (with 95% confidence interval [CI] in the certain cases) for qualitative data. To evaluate statistically significant differences in the mean values of quantitative attributes subject to the law of normal distribution, parametric methods of evaluation in dependent groups were used (Student's T-test). For the comparative analysis of qualitative parameters (frequency distributions) between patient groups, Pearson's chi-squared test (χ^2), in particular with Yates correction, was used. The differences in the results were considered statistically significant at $p < 0,05$, which provides a 95% probability level.

RESULTS

The studied groups were comparable by average age (group 1: 63,4±14,4 years; group 2: 57,0±16,7 years [p=0,091]) and gender (males/females: group 1 – 48/4; group 2 – 21/3 [p=0,805]).

There were no cases of intraoperative complications among the patients included in the study. There was no statistically significant difference in terms of intraoperative blood loss. Mean blood loss in the first group was 46,0±14,0 ml, and in the second group – 46,0±15,0 ml (p=0,929).

Also, there was no statistically significant difference in the operating time between two groups. The average operating time in the first group was 108,7±44,0 minutes compared to the second group – 102,9±41,2 minutes (p=0,592).

The method of anesthesia in perioperative period in both groups did not differ. Principles of multimodal analgesia were respected. Inhaled anesthetic sevoflurane was used during surgical intervention. In postoperative period, patients received a combination of paracetamol up to 3000 mg per day and dexketoprofen up to 150 mg per day.

Preoperative pain score according to VAS in the first group was in the range from 1 to 4 points, with an average value of 2,0±0,9 points, and in the second group – from 1 to 3 points, with an average value of 2,0±0,6 points (p=0,697).

There was also no statistically significant difference in pain scores on postoperative day 0: the mean score in the first group was 4,3±1,2 and 4,3±0,9 in the second group (p=0,814). However, 1 patient (1,9%) from the first group required the administration of opioid analgesics on postoperative day 0.

Similar data between the groups were obtained on postoperative day 1, 2, and 3. According to VAS, the mean score in groups 1 and 2 was as follows: day 1 – 2,9±1,0 and 3,0±0,7 (p=0,486), day 2 – 2,5±0,8 and 2,4±0,8 (p=0,685), day 3 – 2,0±0,7 and 1,9±0,7 (p=0,706).

However, starting from the 7th postoperative day, we observed a statistically significant difference with respect to the severity of pain. According to VAS, the average score in groups 1 and 2 was as follows: day 7 – 1,8±0,6 and 1,4±0,7, respectively (p=0,026), day 30 – 0,8±0,7 and 0,2±0,4, respectively (p=0,001). Thus, in cyanoacrylate glue group less pronounced pain syndrome was observed on the 7th and 30th postoperative day.

Characteristics of the severity of postoperative pain according to VAS among patients by groups are summarized in Table 1.

When assessing the functional activity preoperatively according to the MCCS, no statistically significant difference was found between the groups. The mean total score in the first group was 35,0±10,7 points, and in the second group – 31,1±9,6 points (p=0,135). In terms of percentage, the values were on average 46,6±14,3% in the first group and 41,4±12,8% in the second group (p=0,680), which corresponded to the category of patients' quality of life as "unsatisfied" (>30% … ≤60%).

The minimum score in the first group was 15 points, the maximum – 66 points, and in the second group – 17 points and 61 points, respectively.

Preoperative characteristics of the functional activity of patients according to MCCS data are presented in Table 2. In particular, the studied groups were comparable by the distribution of the MCCS categories (p=0,269; Table 2).

Table 1. Characteristics of the severity of postoperative pain according to VAS

Postoperative day	Average score		p
	Group 1 (n=52)	Group 2 (n=24)	
0	4,3±1,2	4,3±0,9	0,814
1	2,9±1,0	3,0±0,7	0,486
2	2,5±0,8	2,4±0,8	0,685
3	2,0±0,7	1,9±0,7	0,706
7	1,8±0,6	1,4±0,7	0,026
30	0,8±0,7	0,2±0,4	0,001

Table 2. Preoperative characteristics of patients' functional activity at the preoperative stage according to MCCS*

Groups	«Very satisfied» (≤5%)		«Generally satisfied» (>5% ... ≤30%)		«Unsatisfied» (>30% ... ≤60%)		«Very unsatisfied» (>60%)	
	n (%)	M±SD	n (%)	M±SD	n (%)	M±SD	n (%)	M±SD
1 (n=52)	0	-	6 (11,5%)	19,8±2,6	37 (71,2%)	33,1±5,4	9 (17,3%)	52,9±7,1
2 (n=24)	0	-	4 (16,6%)	18,8±1,5	19 (79,2%)	32,1±5,8	1 (4,2%)	61

Note: * – Total number of patients: n = 76; total mean score: 33,7±10,5; mean score in group 1: 35,0±10,7; in group 2: 31,1±9,6 (p=0,135).

Table 3. Characteristics of patients' functional activity on the postoperative day 30 according to CCS*

Groups	«Very satisfied» (≤5%)		«Generally satisfied» (>5% ... ≤30%)		«Unsatisfied» (>30% ... ≤60%)		«Very unsatisfied» (>60%)	
	n (%)	M±SD	n (%)	M±SD	n (%)	n (%)	M±SD	n (%)
1 (n=52)	0	-	44 (84,6%)	20,9±5,1	8 (15,4%)	36,3±3,9	0	-
2 (n=24)	0	-	24 (100%)	13,3±4,2**	0	-	0	-

Notes: * – Total number of patients: n = 76; total mean score: 20,1±8,0; mean score in group 1: 23,3±7,4; in group 2: 13,3±4,2 (p=0,001); ** – p<0,001 (vs. group 1).

A statistically significant difference in functional activity and satisfaction with the results of surgical treatment was found on the postoperative day 30 after surgery using the CCS questionnaire. Thus, the average total score in the first group was 23,3±7,4 points, and in the second group – 13,3±4,2 points (p=0,001), which in general, in terms of percentage, corresponded to the category of patient quality of life as "generally satisfied" (>5% … ≤30%) in both groups. The minimum score in the first group was 12 points, the maximum was 44 points, and in the second group – 7 points and 28 points, respectively. In the second group, all of 24 patients (100%) fell into the category of "generally satisfied" (>5% … ≤30%) with a mean score of 13,3±4,2.

Characteristics of the functional activity of patients on the postoperative day 30 according to CCS questionnaire are presented in Table 3. In particular, the frequency of «unsatisfied» (>30% ... ≤60%) category was numerically, but non-significantly higher in group 1, as compared to the group 2: 15,4 % (95 % CI [6,8-26,6 %]) vs. 0 (95 % CI [0-7,7 %]), respectively (p=0,103).

In addition, there was no statistically significant difference in the hospital stay duration between the two groups. The average length of stay in the first group was 4,1±2,4 days compared to 3,8±2,7 days in the second group (p=0,694).

In the first group, 4 (7,7%) patients developed postoperative wound seroma formation within 30 days of surgery, and in the second group, seroma occurred in 2 (8,3%) patients, with no statistically significant difference (p=0,718). All patients were treated conservatively.

No cases of other complications were observed in both groups during the study period.

DISCUSSION

Pain syndrome in the early postoperative period is the most significant factor that affects the satisfaction with functional outcomes and quality of life after inguinal hernia repair [7]. The mechanism of development of early postoperative pain can be nociceptive in nature and provoked by the release of neurotransmitters due

to tissue injury and the development of an inflammatory reaction in the area of surgery or the installed mesh. On the other hand, it can be the result of mechanical damage to the nerve branches of the genitofemoral, lateral femoral cutaneous and ilioinguinal nerves caused by the capture of nerve fibers with sutures or staples (neuropathic pain) [8].

The difference between both variants of pain syndrome is reflected in its duration, and in the case of mechanical nerve damage, it often causes the development of CPIP [9].

Previously, the main focus was on the reliability of fixation of prosthetic material, but modern views on the results of planned inguinal hernia reconstruction emphasize the importance of achieving acceptable functional results [10].

Among the various methods of preventing the development of postoperative inguinal pain, it has been proposed to use synthetic cyanoacrylate glue to fix the mesh. Previous studies didn`t demonstrate any statistically significant difference in the incidence of recurrence and reoperation when using adhesive fixation compared to stapling techniques. At the same time, when fixing the mesh with cyanoacrylate glue, a significantly lower severity of pain was noted in the early postoperative period, which allowed for early functional activity of the patient [4, 11].

Since most studies focus on long-term outcomes, we set out to evaluate the early results of the postoperative period after inguinal hernia repair using cyanoacrylate glue to fix the prosthetic material compared with the traditional staple fixation technique, including assessment of pain, functional activity, and quality of life.

There was no significant difference in the levels of blood loss, operating time, hospital stay, and incidence of postoperative complications among the groups, which corresponded to the general statistics [12].

According to a preliminary summary of the results, a significant difference in pain intensity was found starting on postoperative day 7, which also correlated with existing studies [13].

The assessment of functional activity in the postoperative period demonstrated significantly better results in cyanoacrylate glue group and higher levels of satisfaction with the operation outcomes. The most likely potential factor that influenced the level of functional activity and, accordingly, the quality of life of patients in the early postoperative period was pain syndrome, the severity of which, according to VAS, has a direct correlation with the results of the CCS survey. In support of the uniqueness of the results obtained, we have not found any original randomized trials over the past 10 years that would evaluate the functional outcomes and pain severity during physical activity after inguinal hernia repair using cyanoacrylate glue.

It is important to note that in order to exclude other factors that could potentially affect the results of the study, perioperative management of all patients was carried out exclusively within the ERAS program.

Further studies on the basis of the obtained data will potentially allow us to analyze the correlation between early and long-term anatomical and functional outcomes.

CONCLUSIONS

Fixation of the mesh with cyanoacrylate glue during laparoscopic transabdominal preperitoneal inguinal hernia repair is a safe and effective technique that helps to reduce the level of postoperative pain and provides better functional results in patients in the early postoperative period compared to the method of fixing the mesh with the AbsorbaTack fixation device.

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This study was carried out within the scope of the research work «Aesthetic and reconstructive surgery of defects and deformations of the abdominal wall» (state registration number 0123U103218; term: 2024-2026), at the place of study – the State Scientific Institution «Center of innovative medical technologies of the National Academy of Sciences of Ukraine», Kyiv, Ukraine, and also as a fragment of PhD thesis «Laparoscopic transabdominal preperitoneal inguinal hernia repair using innovative methods of tissue fixation (clinical and experimental study)» at State Scientific Institution «Center of innovative medical technologies of the National Academy of Sciences of Ukraine», Kyiv, Ukraine (term: 2023-2027).

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Artem V. Trots
Center for Innovative Medical Technologies of the
National Academy of Sciences of Ukraine
22 Voznesens'kyy Descent, 04053 Kyiv, Ukraine
e-mail: trotsartem22@gmail.com

ORCID AND CONTRIBUTIONSHIP

Artem V. Trots 0009-0004-8456-0538 A B C D
Oleksandr V. Perekhrestenko 0000-0002-8240-7095 E F

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

RECEIVED: 07.07.2024
ACCEPTED: 28.10.2024

ORIGINAL ARTICLE

CONTENTS

Modelling business processes in palliative care management: ways to optimise resource management

Anatolii V. Tsarenko[1], Oleksandr O. Kovtun[2], Zoya V. Maksymova[1]
[1]SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE
[2]INTERNATIONAL HUMANITARIAN UNIVERSITY, ODESA, UKRAINE

ABSTRACT

Aim: To evaluate the impact of corrective coefficients on the income of multidisciplinary mobile palliative care teams (MMPCT) in Ukraine under different scenarios, such as new contracts, patient count, and service cost variability, to identify optimization pathways for resource management in palliative care.

Materials and Methods: A comprehensive literature review was conducted, including sources from JAMA Scholar and PubMed. The Monte Carlo method simulated 10 000 scenarios of uncertainty, such as patient count and diagnoses, to assess the effect of corrective coefficients. Income was modeled based on the National Health Service of Ukraine (NHSU) base rate of 69 326,04 UAH, with upward (1; 1,2; 1,5; 2) and downward (0,5; 0,7; 0,9) coefficients. Calculations were done using Python (NumPy, Matplotlib).

Results: Income varied significantly with NHSU corrective coefficients. With increasing coefficients, average annual income reached 1 137 498,89 UAH, while with downward coefficients, it decreased to 887 387,18 UAH. Adding the new contract coefficient (0,9) further reduced income to 799 786,52 UAH.

Conclusions: The NHSU base rates for MMPCT in Ukraine are underestimated, not reflecting actual palliative care costs. Increasing coefficients boost revenue, while downward adjustments and contract discounts reduce profitability. Adjustments in fixed and variable costs, along with payroll taxes, are necessary to meet the financial needs of palliative care services in Ukraine.

KEY WORDS: palliative and hospice care (PHC); multidisciplinary mobile palliative care teams (MMPCT); National Health Service of Ukraine (NHSU); business process optimization, business process modeling

Wiad Lek. 2024;77(11):2271-2276. doi: 10.36740/WLek/197110 DOI

INTRODUCTION

World Health Organization (WHO) defines palliative care as an approach that improves the quality of life of patients – adults and children – and their families who are facing problems associated with life-threatening illness. It prevents and relieves suffering through the early identification, impeccable assessment and treatment of pain and other problems, whether physical, psychosocial, or spiritual. Palliative care improves the quality of life of patients - physical, psychological, social and spiritual. The quality of life of caregivers improves as well. In 2014, the first ever global resolution on palliative care, World Health Assembly resolution WHA67.19, called upon WHO and Member States to improve access to palliative care as a core component of health systems, with an emphasis on primary health care and community/home-based care [1].

WHO and authoritative international professional organizations define Palliative and Hospice Care (PHC) as an integral part of the Health Care System of the country. The development of the PHC System is one of the priority medical, social and humanitarian tasks of governments and societies in the most leading countries of the world during last 50-60 years. This is due to the rapid aging of population, the ever-increasing number of patients with chronic incurable diseases, including those with limited life expectancy around the world. According to a WHO survey relating to noncommunicable diseases conducted among 194 Member States in 2019, funding for palliative care was available in 68% of countries and only 40% of countries reported that the services reached at least half of patients in need [1–6].

According to the European Association for Palliative Care (EAPC), each year, an estimated 4,4 million people in the WHO European Region need palliative care. People living in low- and middle-income countries are less likely to receive palliative care. Palliative care should be available in hospitals, palliative care units, hospices and in primary care through home care teams. EAPC recommends 2 specialized palliative care services per 100 000 inhabitants, consisting of 1 home care team and 1 hospital team [1].

Multidisciplinary mobile palliative care teams (MMPCT) is an important component of the Palliative Care System in Ukraine's healthcare sector. This has become particularly relevant over the past decade, as the country has faced insidious aggression from the Russian Federation, the challenges of the COVID-19 pandemic, and healthcare reforms, among other factors. Funding for MMPCT is a complex process due to the varying needs of patients requiring palliative care and the impact of corrective coefficients from the NHSU package for MMPCT on service costs. This study modeled the annual income of a MMPCT according to the NHSU requirements for 2025.

AIM

The objective of the study was to assess the impact of the National Health Service of Ukraine (NHSU) corrective coefficients on the income of multidisciplinary mobile palliative care teams (MMPCT) under various operational scenarios and uncertainties, such as new contracts, patient count, and service cost variability, with the goal of identifying pathways for optimizing resource management.

MATERIALS AND METHODS

During the study, a literature search was conducted in JAMA, Scholar, and PubMed databases, as well as in domestic and foreign sources.

To model the annual income of MMPCT, the following methods were used: mathematical modeling to forecast income; the Monte Carlo method to simulate the distribution of 10 000 uncertainties (patient count and diagnoses, to apply corrective coefficients); and data analysis to summarize the results. The input data for the calculations were as follows: the NHSU package base rate: 5 777,17 UAH per patient per month (69 326,04 UAH per patient per year); increasing coefficients (1; 1,2; 1,5; 2) and decreasing coefficients (0,5; 0,7; 0,9); average number of patients: 12 (±2 patients based on labor statistics with maximum workload); new contractor coefficient = 0,9 for the first year. Modeling was performed using the Python programming language with NumPy and Matplotlib libraries for calculations and visualization.

RESULTS

The analysis of the scientific literature indicated that discussions on the need to introduce and develop palliative, hospice, and medical-social care for incurable patients with malignant neoplasms and elderly individuals with severe chronic diseases, who had lost the ability to care for themselves, began in Ukraine at the end of the 20th and early 21st centuries. It was proposed that this be implemented through the creation of hospices, medical-social centers, or palliative and hospice care (PHC) departments in healthcare facilities (HCFs), mobile PHC services, and MMPCTs, as well as through the development of social services for palliative care in social protection institutions [7–15].

The urgent need for the creation and development of a PHC System in Ukraine amidst healthcare reforms, the directions for improving the legal framework, and proposals for optimizing the organization of PHC and medical-social care for different categories of patients were discussed over the past decades by Ukrainian researchers [7–15]. The priorities for the development, structural components of the medical and social services of the PHC System, and the management and organizational features of its staffing, financial, and information support, as well as the levels and scope of medical and social care for palliative patients, were identified in the works of many Ukrainian authors. These problems have become especially relevant since the large-scale invasion of the Russian Federation, as during the war, a significant number of palliative patients, particularly elderly individuals, are often left alone without the support of medical professionals, social workers, family, or community.

The simulation results showed that the annual income of the NHSU package for MMPCT, when only increasing coefficients were used, reached 1 137 498,89 UAH, with a standard deviation of 218 077,93 UAH. After applying decreasing coefficients, the average annual income decreased to 887 387,18 UAH, with a standard deviation of 190 650,16 UAH. Additionally, a coefficient of 0,9 was applied for new contracts, which further reduced the annual income to 799 786,52 UAH, with a standard deviation of 170 598,10 UAH. This demonstrates the significant impact of coefficients on MPC income (Fig. 1, Fig. 2, Fig. 3).

The study concluded that the annual income of the NHSU package for MMPCT in Ukraine varies depending on the corrective coefficients. Income calculated based on the minimum staffing requirements of the NHSU shows that increasing coefficients significantly boost income, while decreasing coefficients and discounts for new contracts reduce it.

Monte Carlo distribution modeling showed that the average annual income of the MMPCT ranges between 799 000,00 and 1 137 000,00 UAH. However, these calculations do not take into account the fixed and variable costs of HCFs, as well as payroll taxes, which could significantly reduce the net income. As a result,

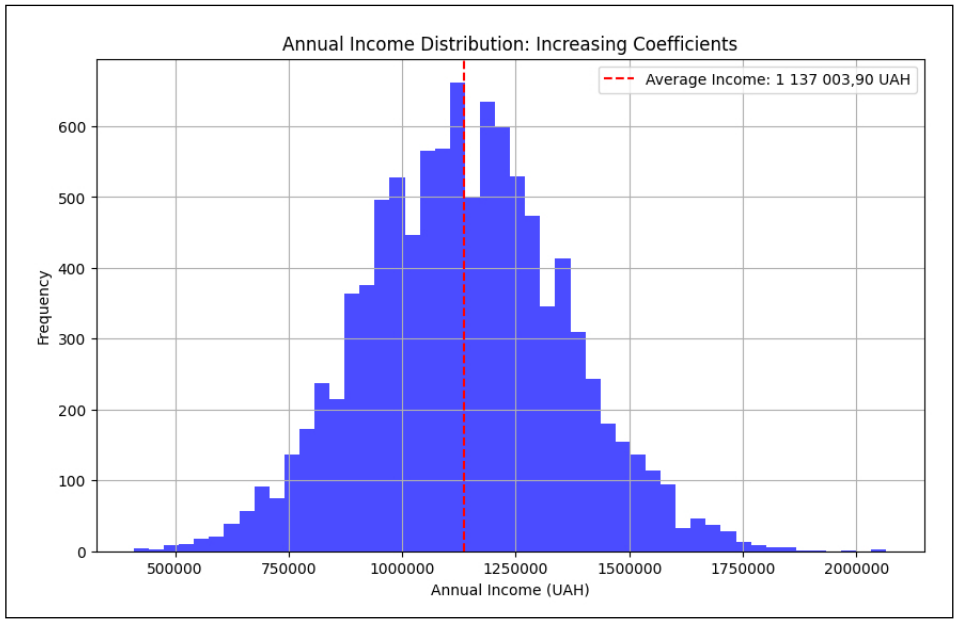

Fig. 1. Annual income distribution (with increasing coefficients).

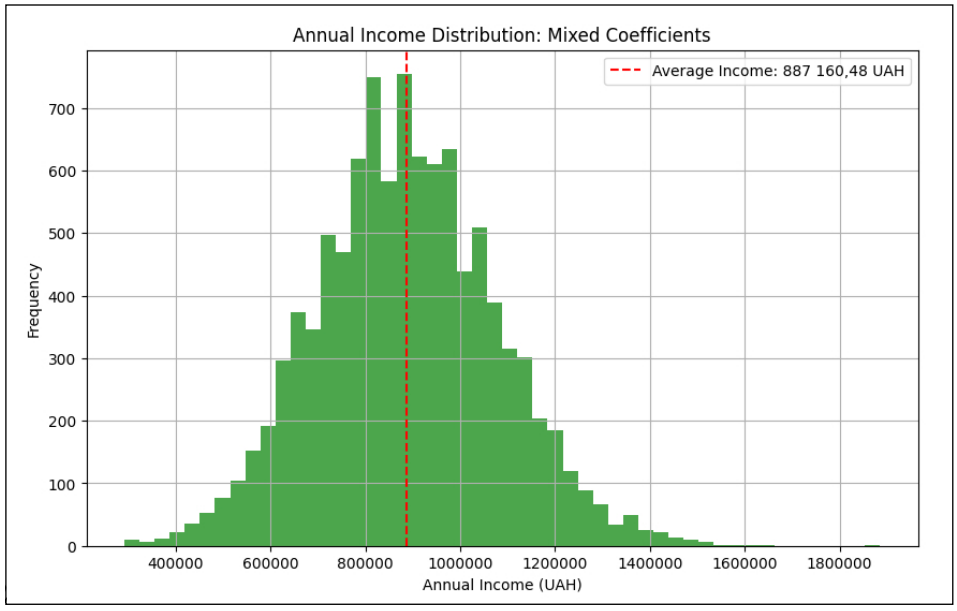

Fig. 2. Annual income distribution (with decreasing coefficients).

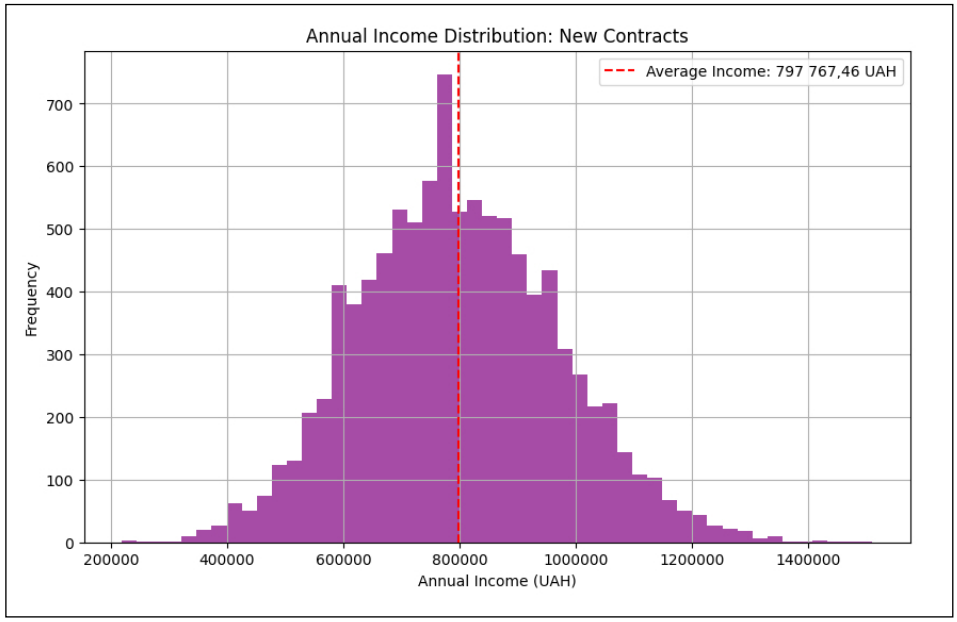

Fig. 3. Annual income distribution (with new contracts).

the NHSU "Mobile Palliative Medical Care for Adults and Children" package is significantly undervalued and does not reflect the actual costs of providing quality care to palliative patients, which may lead to abuses by contractors.

DISCUSSION

For comparison, the analysis of palliative care funding in various countries shows a significant difference in funding levels and approaches. According to recent reports, palliative care funding in Canada depends on the healthcare system of the respective province. For example, in the provinces of Ontario and Alberta, the average cost of palliative care can range from CAD 54 000 to 100 000 (approximately 2,5–4,6 million UAH) per year, depending on the intensity of care [16].

In Germany, palliative care is financed by the health insurance system, where costs can amount to €36 500 – 54 750 per year per patient (approximately 1,7–2,5 million UAH) [17].

In the NHS system of the United Kingdom, palliative care costs can range between £10 000 – 15 000 per year (approximately 0,5–0,8 million UAH), with possible variations depending on the region and the level of medical care [16]. The State funding covers only part of the costs. Charitable organizations in the UK play a key role in funding mobile palliative care. Organizations such as Sue Ryder, Marie Curie, Hospice UK, Together for Short Lives, and Macmillan Cancer Support and others raise funds to provide palliative medical services at home or for palliative mobile teams, supplementing NHS funding. For example, the Hospice UK report notes that hospices providing mobile and inpatient palliative care often receive less financial support from the State than from private sources. In some regions, state funding amounts to only 23 pence per person per year. There is also inequality in funding between regions, where amounts can vary significantly. Some regions receive only £10,33 per year per person for mobile palliative care, which is far below the actual needs. This means that in such regions, PHC services rely heavily on charitable donations and private support.

The figures presented show significantly higher levels of funding compared to Ukraine, where the annual tariff per MMPCT patient is 69 326,04 UAH [17], which is 20-30 times less than in the aforementioned countries. However, this amount does not solely consist of state funding, as it also includes contributions from other sources—such as charitable organizations or insurance funds. This difference in funding affects the quality and availability of PHC.

In Ukraine, the main funding for MMPCT comes from the state budget through the NHSU package, amounting to 69 326,04 UAH per patient per year [18], and the centralized State Funding ensures government support for palliative care services across all regions. This funding provides some stability in service provision. Charitable organizations in Ukraine play a smaller role compared to the UK, due to lower levels of private donations.

Funding is the foundation for maintaining the stability of the Healthcare System, but optimizing financial flows may become a key task at this stage. Therefore, optimizing management in the context of PHC can significantly improve the efficiency and accessibility of services, especially given the limited funding in Ukraine.

Optimization of management from a mathematical perspective involves minimizing costs and maximizing the efficiency of processes through mathematical models and algorithms. Functions are used to predict the costs of PHC depending on the number of patients, staff workload, and variable costs. Linear and nonlinear optimization models are applied to minimize total costs, considering fixed constraints (e.g., mandatory visits).

The second possible direction of management optimization is resource optimization, especially regarding the allocation of personnel and route logistics. This is crucial for ensuring that each healthcare personnel serve an optimal number of patients with minimal workload. The goal of the model is to balance the number of palliative care patients per doctor or nurse, minimizing staff overload while ensuring proper service quality. Route logistics helps reduce travel time and increase the number of palliative care patients a team can visit in a day. Resource optimization through such mathematical models can promote the rational use of limited healthcare resources in Ukraine at the current stage.

CONCLUSIONS

1. The study found that the annual income of the NHSU "Mobile Palliative Medical Care for Adults and Children" for MMPCT varies depending on corrective coefficients. The income calculated based on the minimum NHSU staffing requirements shows that increasing coefficients significantly increase income, while decreasing coefficients and discounts for new contracts reduce it considerably.
2. Stochastic modeling showed that the average annual income of MMPCT ranges between 799 000,00 and 1 137 000,00 UAH. However, these figures should be adjusted for the fixed and variable costs of healthcare facilities, as well as payroll taxes, which may significantly reduce the net income.
3. As a result, the NHSU package for "Mobile Palliative Medical Care for Adults and Children" is significantly underestimated and does not reflect the actual costs of providing quality care to patients, which may lead

to abuses by contractors. Optimizing the management of MMPCTs activities in these circumstances must include resource allocation and route planning, as well as proper and timely documentation of medical episodes. This is particularly important for Ukraine, where the Healthcare Budget is limited, and expenses often exceed allocated resources.

4. Centralized NHSU funding guarantees the Government support for PHC services across all regions and communities. This funding provides some stability in service provision, but there is a need to develop cooperation between charitable, non-governmental, and insurance funds and organizations, which could also be a tool for improving funding.

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The work was carried out within the framework of research works: The Department for Palliative and Hospice Medicine of the Shupyk National Healthcare University of Ukraine "Scientific substantiation of the concept of organizational, medical and psychological support of primary health care specialists for the provision of palliative care to patients and their families in an outpatient setting" (state registration number 0122U000637, deadline: 2021-2023) with the use of research methods of O.O. Kovtun's dissertation on the topic: "Optimization of enterprise business process management".

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Anatolii V. Tsarenko
Shupyk National Healthcare University of Ukraine
9 Dorohozhytska St., 04112 Kyiv, Ukraine
e-mail: atsarenko@gmail.com

ORCID AND CONTRIBUTIONSHIP
Anatolii V. Tsarenko: 0000-0002-1617-3673 A D E F
Zoya V. Maksymova: 0000-0002-1666-3138 B C D
Oleksandr O. Kovtun: 0009-0009-9140-3625 B C D

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

RECEIVED: 19.06.2024
ACCEPTED: 21.10.2024

ORIGINAL ARTICLE

CONTENTS

Significance of oxidative stress in liver damage of male and female rats with simulated ethanol hepatitis

Olha V. Denefil[1], Anna A. Kostiuk[2], Viktoria A. Miroshnyk[1], Svitlana V. Kucher[1], Yuliia A. Sverediuk[1], Ruslan S. Usynskyi[1]

[1]IVAN HORBACHEVSKY TERNOPIL NATIONAL MEDICAL UNIVERSITY OF THE MINISTRY OF HEALTH OF UKRAINE, TERNOPIL, UKRAINE
[2]BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

ABSTRACT

Aim: to find out the gender characteristics of oxidative mechanisms of liver damage in rats with ethanol hepatitis (EH).

Materials and Methods: The study was performed on 48 white male and female rats: 1 – control, 2 – acute EH (12,5 ml/kg of a 40% ethanol solution prepared on 5% solution of glucose for 7 days).The diene and triene conjugates (DC, TC), Schiff's bases (SB), TBA-active products (TBA-ap), activity of superoxide dismutase (SOD) and catalase (CAT) were determined in the blood serum. A morphological study of liver was carried out.

Results: In control males higher values of DC by 8,8%, TC by 7,0%, SB by 7,6%, and SOD by 31,3% were found, females – CAT by 33,8%. An increase in DC, TC, TBA-ap and SB in blood serum of rats was noted during ethanol hepatitis. Thus, the content of DC in male rats increased by 2,6 times, TC – by 65,7%, TBA-ap – by 2,7 times, SB – by 2,8 times; in female, respectively, by 2,3 times, 83,4%, 2,2 times, and 3.4 times. In males with hepatitis, only DC indicators exceeded those in females by 17,6%. In males, the SOD increased by 55,1 %, and in females by 2,0 times. CAT increased 77,0 % in males and 88,9 % in females. Morphological changes confirm more damage to the liver of female rats than males.

Conclusions: In rats with simulated EH, liver damage occurs by an oxidative mechanism, which depends on gender, and more expressed in the female's liver.

KEY WORDS: ethanol hepatitis, rats, oxidative stress

Wiad Lek. 2024;77(11):2277-2282. doi: 10.36740/WLek/197111 DOI

INTRODUCTION

Many diseases, traumatic injuries, and deaths show a direct connection with the volume of alcohol consumption [1]. According to the World Health Organization, as of 2019, 400 million people over the age of 15 are living with an alcohol use disorder, and approximately 209 million suffer from alcohol dependence. It is known that the prevalence of this problem is increasing, and from 2010 to 2019, the number of deaths related to alcohol consumption per 100,000 population increased by 20,2% [2].

Once in the body, alcohol is absorbed in the gastrointestinal tract and metabolized, mainly, by hepatocytes of the liver [3]. Therefore, this organ suffers the most from excessive alcohol consumption, which is associated with the development of oxidative stress, accumulation of acetaldehyde and lipopolysaccharides [4]. Alcoholic liver disease (ALD) unites pathological processes of damage to the parenchyma of the organ caused by alcohol abuse and which can vary from steatosis to the terminal stages of the disease [5].

Alcoholic hepatitis is one of the clinical forms of ALD and has a high short-term mortality risk [6]. It is known that there is a clear correlation between the duration of alcohol consumption and the development of alcoholic hepatitis [7]. However, only 10-20% of people who chronically drink alcohol develop and progress ALD, which indicates the influence of other factors on the course (for example, genetic factors [8], obesity [9], changes in the intestinal microbiome [10], the presence of other liver diseases [11], etc.). Gender also plays an important role in the development of alcoholic hepatitis. Men consume much more alcohol than women, which explains why liver damage is nine times more common in them [12]. However, with the same alcohol consumption, women are considered to be more vulnerable to the development of alcoholic hepatitis, which is probably related to the lower activity of alcohol dehydrogenase and the greater amount of adipose tissue in the body, as well as the difference in sex hormones [13].

Considering that the mortality from an episode of alcohol-associated hepatitis that required hospitaliza-

tion is from 20 to 50% [14], as well as the pathogenesis of alcoholic hepatitis is insufficiently studied, the study of gender characteristics of the development of oxidative mechanisms of liver damage is an urgent task, the solution of which would help in selection of adequate correction methods.

AIM

The aim of the study was to find out the gender characteristics of oxidative mechanisms of liver damage in rats with ethanol hepatitis.

MATERIALS AND METHODS

The work was performed at the Central Research Laboratory of Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine. Morphological investigation of liver were carried out at the Department of Pathologic Anatomy, Autopsy Course and Forensic Pathology of Ivan Horbachevsky Ternopil National Medical University.

All experiments were performed in the first half of the day in a specially designated room at illumination of 250 lux, a temperature of 18-22°C and relative humidity of 40-60%. Animals were kept and experiments on them were conducted in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for research and other scientific purposes. The Commission on Bioethics of Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine (protocol No 77 from April 18, 2024) did not find any violations of ethical norms during the conduct of research work.

The research was carried out on 48 white sexually mature rats of different sexes, aged 4 months, weighing from 180 to 200 g. All animals were divided into 2 experimental groups (Table 1).

Control group rats were kept in standard vivarium regime with free access to food and water.

Simulation of acute ethanol hepatitis was carried out by intragastric administration to animals of 12,5 ml/kg of a 40% ethanol solution prepared on 5% solution of glucose for 7 days. Rats were housed in standard vivarium conditions with free access to water and food [15].

Euthanasia of rats was performed by total bleeding from the heart after previous thiopental-sodium anesthesia (60 mg/kg^{-1} of the animal's body weight intraperitoneally).

In the blood serum of animals, the concentration of diene and triene conjugates (DC, TC), TBA-active products, Schiff's bases (SB), superoxide dismutase (SOD) and catalase (CAT) activity were determined.

The concentration of DC, TC and SB was determined according to the method [16], which is based on the fact that hydroperoxides extracted with a heptane-isopropyl mixture have a certain absorption maximum for DC at $\lambda = 232$ nm, for TC – at $\lambda = 278$ nm, for SB – at $\lambda = 400$ nm. The content of diene and triene conjugates, Schiff bases was expressed in units/g. TBA-active products was determined at a wavelength of 535 nm according to the method [16], expressed in micromoles per litre (μmol/L).

SOD activity in blood serum was determined according to the method [16], expressed in conventional units per 1 mL. CAT activity in blood serum was determined according to the method [16], expressed in mcat/L.

Upper part of the liver was taken immediately after decapitation of the animal and fixed in a 10% solution of neutral formalin. No sooner than two weeks later, the preparations were washed in running tap water and carried out in alcohol, poured into paraffin blocks. Sections were stained with hematoxylin-eosin and viewed under a light microscope [17].

Statistical processing of digital data was carried out using the software "Exel" ("Microsoft", USA) and "STATISTICA" 7.0 ("Statsoft", USA) using parametric and non-parametric methods of evaluating the obtained data. For all indicators, the value of the arithmetic mean of the sample (M), its variance and error of the mean (m) was calculated. The reliability of the difference in values between independent quantitative values was determined in the case of a normal distribution according to the Student's test, in other cases – using the U test of Mann-Whitney. The difference was considered statistically significant at $p<0,05$.

RESULTS

Changes in indicators of lipid peroxidation were detected (Table 2). In control males, compared to females, higher values of DC by 8,8% ($p<0,001$), TC by 7,0% ($p<0,01$) and SB by 7,6% ($p<0,01$) were found.

An increase in DC and TC in blood serum of rats was noted during ethanol hepatitis. Thus, the content of DC in male rats increased by 2,6 times ($p<0,001$), TC – by 65,7% ($p<0,001$), in female animals, respectively, by 2,3 times ($p<0,001$) and 83,4% ($p<0,001$). The development of ethanol hepatitis was also accompanied by an increase in the content of TBA-active products and SB in animals of both sexes. In males, the content of TBA-active products increased, compared to the control, by 2,7 times ($p<0,001$), in females – by 2,2 times ($p<0,001$). SB indicators increased by 2,8 times ($p<0,001$) in males and 3,4 times ($p<0,001$) in females, compared to controls. No statistically significant difference between the parameters of the animals was found. In males with simulated

Table 1. Design of experiment – division of experimental animals

Group number	Investigation condition	Quantity of animal	
		Male	Female
I	Control	12	12
II	Ethanol hepatitis	12	12
Total		48	

Table 2. Changes in indicators of lipid peroxidation in blood serum of rats of different sexes caused by ethanol hepatitis (M±m, n=12)

Group	Index			
	DC, unit/mL	TC, unit/mL	TBA-active products, μmol/L	SB, unit/mL
Males				
Control	1,017 ± 0,008	0,868 ± 0,012	2,188 ± 0,074	0,594 ± 0,009
Ethanol hepatitis	2,609 ± 0,072*	1,438 ± 0,074*	5,855 ± 0,262*	1,678 ± 0,175*
Females				
Control	0,927 ± 0,011**	0,802 ± 0,009**	2,372 ± 0,076	0,549 ± 0,011**
Ethanol hepatitis	2,149 ± 0,075*,**	1,471 ± 0,068*	5,322 ± 0,350*	1,842 ± 0,213*

Notes: * – indicators are reliable (p<0,01-0,001), compared to the control;
** – indicators are reliable (p<0,001), compared to males of the corresponding group.

Table 3. Changes in the enzymatic activity of antioxidants in the blood serum of different sexes animals with ethanol hepatitis (M±m, n=12)

Group	Index	
	SOD, units/mL	CAT, mcat/L
Males		
Control	0,185 ± 0,006	0,074 ± 0,004
Ethanol hepatitis	0,287 ± 0,018*	0,131 ± 0,023*
Females		
Control	0,127 ± 0,005**	0,099 ± 0,003**
Ethanol hepatitis	0,258 ± 0,014*	0,187 ± 0,032*

Note: * – indicators are reliable (p<0,001), compared to the control;
** – indicators are reliable (p<0,001), compared to males of the corresponding group.

ethanol hepatitis, only DC indicators exceeded those in females by 17,6% (p<0,001).

Differences in the indicators of the antioxidant system were also found in rats of different sexes (Table 3). In the blood serum of males of the control group, the level of SOD is higher by 31,3% (p<0,001), compared to females. However, females have higher CAT activity by 33,8% (p<0,001).

SOD and CAT activity increased in animals of both sexes with simulated ethanol hepatitis. So, in males, the SOD indicator increased by 55,1 % (p<0,001), and in females by 2,0 times (p<0,001). CAT activity also increased 77,0 % (p<0,001) in males and 88,9 % (p<0,001) in females. Differences in the indicators of the antioxidant system were not found in ethanol hepatitis rats of different sexes.

Morphological changes in the liver were observed in rats with ethanol hepatitis. Thus, histologically, the liver structure of males was similar to the control group. The traditional lobule structure was preserved. However, hemodynamic disorders were noted, which were manifested by uneven venous-arterial blood filling. At the same time, similar disorders manifested mainly in the portal tracts. Hyperemia was accompanied by blood stasis, erythrocyte sludge, edema of perivasal and periportal spaces.

Hepatocytes became more heterogeneous compared to the control. Among them, cells with granular cytoplasm or protein granules of various sizes were found, sometimes with small fat vacuoles. The contours of individual cells became indistinct. Individual leukocytes were visualized in the perisinusoidal spaces. Stellate macrophages are swollen, some of them are exfoliated into the lumen of the capillary. Round cell inflammatory infiltration took place mainly in the periportal tracts or near the central veins (Fig. 1).

Histological examination of the liver of females with simulated ethanol hepatitis shows somewhat deeper structural

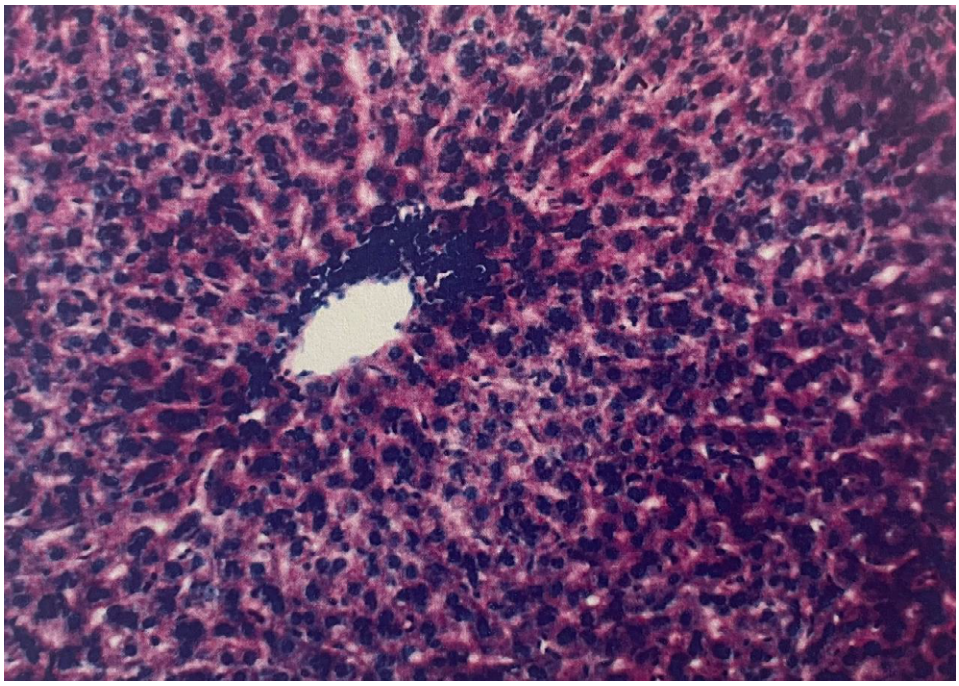

Fig. 1. A fragment of male rat liver with ethanol hepatitis. Inflammatory infiltrate near the central vein. Hematoxylin and eosin straining. x 100.

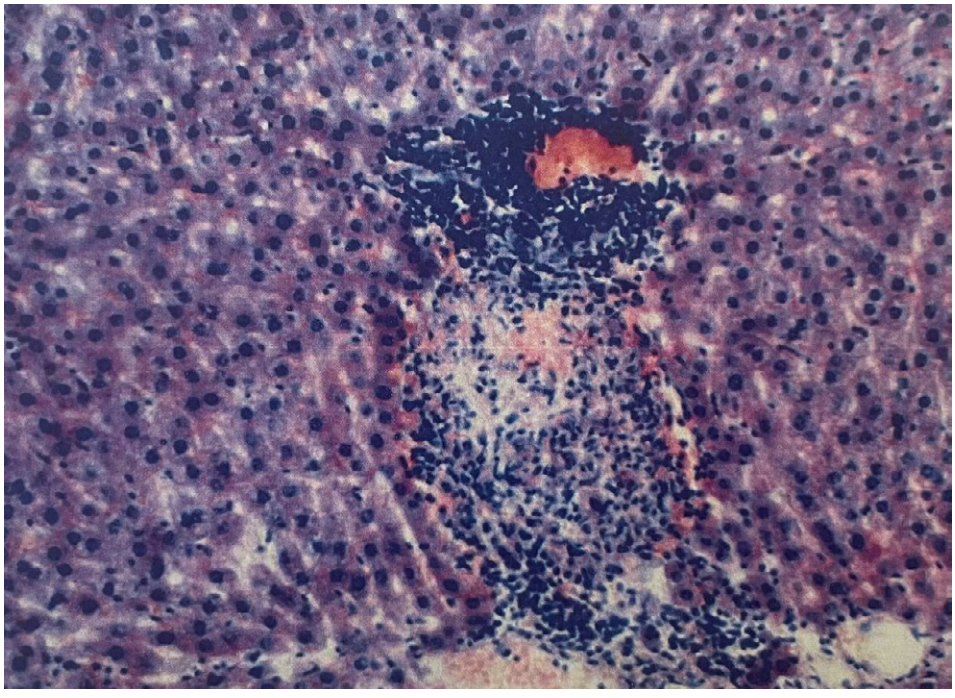

Fig. 2. A fragment of the liver of a female rat with ethanol hepatitis. Intense inflammatory infiltration in the liver parenchyma with its spread along the interlaminar spaces and necrosis of hepatocytes in the center of the infiltrate. Hematoxylin and eosin straining. x 100.

changes. Blood circulation disorders were identical, relative to males, but acquired a systemic character. There is lymphocytic infiltration along the liver plates, occasionally neutrophils were detected. The number of stellate macrophages increased. Lymphocytes formed a tight clutch around the walls of the central veins. The most pronounced round-cell inflammatory infiltration was observed along the course of the portal tracts. Local inflammatory infiltrates with necrosis of liver cells in their thickness were also detected (Fig. 2). The beam-radial structure of the particles prevailed. In many fragments of liver plates, hepatocytes are in a state of protein dystrophy, but there were many intact cells among them.

DISCUSSION

In the blood serum of females of the control group, the concentration of DC, SB, SOD is lower, and TBA-active products and CAT are higher. Taking into account the participation of CAT in the accelerated utilization of ethanol [18], it is possible to think about a better readiness for ethanol inactivation in females, since the CAT pathway helps in the accelerated removal of alcohol from the body. CAT knockout mice have been shown to be incompetent in alcohol metabolism and hydrogen peroxide clearance and are more susceptible to alcohol-induced liver damage [18]. According to the obtained data, females should have less

liver damage compared to males. Males of the same group, despite the higher values of DC and SB, which indicate the predominance of oxidative processes, have greater SOD activity, which indicates the neutralization of the products of lipid peroxidation, but it occurs more slowly, compared to females, that is, according to different mechanisms, which depends on gender. It is possible that females have higher values of TBA-active products, indicating that the accumulation of lipid peroxidation products in them occurs by different mechanisms involving the CAT pathway.

Activation of lipid peroxidation was noted in rats of both sexes with simulated acute ethanol hepatitis. In the blood serum of males, only a higher content of DC is noted, compared to females. It is obvious that the greater growth of DC in males is due to more intensive metabolic processes. But at the same time, a significantly higher content of TC, SB, TBA-active products and antioxidant activity was not noted, compared to females. According to the obtained results, despite the absence of a statistically significant difference, DC, TBA-active products increased to a greater extent in males, and TC, SB increased in females. SOD activity was significantly activated in females, which caused the accumulation of neutralization of primary and intermediate products of lipid peroxide oxidation with the accumulation of final metabolites. As for CAT activity, it increased more in females, which prevented significant morphological damage to their livers.

The morphological picture in the liver of males indicates the development of hepatitis, with signs of inflammation, the presence of fat vacuoles, and protein granules in hepatocytes. Somewhat deeper structural changes are found in females. Lymphocytic infiltration, local inflammatory infiltrates with necrosis of liver cells in their thickness are revealed. In women, compared to men, when consuming the same amount of alcohol, more liver damage occurs [13]. But in our experiments, greater CAT activity was found in women. There is evidence in the literature that estrogen receptor-alpha has higher CAT activity, which contributes to the generation of more ethanol-derived acetaldehyde, but in the heart of women. The latter is supported by the ability of CAT to block estrogen receptor-alpha or to attenuate oxidative stress and myocardial dysfunction caused by alcohol [19]. As for the liver, no such effect was found. We also obtained more structural damage to the liver of female rats and with non-alcoholic liver damage (steatohepatosis, with initial signs of steatohepatitis) [20].

CONCLUSIONS

In animals of both sexes with simulated ethanol hepatitis, liver damage occurs by an oxidative mechanism, as evidenced by an increase in the products of lipid peroxidation with a compensatory increase in the activity of antioxidants. The mechanism of the damaging effect depends on gender: diene conjugates and TBA-active products accumulate more in males, and triene conjugates, Shiff's bases, superoxide dismutase and catalase activities in females. Morphological changes confirm more damage to the liver of female rats than males.

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The work is a fragment of complex research work of Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine, Ternopil, Ukraine «Pathogenetic features of systemic and organ disorders due to the action of extraordinary factors on the body» (state registration number 0121U100071)».

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Olha V. Denefil

Ivan Horbachevsky Ternopil National Medical University
1 Maidan Voli, 46023 Ternopil, Ukraine
e-mail: denefil@tdmu.edu.ua

ORCID AND CONTRIBUTIONSHIP

Olha V. Denefil: 0000-0002-3606-5215 A E F
Anna A. Kostiuk: 0000-0002-5369-2077 B C
Viktoria A. Miroshnyk: 0009-0001-9195-9537 B C
Svitlana V. Kucher: 0000-0003-1026-9567 D E
Yuliia A. Sverediuk: 0000-0002-6231-6962 C D
Ruslan S. Usynskyi: 0000-0001-9565-1014 B E

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

RECEIVED: 25.06.2024
ACCEPTED: 23.10.2024

ORIGINAL ARTICLE

CONTENTS

Gastroesophageal reflux disease: problematic issues of organising outpatient medical care for the adult population

Oleg L. Zyukov, Oleksandr S. Hubar
STATE INSTITUTION OF SCIENCE «CENTER OF INNOVATIVE HEALTHCARE TECHNOLOGIES» STATE ADMINISTRATIVE DEPARTMENT, KYIV, UKRAINE

ABSTRACT

Aim: To determine the problematic issues of the organization of outpatient medical care for patients with gastroesophageal reflux disease (GERD) at the current stage by analyzing the incidence rates and the structure of visits to doctors of a medical institution over a 5-year period.

Materials and Methods: The reported data on the provision of outpatient medical care by doctors of various specialties to the adult population with GERD (ICD-10: K21, K44) from 01.01.2019 to 31.12.2023 were analyzed – 3203 cases of medical visits, 1270 patients.

Results: The prevalence of GERD among the adult urban population is on average 89,84 (83,17-99,12) cases per 10 000, with a tendency to increase prevailing significantly among women and older adults (60 years and older). An imbalance has been established in the system of medical care for patients with GERD, with the dominant role of gastroenterologists, which creates an additional burden on secondary care physicians.

Conclusions: The system of medical care for patients with GERD in real conditions is focused on specialized care by gastroenterology doctors with secondary involvement of primary care doctors, which is contrary to existing standards and international practice, and requires improvement of the functional and organizational model of outpatient medical care for such patients.

KEY WORDS: gastroesophageal reflux disease, organization of outpatient medical care, prevalence of the disease, structure of visits, adult population structure of visits, adult population

Wiad Lek. 2024;77(11):2283-2288. doi: 10.36740/WLek/197152 DOI

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a chronic disease with a long-term course that significantly worsens the patient's quality of life and has a significant economic and social burden [1-3]. The World Gastroenterology Organization (WGO) has recognized GERD as a disease of the 21st century, which is common worldwide, affects all age and gender groups, and has an upward trend [3-5]. Its prevalence, according to various authors, varies widely and depends on the population, country, GERD diagnostic criteria and data collection method [6-7]. In particular, in a systematic review and meta-analysis of the results of 102 studies covering 37 countries and all regions of the UN geographical scheme (until 2018), it was indicated that the total combined prevalence of GERD in the world was 13,98% (95% confidence interval 12,47-15,56%) and varied significantly depending on the region (from 12,88% in Latin America and the Caribbean, 14,12% in Europe, to 19,55% in North America and country (from 4,16% in China, 21,04% in the USA, up to 22,4% in Turkey) [7]. In Ukraine, according to the statistical reporting of requests for medical help, the prevalence of GERD is 190 cases per 100 000 population [8], and according to the data of individual epidemiological studies through questionnaires (according to typical complaints), the prevalence of GERD among the adult population is 30%, including men – 25,1%, women –39,1% [6].

GERD is one of the most common diseases encountered by primary care physicians, gastroenterologists, and surgeons (in the outpatient setting, GERD accounts for 17,5% of all reported gastrointestinal diseases in the US) [2, 9-10]. The organization of the treatment and diagnostic process in patients with GERD by doctors of different profiles or levels needs to take into account such features as the heterogeneity of patient population in terms of the frequency of typical and atypical manifestations of the disease, the severity of the condition, the effectiveness of acid-suppressive therapy, the occurrence of complications, etc., as well as standardization [11]. In Ukraine, standards of medical care for GERD have been developed on the basis of evidence-based medicine using the best global practices [8, 12], but their implementation in the practical activities of health care institutions has certain problems [13].

AIM

To determine the problematic issues of the organization of outpatient medical care for patients with gastroesophageal reflux disease at the current stage by analyzing the incidence rates and the structure of visits to doctors of a medical institution over a 5-year period.

MATERIALS AND METHODS

The data of the statistics department of the State Institution of Science «Center of innovative healthcare technologies» State Administrative Department (SIS "CIHT" SAD) (until 07.07.2024 – State Institution of Science "Research and Practical Center of Preventive and Clinical Medicine" State Administrative Department) on the provision of outpatient medical care by doctors of various specialties to the adult attached population with GERD (ICD-10: K21, K44 in the part "hernia of the esophageal opening of the diaphragm") from January 1, 2019 to December 31, 2023 were analyzed – 3203 cases of medical visits (continuous sample).

Statistical processing of research data was carried out with the help of software products Microsoft Excel (Microsoft Office, 2013) and MedCalc Statistical Software trial v. 23.0.2 using descriptive and analytical statistical methods. Taking into account the distribution law of quantitative traits (testing according to the Shapiro-Wilk test), the arithmetic mean (M), standard error (±m), standard deviation (SD), Student's test (t) were used for the normal distribution law; in other cases – median (Me) with interquartile range (LQ-UQ) and Mann-Whitney test (U). Categorical variables were presented as frequency (P) with standard error (±m), and comparisons between groups were performed using Pearson's Chi-square test (χ^2). The results were considered statistically significant at $p<0,05$.

RESULTS

Significant fluctuations in the prevalence of GERD among the adult population were established over a 5-year period: a decrease in indicators in 2020 – by 34,4% compared to 2019, in 2022 – by 9,4% compared to 2021, and a significant increase in 2023 – by 92,0% compared to 2022 (Fig. 1). The decrease in indicators is possibly related to the decrease in the number of patient requests for medical help during the COVID-19 pandemic (2020) and at the beginning of the full-scale war in Ukraine (2022). The median level (Me [LQ-HQ]) of GERD prevalence among the adult population over a 5-year period was 89,84 (83,17-99,12) cases per 10 000 population. The primary incidence rate of GERD was low, ranging from 1,72 ‰ (2022) to 10,44 ‰ (2020) with a median of 4,26 (2,37-9,11) per 10 000 population. The specific weight of GERD in the prevalence structure of diseases of the digestive organs was 1,35% in 2022 and 3,67% in 2023.

A comparison of GERD prevalence levels among the population of different sex and age groups showed an increase in indicators among women and people of retirement age compared to men and the working-age population both in individual years (Fig. 2) and on average over the entire observation period: 103,74 (89,40-114,85) against 75,07 (72,23-79,24) per 10 000 female and male population, respectively; 94,5 (89,65-107,41) ‰ among patients 60 years and older compared to 82,91 (73,18-86,27) ‰ among persons of working age.

The absence of statistically significant differences between the specified groups ($p>0,05$ according to the U-criterion) is obviously related to the short observation period (5 years) and the large dispersion of data over the years, because an in-depth analysis of the contingent of patients with GERD – 1270 people, who received medical assistance over a 5-year period, showed a significant predominance of female patients – 61,81±1,36% (n=785) versus 38,19±1,36% (n=485) ($p<0,001$), and older people (60 years and older) – 62,05±1,36% (n=788) versus 37,95±1,36% (n=482) ($p<0,001$) (Fig. 3).

The analysis of the frequency and structure of GERD patients' visits to doctors (specialists) of various profiles made it possible to reveal the peculiarities of the organization of outpatient medical care for this contingent, which actually developed at the time of 2019-2023.

It was established that in the structure of patient visits for outpatient polyclinic care during a 5-year period (n=3203) visits with consultative (56,89±0,88%) and medical and diagnostic purposes (27,97±0,79%) dominated (Table 1). At the same time, the number of visits to doctors (specialists) for consultation regarding GERD ranged from 1 to 18 and averaged 2,17±0,07 cases per 1 patient, for treatment and diagnostic measures – from 1 to 11 visits, an average of 1,68±0,06 visits per 1 patient over 5 years ($p<0,001$ by t-test).

During 5 years the largest flow of patients with GERD received medical care from gastroenterologists (n=847; 66,69±1,32%) and family doctors (n=637; 50,16±1,40%). Moreover, the total burden of providing medical care for such patients (the total number of visits) at gastroenterologists was 1,4 times higher than at primary care physicians – 57,2±0,87% of all visits versus 40,77±0,87% respectively ($p<0,001$) (Table 2). The share of referrals to surgical specialists did not exceed 2%.

An important feature of the organization of the process of providing medical care to patients with GERD is close interaction between doctors (specialists) of various profiles. The analysis of visits showed that the majority of patients (n=1027; 80,87±1,1%) sought

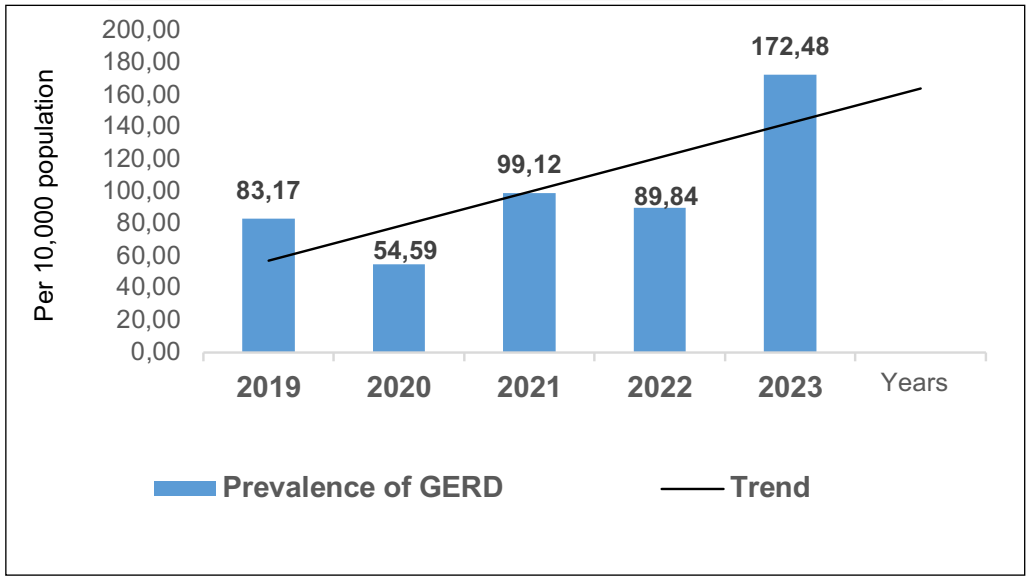

Fig. 1. Dynamics of the prevalence of GERD among the adult population over a 5-year period (2019-2023).

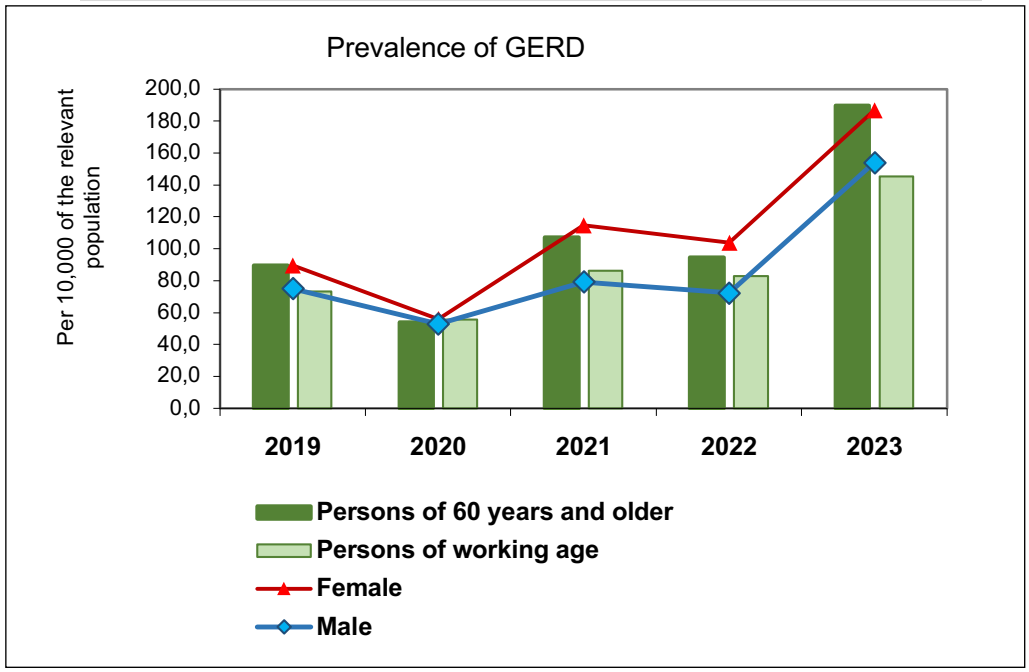

Fig. 2. Dynamics of the prevalence of GERD among the adult population of different sex and age groups over a 5-year period.

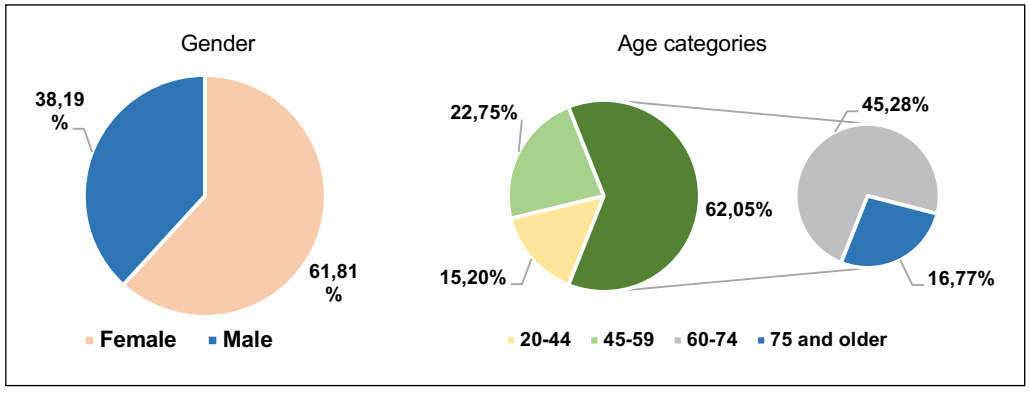

Fig. 3. Distribution of the studied contingent of patients with GERD by gender and age.

medical help only from specialists of one profile within 5 years. This was mostly characteristic of gastroenterologists (n=610; 72,02±1,54%) and primary care physicians (n=400; 62,79±1,92%) with p<0,001 between indicators (Fig. 4). Another 17,87±1,08% (n=227) of patients sought medical help from 2 specialists, mainly in the group "family doctor – gastroenterologist" –17,24±1,06% (n=219).

Table 1. The structure of medical visits depending on the purpose of the patient seeking medical help during a 5-year period

Indicator	The purpose of contacting a doctor (specialist)				
	Preventive examination	Advice	Therapeutic and diagnostic measures	Including surgical intervention	Dispensary observation
Number of patients (n=1270), n/ P±m, %	72/ 5,67±0,65	838/ 65,98±1,33	533/ 41,97±1,38	17/ 1,34±0,32	224/ 17,64±1,07
Number of medical visits (n=3203), n/ P±m, %	88/ 2,75±0,29	1822/ 56,89±0,88	896/ 27,97±0,79	17/ 0,53±0,13	380/ 11,86±0,57
Average number of visits per 1 patient, M±m (SD)	1,22±0,05 (0,45)	2,17±0,07 (1,98)	1,68±0,06 (1,32)	1,0±0,0 (0,0)	1,70±0,09 (1,30)

Table 2. The structure of visits by patients with GERD to doctors (specialists) departments during a 5-year period

Indicator	Doctors (specialists) of departments				
	Family doctor (outpatient clinic)	Gastroenterologist	Otolaryngologist	Surgeon (polyclinic)	Surgeon (inpatient)
Number of patients (n=1270), n/ P±m, %	637/ 50,16±1,40	847/ 66,69±1,32	7/ 0,55±0,21	24/ 1,89±0,38	17/ 1,34±0,32
Number of medical visits (n=3203), n/ P±m, %	1306/ 40,77±0,87	1832/ 57,2±0,87	8/ 0,25±0,09	40/ 1,25±0,20	17/ 0,53±0,13
Average number of visits per 1 patient, M±m (SD)	2,05±0,07 (1,72)	2,16±0,07 (1,96)	1,14±0,14 (0,38)	1,67±0,34 (1,69)	1,0±0,0 (0,0)

The participation of surgical doctors in the provision of medical care to the mentioned contingent was mostly of a consultative/ therapeutic nature as part of multidisciplinary teams of specialists (family doctor, gastroenterologist, surgeon) – 73,17±6,92 % (n=30).

The majority of patients with GERD who sought medical care directly from otolaryngologists (n=6; 85,7±13,2%) were not subsequently referred to primary care physicians or gastroenterologists.

DISCUSSION

Taking into account the existing imperfection of statistical reporting in Ukraine regarding certain nosological forms, in particular GERD (incomplete data registration due to different subordination of health care institutions) and the lack of large-scale scientific studies, the results obtained in our study complement the objective picture of the prevalence of this disease among the adult population of the city [11]. It was established that the average prevalence rate of GERD over a 5-year period was 89,84 (83,17-99,12) cases per 10 000 population and tends to increase, the rate of primary morbidity is 4,26 (2,37-9,11) per 10 000. At the same time, the decrease in incidence with a simultaneous increase in prevalence indicates an increase in the number (accumulation) of patients with this pathology [11]. Discrepancies between indicators obtained from reporting data and epidemiological studies [7] may, on the one hand, indicate insufficient diagnosis and/ or registration of the disease, including by clinicians of other profiles (cardiologists, otolaryngologists, pulmonologists, dentists etc.) with similar symptoms; on the other hand – to testify to the low level of requests for medical help among these patients, especially in the case of an unexpressed clinical disease [14].

Our study also confirmed the predominance of women and older adults (60 years and older) among GERD patients, which is consistent with literature data [3, 6, 15]. In addition, gender and age differences may be related both to the greater attachment of such patients to their health and to the implementation of the doctor's recommendations, and to the presence of comorbid pathology, against the background of which the main symptoms of GERD acquire a clinical manifestation. The literature now talks about the increased medical activity of patients associated with their own "experience" regarding medical care [16-17].

The data we obtained regarding the dominance of the participation of gastroenterologists in the system of providing medical care to the adult population with GERD (57,2±0,87% of visits to gastroenterologists versus 40,77±0,87% to primary care physicians; $p<0,001$) can be explained both by the preserved free access of patients at the level of secondary and tertiary medical care and mainly by the patient's independent formation of his/her route (the experience of the patient's visits to medical institutions, loyalty to his doctor), and by the limitations

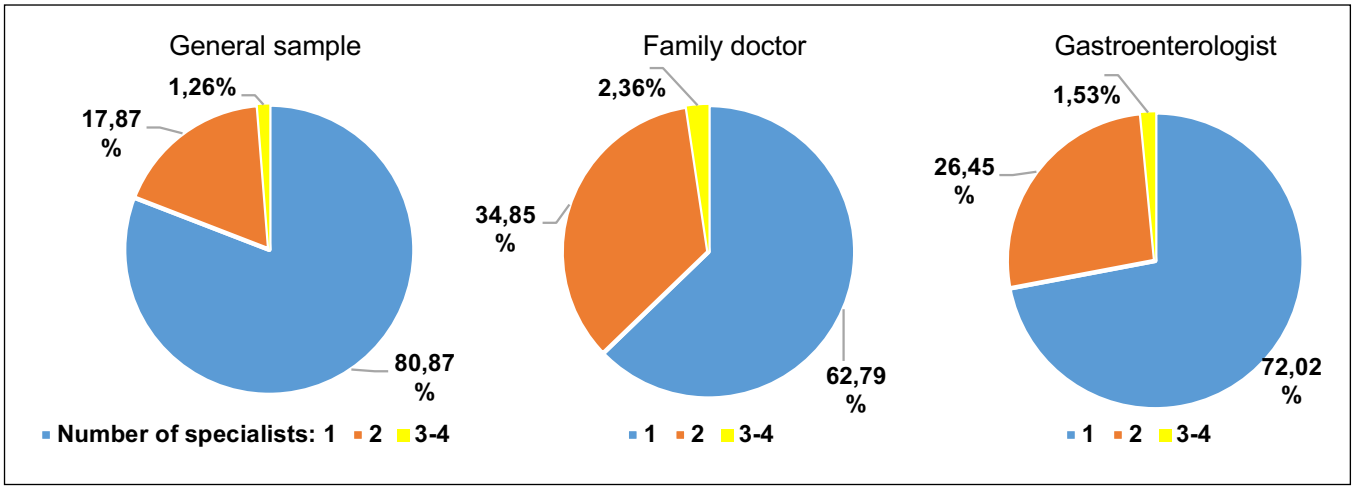

Fig. 4. Distribution of patients who sought medical help within 5 years, according to the number of involved doctors (specialists) of departments.

of primary care doctors regarding medical care for patients with this pathology (insufficient qualification and resource provision, low motivation, etc.) [18]. That is, in real conditions, there is an imbalance in the system of medical care for patients with GERD with the dominant role of gastroenterologists, mostly due to the existing stereotypes of the consumer of medical services and insufficient readiness of primary care doctors to take responsibility for solving the patient's problems. This creates an additional burden on secondary care doctors and, as a result, reduces the availability of patients with other pathologies (increasing the wait to 2-4 weeks).

CONCLUSIONS

1. The problem of medical care for patients with GERD is relevant for the adult population of Ukraine of all sex and age groups. This mostly applies to older patients due to the high frequency of comorbid conditions and increased medical activity.
2. The system of medical care for patients with GERD in real conditions is focused on specialized care by gastroenterology doctors with secondary involvement of primary care doctors, which is contrary to existing standards and international practice, which emphasizes the activity of primary care in this process.
3. An interdisciplinary and interlevel approach to the medical care of patients with GERD requires improvement of the system of registration and statistical accounting of this contingent, ensuring its standardization and compliance with real volumes.
4. Taking into account the interdisciplinary and interlevel approach to the medical care of patients with GERD, in order to improve the manageability of this problem, an in-depth study of the prevalence, structure and risk factors of GERD, conducting a clinical audit, improving the functional and organizational model of outpatient medical care for such patients and its implementation in medical practice is necessary according to medical standards.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Oleksandr S. Hubar
State Institution of Science
«Center of innovative healthcare technologies»
State Administrative Department
5 Verkhnia St., Kyiv 01014, Ukraine
e-mail: alex_gubar@ukr.net

ORCID AND CONTRIBUTIONSHIP

Oleg L. Zyukov: 0000-0003-1190-2571 A E F
Oleksandr S. Hubar: 0000-0003-4486-2377 B C D

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

RECEIVED: 27.06.2024
ACCEPTED: 26.10.2024

ORIGINAL ARTICLE

CONTENTS

Practices of trauma-informed learning and their influence on the level of situational and personal anxiety of students who are studying face-to-face during hostilities

Tetiana I. Miyer[1], Borys I. Palamar[2], Svitlana P. Palamar[1], Olga V. Kotenko[1], Hennadii L. Bondarenko[1], Nataliia V. Vyshnivska[1], Roksolana I. Shpitsa[1]

[1]BORYS GRINCHENKO KYIV METROPOLITAN UNIVERSITY, KYIV, UKRAINE

[2]BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

ABSTRACT

Aim: To reveal the essence of trauma-informed learning in the context of the functioning of a person's inner world and the impact on changing the levels of situational and personal anxiety.

Materials and Methods: The study involved 900 people who are pursuing higher education during the military operations on the territory of Ukraine. Two parts of the Spielberger State-Trait Anxiety Inventory (STAI) questionnaire were used to determine low, medium, and high levels of situational and personal anxiety.

Results: Two groups of education seekers are studying face-to-face during hostilities. The first group includes students with a high level of creativity development who quickly adjust to the learning process after the "Alarm Termination" signal and show interest in communicating with the lecturer and classmates. The second group includes students with a low level of creativity, who, after the "Alarm Termination" signal, seek solitude, withdraw from studies, are depressed for a long time, and are quickly distracted by extraneous sounds.

Conclusions: The repetitiveness of the process of experiencing situational anxiety without external supporting influence causes the formation of personal anxiety as a stable personal tendency in students. The use of preventive practices of trauma-informed learning (practices of focusing attention on breathing with a creative supplement, practices of supportive communication, practices of avoiding retraumatization, practices of "Creative pauses") helps to reduce the levels of situational and personal anxiety among students in face-to-face training during military operations.

KEY WORDS: the inner world of a person, restructuring, anxiety, synergistic approach, preventive educational practices

Wiad Lek. 2024;77(11):2289-2295. doi: 10.36740/WLek/197112 DOI

INTRODUCTION

In this study, we use the terms anxiety (propensity of education seekers to experience anxiety) and worry (an emotional state that arises in situations of uncertain danger, manifested in anticipation of unfavorable development of events during Russia's military actions on the territory of Ukraine). Also, during the research, we consider the distinction in scientific sources [1-4] of two types of anxiety: 1) situational anxiety is the state of the subject at the moment, which is related to a specific external situation; 2) personal anxiety is a state of increased tendency to feel anxious about both real and imagined danger.

Experiencing situational anxiety can cause different states in different subjects. In particular, these are [3, 5, 6]: the state of perceiving anxiety as a warning about probable danger, a state that triggers the process of purposeful search for this danger, the state of active research of the surrounding environment for the specification of risk and its avoidance. It is important to emphasize that a feeling of helplessness, self-doubt, powerlessness in the face of the threatening nature of external factors, general disorganization of activities, and violation of the direction of actions and productivity can accompany the state of experiencing anxiety. Experiencing anxiety can take on external manifestations. It can be [7]: verbal aggression (expression of negativity through the form (raised tone of voice) or content of statements; indirect aggression (generation of gossip, jokes); negativism (demonstrating an oppositional form of behavior); irritation (readiness at the slightest excitement to demonstrate hot temper, harshness, rudeness); resentment (manifestation of envy and hatred towards others).

If the experience of one or another state of situational anxiety is constantly repeated, then with a high prob-

ability, personal anxiety is formed as a stable property of the student of education, that is, a state of increased tendency to feel anxiety about both real and imagined danger develops.

The Ukrainian scientists [1] not only established the connection between personal anxiety and alexithymia but also emphasized that the accumulation of resentment and anger in combination with indirect verbal aggression and a low level of aggressiveness lead to negative experiences and are the impetus for the development of psychosomatic disorders.

In this study, we assume that learning is mental work. Various factors determine the level of its tension. Among the significant factors we include: the content of the educational material; didactic tools used by the lecturer; the presence of education seekers with internal motives for effective mental work; external conditions. Focusing attention on external conditions (for the third year in a row, Russian military operations continue on the territory of Ukraine), we consider the introduction of face-to-face training in the context of two aspects:

The first aspect is a response to the need of education seekers to learn in direct interaction with the teacher and other students (as scientists prove [8]), because mental overload occurs during e-learning. According to the generalizations of scholars [8], mental overload during e-learning occurs when: 1) students on the following skills are formed at a low level: the ability to work with high-tech equipment, such as a computer and the Internet [9]; the ability to work with IC technologies [10]; the ability to manage one's study time without outside help and manage one's own learning rhythms [11]; the ability to separate primary and auxiliary information [12]; 2) it is difficult for students to study in a self-disciplined way [13]; 3) students lack motivation for instrumental use [7]; 4) students perceive the educational system as complex and confusing [14]; 5) the lecturer does not add methodological support to facilitate students' independent work [15].

The second aspect is considering face-to-face training during military operations as intense mental work since training is organized in situations of uncertain danger (shelling, destruction, etc.). During long-term stress, systemic morphofunctional disorders develop in the body, which underlie the mechanism of development of chronic fatigue [16]. In the case of the organization of face-to-face training without taking into account situations of uncertain danger, a high level of probable increase in psycho-emotional stress arises, which, in turn, contributes to the development of chronic fatigue, leads to uneven rates of growth in the severity of symptoms and causes the appearance of reverse processes (intensification of physiological processes, emergence of pre-pathological processes), and irreversible (formation of pathological processes).

AIM

To reveal the essence of trauma-informed learning in the context of the functioning of a person's inner world and the impact on changing the levels of situational and personal anxiety; to summarize the data on the practices of trauma-informed learning, which, according to the results of the study, effectively affect the reduction of the level of situational and personal anxiety of the students of education who are studying face-to-face during military operations.

MATERIALS AND METHODS

A total of 900 participants were included in the study. These are students pursuing higher education at Borys Grinchenko Kyiv Metropolitan University (in particular, future primary school teachers, English language teachers, and future managers of the quality system of primary education) and students of higher education and at the Bogomolets National Medical University (in particular, these are future public health specialists). The study participants studied face-to-face during hostilities; that is, they were negatively affected by war's stressful and destructive factors.

To determine the levels of situational and personal anxiety, two parts of the Spielberger Anxiety Scale questionnaire (State-Trait Anxiety Inventory, STAI) [17] were used. Questionnaire questions contributed to the assessment of situational anxiety (how students of education felt at the moment of full-time study) and personal anxiety (how students of education usually feel). The levels of situational and personal anxiety were determined as a result of the analysis of the answers of the students to the questions of the questionnaire with an emphasis on the degree of intensity (regarding personal anxiety) and with a focus on the frequency of repetition of the feeling of anxiety (regarding situational anxiety). Determining the levels of situational and personal anxiety was carried out using the following scale: low level of anxiety – up to 30 points; average level of anxiety – 31–45 points; high level of anxiety – more than 45 points.

In this study, the authors adhered to the Ethical Principles for Medical Research Involving Human Subjects outlined in the World Medical Association's Declaration of Helsinki (VMA, 1964 p.) and current Ukrainian regulations. The local ethics committee approved the study protocol.

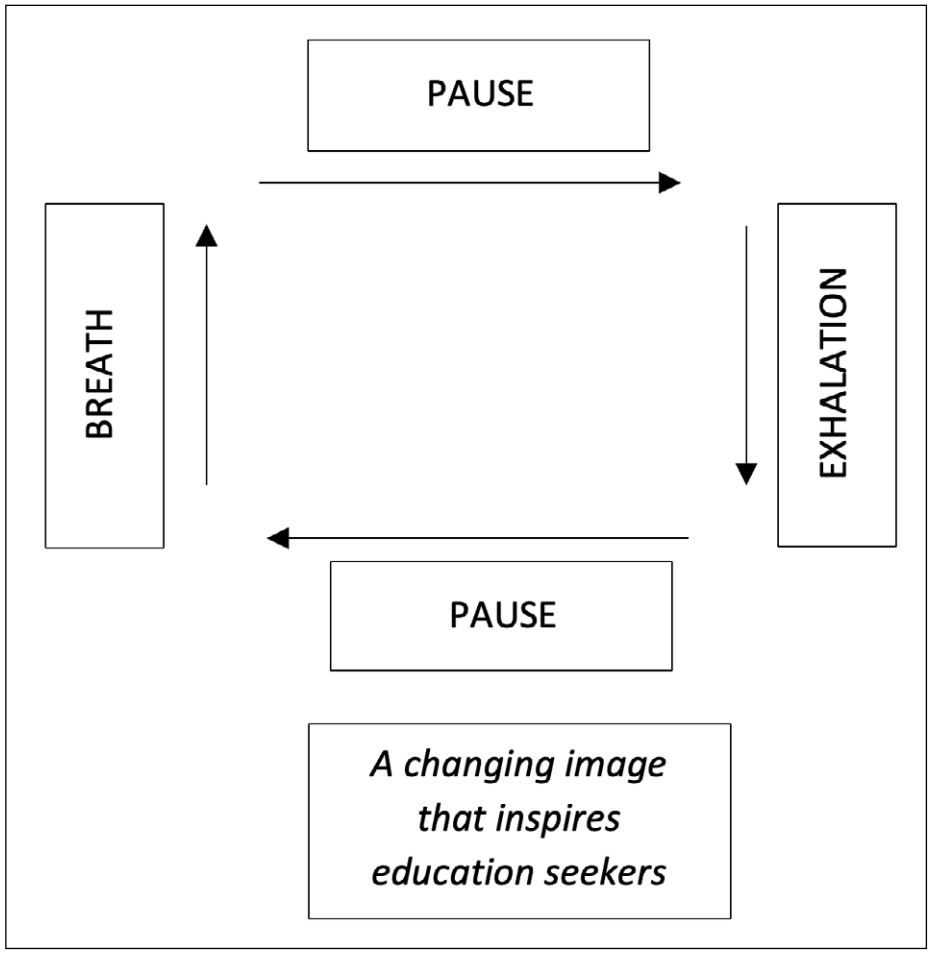

Fig. 1. Self-controlled breathing according to the square.

RESULTS

One of the components of the theoretical basis for the implementation of trauma-informed education of students is a synergistic approach to understanding the nature of the psyche and the functioning of structures in the inner world of a person. We can talk about the stable state of the structures of the inner world of a person only in two cases:

1. Presence of correspondence between the structures of the inner world and external conditions (In the absence of this correspondence, a person feels psychological discomfort. The appearance of this feeling proves that the existing structure no longer corresponds to the new conditions).
2. The presence of correspondence between the structures of the inner world and the person's self (In the absence of this correspondence, the basis for psychotrauma is created).

The organization of education in situations of uncertain danger (shelling, destruction, etc.) disrupts the stable state of the structures of the inner world of education seekers and starts the process of restructuring. The course of the process of restructuring the inner world of education seekers without various complications for their health is facilitated by training, which provides:

1. Considering that the influence of mental states manifests at the cellular level of the organism [18].
2. Increasing the level of resilience of students for full functioning during martial law [3].
3. Organization of training based on health-preserving didactics [20, 21].
4. Taking into account the dynamics of productivity, which is manifested in the sequential change of five stages:

1) the stage of entry into work;
2) the stage of relative working capacity;
3) the stage of inefficiency as a norm of efficiency;
4) the stage of gradual decline in working capacity;
5) final pulse stage.

These stages take place in the educational process of different durations, in particular during one educational session (lecture, seminar, etc.), as well as during one academic day, one semester, one academic year, or period of study at a higher educational institution [22].

5. Organization of higher education based on trauma-informed learning.

Based on the analysis of data from the questionnaires of lecturers and students of education, it was established that when organizing face-to-face training during military operations, various practices of

Table 1. Correspondence between the phrases of judgmental and supportive communication, which cause a specific effect on situational and personal anxiety, and the choice of words by students of education (%)

Phrases of supportive communication	Positive influence on education seekers, %	Phrases of reprehensible communication	Negative impact on education seekers, %
I see you have trouble concentrating, suggests a breathing exercise	95% cases	As always, you can't concentrate	99% cases
I see that it is difficult for you. How can I help you?	92% cases	Why is this such a difficult task for you?	97% cases
Did I notice something bothering you? Do you want to talk about it?	90% cases	What is happening to you lately? What happened to you?	95% cases
Let's agree on the rules of communication that will be comfortable for both of us.	87% cases	You must respect me as a teacher	94% cases

trauma-informed training contribute to reducing the levels of both situational and personal anxiety. During the research, the most effective preventive practices were classified as:

1. PRACTICES OF FOCUSING ATTENTION ON BREATHING WITH A CREATIVE ADDITION

Fig. 1. shows the practice of self-controlled breathing by the square. Students supplement this practice in this way: in the middle of the square, they add an image (photo) that inspires them in this particular situation on this specific day. According to the results of the study, students most often used photos depicting the sea (92% of cases), mountains (67% of cases), flowers (49% of cases), and funny cases with animals (32% of cases).

In the questionnaires, the students noted that the implementation of this practice contributed to switching their attention from external events, information, and sounds to breathing (in 96% of cases), emergence of a state of concentration (in 68% of cases); emergence of a sense of community (in 56% of cases); ease in thoughts (in 44% of cases); commonality with others (in 32% of cases), the emergence of a feeling of security (in 30% of cases).

2. PRACTICES OF SUPPORTIVE COMMUNICATION

In the first column of Table 1, there is a list of phrases that contribute to the reduction of indicators of situational and personal anxiety among students. Also indicated is the percentage of those students who felt a release from the state of anxiety by focusing their attention on the content of the lecturer's phrase. The second column of Table 1 lists the words of judgmental communication. According to the results of the study, the use of these phrases caused a significant increase in the indicators of situational and personal anxiety of students who are studying face-to-face during hostilities.

Table 1 shows the results obtained at the end of the study. It should be emphasized that students, in 68% of cases, ignored them at the beginning of the use of supportive communication phrases. Instead, phrases of judgmental communication had an equally adverse effect on them both at the start of the study and at the end of its conduct. According to the questionnaire data, the students of education reacted more actively to the tone with which a supportive or condemning phrase was said.

3. PRACTICES TO AVOID RETRAUMATIZATION

Trauma-informed education provides for the exclusion from the educational process of situations that directly or indirectly remind students of the trauma, that is, cause retraumatization. In the context of the impact of stressful and destructive factors of war on the inner world of education seekers, situations that cause retraumatization were determined. Based on the processing of the questionnaires, the following generalizations were made: retraumatization can be caused by the following situations:

- specific sounds (in 100% of cases);
- isolation (in 88% of cases);
- decrease or absence of lighting (in 88% of cases);
- public criticism (in 88% of cases);
- downplaying or denying the lived experience (in 80% of cases).

4. "CREATIVE PAUSES" PRACTICES

Also, practices were added to the educational process to develop student creativity. During the conduct of this study, we found that during the announcement of the "Air Alarm" signal and after the announcement of its end, students with different levels of creativity behaved differently, namely:

- students with a high level of creative development in the conditions indicated above quickly adjusted to the

learning process and communicated interestedly with the lecturer and fellow students;
- under the same conditions, students with a low level of creativity development did not show quick adaptation to new learning circumstances; they were depressed for a longer time, quickly distracted by extraneous sounds, and their detachment from learning and loneliness were observed. A short-term outburst of anger was also recorded when responding to the words of classmates or the lecturer. This testified that this category of education seekers needed external help, as experiencing a traumatic experience caused an inadequate perception of the environment and events, which, in turn, significantly increased the indicators of situational (in 47% of cases) and personal (in 16% of cases) anxiety.

To conduct the "Creative Pause" practices, students were offered to choose one of three methods of performing the practice. The method provided for the continuation of the creative idea proposed by the lecturer. During the implementation of the II method, the education students proposed their creative ideas—the third method provided for competition among students for the originality of creative ideas or formulas for creativity.

Next, we give examples of "Creative pauses" practices:

1. Based on creative ideas (1. How to bring a pencil to life. 2. How to combine a textbook and a clock. 3. How to teach a notebook to speak. 4. How to combine a ruler and an alphabet etc.).
2. Based on the application of the creativity formula.

Creativity formula: *Subject + Subject = New subject.*

Option to implement the formula of creativity: *Jacket + backpack = backpack with a hood.*

Options for continuing the implementation of the creativity formula:

Typewriter + TV = ? Book + TV = ? Blackboard + TV = ? Pencil + gouache = ?

DISCUSSION

In the context of a synergistic approach, scientists single out endlessly migrating structures in the ordered, structured, hierarchical space of the psyche. The organization of trauma-informed training contributes to the reduction of the negative impact of situations of uncertain danger on the health of students. The theoretical basis for implementing this training is the work of scientists [5, 6, 17] about the inner world of a person. In this study, we proceed from the fact that the essence of the concept "inner world of a person" is specified by two contexts, namely:

1) the semantics of the concept "world" – in this context, the inner world of a person is characterized as complex, non-linear, structured, capable of productivity, development and self-development;

2) the semantics of the concept of "personality" – according to this context, a person's inner world is characterized as self-directed.

To detail such a characteristic of a person's inner world as self-control, we refer to the scientific works of M. Papycha [17, 18]. The scientist emphasized that a person acts as a subject of his inner world, which he constantly structures and restructures. A person realizes specific behavior through his inner world and performs managerial and regulatory actions. However, these actions do not always occur with the same efficiency and intensity. In the period of age and life crises, psychotraumas, and stressful and destructive factors of war, these processes generally stop because what is not covered by structuring is perceived as alien to the inner world of a person and something that confuses and traumatizes them.

These structures represent processes that are localized in a specific time and space. Moving, the structures form temporary structural connections among themselves and with various components of the psyche. Different thoughts, emotions, feelings, goals, and ideas arise during the movement of structures, and human behavior is formed. Structures appear either slowly and gradually or instantaneously. They move in the inner world of a person, and during this movement, they change it and then disappear, leaving behind experience. Memory stores this experience and can reproduce any structure at any time.

According to scientists [4, 5, 6, 20], the processes of ordering, development, complication, and restructuring of structures in the inner world of a person can be triggered by various factors (experiencing various events (vital, extreme, etc.); experiencing one's own mental states, emotions, impressions; staging and achievement of one's own goals and motives; ontogenetic development; interaction with the environment; internal processes in the "personality" system).

CONCLUSIONS

The results of the conducted research provide grounds for forming the following conclusions:

1. Research results indicate that situational anxiety is observed in students who study during military operations both face-to-face and remotely.
2. The repetitiveness of the process of experiencing situational anxiety without external supporting influence causes the formation of personal anxiety as a stable personal tendency in students of education.
3. As a result of the conducted research, it was established that students with different levels of creative development react differently to the notification

of the "Air alarm" signal and the termination of this signal. Students with a high level of creativity develop quickly after the "Alarm Termination" signal, promptly tune in to the learning process, and show interest in communicating with the lecturer and fellow students. On the other hand, students with a low level of creativity seek solitude, withdraw from studies, are depressed for a long time, and are quickly distracted by extraneous sounds.

4. The use of preventive practices of trauma-informed training during training contributes to the reduction of the levels of situational and personal anxiety among students in face-to-face training during military operations.
5. As a result of the conducted research, the effectiveness of such preventive practices of trauma-informed learning was proven, such as focusing attention on breathing with a creative addition, supportive communication practices, retraumatization avoidance practices, and "Creative pauses" practices.

PROSPECTS FOR FURTHER RESEARCH

We plan to direct further research to establish the relationship between the reduction of personal anxiety indicators and the purposeful interaction of students of education with each other and with the lecturer in the conditions of face-to-face training during military operations.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Borys I. Palamar
Bogomolets National Medical University
13 Taras Shevchenko Blv, 01601 Kyiv, Ukraine
e-mail: palamar.bi@ukr.net

ORCID AND CONTRIBUTIONSHIP

Tetiana I. Miyer: 0000-0002-2874-2925 A B C D E F
Borys I. Palamar: 0000-0003-2510-0713 A B C D E F
Svitlana P. Palamar: 0000-0001-6123-241X A B C D E F
Olga V. Kotenko: 0000-0001-8967-8130 C E
Hennadii L. Bondarenko: 0000-0001-5978-5138 C E
Nataliia V. Vyshnivska: 0000-0001-7509-2841 C E
Roksolana I. Shpitsa: 0000-0002-9354-0641 C E

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

RECEIVED: 11.07.2024
ACCEPTED: 24.10.2024

ORIGINAL ARTICLE

CONTENTS

The effect of the treatment and prevention complex on the activity of the antioxidant protection system in experimental periodontitis combined with stress

Galyna F. Biloklytska[1], Oleksandr Yu. Gutnyk[1], Iryna K. Novytska[2], Olena V. Tretiakova[3]

[1]SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE

[2]STATE ESTABLISHMENT "INSTITUTE OF STOMATOLOGY AND MAXILLO-FACIAL SURGERY NATIONAL ACADEMY OF MEDICAL SCIENCES OF UKRAINE", ODESA, UKRAINE

[3]STATE ENTERPRISE UKRAINIAN RESEARCH INSTITUTE FOR MEDICINE OF TRANSPORT, MINISTRY OF HEALTH OF UKRAINE, ODESA, UKRAINE

ABSTRACT

Aim: To investigate the periodontal protective properties of the developed therapeutic and prophylactic complex in terms of pro- and antiradical protection system on an experimental toxic calcium-deficiency model of periodontitis under noise and cold stress.

Materials and Methods: Calcium-deficiency toxicity was modelled in animals with the addition of noise and cold stress. The effect of the developed therapeutic and prophylactic complex consisting of the oral gel and the food supplement on the indicators of pro- and antioxidant protection in the blood and periodontal tissues of rats was studied.

Results: The results of our biochemical study indicate significant disturbances in the blood and periodontium of experimental animals with modelled periodontitis with the addition of noise and cold stress, the balance of pro- and various links of the antioxidant system. Thus, in animals with experimental periodontitis, an increase in radical oxidant system and an imbalance in the antioxidant system were detected – a multidirectional change in the activity of catalase and superoxide dismutase, a decrease in the activity of both in the blood and in periodontal tissues, compared to intact animals. This resulted in an increase in the malondialdehyde/glutathione peroxidase ratio, indicating a state of depletion of this protection system and the development of oxidative stress.

Conclusions: The treatment and prophylactic complex developed by us provides a high level of antiradical protection, both at the local (in periodontal tissues) and systemic levels, which indicates its pathogenetic orientation.

KEY WORDS: experiment, antioxidant protection, periodontitis, stress

Wiad Lek. 2024;77(11):2296-2302. doi: 10.36740/WLek/197116 DOI

INTRODUCTION

According to the World Health Organization, 95% of the world's adult population has periodontal disease. In modern Ukraine, according to epidemiological studies, the prevalence of periodontal disease among the working-age population aged 25–60 years ranges from 80% to 98%, and generalized periodontitis is the main cause of tooth loss in people over 30 years of age [1, 2]. The debate on the pathogenesis of generalized periodontitis in the scientific community has been going on for more than 50 years, and stress has been identified as one of the various causes of this pathological process, especially in recent years [3, 4]. The data of experimental and clinical studies show that a short-term exposure to negative emotions leads to a long-term increase in vascular tone. At the same time, the level of glucocorticoids in the blood increases, which have a catabolic effect, leading to a slowdown in the recovery processes in cells, which activates lipid peroxidation and increases the content of free radicals, which are known to have a toxic effect. Under the influence of chronic stress in the periodontium, metabolic disorders occur, primarily of lipids, and the formation of lipid peroxides increases, which leads to accelerated involution and the development of hypoxia in periodontal tissues [5-8].

The study of the effect of stress on the development of inflammatory and dystrophic process in periodontal tissues is of particular relevance today in modern Ukraine, which is under full-scale invasion and almost the entire population is under the influence of permanent stress [4].

All of the above indicates that the prevention of periodontal disease, which has been relevant at all times, is becoming increasingly important today and requires consideration of the ever-present stress factors. One of the possible ways to solve this problem is to develop

new pathogenetically targeted therapeutic and prophylactic agents of local and general action with their preliminary testing in the experimental sector.

AIM

The aim of the study is to investigate the periodontal protective properties of the developed therapeutic and prophylactic complex in terms of pro- and antiradical protection system on an experimental toxic calcium-deficiency model of periodontitis under noise and cold stress.

MATERIALS AND METHODS

The experimental studies were performed in accordance with the Law of Ukraine "On Protection of Animals from Cruelty" and the national "General Ethical Principles for Animal Experiments" [10], which are consistent with the provisions of the "European convention for the protection of vertebrate animals used for experimental and other scientific purposes (1985)" [11]. Animals were withdrawn from the experiment by decapitation after preliminary anaesthesia (injections into the abdominal cavity of 2.5% 2,2,2-triethromethanol (Aldrich in 2-methylbutanol; 1:50 in PBS; 300 mg/kg).

The experiments were conducted on 40 white male laboratory rats weighing 115–166 g, which were kept on a standard diet with free access to water and food [9]. All animals were divided into 5 groups of 8 animals each:

Group 1 – control – intact animals;

Group 2 – experimental model of periodontitis with added stress (hereinafter referred to as the model) – administration of warfarin and 2% EDTA solution with modelling of noise and cold stress for 7 days, followed by observation in vivarium conditions for the next 14 days;

Group 3 – model and placebo gel – administration of warfarin and 2 % EDTA solution with modelling of noise and cold stress and daily application of placebo gel to the alveolar mucosa starting from day 7 and for 14 days;

Group 4 – model and developed oral gel «Periogutgel» – administration of warfarin and 2% EDTA solution and daily application of the gel to the mucous membrane of the alveolar ridge starting from day 7 for 14 days;

Group 5 – main – the model and the developed treatment and prevention complex – administration of warfarin and 2% EDTA solution and, starting from day 7, daily, for 14 days, application of the oral gel «Periogutgel» to the mucous membrane of the alveolar ridge and intragastric administration of the mineral complex «Minerol».

Periodontitis was modelled by inducing a toxic calcium-deficiency state in animals with additional exposure to noise and cold stress according to the following scheme: for 21 days, animals were given a 2% EDTA solution (a complex that can bind metal ions, including calcium) daily with drinking water and administered *per os* the drug warfarin. Warfarin is a vitamin K antagonist that blocks the synthesis of vicasol-dependent clotting factors in the liver and in high doses leads to haemorrhages and bleeding, including in the gums. This drug has a cumulative effect and its maximum effect is manifested on days 5 to 7, so the first week the dose of its administration *per os* was 0,1 mg/kg (in terms of the active ingredient warfarin sodium), in the second week the subsequent dose was ½, in the third week was¼ of the initial dose, respectively [12].

To model noise and cold stress, animals of groups 2-5 were kept in a special cold room for 7 days around the clock at a temperature of 5^0 C, relative humidity of 83–91%, with the addition of a noise load of 92-95 dB for 15 minutes, 4 times a day (noise intensity of more than 85 dB leads to physiological and psychological negative effects on the nervous system, sleep, emotions and performance).

The gel was applied to the oral mucosa at a dose of 0,15 mg/kg (which corresponds to the dosage of the drug when used according to the instructions).

«Periogutgel» is a vitamin and mineral phytogel for the oral cavity containing sage extract, aevite, chlorophyllipte and «Minerol» dietary supplement (Expert conclusion No. 972/16 of 08.07.2024).

The mineral complex «Minerol» was administered daily at a dose of 0,08 mg/kg 30 min before feeding. «Minerol» is a dietary supplement with absorbent properties based on natural minerals, which includes silicon, calcium, iron, magnesium, sulfur, zinc, copper, manganese, molybdenum, chromium, iodine, selenium, cobalt, etc. in a naturally balanced form (TU U21640172 – 1 – 2001, Conclusion of the State Sanitary and Epidemiological Expertise 12.2-18-2/27340 of 08.12.2020, manufactured by Gobor, Ukraine, Bucha, B. Khmelnytsky Blvd. 4/220).

To study the state of the pro- and antioxidant protection system in the blood serum and periodontal tissues of animals, the content of diene conjugates (DC), malondialdehyde (MDA), the activity of key enzymes – superoxide dismutase (K.F.1.5.1.5 – SOD), catalase (K.F.1.11.1.6 – CAT), glutathione peroxidase (K.F.1.11.1.7 – GP), and their ratio. The activity of enzymes in periodontal tissue homogenates was expressed in units that characterize the amount of nmol (μmol, mmol) of substrate or reaction product converted per min (time) in terms of mg (g) of protein in the homogenate, which was determined by the Lowry-Folin method [13, 14].

Statistical processing was performed using the Microsoft® Office Excel 2003 package (license № 74017–640–0000106–57490) and EZR v. 1.68. The continuous variables

Table 1. The parameters of pro- and antioxidant systems in blood serum in experimental animals (rats), Me (Q1-Q3)

Indicators / ratios	Group 1 – control (intact animals) n=8	Group 2 – model n=8	Group 3– model + placebo gel n=8	Group 4 – model + «Periogutgel» gel n=8	Group 5 – main group model + «Periogutgel» gel + «Minerol» complex n=8
DC, a.u./ml	1,19 (1,13-1,24)	2,15 (2,02-2,31) p<0,01	2,17 (2,01-2,54) p<0,01	2,18 (1,93-2,30) p<0,01	1,24 (1,14-1,36) p*<0,01/2,3,4
MDA, μmol/ml	0,39 (0,37-0,41)	0,55 (0,51-0,58) p<0,01	0,50 (0,48-0,51) p<0,05	0,48 (0,44-0,49) p<0,01	0,39 (0,38-0,41) p*<0,01/2 p*<0,05/3,4
SOD, U/ml x min	166,7 (157,8-171,1)	264,9 (239,6-272,4) p<0,01	241,1 (230,7-261,9) p<0,05	227,7 (214,3-236,6) p<0,01	169,7 (154,8-181,6) p*<0,01/2,4 p*<0,05/3
CAT, a.u./ml x min	0,41 (0,38-0,46)	0,32 (0,30-0,34) p<0,05	0,32 (0,28-0,35) p<0,05	0,34 (0,28-0,37)**	0,50 (0,47-0,52) p*<0,01/2,3,4
SOD/CAT, a.u.	410,0 (387,9-427,9)	816,1 (738,8-910,1) p<0,01	824,7 (667,1-900,4) p<0,01	750,2 (603,7-816,9) p<0,01	343,0 (297,1-384,5) p*<0,01/2,3,4
GP, μmol of glutathione oxidase/ ml x min	1,12 (1,08-1,21)	0,85 (0,78-0,86) p<0,05	0,85 (0,76-0,87) p<0,05	0,87 (0,82-0,89) p<0,05	1,20 (1,11-1,30) p*<0,01/2,3,4

Notes: p – the significance of changes vs. control (group 1); p* – the significance of changes vs. the corresponding studied group (/2, 3, 4); ** – p = 0,063.

were presented as median (Me) and interquartile range (Q_1 – Q_3). The comparison of indicators in the studies groups was carried out by the use of Kruskal-Wallis H test, with the following Mann-Whitney U-test for *post hoc* comparisons. A 2-tailed p<0,05 was considered statistically significant (considering the Bonferroni correction) [15].

RESULTS

The development of pathological conditions of various etiologies is often accompanied by the activation of lipid peroxidation processes due to the formation of free radicals, which lead to a violation of the integrity of biological membranes. This initiates a number of shifts in energy, carbohydrate, protein, and mineral metabolism.

Thus, an important indicator of the activity of metabolic processes in the body is the determination of the intensity of lipid peroxidation in both blood serum and tissues. The study of various components of protective antioxidant systems, which ensure the balance between the formation and metabolism of reactive oxygen species in cells, is also a necessary step in the investigation of the pathogenetic mechanisms of various types of pathology, including periodontitis.

As a result of the study, it was determined that in the modeling of periodontitis by developing a toxic calcium-deficient state in animals with additional exposure to stressors (noise and cold stress), by the end of the experiment, animals in groups 2 and 3 showed an increase in the level of DC, the initial stage of lipid peroxidation, in the blood serum by more than 80,0% (Table 1). The level of MDA significantly increased in these groups by 28,2-41,0% compared to the control group. In animals of group 4, which were treated only with «Periogutgel» gel, the corresponding values of DC and MDA exceeded the control values by 83,2% and 23,1%, respectively. In the main group 5, which received complex treatment with «Periogutgel» gel and the «Minerol» dietary supplement, stabilization of these indicators was noted, which did not differ significantly from the values of intact animals in the control group but showed reliable differences from groups 2-4, with a reduction in DC and MDA levels by more than 42,0% and 19,0%, respectively (Table 1).

The changes in the activity of antioxidant enzymes are sensitive indicators that characterize the lability of various links of the body`s nonspecific resistance and are gradually (or urgently) involved in metabolic processes in various types of pathology. The study of the key enzyme SOD (Table 1), which belongs to the primary (emergency) link of antioxidant protection, showed its increase in groups 2-3 by 58,9 % and 44,6 %, respectively (vs. control), which clearly indicates the activation of superoxide anion production in the body.

Table 2. The parameters of pro- and antioxidant systems in periodontal tissues in experimental animals (rats), Me (Q1-Q3)

Indicators / ratios	Group 1 – control (intact animals) n=8	Group 2 – model n=8	Group 3– model + placebo gel n=8	Group 4 – model + «Periogutgel» gel n=8	Group 5 – main group model + «Periogutgel» gel + «Minerol» complex n=8
MDA, μmol/ g of tissue	27,9 (26,3-28,4)	37,9 (36,7-39,3) p<0,01	38,4 (36,2-39,5) p<0,01	29,3 (26,6-30,4) p*<0,01/2,3	29,3 (27,7-30,0) p*<0,01/2,3
SOD, U/mg of proteinxmin	127,7 (123,1-136,3)	173,4 (169,5-181,1) p<0,01	164,7 (156,4-178,8) p<0,01	139,3 (133,1-150,2) p*<0,01/2 p*<0,05/3	140,3 (136,4-147,4) p*<0,01/2 p*<0,05/3
CAT, U/mg of protein x min	3,16 (2,97-3,23)	2,77 (2,66-2,90) p<0,05	2,65 (2,39-2,94)**	3,12 (3,02-3,26) p*<0,05/2 p*<0,01/3	3,31 (3,19-3,35) p*<0,05/2 p*<0,01/3
SOD/CAT, a.u.	42,0 (39,8-42,9)	61,8 (59,4-66,4) p<0,01	64,2 (56,5-69,0) p<0,01	44,1 (41,6-47,8) p*<0,01/2,3	43,7 (40,4-45,5) p*<0,01/2,3
GP, μmol of oxidized glutathione/ mg of protein x min	2,31 (2,09-2,46)	1,41 (1,32-1,68) p<0,01	1,27 (1,17-1,46) p<0,01	2,79 (2,62-3,15) p<0,05 p*<0,01/2,3,5	3,83 (3,49-4,12) p<0,01 p*<0,01/2,3,4
MDA/GP, a.u.	11,6 (11,0-13,4)	26,2 (24,2-28,5) p<0,01	29,9 (24,9-34,0) p<0,01	10,4 (8,9-11,4) p*<0,01/2,3 p*<0,05/5	7,3 (7,2-8,2) p<0,01 p*<0,01/2,3 p*<0,05/4

Notes: p – the significance of changes vs. control (group 1); p* – the significance of changes vs. the corresponding studied group (/2, 3, 4, 5); ** – p = 0,074.

At the same time, a significant decrease in CAT activity in these groups by almost 22,0 % was determined.

In the 4th group of animals treated only with «Periogutgel» gel, the activity of SOD in the blood serum remained elevated by 36,5%, with a decrease in the activity of CAT by 17,1 %.

In the main group of animals, group 5, which received complex treatment with the gel and mineral supplement, these indicators did not differ significantly from the control values, at the same time, they had reliable differences from the values groups 2-4 – decreased SOD activity by more than 25.0% and increased CAT activity by about 50%. (Table 1).

The ratio of SOD/ CAT activity is important for ensuring cell viability, since an increase in SOD, without appropriate catalase and peroxidase activation, is cytotoxic. When calculating the SOD/CAT ratio, it was found (Table 1) that this indicator in groups 2 – 4 was 80-100% higher than in the control group, and in group 5, the main group, it practically did not differ from the indicators of intact animals in the control group, but reliably differed from groups 2-4, which indicated the stabilization of the protection system.

Another link in the antioxidant system, GP, detoxifies not only hydrogen peroxide but also organic peroxides (peroxides of unsaturated fatty acids) without the formation of radical products. Studies have shown that the activity of the key enzyme GP in the blood serum was significantly reduced only in groups 2-4 – by 22,0-24,0 % (Table 1). In group 5, the main group of animals treated with the gel and injected with the «Minerol» complex, serum GP did not differ significantly from the control values, but was about 40% higher compared to groups 2-4 (Table 1).

More informative indicators of the development of the pathological process and the evidence base for the effectiveness of the use of the studied drugs for the treatment is the study of the state of pro-antioxidant systems directly in periodontal tissues. The data presented in Table 2 showed that in the modeling of periodontitis against the background of stress factors in animals of groups 2-3 (vs. control), the content of MDA increased by 36,9% and 37,6%, respectively, with a simultaneous activation of SOD by 35,8% and 28,9%, respectively, and a reduce in CAT activity by 12,3% and 16,1%, respectively. At the same time, the SOD/CAT ratio changed significantly, increasing by approximately 50%, which may cause additional development of cytotoxic effects due to the accumulation of hydrogen peroxide in the tissues (Table 2).

In the case of experimental animals of groups 4 and 5, the MDA content in the periodontal tissues did not show significant differences compared to the control, but it was significantly lower than in groups 2 and 3 by approximately 23,0% (Table 2). In group 4, the activity of SOD and CAT did not show significant differences from the control values, but there were significant differences compared to groups 2 and 3. In group 5, which received complex treatment, stabilization of all the studied parameters of the pro-antioxidant protection system in the periodontal tissues was also observed, with a compensatory increase in CAT activity compared to both groups 2 and 3. This was confirmed by the SOD/CAT ratio, which did not differ from the control but was significantly lower compared to groups 2 and 3. In the experimental animals of groups 4-5, the activity of SOD and CAT did not differ significantly from the control values, and their ratio fluctuated within the control range (Table 2).

The study of the key enzyme of the glutathione-antioxidant system (Table 2) also revealed a decrease in its activity in the groups 2-3 of experimental animals, that did not receive an appropriate treatment, by 39,0-45,0 % in contrast to control group. At the same time, the ratio of MDA/GP more than doubled, indicating a state of depletion of this protection system. In the group 4, which was treated with «Periogutgel» only, the activity of GP was reliably increased from the control group by almost 21,0 %, and from groups 2-3 – by around 100 %. In the main group 5, which received complex treatment, signs of adaptive activation of the protective system – a significant enzyme activity both relative to the control group and to groups 2-4. Moreover, the ratio MDA/GP in 4th and 5th groups significantly decreased as to the groups 2-3 more than в 2,5-4,0 times, and in the 5th group, in contrast to control intact group 1, – by 37,1 %. In addition, the level of MDA/GP in the group 5 was significantly lower as compared to the group 4 by almost 30 % (Table 2).

DISCUSSION

This experimental study is one of the stages of a comprehensive study of the periodontal protective properties of the therapeutic and prophylactic complex developed by us on a model of toxic calcium-deficient periodontitis in rats under the influence of noise and cold stress by indicators characterizing the state and ratio of the main links of the prooxidant (ROS) and antioxidant systems (activity of the antioxidant enzymes).

We have developed a mineral and vitamin phytogel for the oral cavity, «Periogutgel», for topical use, and a mineral complex, «Minerol», for general treatment and maintenance of the body's trace element status.

Many previous studies have provided convincing evidence of a significant relationship between periodontal health and the effects of stressors [3-6], with particular emphasis on their impact on the development of oxidative imbalance, which provokes the inflammatory process. It is known that lipid peroxidation in animals occurs at a certain physiological level and is of great functional (regulatory) importance. Mitochondrial membranes maintain a certain level of lipid peroxidation, which reflects the degree of molecular oxygen exposure to mitochondrial lipids under normal physiological conditions. The role of peroxidation processes is determined by their ability to regulate the structural and functional state of membranes, which is crucial for the functioning of enzyme systems [5-8].

The results of our biochemical study indicate significant disturbances in the blood and periodontium of experimental animals with modelled periodontitis with the addition of noise and cold stress, the balance of pro- and various links of the antioxidant system. Thus, in animals with experimental periodontitis, an increase in ROS and an imbalance in the antioxidant system were detected – a multidirectional change in the activity of SOD and CAT, a decrease in the activity of GP both in the blood and in periodontal tissues, compared to intact animals. This resulted in an increase in the MDA/GP ratio, indicating a state of depletion of this protection system and the development of oxidative stress. This may indicate the predominance of damage mechanisms over protection mechanisms and increased inflammatory processes in tissues and the body as a whole. It is worth noting that some authors believe that activation of lipid peroxidation processes causes destruction of cell membranes, which leads to a decrease in immunity and the development of inflammation due to the activation of periodontal pocket microorganisms [3, 4, 7].

The use of the oral gel «Periogutgel» almost did not contribute to the improvement of blood biochemical parameters, but at the same time, according to the obtained indicators, a local therapeutic effect was determined in periodontal tissues. In animals treated with the full therapeutic and prophylactic complex, signs of adaptive activation of the antioxidant system in periodontal tissues were found in terms of the MDA/GP ratio compared to the control group.

Thus, we found that using a phytogel and the therapeutical-prophylactic complex in rats with a periodontitis model and stress has a positive effect on the state of a blood serum and periodontal tissues. This positive effect is due to the normalization of the lipid peroxidation activity (decrease in the content of MDA) and the restoration of the activity of enzymes (SOD and CAT)

and glutathione-antioxidant systems of antiradical protection.

CONCLUSIONS

1. In the periodontal tissues of animals with experimental periodontitis modelled by the development of a toxic calcium-deficient state, under the additional influence of noise and cold stress, lipid peroxidation processes are activated, accompanied by a decrease in the activity of antioxidant protection enzymes, especially its glutathione link.
2. The use of the developed therapeutic and prophylactic vitamin-mineral gel for the oral cavity in animals with experimental periodontitis helps to stabilize the indicators of antiradical protection in periodontal tissues, indicating its periodontal protective properties.
3. The use of the proposed therapeutic and prophylactic complex (gel and oral dietary supplement) in animals with experimental periodontitis under noise and cold stress provides a high level of antiradical protection, both at the local (in periodontal tissues) and systemic levels, indicating its pathogenetic orientation.

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The study was performed as a fragment of the complex initiative scientific research projects of the Department of Therapeutic Dentistry (Shupyk National Healthcare University of Ukraine) «Clinical and pathogenetic substantiation of diagnostics, prevention and treatment of dental diseases in patients with comorbid conditions» (state registration number 0124U000633; term: 2024-2028).

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Galyna F. Biloklytska
Shupyk National Healthcare University of Ukraine
9 Dorohozhytska St, 04112 Kyiv, Ukraine
e-mail: beloklitska@ua.fm

ORCID AND CONTRIBUTIONSHIP
Galyna F. Biloklytska: 0000-0002-3039-0500 A B D E
Oleksandr Yu. Gutnyk: 0009-0003-5561-1837 A B C D E F
Iryna K. Novytska: 0000-0002-5768-5958 A B D E
Olena V. Tretiakova: 0000-0002-4843-729 A B C D

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

RECEIVED: 12.07.2024
ACCEPTED: 27.10.2024

ORIGINAL ARTICLE

CONTENTS

Ultrastructural and morphometric study of regeneration dynamics in the rabbit lower jaw after B-tricalcium phosphate transplantation

Ilona V. Chelpanova
DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE

ABSTRACT

Aim: The aim of this study is to determine the dynamics of histoarchitectural changes in the bone-ceramic regenerate after transplantation β-tricalcium phosphate into an experimental defect in the rabbit mandible.

Materials and Methods: Adult male rabbits aged 6-7 months and weighing 2.5-3 kg were used for the study. The control group consisted of animals with a bone defect that healed under a blood clot. The experimental group consisted of rabbits in which the bone defect was filled with β-tricalcium phosphate. Post-traumatic monitoring was conducted over 84 days using scanning electron microscopy and morphometric analysis of three parameters of the regenerate.

Results: Studying the surface relief characteristics of the experimental bone defect in the lower jaw after implantation of the β-tricalcium phosphate material revealed numerous regenerative changes that occurred after the injury and correlated with the dynamics of changes in the relative volume of bone tissue, osteoplastic material, and connective tissue in the regenerate. Morphometric analysis of the regenerate showed a phased character of the dynamics of changes.

Conclusions: The study found that the relative volume of bone tissue in the regenerate increased over time, and at the end of the experiment, it was statistically similar to the control group.

KEY WORDS: bone regeneration, mandible/lower jaw, β-tricalcium phosphate morphometry, scanning electron microscopy (SEM)

Wiad Lek. 2024;77(11):2303-2310. doi: 10.36740/WLek/197118 DOI

INTRODUCTION

Bone defects and injuries, arising from trauma, diseases, or surgical interventions, are a prevalent issue in modern healthcare. Therefore, the development of effective bone tissue regeneration methods that accelerate healing, reduce the risk of complications, and improve patients' quality of life is a crucial task for contemporary dentistry and traumatology [1,2]. Despite the natural regenerative capacity of bone, substantial defects, pathological fractures, or infections can impede the healing process. According to research by Ferraz MP., in such cases, surgical intervention with the use of bone substitutes is necessary [3]. It is known that the choice of material for bone grafting depends on many factors, such as availability, defect size, biomechanical properties, ease of processing, cost, ethical considerations, biological properties, and potential complications [4]. The «gold standard» for bone grafting of large defects is considered to be the use of autologous bone from the iliac crest. However, this method has its drawbacks [4–6]. Optimal bone grafts should meet the following key criteria: stimulate osteogenesis, promoting healing and restoration of bone structures, not elicit an immune response and promote rapid revascularization, stimulate osteoinduction and osteoconduction for high-quality replacement of the graft with bone tissue, analogous to the tissues of the host organism [4, 6]. Recent research has highlighted the potential of synthetic bone substitutes, particularly bioactive ceramics, to stimulate bone cell proliferation, differentiation, and regeneration [7]. Among these, β-tricalcium phosphate (β-TCP) stands out as a widely used and effective synthetic bone graft substitute. β-TCP is synthetic, possesses excellent osteoconductive and osteoinductive properties, degrades rapidly in the body, and its structure is readily replaced by natural bone tissue. These characteristics make β-TCP a highly promising bone graft substitute [8]. While β-tricalcium phosphate is already widely employed in clinical osteoplasty, its outcomes can be unpredictable. A comprehensive understanding of complete and high-quality bone regeneration in the maxillofacial region, along with its mechanisms and dynamics, necessitates further detailed investigation [8, 9].

AIM

The aim of this study is to determine the dynamics of histoarchitectural changes in the bone-ceramic regenerate after transplantation of β-tricalcium phosphate into an experimental defect in the rabbit lower jaw.

MATERIALS AND METHODS

The study involved 45 adult male rabbits, aged 6-7 months, with a weight range of 2.5-3 kg. The animals were divided into control and experimental groups (20 animals in each). An additional 5 intact animals were used to study the normal structure of bone tissue in the studied area of the lower jaw.

Following general anesthesia administered via intraperitoneal injection of Thiopenate ("Brofarma", Ukraine) at a dosage of 25 mg/kg of body weight, both control and experimental groups underwent bone defect creation. This involved drilling a 4 mm deep and 3 mm wide cavity in the edentulous region of the mandibular part of the lower jaw using a dental burr. The control group consisted of animals with a bone defect that healed under a blood clot. The experimental group consisted of rabbits in which the bone defect was filled with Synthetic β-tricalcium phosphate CerasorbM B-TCP (Inc. in North Carolina, USA) (β-TCF). The study of the condition of bone tissue in the area of the inflicted defect was performed at 1, 7, 14, 21, 28, 35, 56 and 84 days after the injury was inflicted.

The dynamics of histomorphological remodeling of bone tissue in the area of the experimental defect in the lower jaw were studied using scanning electron microscopy, performed on a JEOL T220A scanning electron microscope at the Laboratory of Physical Research Methods in Geology, Ivan Franko Lviv National University . To obtain micrographs of interdental area fragments from the alveolar part of the lower jaw, samples were fixed in a 2.0% glutaraldehyde solution and then washed three times with distilled water (10 minutes each wash). After washing, a sublimation drying procedure was carried out, with freezing of the wet samples and their subsequent placement in a vacuum chamber for sublimation of moisture for 1.5-2 hours until complete evaporation. Dried tissue samples were glued to cylindrical copper holders, after which their surface was metallized by thermal deposition of a thin layer of copper (up to 20 nm). Spraying was carried out in a VUP-5 sputterer. The surfaces of the samples were photographed on x 15-200 magnification, the accelerating voltage in all experiments was 20 kV [10,11].

To prepare histological slides, fragments of the mandibular bone incorporating osteotropic materials were fixed in a freshly prepared 10% formalin solution. Demineralization was then carried out using a 10% aqueous solution of nitric acid (HNO3), ensuring optimal preservation of the regenerate structures. Then, the bone fragments were passed through ascending concentrations of alcohols followed by embedding in paraffin. Sections with a thickness of 5-7 μm were made from the obtained paraffin blocks using a microtome type MS-2 TU-64-1-1629-78. The obtained sections were placed on glass slides, deparaffinized and stained with hematoxylin and eosin according to a standard method [12], visualized using a UlabXSP –137TLED light microscope (China) and photographed with an XCAM-1080 P camera (China). To determine changes in the composition of the regenerate, the relative volume of bone tissue; osteoplastic material; connective tissue in the regenerate, which were determined on demineralized histological sections of fragments of the lower jaw, stained with hematoxylin and eosin, were counted. The morphometric parameters of the bone tissue of the control group animals were compared with those of the animals treated with β-TCF.

To determine compliance of the obtained data to the normal distribution law, an analysis of the distribution histogram, asymmetry indicators and extinction coefficients and the Shapiro-Wilk test were used. The results of each group at different time points followed a normal distribution law, and are presented in the form of M±m, where M – is the arithmetic mean, m – is the standard deviation of the mean. To determine the probable differences between the average values of the indicators at different times of the experiment, as well as to compare the data of the control group with the experimental group at the same observation times, the Student's t-test was used. The difference between the groups was considered significant at $p<0.05$.

Committee on bioethics of Lviv Danylo Halytskyi National Medical University (protocol No. 3 dated March 11, 2020) established that all animals were housed in a vivarium, adhering to established protocols for cleaning, inspection, marking, and all other manipulations in accordance with the outlined guidelines of the «European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes» (Strasbourg, 1985), the «General Ethical Principles of Experiments on Animals» adopted by the First National Congress on Bioethics (Kyiv, 2001), the Law of Ukraine No. 3447-IV «On the Protection of Animals from Cruel Treatment» in accordance with the Directive of the Council of the European Union 2010/63/EU on compliance with the regulations, laws, and administrative provisions of the EU Member States on the protection of animals used for scientific purposes [13,14].

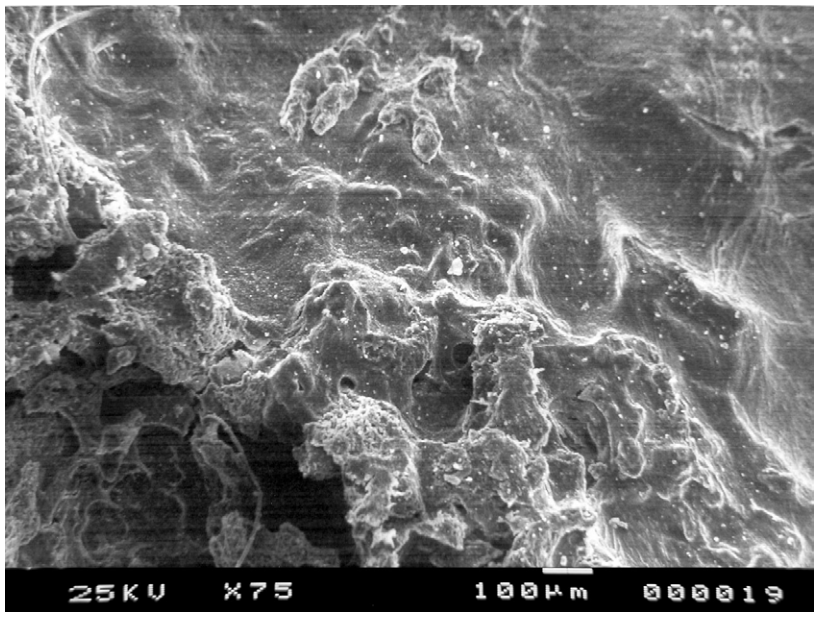

Fig. 1. Electron scanogram of the bone defect zone of the rabbit mandible 1 day after trauma and filling the defect with β-TCP material. ×150. 1 – defect of the surface of the outer bone plate with damaged microvessels; 2 – focus of perivascular edema; 3 – hemorrhage in the damaged interdental region of the alveolar part.

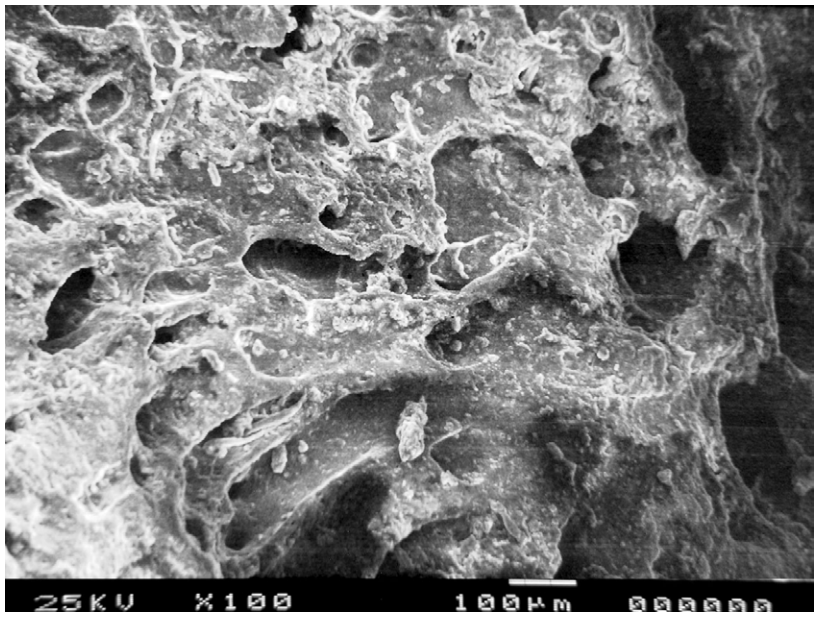

Fig. 2. Electronic scan of the bone defect zone of the lower jaw of a rabbit 3 weeks after injury and filling of the defect with β-TCF material. ×75. 1 – the inner surface of the bone defect; 2 – bumpiness; 3 – heteromorphic osteoid growths.

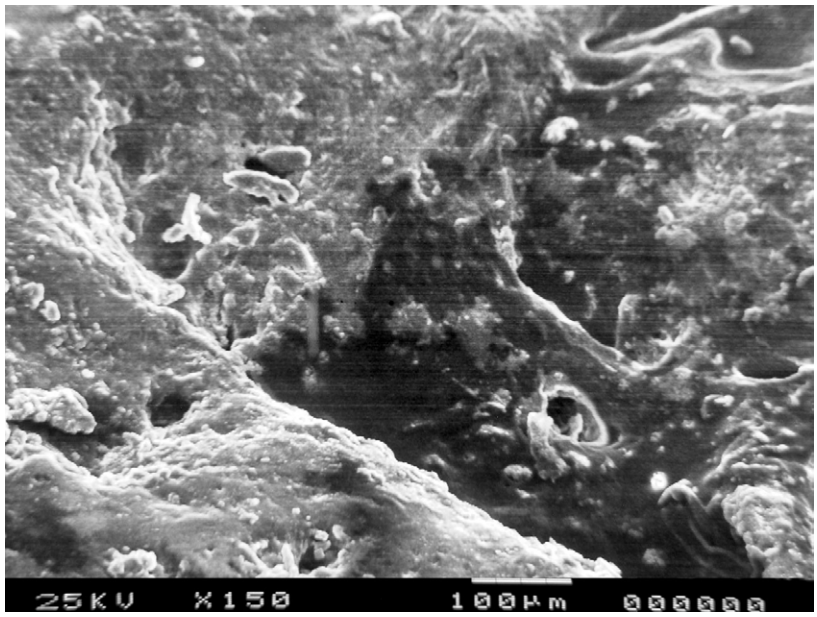

Fig. 3. Electron scanogram of the bone defect zone of the rabbit mandible 8 weeks after trauma and filling the defect with β-TCP material. ×100. 1 – newly formed bone trabeculae of the deep zone of the regenerate; 2 – osteoclast; 3 – intertrabecular spaces.

RESULTS

Scanning electron microscopy used to study the microrelief features of an experimental mandibular bone defect after implantation of β-TCP material revealed numerous regenerative reconstructions that occurred after injury. Specifically, in the compact cortical plate, 1 day after the experimental intervention, damaged blood microvessels with thrombi in the lumen were observed on the relatively smooth surface of the trepanation hole. Small hemorrhages were visualized around the vessels. Between the fractured trabeculae and spicules of the cancellous bone tissue at the edges of the native bone defect, a moderate number of small fragments of varying shapes were observed alongside perivascular accumulations of blood cells (Fig.1). Pronounced signs of perivascular and intracellular edema were observed in tissue structures near the experimental defect. Significant accumulation of exudate was also observed at the edges of the implant between fragments of the implanted β-TCP material.

During the first 3 weeks of the experiment, active formation of numerous osteoid mounds and outgrowths occurred on the surface of the tissue of damaged osteons and bone trabeculae of the native bone. These structures varied significantly in configuration and size, covering the inner surface of the defect and trabeculae (Fig. 2). Signs of inflammation (perivascular edema, leukocyte infiltration) in the defect zone were reduced within the first two weeks after the intervention and were not detected after 3 weeks of the experiment. In numerous small foci of membranous osteogenesis, fibroblast-like cells were distributed diffusely and, unlike the control group, were visualized in large quantities. At the periphery of the regenerate, newly formed trabeculae between fragments of the implanted β-TCP material merged with the nodular trabeculae of the parent cancellous bone. In the deep areas of the regenerate, a moderate number of microvessels were identified surrounded by active fibroblasts and dense spicule-like connective tissue outgrowths.

Four and five weeks after implantation of the β-TCP material, polymorphonuclear osteoid trabeculae with a predominant radial orientation and dense anastomosis were observed at the periphery of the regenerate. Small fragments of the material were rarely detected. In the inner zones of the experimental defect, significant areas of coarse-fibered bone tissue were observed, within which a dense network of primitive hemocapillaries was located, as well as remnants of resorbed β-TCP material. A significant number of osteoblasts formed numerous clusters between osteogenic islands and microvessels. In some small areas of the deep zone of the regenerate, around the implant particles, foci of desmal osteogenesis were observed with a significant number of fibroblasts. In the peripheral trabeculae of the regenerate, between the small remnants of the material, osteoblasts were quantitatively predominant, however, the moderate number of osteocytes significantly exceeded the content of these cells when examining bone samples of the control group. The surface of most newly formed trabeculae displayed a clearly defined heteromorphic bumpiness. Osteoclasts were predominantly found at the border of the bone-ceramic regenerate with the lamellar bone tissue of the cortical plate of the maternal bone. Unlike the previous observation period, foci of desmal osteogenesis were not identified in the superficial localization. A clear border was observed, where the woven bone tissue of the newly formed trabeculae closely adjoined the osteons of the native bone. Some activated osteoclasts, measuring approximately 100 μm, were visualized on the surface of bone trabeculae as well as near particles of the implanted material, indicating intense bioresorption within the regenerate. The total number of osteoclasts was significantly higher compared to both the 5th week of the experiment and the control group animals.

After 8 weeks of the experiment, the polymorphic trabeculae within the bone-ceramic regenerate showed significant variations in their histoarchitecture (Fig.3). Specifically, in the depth of the applied bone defect in the jaw, at the periphery of the regeneration zone, the newly formed trabeculae consisted predominantly of lamellar bone tissue. The content of secondary osteoblasts and osteoclasts was relatively insignificant, and the boundary between the trabeculae of the regenerate and the native cancellous bone was not visualized. Within the regenerate, a heteromorphic cancellous structure was observed, which consisted of primitive bone plates and also contained a notable amount of woven bone tissue with an admixture of partially resorbed fragments of the implanted material. Among the osteogenic cells, diffusely located osteoblasts prevailed in a moderate amount. In contrast to the observations in the control group of animals, a limited number of osteoclasts ware detected only in a small part of the bone trabeculae of the woven bone tissue, which indicates remodeling of these areas. Signs of moderately developed lacunar-canalicular architecture were observed at the boundary of the regenerate with the outer bone plate of the native bone, where newly formed primitive osteons were identified.

After 12 weeks of β-TCP material implantation, the trabeculae of the cancellous bone regenerate of the alveolar process consisted predominantly of lamellar bone tissue. In some deep areas of the regenerate, foci of woven bone tissue were encountered, which contained

Table 1. Dynamics of changes in the relative volume of bone tissue, osteoplastic material and connective tissue in the regenerate (%), M±m

Terms	Bone tissue		Material	Connective tissue	
	Control	β-TCP	β-TCP	Control	β-TCP
1 day	3,7±0,6	4,5±0,7	72,4±5,6	6,9±0,4	6,5±0,4
7 days	5,6±0,8#	7,2±0,8#	64,5±4,1#	14,7±1,1#	16,8±1,8#
14 days	15,4±1,2#	22,5±1,6*#	53,8±4,7#	30,5±2,4#	25,7±2,1*#
21 days	24,7±1,8#	32,5±2,7*#	33,1±2,5#	56,1±3,5#	33,1±3,7*#
28 days	41,5±3,4#	48,4±3,6*#	28,7±2,1	41,8±3,1#	20,6±2,6*#
35 days	54,8±3,7#	62,1±4,9*#	25,3±1,9	34,2±2,3#	13,2±1,8*#
56 days	68,2±4,5#	67,3±5,0	20,8±1,7#	20,7±1,7#	11,2±1,7*
84 days	70,3±4,8	72,1±5,4	15,6±1,2#	24,5±2,0	10,4±1,6*

Note: * - the difference is statistically significant when compared to the control group; # - the difference is statistically significant when compared to the previous time point of the experiment.

a moderate number of activated osteoclasts. Individual small fragments of the implanted material were rarely encountered. In the peripheral areas of the regenerate, near the intact native cancellous bone that was not damaged by the trauma, the microrelief of the surface of most trabeculae was relatively smooth, without signs of new osteoid formation on the surface. The number of osteogenic cells and osteoclasts within the regenerate at this location was lower than at the eighth week of the experiment and approached the characteristics of the undamaged alveolar process bone. Overall, the osteocytic lacunar-canalicular system that formed after implantation of the material acquired features of typical structure. Unlike the control group of animals, in the zone of the outer bone plate after application of β-TCP material, the osteons of the regenerate did not differ in structure and geometry from the typical structure of the native bone. Foci of incomplete osteogenesis were not detected.

In the morphometric study of changes dynamics of the relative volume of bone tissue in the regenerate of the experimental defect in the control group, active growth of the parameter was determined from the first to the fifth week after the defect was created (Table 1). Specifically, the relative content of bone tissue in the regenerate after 2 weeks of the experiment was 2.75 times ($p<0.05$) higher than the value at the 1st week; after 3 weeks, the parameter increased by 60.4% ($p<0.05$) relative to the level of the previous period; after 4 and 5 weeks - by 68.0% ($p<0.05$) and 32.0% ($p<0.05$), respectively. From the 5th to the 8th week, the increase in the relative volume of bone tissue in the regenerate that formed under the blood clot was 24.5% ($p<0.05$). Subsequently, until the 12th week of the experiment, the changes in the indicator were not statistically significant compared to the value of the 8th week, stabilizing at the level of (70.3±4.8)%.

Morphometric analysis revealed that after the application of β-TCP material, there was active growth in the relative volume of bone tissue in the regenerate. The dynamic of this growth was similar to that observed in the control group, but the intensity of changes significantly exceeded the control group between the 2nd and 5th week. So, 1 week after implantation, the parameter value was 60.0% ($p<0.05$) higher than its level on the first day of the experiment, but didn`t differ statistically significantly from the control group indicator. After 2 weeks of β-TCP implantation, the parameter value increased 3.1 times ($p<0.05$) compared to the value of the first week, which was 46.1% ($p<0.05$) higher than the control indicator. Subsequently, active growth in the relative volume of bone tissue in the regenerate also occurred: after 4 weeks - by 48.9% ($p<0.05$) relative to the third week, after 5 weeks - by 28.3% ($p<0.05$) relative to the fourth week, and in both of these periods, the parameter of the experimental group statistically significantly exceeded the corresponding control values. From the 5th to the 12th week after implantation of the material, the relative volume of bone tissue stabilized at the level of 62-73% of the total volume of the bone-ceramic regenerate and at the end of the experiment did not differ statistically significantly from the indicators of the control group.

Analysis of the relative volume of osteoplastic material in the regenerate during the stages of augmentation of the experimental defect in the group with β-TCP implantation revealed gradual activity of bioresorption in the first two weeks of the experiment. Specifically, within the first week after implantation, the parameter under study decreased by 26.8% ($p<0.05$) relative to the value after 1 day. Within the second week, the decrease in the relative content of the material was 16.6% ($p<0.05$) compared to the value of the first week. During the 3rd week, there was a more significant decrease in the parameter - by 38.5% ($p<0.05$) relative to the value after 2 weeks, however, by the end of the 5th week, the indicator of the relative volume of osteoplastic material

in the regenerate didn`t have statistically significant changes compared to the previous periods. However, at the end of the experiment, the rate of β-TCP bioresorption accelerated again. Specifically, after 8 weeks, the parameter decreased by 17.8% ($p<0.05$) relative to the level of the 5th week, after 12 weeks - by 25.0% ($p<0.05$) relative to the level of the 8th week, reaching a value of (15.6±1.2)%.

In the first three weeks of the experiment, active growth in the content of the connective tissue component in the regenerate was observed. Specifically, 1 week after the trauma was inflicted, the parameter increased 2.1 times ($p<0.05$) relative to the value after 1 day; after 2 weeks of the experiment - by 107.5% ($p<0.05$) compared to the value of the 1st week; after 3 weeks, the parameter increased by 83.9% ($p<0.05$) relative to the level of the previous period and reached the highest value (56.1±3.5)%. Morphometric study of the relative volume of connective tissue in the regenerate of the experimental defect in the control group revealed a phased nature of the dynamics of parameter changes. In the subsequent dynamics of changes in the content of connective tissue, a gradual decrease in the indicator was observed: after 4 weeks - by 25.5% ($p<0.05$) relative to the peak value of the 3rd week; after 5 and 8 weeks - by 18.2% ($p<0.05$) and 39.5% ($p<0.05$), respectively, compared to the previous research period. From the 8th to the 12th week, the relative volume of connective tissue in the regenerate that formed under the blood clot did not change statistically significantly and stabilized at the level of (24.5±2.0)%.

In the experimental group of animals where the defect was repaired using β-TCP material, a characteristic phased dynamic of changes in the relative volume of connective tissue in the regenerate was observed, which had significant differences from the changes in the control group. After 1 week of trauma, the parameter value was 2.6 times ($p<0.05$) higher than the indicator of the first day and didn`t differ significantly from the control level. After 2 weeks of β-TCP material implantation, there was a moderate increase in the parameter compared to the value of the first week (by 53.0%; $p<0.05$), however, the degree of this increase was less than in the control group (by 15.7%; $p<0.05$). After 3 weeks, the parameter of the experimental group increased by 28.8% ($p<0.05$) relative to the previous period and reached the highest value for the entire observation period (33.1±3.7)%, which was significantly lower than the control (by 41.0%; $p<0.05$). After the 3rd week, in the experimental group, active reduction of the connective tissue component of the regenerate was observed, the severity of which exceeded the control dynamics. Specifically, 4 weeks after β-TCP material implantation, the parameter under study was 37.8% ($p<0.05$) lower than the value of the 3rd week, and 50.7% ($p<0.05$) lower than the corresponding control value for the same period. After 5 weeks, the parameter in the experimental group was 35.9% ($p<0.05$) lower than the value of the 4th week, and 61.4% ($p<0.05$) lower than the control value. At 8 and 12 weeks after β-TCP implantation, the content of connective tissue in the regenerate didn`t change significantly compared to the indicator of the 5th week, but was significantly lower than the control level: after 8 weeks - by 45.9% ($p<0.05$), after 12 weeks - by 57.6% ($p<0.05$).

DISCUSSION

Bone grafting is a widely used procedure in dentistry. It is relevant in various clinical scenarios, including periodontal surgery, dental implant placement, sinus lift, socket preservation, and many other procedures [15–17]. Synthetic materials present a promising alternative for bone tissue regeneration, offering a less invasive approach compared to autogenous, allogeneic, and xenogeneic bone grafts. Unlike autogenous grafts, synthetic materials do not require additional surgical intervention for taking bone tissue which reduces the risk of infection, scarring, and other complications, and shortens the operative time [18,19]. Despite their popularity and availability, the effectiveness of calcium phosphate materials for bone regeneration in the maxillofacial region needs further investigation as the outcome of their application is not always predictable [8, 9]. Previous studies have reported positive results from the use of trace elements, such as Sr, Si, Mg, and Zn, to enhance the osteoinductive properties of β-TCP. Research has also investigated the use of growth factors as an alternative method to improve osteogenesis. While most results were favorable, growth factors such as BMP-2 and VEGF are not recommended for clinical use due to potentially serious side effects [20].

Therefore, β-TCP is clinically safe, possessing its own osteoinductive properties. Our research has expanded the understanding of the dynamics of histarchitectural features in bone tissue regeneration through detailed ultrastructural analysis. So, the study of the dynamics of histoarchitectural remodeling and ultrastructural features of bone regeneration using β-tricalcium phosphate will allow the development of more effective innovative solutions that improve bone regeneration outcomes in the maxillofacial region. Developing effective methods for bone tissue regeneration remains a key challenge in modern dentistry and traumatology

Modern developments in the production of pure β-TCP, as well as access to research using nuclear mag-

netic resonance, transmission electron microscopy, scanning electron microscopy, x-ray and radiovisiographic methods, tomography, etc., will undoubtedly allow us to find answers to existing questions in the near future, and thus making β-TCP an even more attractive research subject and bone graft substitute [8].

CONCLUSIONS

Determining the dynamics of histoarchitectural remodeling of the bone-ceramic regenerate after β-tricalcium phosphate transplantation into an experimental defect in the rabbit mandible allowed us to establish a consistent increase in the relative volume of bone tissue in the regenerate. This increase was significantly greater than the control group from the 2nd to 5th week, but did not differ statistically significantly from the control group at the end of the experiment. Morphometric analysis of the relative volume of bone tissue, osteoplastic material, and connective tissue in the regenerate of the experimental defect allowed us to establish a phased character of the dynamics of the studied changes, which correlated with the dynamics of numerous regenerative changes in the surface relief of the experimental bone defect of the mandible after β-tricalcium phosphate (β-TCP) implantation. After all, in today's conditions, the time frames for the regeneration of bone defects, their complete and high-quality regeneration, are of particular importance, and the results obtained by us in comparison with literature data provide a clearer understanding of the mechanisms and terms of activity of the regenerative processes of bone defects of the lower jaw.

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The study was conducted within the framework of the initiative research work of the Department of Histology, Cytology and Embryology, Danylo Halytsky Lviv National Medical University: "Morpho-functional and Immunohistochemical Features of Tissues and Organs in Norm and under Pathological Conditions." State registration number: 0122U000168.

CONFLICT OF INTEREST
The Author declare no conflict of interest

CORRESPONDING AUTHOR
Ilona V. Chelpanova
Danylo Halytsky Lviv National Medical University
52 Pekarska St., 79010 Lviv, Ukraine
e-mail: ilona.med75@gmail.com

ORCID AND CONTRIBUTIONSHIP
Ilona V. Chelpanova: 0000-0001-5215-814X A B C D E F

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

RECEIVED: 09.07.2024
ACCEPTED: 28.10.2024

ORIGINAL ARTICLE

CONTENTS

Morphometric assessment of the structural remodeling of endothelial cells in the arterial and venous systems of the testes in experimental animals under ethanol intoxication

Mykhaylo S. Hnatiuk, Serhiy O. Nesteruk, Yaroslav O. Bilyk, Larisa Ya. Fedoniuk, Viktor V. Tverdochlib, Liudmyla V. Tatarchuk, Olena A. Yakymchuk

I HORBACHEVSKY TERNOPIL NATIONAL MEDICAL UNIVERSITY, TERNOPIL, UKRAINE

ABSTRACT

Aim: Using morphometric methods to study the features of endotheliocyte remodeling of the arterial and venous beds of the testicles during long-term ethanol intoxication.

Materials and Methods: Endotheliocytes of the arterial and venous bed of the left and right testes of 60 white male rats, which were divided into two groups, were morphologically studied. The 1 group consisted of 30 intact animals, the 2 - 30 rats, which were daily intragastrically injected with a 30 % ethanol solution at the rate of 2 ml per 100 g of the animal's weight for 28 days. Animals were euthanized by bloodletting under thiopental anesthesia. Histological preparations were made from the testes, which were stained with hematoxylin and eosin, a mixture of acid fuchsin and picric acid, Mallory's mixture, according to Masson, toluidine blue, impregnation with silver nitrate was carried out. The area of the endotheliocyte, its nuclei, the nuclear-cytoplasmic index, and the relative volume of damaged endotheliocytes were determined on the micropreparations of the left and right testes in the arteries and veins. Measurements were performed on vessels of small caliber (outer diameter 26-50 μm). Quantitative values were processed statistically.

Results: It was established that intoxication of white male rats with ethanol led to pronounced remodeling of the endothelial cells of the arterial and venous beds of the left and right testes, which was characterized by a disproportionate decrease in the area of endotheliocytes and their nuclei, a violation of structural cellular homeostasis, and a pronounced increase in the relative volumes of damaged endotheliocytes. Thus, in the arteries of the left testicle, the relative volume of damaged endotheliocytes increased by 17.7 times, in the right - by 14.9 times ($p<0.001$), in the veins, the indicated morphometric parameters changed by 18.8 and 18.4, respectively times ($p<0.001$).

Concluions: Conducted studies and obtained results indicate that intoxication of laboratory sexually mature white male rats with ethanol leads to remodeling of the endotheliocytes of the arterial and venous vessels of the testes. Revealed remodeling of endothelial cells of testicular arteries and veins under the influence of ethanol is characterized by unevenness. a disproportionate decrease in the area of endotheliocytes and their nuclei, a change in the relationship between them, disturbances in structural cellular homeostasis, and an increase in the relative volumes of damaged endotheliocytes. The established structural rearrangement of the endotheliocytes of the vascular bed in the conditions of the simulated pathology dominated in the veins and in the left testicle.

KEY WORDS: endothelial cells, arteries, veins, testes, ethanol, morphometry

Wiad Lek. 2024;77(11):2311-2316. doi: 10.36740/WLek/197119 DOI

INTRODUCTION

Recently, researchers have increasingly focused on studying the structure and function of endothelial cells and their changes in various physiological and pathological conditions [1-3]. The endothelium is a major component of the inner layer (intima) of blood and lymphatic vessels, as well as the inner surface of heart chambers. These cells represent a multifunctional, heterogeneous, dynamic, secretory, and metabolically active system, forming a monolayer of mesenchymal origin [4-6].

Quantitative morphological methods have been widely used in medical-biological studies in recent years, allowing for an objective assessment of structural-functional changes in organs and systems under various physiological and pathological states, significantly expanding research possibilities. Researchers often apply morphometry to enhance the accuracy and reliability of the obtained information [7-9].

Ethanol (ethyl alcohol) primarily enters the body through the gastrointestinal tract, being rapidly absorbed and reaching maximum concentration in the blood within about half an hour. Ethanol is classified as a vascular poison that circulates in blood vessels, damaging endothelial cells. It is worth noting that quantitative morphological changes in the testes and, in particular, in their vascular endothelial cells due to ethanol intoxication are not fully understood [10-12].

AIM

To investigate the characteristics of endothelial cell remodeling in the arterial and venous systems of the testes during prolonged ethanol intoxication using morphometric methods.

MATERIALS AND METHODS

A complex of morphological methods was used to study the endothelial cells of the arterial and venous systems of the left and right testes in 60 laboratory adult male white rats, which were divided into two groups. The first group consisted of 30 intact experimental animals, and the second group included 30 rats that received a daily intragastric dose of 30% ethanol solution at a rate of 2 ml per 100 g body mass for 28 days [11]. The animals were kept in standard vivarium conditions. Euthanasia was performed by bleeding under thiopental anesthesia. Histological preparations were made from the testes, stained with hematoxylin and eosin, a mixture of acid fuchsin and picric acid, Mallory's mixture, Masson's trichrome, and toluidine blue, and silver nitrate impregnation was conducted [13], followed by light optical and morphometric examination. The area of the endothelial cell, its nucleus, the nuclear-cytoplasmic index, and the relative volume of damaged endothelial cells were determined on micro-preparations from the left and right testes in the arterial and venous systems. Measurements were taken on small-caliber vessels (external diameter 26-50 μm) using an Olympus BX-2 light microscope with a digital camera and the "Video Test 5.0" and "Video Size 5.0" software packages. Quantitative data were statistically processed. Results were analyzed in the Department of Systemic Statistical Research at Ternopil National Medical University named after I. Ya. Horbachevsky, Ministry of Health of Ukraine, using the Statsoft STATISTIKA software package (license no. VHR303F737429FA-8). Differences between comparative values were determined using Student's t-test [14, 15].

It is worth noting that the performed experimental studies and euthanasia of laboratory sexually mature white male rats were performed in compliance with the «General Ethical Principles of Animal Experiments» adopted by the First National Congress on Bioethics (Kyiv, 2001) and in accordance with the «European Convention on the Protection of Vertebrate Animals, which are used for research and other scientific purposes» [16].

RESULTS

A comprehensive analysis of the data revealed that prolonged ethanol intoxication in experimental animals resulted in pronounced structural remodeling of endothelial cells in the arterial and venous vessels of the left and right testes, confirmed by the obtained morphometric parameters. For instance, the area of endothelial cells in the arteries of the left testis significantly decreased ($p<0.001$) from (29.70±0.24) μm^2 to (20.60±0.21) μm^2, a reduction of 30.6%. The nucleus area of the mentioned cell in control observations was (7.45±0.06) μm^2, whereas in the second group (ethanol intoxication), it was (7.20±0.05) μm^2. A statistically significant difference was found ($p<0.05$), with the latter morphometric parameter being 3.3% smaller than the previous one.

Disproportionate changes in the morphometric parameters of the cytoplasm of endothelial cells in the arteries of the left testis and their nuclei in experimental animals under ethanol intoxication led to a disruption of the relationship between them, which was adequately demonstrated by the nuclear-cytoplasmic index. The last quantitative morphological indicator in the second group was (0.349±0.003), showing a high degree of statistically significant difference ($p<0.001$) compared to the control indicator (0.251±0.002), a difference of 39%. It was also established that, in the modeled experiment, the relative volume of damaged endothelial cells in the arteries of the left testis increased 17.7 times ($p<0.001$), reaching (37.20±0.18)% (Table 1).

It is also worth noting that the changes in the studied morphometric parameters of the arteries of the right testicle of experimental animals during ethanol intoxication were similar compared to the above data. Thus, the area of the endotheliocyte of the studied vessels decreased statistically significantly ($p<0.001$) by 29.9% compared to the control value, the area of the nucleus – by 2.2% ($p<0.05$).

The nuclear-cytoplasmic index in the arterial endotheliocytes of the right testicle increased by 37.6% with a statistically significant difference ($p<0.001$), and the relative volume of damaged endotheliocytes increased by 14.9 times ($p<0.001$).

It was also established that the changes in the morphometric parameters of the endothelial cells of the arteries of the right testicle were smaller compared to the structural rearrangement of the indicated cells of the left testicle.

The morphometric parameters of the endotheliocytes of the venous vessels of the left and right testicles of experimental animals obtained as a result of the research are shown in Table 2.

A comprehensive analysis of the given quantitative morphological indicators of the studied structures in the indicated table established that under the conditions of ethanol poisoning of experimental animals, they changed significantly.

Table 1. Morphometric characteristics of testicular arteries' experimental animals' endothelial cells (M±m)

Indicator	Group of animals	
	First (n-30)	Second (n-30)
Left testicle		
Endotheliocyte area, μm²	29,70±0,24	20,60±0,21***
Endotheliocyte nucleus area, μm²	7,45±0,06	7,20±0,05*
Nuclear-cytoplasmic index	0,251±0,002	0,349±0,003***
Relative volume of damaged endothelial cells, %	2,10±0,02	37,20±0,18***
Right testicle		
Endotheliocyte area, μm²	29,40±0,24	20,74±0,21***
Endotheliocyte nucleus area, μm²	7,35±0,05	7,15±0,05*
Nuclear-cytoplasmic index	0,250±0,002	0,344±0,003***
Relative volume of damaged endothelial cells, %	2,05±0,03	30,60±0,18***

Note. **-p<0,01: ***-p<0,001 compared to the 1st group.

Table 2. Morphometric characteristics of testicular venous blood vessels endothelial cells of experimental animals (M±m)

Indicator	Group of animals	
	First (n-30)	Second (n-30)
Left testicle		
Endotheliocyte area, μm²	18,54±0,15	15,90±0,12***
Endotheliocyte nucleus area, μm²	10,18±0,08	9,50±0,07**
Nuclear-cytoplasmic index	0,550±0,004	0,598±0,005***
Relative volume of damaged endothelial cells, %	2,28±0,03	42,96±0,51***
Right testicle		
Endotheliocyte area, μm²	18,46±0,18	16,04±0,12***
Endotheliocyte nucleus area, μm²	10,20±0,09	9,40±0,07**
Nuclear-cytoplasmic index	0,552±0,003	0,586±0,004**
Relative volume of damaged endothelial cells, %	2,20±0,03	38,60±0,51***

Thus, the area of the endotheliocyte of the venous vessels of the left testis of laboratory sexually mature white male rats during ethanol intoxication statistically significantly ($p<0.001$) decreased from (18.54±0.15) μm² to (15.90±0.12) μm², i.e. by 14.2%. Under these experimental conditions, the area of the nuclei of the endothelial cells of the studied vessels also decreased.

Thus, in the control observations, the indicated morphometric parameter of the left testicle was equal to (10.18±0.08) μm², and in the 2nd group of observations (ethanol intoxication) – (9.50±0.07) μm². A statistically significant difference ($p<0.001$) was established between the given quantitative morphological indicators. At the same time, the last morphometric parameter turned out to be smaller compared to the previous one by 6.7%.

The nuclear-cytoplasmic index in the endothelial cells of the venous vessels of the left testis in the second group was (0.598±0.005), which showed a statistically significant difference ($p<0.001$) compared to the control value, increased by 8.7%. The relative volume of damaged endothelial cells in the venous vessels of the left testis in the first group was (2.28±0.03)%, and under ethanol intoxication (the second group of rats), it was (42.96±0.51)%. The morphometric parameters showed significant statistical differences ($p<0.001$), with the last indicator increasing 18.8 times compared to the previous one.

In the right testis, the studied morphometric parameters of endothelial cells in the venous vessels also changed similarly under the influence of ethanol. However, the degree of these changes in the right testis was less than that in the left testis. For example, the area of endothelial cells in the veins of the right testis under ethanol intoxication significantly decreased ($p<0.001$) by 13.1%, and the area of the nucleus decreased by 7.8% ($p<0.01$).

The nuclear-cytoplasmic index in the endothelial cells of the venous vessels of the right testis increased by 6.1% with statistically significant difference ($p<0.01$) compared to the control morphometric parameter. The relative volume of damaged endothelial cells in the

venous vessels of the right testis under ethanol intoxication increased significantly ($p<0.001$) from (2.20±0.03)% to (38.60±0.52)%, a rise of 17.5 times.

Light optical examination of the testicular micro-preparations revealed significant disturbances in lymphatic and blood circulation. The venous vessels were primarily dilated, congested, with thinned walls. Observations showed perivascular and stromal edema, foci of dystrophically, necrobiotically, and apoptotically altered endothelial cells, interstitial endocrinocytes, spermatogenic epithelial cells, stromal structures, foci of cellular infiltration, and connective tissue proliferation. The lumens of the venous vessels were deformed, exhibiting alternating constrictions and dilations with sacculations. Endothelial cells exhibited swelling, unclear boundaries, desquamation foci, and proliferation. This proliferation may indicate the presence of hypoxia. More pronounced morphological changes were noted in the left testis of the experimental animals. Some researchers attribute these intensified morphological changes to increased venous pressure in the left testicular veins and difficulty in venous outflow compared to the right [17].

DISCUSSION

The endothelium is a metabolically active tissue characterized by heteromorphism, primarily judged by morphological features: varied shape, size, cell surface relief, organelle arrangement, structure of intercellular contacts, and subendothelial structure [18].

The endothelium produces numerous factors that ensure normal homeostasis: growth stimulators and inhibitors; vasorelaxants and vasoconstrictors; pro- and anti-thrombotic factors; activators and inhibitors of fibrinolysis; cytokines, illustrating the functional diversity of the endothelial monolayer in normal physiology [19, 20]. Intact endothelium modulates the tone of smooth muscle cells, maintains the non-adhesive surface of the vessel lumen, mediates hemostasis, cell proliferation, and inflammatory and immune mechanisms in the vascular wall. The endothelium is viewed as a central mediator of vital information exchange between the cardiovascular and immune systems, as well as a powerful autonomous endocrine organ with paracrine functions regulating vascular tone, coagulation, cell migration, and proliferation, participating in inflammatory responses, tissue regeneration, and fibrosis, determining the kidney's filtration function, and the diffusion of water and ions [21, 22].

Currently, it has been established that the endothelium is a dynamic system that can change, and its structural-functional properties are closely related to numerous local and systemic factors, as well as the peculiarities of structural organization. In different areas of the vascular system, endothelial cells are subjected to varying hemodynamic and metabolic conditions, resulting in differences in shape, size, nuclear and cytoplasmic properties, biochemical specificity, types of receptors, enzyme activity, and orientation relative to the vessel axis [23].

Damage or activation of endothelial cells disrupts normal regulatory mechanisms and leads to phenotypic changes (imbalance between relaxing and constricting factors, anticoagulant and procoagulant mediators, promoters and inhibitors of growth), wdich are named as endotheliail disfunction [6].

In the arterial wall, certain consequences of endothelial dysfunction are directly related to the pathophysiology of atherosclerosis and are manifested by vascular reactivity and angiospasm; increasing permeability for lipoproteins; violation of the regulation of the growth of vascular wall cells (decreased regeneration of the endothelium and increased proliferation of smooth muscle cells); shifts in the hemostatic/fibrinolytic balance (promoting thrombin generation, platelet and fibrin deposition). It is believed that various angiospastic stimuli can lead to endothelial dysfunction with an imbalance of active vascular mediators that are responsible for maintaining normal angiotonus [24]. These same factors can also cause a violation of the antithrombogenic activity of the vessel wall by disrupting cellular metabolism and depleting energy reserves.

The main stimuli of endothelial dysfunction are activation of the endothelium by cytokines, hypercholesterolemia, and oxidation of lipoproteins. To date, there are no definite explanations for the mechanisms of lipid/cytokine-mediated endothelial dysfunction, but it is assumed that they may be oxidative and non-oxidative in nature.

In the case of long-term exposure to damaging factors: hypoxia, toxins, immune complexes, inflammatory mediators, hemodynamic overload, persistent activation or damage of endotheliocytes occurs, which leads to a pathological response even to ordinary stimuli with thrombus formation and cell proliferation [23]. The reaction of the body in response to hemodynamic overload is the activation of circulating endotheliocyte-like stem cells of the bone marrow due to the initiation of the processes of their proliferation, migration and differentiation into functionally complete endotheliocytes. Such stem cells are able to facilitate the repair of the endothelium in the process of angiogenesis *in vivo*.

One of the most significant mechanisms of endothelial dysfunction is the disruption of L-arginine-nitric oxide (NO) metabolism, which is the main effector molecule

produced by endotheliocytes. Changes in the function of the vascular endothelium can be a consequence of both a decrease in the synthesis of NO by endothelial cells and the activation of its degradation processes under conditions of oxidative stress.

Endothelial dysfunction is a violation of the relationship between factors that support homeostasis and that regulate numerous functions of the endothelium. Violations of the functional state of the endothelium have been described in various pathological conditions, such as atherosclerosis, arterial hypertension, hypercholesterolemia, chronic heart failure, and diabetes [25, 26]. At the same time, it was established that the dysfunction of the endothelium is detected already at the initial stages of the disease.

The endothelium plays a major role in regulating vascular architecture, and long-term changes in hemodynamic stress can mediate vascular remodeling. This process involves the restructuring of the components of the vascular wall, i.e. endothelial and smooth muscle cells, collagen matrix. Two main endothelial factors have a pronounced effect in this process: NO, which inhibits lipogenesis and proliferation of smooth muscle cells, and endothelin, which is mitogenic and activates pro-oncogene expression in vascular smooth muscle cells. It is possible that anti-inflammatory cytokines, angiotensin II and norepinephrine contribute to vascular remodeling. These factors stimulate the formation of active forms of metalloproteases - enzymes involved in the destruction of the vascular intracellular matrix. However, the contribution of structural remodeling and reduced elasticity of small vessels in the progression of vascular damage still remains unclear.

CONCLUSIONS

The conducted studies and obtained results indicate that intoxication of laboratory sexually mature white male rats with ethanol leads to pronounced remodeling of the endotheliocytes of the arterial and venous vessels of the testicles. The revealed remodeling of the endothelial cells of testicular arteries and veins under the influence of ethanol is characterized by an uneven, disproportionate decrease in the area of endotheliocytes and their nuclei, a change in the relationship between them, disturbances in structural cellular homeostasis, and an increase in the relative volumes of damaged endotheliocytes.

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The work is a fragment of scientific research work of the Clinical Anatomy and Operative Surgery Department of the I. Horbachevsky Ternopil National Medical University "Structural and functional regularities of the course of adaptive and compensatory processes in organs and systems during surgical interventions on abdominal organs and chest cavity under the influence of toxic endogenous and exogenous factors" (registration number 0122 U 000031).

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Larysa Ya. Fedoniuk
I. Horbachevsky Ternopil National Medical University
9 Valova St, 46000 Ternopil, Ukraine
e-mail: Fedonyuk22Larisa@gmail.com

ORCID AND CONTRIBUTIONSHIP

Mykhaylo S. Hnatiuk: 0000-0002-4110-5568 E F
Serhiy O. Nesteruk: 0000-0003-1573-4480 A B C D
Yaroslav O. Bilyk: 0000-0001-8971-1420 B C
Larisa Ya. Fedoniuk: 0000-0003-4910-6888 A E F
Viktor V. Tverdochlib: 0000-0003-3460-5625 C
Liudmyla V. Tatarchuk: 0000-0002-4678-4205 C D
Olena A. Yakymchuk: 0000-0002-5373-5161 C

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

RECEIVED: 10.07.2024
ACCEPTED: 26.10.2024

ORIGINAL ARTICLE

CONTENTS

ABCC8 polymorphisms rs757110 and rs1801261 association with sulfonylurea therapy of Iraqi type 2 diabetics

Sarah H. Shaalan[1], Muneer Khudhair[2], Noaman Ibadi Mohammed[3], Zubaida Falih Alzubaidi[4]

[1]DEPATMENT OF PHARMACOGNOSY, KUFA UNIVERSITY COLLEGE OF PHARMACY, KUFA, IRAQ

[2]DEPARTMENT OF LAB. INVESTIGATION, FACULTY OF SCIENCE, UNIVERSITY OF KUFA, KUFA, IRAQ

[3]DEPARTMENT OF PHYSIOLOGY, UNIVERSITY OF KUFA FACULTY OF VETERINARY MEDICINE, KUFA, IRAQ

[4]DEPARTMENT OF CLINICAL LABORATORY SCIENCES, FACULTY OF PHARMACY, UNIVERSITY OF KUFA, KUFA, IRAQ

ABSTRACT

Aim: Our study aimed to investigate the connection between the ABCC8 gene polymorphisms (rs1801261 and rs757110) and T2DM in the Iraqi Middle Euphrates region. Patients with type 2 diabetes were chosen because they were treated with glibenclamide and glimepiride.

Materials and Methods: The groups of this case-control study are the control group obviously healthy persons included 400 (235 Male/165 Female) and 400 T2DM group (213 Male/ 187 Female). The FSG, HDL-Ch, Total-Ch, LDL-Ch, and Tgs are measured and genotyping of these two SNPs.

Results: Our findings that FSG augmented in CA and AA codominant styles of rs757110. Moreover, there were decreased insulin and BMI significantly ($p = 0.002$ & 0.026 respectively) in CA and AA codominant patterns of rs757110. Likewise, glucose was high significantly (0.017) and reduced insulin and BMI ($p = 0.001$ & 0.013 respectively) in dominant pattern (CA+AA) of rs757110. As codominant pattern (of rs757110), AA carriers revealed significant ($p = 0.03$) risky (OR = 1.83) when adjustment with age, BMI and sex. Additionally, after controlling for gender, age, and dominant pattern, CA + AA carriers had a significant ($p = 0.02$) risk factor for diabetic disease (OR = 1.45). There was no correlation between rs1801261, and T2DM.

Conclusions: The rs757110 related to less insulin excretion in T2DM of Iraqi Middle Euphrates population who received glimepiride and glibenclamide for diabetes treatment.

KEY WORDS: Polymorphisms, Type 2 diabetes mellitus, sulfonylurea receptors, ABCC8 gene

Wiad Lek. 2024;77(11):2317-2325. doi: 10.36740/WLek/193434 DOI

INTRODUCTION

The sulfonylurea receptors (SURs) are membranous receptors that are the primary targets of the antidiabetic tablets sulfonylurea whose promote pancreas to release insulin from beta cells. More specifically, SURs receptors are monomers of potassium ion channels known as Kir6.x (6.1 and 6.2) inwardly-rectifying potassium channels [1]. The ATP-sensitive K+ channel (KATP channel) is an ion-directing channel that is formed when 4 Kir6.x subunits join with 4 SURs subunits [2]. SUR1, which is encoded by ABCC8 gene (ATP-binding cassette transporter sub-family C member 8) [3], is one type of sulfonylurea receptor. Pancreatic beta cells are sensitive to ATP, which binds to the KATP channel, shutting it down. There is a relative decrease in membrane polarization, which unlocks voltage gated Ca^{+2} channels and causes an increase in intracellular [Ca^{+2}], which in turn promotes insulin secretion [4].

ABCC8 polymorphisms perform a significant function in affecting the risk as well as treatment consequences regarding type 2 diabetes mellitus (T2DM). Research specifies that the rs757110 (Ser1369Ala) variant of ABCC8 gene is linked with different responses to sulfonylurea medicines spent to T2DM management, modifying aspects like fasting glucose concentrations and A1C ratios [5]. Additionally, studies retain that alterations in ABCC8 can change insulin discharge and glucose homeostasis, conceivably heading to T2DM progress. However, a definite common rs1799854 (C49620T) polymorphism of SUR1 genetic material, which is component of ABCC8 gene, was not originate to be accompanying with insulin resistance and excretion, or the progress of gestational diabetes with a previous GDM condition [6]. These findings show up the complex interchange between ABCC8 polymorphisms and T2DM vulnerability and treatment concerns.

ABCC8 expression, a gene combined using sulfonylurea response, changes across different populations and situations. In patients combined with congenital hyperinsulinism (CHI), variations of ABCC8 cause hyperinsulinemic hypoglycemia (HH) [7]. For T2DM individuals, distorted ABCC8 expression is related to

diabetic kidney disease (DKD) [8]. Moreover, shifts in ABCC8 expression have been seen in colorectal cancer. This indicates that it might be a participant or catalyst for the development and growth of cancer [9]. Further, in a murine sepsis model, ABCC8 knockout showed lessened microglial activation and better neurobehavioral results. This suggests its involvement in brain injury response [10]. These highlight the different effects of ABCC8 expression on different conditions and groups of people that can impact their response to sulfonylurea therapy.

A particular rs757110 SNP shows a higher risk of T2DM, especially G-allele in terms of Ala1369Ser polymorphism within ABCC8 gene [11]. Study states that Kurdish people in western Iran are more prone to T2DM due to this genetic variant [12]. In addition, they found certain variants associated with INS-R were connected with lower risk for getting T2DM which implies a safeguarding impact is present too [13]. Moreover, studies that focus on the analysis of genetic variations specific to ancestry have identified genes related to T2DM. This highlights how crucial it is to use function-based and ancestry-specific methods when finding genes linked with polygenic diseases like T2DM [14]. These findings stress on the importance of genetic elements, like SNP rs757110, in affecting the susceptibility towards developing T2DM within distinct populations.

The rs1801261 polymorphism of the ABCC8 gene was associated with several T2DM concerns. For example, evidence suggested that this polymorphism might impact the response to repaglinide treatment in Chinese Han T2DM patients and it has a link with susceptibility to T2DM [15]. More investigations showed those who carry variants similar to this had worse beta-cell function, raising chances for getting Type 2 Diabetes at lower BMIs and younger ages [16]. But this diversity was not found to be linked with T2DM in a cohort from Asian Indian. This could show possible dissimilarity among various ethnic groups [17]. Also, the effect of ABCC8 genetic variation including rs1801261 is associated with the development of cerebral edema in those who have traumatic brain injury. This points to a wider role beyond diabetes [18]. These two variants of ABCC8 gene; rs757110 and rs1801261 have not been studied among Iraq population regarding their connection to sulfonylurea therapy for type 2 diabetics.

AIM

Our study aimed to investigate the connection between the ABCC8 gene polymorphisms (rs1801261 and rs757110) and T2DM in the Iraqi Middle Euphrates region. Patients with type 2 diabetes were chosen because they were treated with glibenclamide and glimepiride.

MATERIALS AND METHODS

ETHICAL APPROVAL

Every participant in this experiment gave their informed consent, and the institutional ethical committee approved the study. The design of this work complies with the 2001 World Medical Association Declaration of Helsinki.

STUDY PARTICIPANTS

The groups of this case-control study are the control group obviously healthy persons included 400 (235 Male/165 Female) and 400 T2DM group (213 Male/187 Female). The T2DM patients detected matching to American Diabetes Associations descriptions [19] at the medical centers of Iraqi Middle Euphrates region. Diabetic type 2 chosen as they be given glimepiride and glibenclamide for diabetes handling. Every specimen that was gathered between April 2023 and April 2024. Participants with heart, renal, hepatic or other illnesses were not allowed to participate in the research. The biochemical parameters that were assessed for the participants, who range in age from 35 to 65 years, including insulin after night fating, total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol as well as serum glucose following overnight fasting (FSG).

CHEMICAL ANALYSES AND SAMPLE COLLECTION

Participants were given a 5-milliliter vein blood draw following fasting; each sample was divided into two milliliter aliquots for the genotyping analysis and three milliliter aliquot portions for the assessments of lipids, insulin, and glucose. The biochemical tests used to spectrophotometrically detect HDL-Ch, FSG, LDL-Ch, Total-Ch, and Tgs using Colorimetric Detection Kits made by Invitrogen-Thermo Fisher Scientific per manufacturer recommendation. The constructor described method was used for computing insulin utilizing the Human Sandwich ELISA kit from Abcam Company (ab100578). Insulin resistance (IR) was assessed with the use of Matthews' Homeostatic Model [20]. HOMA-IR is equal to [FSG (mg/dL) * FSI (μU/mL)]/405, where FSG and FSI stand for fasting serum glucose and insulin, respectively.

GENOTYPING

Two milliliter aliquots of heparinized whole blood were utilized in the Blood DNA Isolation Mini Kit (Catalog No. 46380) manufactured by NORGEN-Biotek Corp.

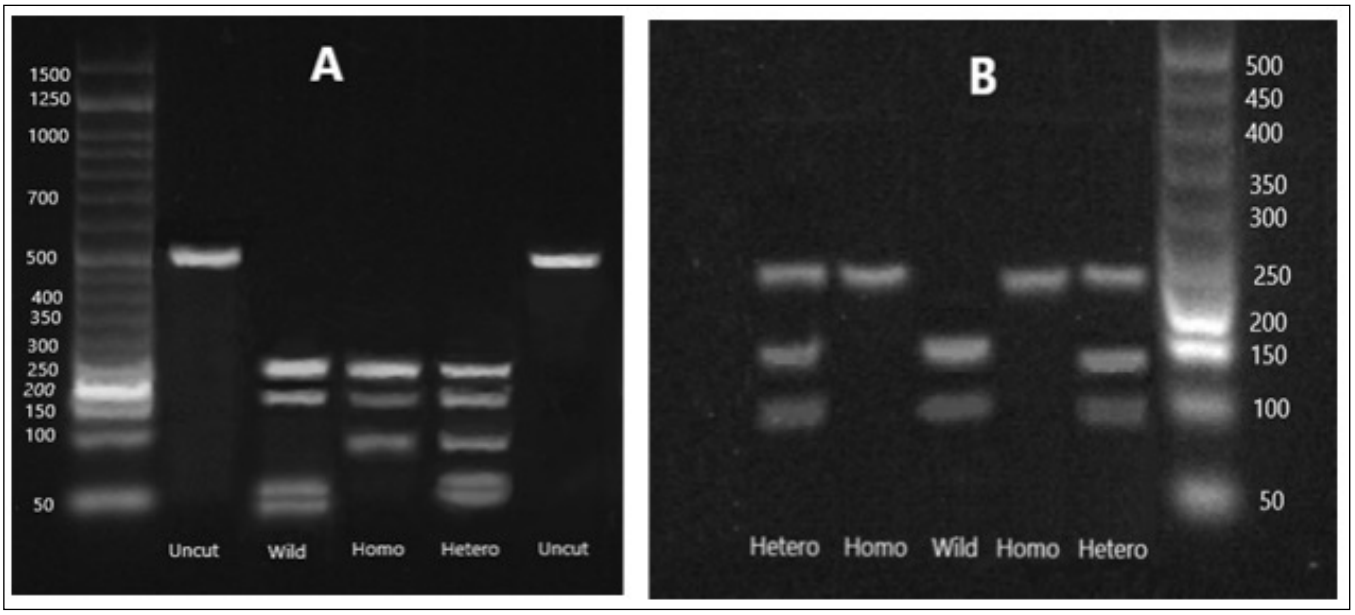

Fig. 1. Gel-electrophoresis (3%) after DNA digestion; A: represent allelic variants of rs757110 SNP. B: represent allelic variants of rs1801261 SNP.

to extract DNA. Obtained DNA was examined using electrophoresis instruments and A260/A280 fraction spectroscopy to determine its magnitude and purity. The RFLP-PCR method handled the copying of DNA and the identification of allele genetic composition. The NCBI accessible sites takes into consideration primers. The rs757110 primer structures that are being examined include AGCACTGGGGGAAGGCAATA for the forward primer and TCCTGAAAACCGAGGCAGAG for the reverse primer. Additionally, the reverse primer for rs1801261 is AGACTGGGTCCAGGGGATTG, and the forward primer is AGGGTGATGTGGCTCCCTTG. A volumes 256 µL of PCR reaction mixtures, 16 µL of sample DNA, 16 µL of each primer (10 pm/6 µL), 156 µL of master mix, and 46 µL of electro-deionization water are all needed. The steps in the thermal PCR method are as follows: a primary denaturing stage that lasts for 5 minutes at 95°C; 35 rounds of denaturing that last for 30 seconds each at 95°C; an annealing phase that lasts for 30 seconds at 54°C (for rs757110) or 56°C (for rs1801261); and an extension phase that lasts for 35 seconds at 72°C. The maximum extension time was five minutes at 72°C. The results of the PCR are 512 b for rs757110 and 237 b for rs1801261, respectively. MwoI (NEB & cat. R0573L), a restriction-enzyme, was employed to cut the segments for C-Allele (165 b – 51 b – 41 b – 255 b) and A-Allele (165 b – 92 b – 255 b) at rs757110 (512 b). The endonuclease-incised wild genotype of rs1801261, which is represented by the BsiE1 (NEB Cat. R0554S) piece full length homo-allele, is 237 b; the length of the wild-allele is (97 b, 140 b), and the hetero-alleles are (237 b, 97 b, 140 b) b segments. The gel-electrophoresis systems' recommended yields, with 3% agarose gel used in each series. Each model's allelic type is classified by a UV transilluminator as shown in figure 1 below, and the final 25% of samples are re-genotyped for approval.

STATISTICAL ANALYSIS

The control group was found to be within Hardy-Weinberg equilibrium using chi-square analysis. For both participant groups, the biochemical parameters Age, BMI and Gender were represented as mean ± Standard Deviation (M±SD). Statistical analysis of the data was done using the SPSS V25.0 software (SPSS Inc., Chicago, IL, US). While Logistic Regression Analysis was utilized to clarify the T2DM risk factor of the two investigated alleles reported as Odd Ratio (OR) with Confidence Interval 95% (95% CI) and p-value < 0.05, Students t-test was employed to investigate continuous variables. The adjustment of OR as a risk factor was examined for the confounders BMI, Age, and Gender.

RESULTS

The biochemical characteristics and anthropometrics measurers of both groups elucidate the significant differences between both groups as shown in table 1. Hardy equilibrium tested for control group using non-parametric χ^2 test, the two SNPs (rs757110 and rs1801261) were within Hardy equilibrium with p-value 0.902 and 0.575 respectively. Minor Allele Frequency (MAF) of rs757110 was higher in T2DM (0.33) than control group (0.27), and MAF of rs1801261 also was higher in T2DM (0.05) than in control (0.03). The genotype and allele frequency of rs757110 tested by logistic regression

Table 1. Biochemical parameters and anthropometrics features of both groups' differences

Biochemical Parameters	Control (400)	T2DM (400)	p-value
	Mean ± SD	Mean ± SD	
Sex of Participant	1.41 ± 0.49	1.47 ± 0.5	0.12
Age of Participants	47.38 ± 7.65	49.63 ± 8.9	< 0.001
BMI	24.42 ± 2.62	26.6 ± 1.53	< 0.001
FSG (mg/dl)	87.13 ± 4.57	233.79 ± 24.53	< 0.001
Triglycerides (mg/dl)	116.69 ± 20.38	165.71 ± 14.52	< 0.001
Total-C (mg/dl)	137.81 ± 35.8	199.11 ± 29.11	< 0.001
HDL-C (mg/dl)	47.1 ± 9.82	42.25 ± 10.09	< 0.001
VLDL-C (mg/dl)	23.34 ± 4.08	33.14 ± 2.9	< 0.001
LDL-C (mg/dl)	68.09 ± 37.38	123.72 ± 31.32	< 0.001
Insulin (µU/ml)	2.56 ± 0.87	7.54 ± 1.44	< 0.001
HOMA-IR	0.55 ± 0.19	4.34 ± 0.91	< 0.001

Table 2. Genotype of the two SNPs (rs757110 and rs1801261) expressed as codominant, dominant and recessive models

ABCC8 Polymorphisms	Cases	Controls	Crude Model OR (95% CI)	p-value	Adjusted Model* OR (95% CI)	p-value
rs757110 C > A	n = 400	n = 400				
Allele (%)						
C	538 (67.25)	590 (73.22)				
A	262 (32.75)	210 (26.88)	1.33 (1.1 ± 1.64)	**0.01**		
Genotype (%)						
CC	183 (45.75)	220 (55)				
CA	172 (43)	145 (36.25)	1.43 (1.06 ± 1.92)	**0.02**	1.37 (0.98 ± 1.91)	0.07
AA	45 (11.25)	35 (8.75)	1.55 (0.95 ± 2.51)	0.08	1.86 (1.05 ± 3.28)	**0.03**
Dominant model (%)						
CC	183 (45.75)	220 (55)				
CA + AA	217 (54.25)	180 (45)	1.45 (1.1 ± 1.92)	**0.01**	1.45 (1.06 ± 1.99)	**0.02**
Recessive model (%)						
CC + CA	355 (88.75)	365 (91.25)				
CC	45 (11.25)	35 (8.75)	1.32 (0.83 ± 2.11)	0.24	1.61 (0.93 ± 2.79)	0.09
rs1801261 G > A	n = 400	n = 400				
Allele (%)						
G	759 (94.87)	722 (96.5)				
A	41 (5.13)	28 (3.5)	1.49 (0.91 ± 2.43)	0.11		
Genotype (%)						
GG	361 (90.25)	373 (93.25)				
GA	37 (9.25)	26 (6.5)	1.47 (0.87 ± 2.48)	0.15	1.33 (0.74 ± 2.4)	0.35
AA	2 (0.5)	1 (0.25)	2.07 (0.19 ± 22.89)	0.55	3.88 (0.16 ± 92.88)	0.4
Dominant model (%)						
GG	361 (90.25)	373 (93.25)				
GA + AA	39 (9.75)	27 (6.75)	1.49 (0.9 ± 2.49)	0.13	1.38 (0.77 ± 2.47)	0.28
Recessive model (%)						
GG + GA	398 (99.5)	399 (99.75)				
AA	2 (0.5)	1 (0.25)	2.01 (0.18 ± 22.2)	0.57	3.8 (0.16 ± 90.92)	0.41

* Adjusted for age, sex, BMI.

* Adjusted for age, sex, BMI.

Table 3. Analysis of Variance of rs757110 as Codominant Model

Biochemical Parameters	Mean ± SD			p-value
	CC (183)	CA (172)	AA (45)	
Sex	1.45 ± 0.5	1.49 ± 0.5	1.44 ± 0.5	0.765
Age (yr.)	50.92 ± 9.04	51.58 ± 8.78	49 ± 10.32	0.236
BMI	26.71 ± 1.7	26.38 ± 1.46	26.11 ± 1.2	**0.026**
FSG (mg/dl)	231.2 ± 23.34	236.54 ± 26.3	239.3 ± 22.96	**0.046**
Triglycerides (mg/dl)	165.01 ± 14.88	166.32 ± 14.5	168.12 ± 11.73	0.385
Total-C (mg/dl)	201.33 ± 29.06	200.89 ± 30.77	198.73 ± 25.17	0.868
HDL-C (mg/dl)	42.29 ± 10.05	42.71 ± 9.95	43.97 ± 10.92	0.605
VLDL-C (mg/dl)	33 ± 2.98	33.26 ± 2.9	33.62 ± 2.34	0.385
LDL-C (mg/dl)	126.04 ± 30.52	124.92 ± 31.81	121.14 ± 25.76	0.629
Insulin (µU/ml)	7.84 ± 1.21	7.43 ± 1.41	7.15 ± 1.83	**0.002**
HOMA-IR	4.48 ± 0.82	4.35 ± 0.99	4.23 ± 1.16	0.202

Table 4. Analysis of Variance of rs757110 as Dominant Model

Biochemical Parameters	Mean ± SD		p-value
	CC (183)	CT+TT (217)	
Age of Participants	50.92 ± 9.04	51.05 ± 9.16	0.889
BMI	26.71 ± 1.7	26.32 ± 1.41	**0.013**
FSG (mg/dl)	231.2 ± 23.34	237.11 ± 25.61	**0.017**
Triglycerides (mg/dl)	165.01 ± 14.88	166.69 ± 13.97	0.245
Total-C (mg/dl)	201.33 ± 29.06	200.45 ± 29.65	0.764
HDL-C (mg/dl)	42.29 ± 10.05	42.97 ± 10.15	0.502
VLDL-C (mg/dl)	33 ± 2.98	33.34 ± 2.79	0.245
LDL-C (mg/dl)	126.04 ± 30.52	124.14 ± 30.64	0.536
Insulin (µU/ml)	7.84 ± 1.21	7.37 ± 1.51	**0.001**
HOMA-IR	4.48 ± 0.82	4.32 ± 1.03	0.107

analysis that showed significant difference between T2DM and control (p = 0.01) with a risk value (OR) 1.33 (1.1 ± 1.64). As codominant model, AA carriers had significant (p = 0.03) risky (OR = 1.83) after adjustment with age, BMI and sex. Following adjustment for BMI, age, and gender, the dominant model also exhibited a significant (p = 0.02) risk diabetic factor (OR = 1.45). Table 2 indicates that there was no statistically significant variation observed in the remaining models of rs757110 SNP or any of the rs1801261 SNP models. The CA and AA codominant models of rs757110 exhibited a significant (p = 0.046) increase in the Fasting Glucose analysis of variance. In addition, the CA and AA codominant models of rs757110 showed a significant decline in Insulin and BMI (p = 0.002 & 0.026, respectively). In similar way, Glucose was increased significantly (0.017) and decreased Insulin and BMI (p = 0.001 & 0.013 respectively) in dominant model (CA+AA) of rs757110 as shown in table 3, table 4). The second SNP (rs1801261) shown no significant differences in all genotypes models data not mentioned.

DISCUSSION

Our findings that FSG augmented in CA and AA codominant styles of rs757110. Moreover, there were decreased insulin and BMI significantly (p = 0.002 & 0.026 respectively) in CA and AA codominant patterns of rs757110. Likewise, glucose was high significantly (0.017) and reduced insulin and BMI (p = 0.001 & 0.013 respectively) in dominant pattern (CA+AA) of rs757110 as revealed in table 3, table 4). As codominant pattern (of rs757110), AA carriers revealed significant (p = 0.03) risky (OR = 1.83) when adjustment with age, BMI and sex. Also, as dominant pattern CA + AA carriers included significant (p = 0.02) risk factor for diabetic (OR = 1.45) following adjustment for age, BMI and gender.

Sulfonylureas are arranged according to KATP channel multipart they adhere to. There are three binding sites: A, B, and A + B (both A and B sites). Positioned on SUR1 subunit in its second transmembrane domain is the A site. Two KATP channel regions are present in the B site: one is located in the SUR1 subunit and the other in the Kir6.2 subunit [21]. Nonetheless, polymorphisms in

the A + B site of glimepiride and glibenclamide, which both bind to this site, have an impact on the clinical effects of rs757110 on the hypoglycemic effect [22]. It was hypothesized that SUR1 subunit polymorphism and T2DM are related. The response to sulfonylureas and predisposition to T2DM were linked to genetic variants in ABCC8 [23, 24]. The rs757110 polymorphism, located in exon 33 of ABCC8 gene, was one of them. It resulted in the substitution of Ser1369Ala (TCC>GCC). We showed in this research that the distribution of the rs757110 (Ser1369Ala) genotype in diabetic group was similar to the results observed in patients with diabetes from East Asia [25]. Furthermore, it was shown that the T2DM population had a higher frequency of the rs757110 variant than the other population. It was suggested that the Ala1369 allele may increase the risk of Type 2 diabetes [11]. Ser1369Ala variant was found to increase gliclazide efficacy by Feng et al. [26]. It's interesting to note that the variant that had been linked to a higher risk of T2DM reduced the effect of gliclazide, which attaches to the A position of the KATP channel, on glucose metabolism. It is still unknown whether the rs757110 polymorphism and insulin secretion are related. According to Florez et al., subjects who were homozygous carriers of the AA allele had lower insulin secretion functions and poorer glucose tolerance than subjects who had the CC allele [27]. However, Rissanen et al. found no significant correlation between the rs757110 polymorphism and the function of insulin secretion in non-diabetic persons [28].

It is possible that glimepiride and glibenclamide therapies, which both bind to the A + B site, are the reason for the significant differences observed between the genotypes of the rs757110 variant in this study. In vitro investigation [28] that showed the rs757110 polymorphism did not significantly alter the KATP channel's glibenclamide sensitivity is consistent with our results. Sato et al. clarify that the total number of AA allele carriers may were higher in the hypoglycemic category if the rs757110 variant had affected the glucose-lowering effect of glimepiride and glibenclamide, similar to gliclazide. In fact, only a small number of individuals in the hypoglycemic class had the rs757110 variant homozygously (AA) [30]. Alongside the ABCC8 gene, the KCNJ11 gene, which codes for the extra subunit of KATP (Kir6.2), has also drawn attention. Rare KCNJ11 alterations, many of which were seen on the B site, are known to be the cause of newborn hyperglycemia or neonatal diabetes. It has been identified that KCNJ11 stimulating mutations in Kir6.2 cause either transient or permanent neonatal diabetes [31]. On the other hand, modest changes in the function of the KATP channel may result in an insulin shortage in young people, and serious diabetes in middle age. [32]. Previous research has established that the E23K variant coexists with the rs757110 polymorphism in significant linkage disequilibrium with respect to frequent SNPs of KCNJ11 [33]. The AA allele in the rs757110 of ABCC8 is essentially carried permanently by the K transporters in the E23K. According to a German study, the E23K variant lowers the risk of severe hypoglycemia brought on by sulfonylureas (after glimepiride and glibenclamide, which bind to the A + B site) treatment. Additionally, compared to the control group, the sulfonylurea-stimulated hypoglycemia group had a lower prevalence of the E23K mutation. This result suggests that, along with E23K, there will be fewer recurring copies of the rs757110 variant in the hypoglycemic group, in accordance with the linkage disequilibrium [34].

Nevertheless, the genotype and biochemical parameter variance of the ABCC8 rs1801261 SNP did not differ significantly according to our findings. The frequency regarding 'A' allele of the rs1801261 SNP was found to differ between groups with glucose tolerance and those with T2DM, according to Radha et al.'s analysis of the link between this SNP in an Asian Indian community from south India. There has been no significant difference in the genotypic incidence between Glucose tolerance and T2DM groups. It was determined that there was no significant difference in the genotypic incidence or allele frequency of the rs1801261, polymorphism between the glucose tolerance and T2DM groups [17]. Zhou X et al., however, found that T2DM patients had a higher incidence of the ABCC8 rs1801261 allele than did the control group. Repaglinide treatment resulted in significant reductions in Fasting Glucose and HbA1c in T2DM allele carriers compared to CC carriers. This study examined the connection between ABCC8 rs1801261, and repaglinide effectiveness. In T2DM with the GA genotype of rs1801261, the repaglinide act ion was less effective at lowering fasting glucose. Patients with the CC genotype of rs1801261 showed a significant decrease in fasting glucose levels compared to those with the GA genotype, indicating that repaglinide is less efficient in reducing postprandial glucose in T2DM patients with the A-allele. [15]. ABCC8 gene's exon region contains the ABCC8 rs1801261 SNP. The mRNA of such SNP with the C allele changes to the T allele (ACC →ACT), while the threonine at 759 residues (Thr759Thr) remains unchanged. Drug metabolisms are influenced by a number of genes as well as their polymorphisms, including cytochrome P450 (CYP) 2C8 and the drug metabolism of organic anion-transporting polypeptide (OATP1B1) [35]. Those two genes might have an impact on how well repaglinide works to prevent these contradictory outcomes [36].

These two SNPs of the study may affect the Sulfonylurea therapy glibenclamide and glimepiride of our T2DM patients. A significant relationship between the SNP of ABCC8 gene and response to the sulfonylurea drugs has been discovered by a unique association screening system built on comprehensive likelihoods in Mexican [37]. The rs757110 polymorphism in the ABCC8 gene can impact the therapeutic effectiveness of glimepiride and glibenclamide on lowering glucose levels [30]. The rs1801261 polymorphism was related with the possibility of T2DM in Danish and Canadian residents. In contrast, two earlier studies encompassing Finnish and North Indian inhabitants have deposited onward that the *ABCC8* gene with rs1801261 variants is not concomitant with a threat to T2DM.

CONCLUSIONS

The gene polymorphism of ABCC8 involved rs757110 is more frequent in T2DM than in healthy group. Moreover, T2DM who received glimepiride and glibenclamide for diabetes treatment, the fasting glucose higher in CA and AA codominant styles of rs757110, and there were decreased insulin and BMI significantly in CA and AA codominant patterns of rs757110. Likewise, glucose was high significantly and reduced insulin and BMI in dominant pattern (CA+AA) of rs757110. Similarly, the codominant pattern with AA carriers exposed significant risky in T2DM when adjustment with age, BMI and sex. Also, the dominant pattern of CA + AA carriers shown significant risk factor for diabetic after adjustment for age, BMI and gender. The rs1801261 showed no association with T2DM and factors involved in its complication and treatment with glimepiride and glibenclamide drugs. These polymorphisms are situated at Nucleotide Binding Domains which is significant for SUR1 function and in this manner KATP channel.

Genetic examination has been devoted to assess the interindividual changeability in the responding to oral glucose-lowering therapies, and in current times, many pharmacogenetic investigations of relatives between genetic variations and hypoglycemic drug responding have been issued. Certainly, the control of T2DM needs an individualized method because of the detail that the syndrome is heterogeneous, changes in pathophysiological and molecular pathways of glucose control fluctuate between subjects, and the flexible special effects of present therapies mark it hard to expect individual responding to hypoglycemic medications.

The usual methodology for T2DM treatment includes the step by step adding of drugs to lifestyle interferences, typically start with a one oral medication and progress to mixture therapy, developed by the supplement or exchange of insulin, established on the gradual collapse of the drugs to provide satisfactory glucose mechanism. In the setting of marked or precision medication, pharmacogenetic knowledge may be beneficial for patient lamination with the purpose of recognize respondents and to steadiness the advantages of hypoglycemic treatments with their probable risks.

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The authors extend their sincere thanks to all participants in the study. We also extend our thanks and gratitude to the health staff in health institutions for providing assistance in completing the number of samples required for research. We also do not forget the people who provided any kind of assistance in completing the research requirements, especially those working at the University of Kufa.

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Zubaida Falih Alzubaidi
University of Kufa
299G+HPX, Kufa Street, Kufa, Najaf Governorate, Iraq
e-mail: zubaidahf.mezaal@uokufa.edu.iq

ORCID AND CONTRIBUTIONSHIP
Sarah H. Shaalan: 0009-0003-2419-9958 E F
Muneer Khudhair: 0000-0002-8234-8507 C D
Noaman Ibadi Mohammed: 0000-0003-4495-6584 A B
Zubaida Falih Alzubaidi: 0000-0003-3784-4437 E F

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

RECEIVED: 03.07.2024
ACCEPTED: 16.09.2024

ORIGINAL ARTICLE

CONTENTS

The impact of bladder symptoms on Relapsing Remitting Multiple Sclerosis patients' Quality of Life – a pilot study

Anna Walawska-Hrycek[1], Marcin Dudek[2], Tomasz Fura[2], Julia Silldorff[2], Joanna Siuda[1]

[1]DEPARTMENT OF NEUROLOGY, FACULTY OF MEDICAL SCIENCES IN KATOWICE, MEDICAL UNIVERSITY OF SILESIA, KATOWICE, POLAND

[2]STUDENTS' SCIENTIFIC ASSOCIATION, DEPARTMENT OF NEUROLOGY, FACULTY OF MEDICAL SCIENCES IN KATOWICE, MEDICAL UNIVERSITY OF SILESIA, KATOWICE, POLAND

ABSTRACT

Aim: Bladder disorders are one of the most troubling symptoms of Multiple Sclerosis (MS), which impact patients quality of life (QoL). Therefore the aim of this pilot study was to assess this problem.

Materials and Methods: 47 patients (28 females and 19 males, aged 40,8±12,2 years; EDSS 3,2±1,9) diagnosed with Relapsing Remitting Multiple Sclerosis (RRMS) during disease modifying treatment (DMT) were enrolled to our study. Patients were asked to fill 8-item Actionable Bladder Symptoms Screening Tool (ABSST), Multiple Sclerosis Quality of Life Inventory (MSQLI) and King's Health Questionnaire (KHQ).

Results: Bladder problems were reported by 24/47 (51%) of patients, but only 7/24 (36,8%) were treated. The results of ABSST (2,3±1,5) and Incontinence Impact (II) domain of KHQ (40,3±20,5) indicated influence of the bladder disorders on patients QoL. Besides bladder control, the most important impact on QoL from MSQLI are fatigue and mental health issiues.

Conclusions: Bladder symptoms in MS have significant impact on patients QoL, and therefore should be a part of clinical evaluation and must be actively treated.

KEY WORDS: multiple sclerosis, bladder dysfunction, quality of life

Wiad Lek. 2024;77(11):2326-2331. doi: 10.36740/WLek/195752 DOI

INTRODUCTION

Multiple sclerosis (MS) is an autoimmune inflammatory disorder of central nervous system (CNS) of unknown etiology, characterized by demyelination leading to oligodendrocyte degeneration, axonal loss and neuronal destruction [1]. MS affects multiple CNS regions what cause a wide range of urodynamic abnormalities. Bladder disorders encompass both storage and voiding symptoms. Storage symptoms consist of overactive bladder syndrome, characterized by urinary urgency and increased urinary frequency, as well as urinary incontinence (UI). Nocturia can contribute to decreased sleep quality. Voiding symptoms comprise urinary hesitancy, urinary retention and the sensation of incomplete emptying [2].

Over 50-80% MS patients complains from bladder dysfunction [3, 4]. Approximately 96% of MS patients will have bladder dysfunction after 10 years from the disease onset [1, 3, 5]. A higher prevalence of neurogenic bladder symptoms is observed in patients with higher disability, in older age and with longer disease duration. Bladder symptoms increase incidence of urinary tract infections, falls and fall-related injuries, which requires hospitalization and nursing home admission [3].

Bladder and bowel dysfunction may occurred at any time during the course of MS [5]. Several studies have shown that they can be the source of significant disability and result in a reduction of Health-Related Quality of Life (HRQoL) [6]. Commonly used EDSS scale (Expanded Disability Status Scale) is not enough for evaluating this symptoms [7]. A specific screening tool for patients with bladder problems related to MS is the Actionable Bladder Symptom Screening Tool (ABSST) [4]. The ABSST is designed to identifying patients who need treatment for MS- related urinary symptoms or urologic evaluation [4].

The King Health Questionnaire (KHQ) is a tool designed to evaluate the level of impact of urinary symptoms to patient's QoL [8]. The lower scores indicate patient wellbeing and higher scores mean difficulty in voiding [8]. According to Nortvedt et al bladder dysfunction in MS patients leads to reduction in QoL [9,10]

especially in domains such as emotional well-being and social function [7,11]. Multiple Sclerosis Quality of Life Inventory (MSQLI) describe HRQoL specifically for MS patients [12]. This instrument consisting of a widely-used generic measure, the Health Status Questionnaire (SF-36), supplemented by nine symptom-specific measures (covering fatigue, pain, bladder and bowel, emotional status, perceived cognitive function, visual function, sexual satisfaction, and social relationships) [12, 13].

AIM

Although the number of MS patients has increased in Poland, there are limited studies on bladder dysfunction in this population. Therefore we have designed this study to point out the problem and investigate whether bladder dysfunction has an negative impact on the QoL in MS patients

MATERIALS AND METHODS

PARTICIPANTS

The study was carried out among 47 adult patients diagnosed with Relapsing Remitting Multiple Sclerosis (RRMS) during disease-modifying treatment (DMT) in the Department of Neurology, Central University Hospital of the Medical University of Silesia in Katowice. Demographic data are presented in the Table 1.

Patients were asked to fill the 8-item ABSST, KHQ and MSQLI. 54 stationary surveys, ABSST, KHQ and MSQLI were distributed, 47 (87%) of them were completed and returned. Patients were also evaluated for overall disability using the EDSS by a MS specialist.

The ABSST questionnaire contains 8 questions ranging from 0 to 3 in which 0 means no symptoms at all and 3 represents the most severe symptoms or impact, there is also a 9th yes or no question asking about the need of medical help for bladder symptoms and is not involved in the final score. The cut-off of total score ≥3 indicates the necessity of further urological assessment [14]. It was used to determine a group of patients suffering from urinary tract symptoms with a threshold of 1 point.

The KHQ consists of 21 questions investigating 9 domains: General Health Perceptions (GHP), Incontinence Impact, Role Limitations, Physical Limitations (PL), Social Limitations, Personal Relationships, Emotions, Sleep/Energy and Severity Measures. Domains scores range from 0 (best) to 100 (worst), except for the Severity Measures where scores range from 0 (best) to 30 (worst). Only patients who reported urinary disorders were asked to fill it and it was used to evaluate the level of impact on QoL in this group [8].

For the purpose of this study we have used only those domains from MSQLI that would be relevant to the studied bladder problem. The domains used were: SF-36, Modified Fatigue Impact Scale (MFIS), Bladder Control Scale (BLCS), Bowel Control Scale (BWCS), Mental Health Inventory (MHI).

Statistical analysis was conducted using Statistica 13.1. To assess the differences in variable levels among groups, we employed the U-Mann Whitney test for data which does not meet the assumptions of normality required for parametric tests. Meanwhile, for variables that have a normal distribution we used the t-student

Table 1. Characteristics of enrolled patients.

Variables	Results
Sex, n (%)	
Male	19 (40,4%)
Female	28 (59,6%)
Age, mean ± SD	40,2±12,7
Age at disease onset, mean±SD	29,5±11,3
Age at diagnosis, mean±SD	31,5±11
Disease duration mean±SD	9,3±6,3
Marital Status n (%)	
Single	4 (8,5%)
Married	27 (57,4%)
Cohabitation	11 (23,4%)
Divorced	5 (10,7%)
Education, n (%)	
≤12 years	25 (53,2%)
>12 years	22 (46,8%)
Urinary symptoms	
present	24 (51%)
non-present	23 (49%)

Table 2. Sample characteristics by group

	Urinary symptoms reported	Urinary symptons non-reported	p-value
Population, n (%)	24 (51%)	23 (49%)	-
Male, n (%)	9 (37,5%)	10 (43,5%)	-
Female, n (%)	15 (62,5%)	13 (56,5%)	-
Age	40,8±12,2	39,6±13,4	p>0.05
Age at disease onset, mean±SD	29,4±11,21	29,6±11,56	p>0.05
Age at diagnosis, mean±SD	31,9±11,14	31,1±11,16	p>0.05
Disease duration, mean±SD	9,22±6,4	9,39±6,42	p>0.05
Education, n			
≤12 years	15 (62,5%)	10 (43,5%)	-
>12 years	9 (37,5%)	13 (56,5%)	-
EDSS	3,2±1,9	2,4±1,62	p>0.05
Duration of MS until dysuric symptoms	10,6±16,8	-	-
ABSST	2,3±1,5	-	-

Table 3. KHQ findings

Domain	Mean score ± SD
General Health Perceptions	34,4±19,2
Incontinence Impact	40,3±29,5
Role limitations	22,2±24,9
Physical Limitations	34±31,2
Social Limitations	19,9±25,4
Personal relationships	22,8±38,4
Emotions	23,6±26,5
Sleep/ Energy	18,8±22,1
Severity Measures	19,2±19,1
Symptom Severity Scale*	8,5±5,7

*Symptom Severity Scale ranges from 0 to 30

test. In all our analyses, a p-value less than 0,05 was regarded as indicating statistical significance.

RESULTS

In our group we didn't find any difference in such aspects as age at disease onset, duration and EDSS score between patients with or without urinary symptoms (Tabel 2).

In our study 24/47 (51%) of patients reported at least one moderate or severe symptom via ABSST (mean 2,3±1,5). 10/47 (20,4%) patients requires further urological assessment (ABSST score >3). The KHQ findings show significant impact of subjective perception of the bladder disorders to the patients QoL (Incontinence Impact domain 40,3±29,5), however urinary frequency occurred more often (Table 3).

13/47 (27%) patients reported the negative influence on their sleep by their bladder dysfunctions. The total list of presented symptoms, including Symptoms Severity Scale were shown in Table 4.

In order to analyze the presence of a relationship between urinary symptoms and QoL,patients completed the MSQLI questionnaire. Table 5 shows the results divided into patients with and without bladder symptoms. Patients with urinary symptoms had more

Table 4. Symptoms presented in KHQ

Symptoms	Findings, n (%)*
Urinary frequency	18 (75%)
Nocturia	13 (54,2%)
Urgency	17 (70,8%)
Urge Incontinence	10 (41,7%)
Stress incontinence	8 (33,3%)
Nocturnal Enuresis	3 (12,5%)
Intercourse incontinence	4 (16,7%)
Waterworks infections	11 (45,8%)
Bladder Pain	8 (33,3%)
Difficulty in passing urine	9 (37,5%)

*Number and percentage of patients reporting each symptom

Table 5. MSQLI results by groups

	Urinary symptoms present	Urinary symptoms nonpresent	p value
SF-36 PCS	41,1±12,8	47,8±11,8	p>0,09
SF-36 MCS	44,9±12,7	49,5±7,6	p>0,23
MFIS	36,9±24,3	21,1±14,7	**p<0,03**
BLCS	4,8±5,3	0,9±1,8	**p<0.004**
BWCS	3,6±4,5	0,8±1,2	**p<0,04**
MHI	59,4±21,7	72,9±15,1	**p<0.02**
MSSS	77,2±28,1	90,8±12,9	p>0,09

often bowel problem (BWCS) which impact their everyday activities (BLCS). Moreover, this group of patients complained also on deterioration in mental health including: anxiety, depression, behavioral control (MHI). Fatigue significantly affected daily activities.

Only 7 patients with bladder disorders 7/24 (36,8%) were treated: 4 with oral treatment, 2 with botulinum toxin injections and 1 with physiotherapy. 10/24 (42%) patients were actively looking for more information about treatment their bladder dysfunction.

DISCUSSION

In our pilot study half of the respondents reported at least one moderate or severe urinary symptom. High frequency of bladder dysfunctions in MS patients were also described by Hennessey et al 84/190 (44%) [15], Mahajan et al 6263/16858 (65%) [16], Lin et al 105/135 (77,8%) [17]. First symptoms of dysuria occurred on average 10 years after the onset of MS [3,5,18]. Similarly, in the participants of our study, dysuria appeared nearly 10 years after the onset of MS. The EDSS score in our patients with bladder dysfunction indicated more advanced disability. Moreover they had longer disease duration similarly to Azadvari et al, Vitkova et al, Browne et al studies [6, 7, 18].

Only one-third of MS patients who experiencing UI were actively looking for medical help [3]. In our study only 36,8% of patients received treatment. Based on our results obtained on the ABSST scale (2,3±1,5), 20% of enrolled patients required further urological assessment. Data from the literature indicate varying degree of dysuria symptoms, e.g. Carotenuto et al revealed bladder dysfunction in 60/150 (40%) of enrolled patients with average ABSST value 2,7±2,83 [19]. Based on ABSST results the further urological assessment is required in most of MS patients in studies presented by Akhou (4,37±3,78), Azadvari (6,8±5,7) [18]. Lin et al showed that a cut –off ABSST > 6 was found in 74,4% of responders [17]. A significant relationship between EDSS and ABSST were confirmed, with little effect of age [17].

Our results indicate that patients health problems described in KHQ domains such as UI, GHP and PL have the most impact on QoL. Based on Vitkova et al, bladder dysfunction are associated with a poorer HRQL in MS patients even if they have suffered from MS for a relatively short time [20]. Filippo et al found an association between irritative urinary symptoms and the MS QoL in the whole clinically isolated patients (CIS) cohort i.e. even at an early stage of the disease, which may also have prognostic significance [7].

In our study the most important impact of QoL have fatigue, well-being, bowel and bladder disorders. It is shown as a results of appropriate subscales: MFIS, BLCS, BWCS, MHI from MSQLI tool. Patients with urinary symptoms were experiencing fatigue (MFIS) which is a common disabling and reducing the QoL symptom [11,21]. Stanton et al, reported problem with fatigue in 64% of checked patients [22].

MHI is an instrument to provide an assessment of mental health including anxiety, depression, behavioral control, positive affect and general distress. In our study MS patients who suffering from bladder dysfunction had a significantly worse well- being what is seen as a results of the MHI (see Tabel 6, 59,4±21,7 *vs.* 72,9±15,1, p<0,02). According to Hamad Al Khader et al the prevalence of cognition and mental health impairment in MS patients are 23,3% and 14,1% respectively [23]. UI is also related to depressive symptoms in older adults [24]. Frequent awakenings due to nocturia, urgency or even nocturnal enuresis strongly impact quality of sleep resulting in increased fatigue [25].

Our results from BLCS and BWCS support earlier described findings from ABSST and KHQ about impact on QoL by bladder dysfunction [26, 27].

The significant impact on the quality of life and concern for general health require planning research on a wider group of MS patients. Simple tests assessing dysuria disorders should be used periodically during medical visits.

CONCLUSIONS

The bladder symptoms in MS have significant impact to patients QoL, especially on mental health, physical functioning and fatigue. Healthcare professionals should pay more attention to these symptoms in MS patients. More research is needed to evaluate and select new tools to more accurately assess bladder symptoms in MS patients.

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ORCID AND CONTRIBUTIONSHIP

Anna Walawska-Hrycek: 0000-0003-4250-8710 A B C D E F
Marcin Dudek: 0009-0003-6762-0124 B C D
Tomasz Fura: 0009-0008-0947-8058 B C D
Julia Sildorff: 0009-0002-9223-2089 B C D
Joanna Siuda: 0000-0002-0340-660X A E F

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Anna Walawska-Hrycek
Department of Neurology, Faculty of Medical Sciences in Katowice,
Medical University of Silesia in Katowice, Poland
e-mail: awalawska-hrycek@sum.edu.pl

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

RECEIVED: 30.09.2024
ACCEPTED: 04.11.2024

REVIEW ARTICLE

CONTENTS

The connection of systemic inflammation and atherosclerosis: What do we know nowadays?

Yuliia O. Bielikova, Amir M. Khranovskyi, Tetiana M. Motsak, Andrii A. Kuzminets
BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

ABSTRACT

Aim: To summarise what is known about the inflammatory nature of atherosclerosis to date**.**

Materials and Methods: We conducted the search using Google Scholar and Medline and selected state-of-the-art articles that were consistent with the aim of study.

Conclusions: To date, there is sufficient evidence that atherosclerosis is driven by the inflammatory process. Evidence suggests that atherosclerosis is fundamentally an inflammatory disease, with local systemic inflammation and both being a key drivers of plaque formation and progression. It involves multiple inflammatory mechanisms at all stages, including multiple inflammatory mediators and cytokines (such as IL-1β, IL-6, and TNF-α), CD4+ and CD8+ T-lymphocytes, B-lymphocytes and their subtypes, multiple modes of macrophage activation (including NF-κB-mediated), and neutrophils that undergo NETosis, which exacerbates and builds up inflammation. Inflammatory processes are involved in both plaque formation and plaque destabilization leading to rupture and subsequent thrombosis. Many of these mechanisms have been shown to be applicable as biomarkers of the atherosclerotic process, its severity and its risk level. Currently, a number of antiatherosclerotic therapies targeting inflammation have been developed, but most have not yet been introduced into general medical practice. There is a need to increase awareness of the inflammatory mechanisms underlying atherosclerosis and to apply the knowledge gained in this area to improve the diagnosis, risk stratification and prevention or treatment of this pathology.

KEY WORDS: inflammation, atherosclerosis, extracellular traps, anti-inflammatory agents

Wiad Lek. 2024;77(11):2332-2339. doi: 10.36740/WLek/197122 DOI

INTRODUCTION

Chronic inflammation becomes widely acknowledged as a significant driver of cardiovascular diseases (CVDs). Previously considered secondary to traditional risk factors such as hypertension, dyslipidemia, and diabetes, inflammation is now understood to play a primary role in the pathogenesis of a range of cardiovascular conditions. Atherosclerosis is the proximate and common cause of life-threatening cardiovascular conditions. This review sheds light on the connection between underlying mechanisms of chronic inflammation and risks of the initiation and exacerbations of atherosclerosis.

AIM

The aim is to summarise what is known about the inflammatory nature of atherosclerosis to date**.**

MATERIALS AND METHODS

We conducted the search using Google Scholar and Medline and selected state-of-the-art articles that were consistent with the aim of study.

REVIEW AND DISCUSSION

GENERAL IMPACT OF SYSTEMIC INFLAMMATION ON CARDIOVASCULAR SYSTEM

With the modern pace of life cardiovascular diseases get more and more attention of all the doctors around the world. Recent studies show that diseases of the cardiovascular system are still number one in the list of causes of mortality worldwide [1]. The search of causes of initiation, progression and development of cardiovascular diseases becomes inevitable. Aside from well-known causes of the cardiovascular diseases such as using of tobacco, high blood pressure, high blood glucose, lipid abnormalities, obesity, and physical inactivity [2], there is another cause that is worth detailed studies, and this cause is systemic inflammation.

Systemic inflammation exerts a substantial impact on the condition of the endothelium. In particular, existing research indicates that both local and systemic inflammation aside from other causes (Table 1) lead to endothelial dysfunction [3].

The general state of the cardiovascular system is impacted by systemic inflammation both directly and indirectly. Concretely, current scientific literature shows that the human brain is capable of processing stress-related signals. Thus, it results in imbalance of the autonomic nervous system, which in turn impairs the condition of endothelium by decreasing nitric oxide (NO) generation and increasing endothelin-1 (ET-1) release [3].

Based on the information at hand, it can be concluded that systemic inflammation is a significant detrimental element of the cardiovascular system condition. Therefore, this relationship warrants further comprehensive investigation in the future.

THE INTERCONNECTION OF PSORIASIS AS A SYSTEMIC INFLAMMATORY DISEASE AND THE PROGRESSION OF ATHEROSCLEROSIS

A more comprehensive understanding of the relationship between systemic inflammation and CVDs can be achieved by establishing a connection between a pathology that is characterized by systemic inflammation and inflammation-mediated cardiovascular disease. Particularly, we can investigate the relationship between Psoriasis and Atherosclerosis.

Modern scientific literature demonstrates that patients with Psoriasis are more susceptible to dyslipidemia, which subsequently contributes to the advancement and onset of Atherosclerosis. Particularly, elevated levels of oxidized low-density lipoproteins (oxLDL), which accumulate in the endothelium represent an early stage in the sequence of inflammation-mediated cascade essential to the development of atherosclerosis [4].

OxLDL causes the most damage as it activates endothelial and mononuclear cells, causing release of cytokines and inflammatory mediators. In particular, the Apolipoprotein B (ApoB) peptide component of oxLDL, which is an amphipathic protein critical for lipoprotein metabolism, that is mostly produced by cells in the liver and intestines [5], functions as a self-antigen that CD4+ T cells identify by presenting it to major histocompatibility complex (MHC) class II [4]. Consequently, these substances provide significant contributions to the inflammatory cascade, plaque development, and instability [4].

Following this, antigen-presenting cells (APCs) identify antigens and stimulate CD4+ T cells, which then undergo differentiation into T helper 1 (Th1) and Treg cells, each with distinct biological functions [4] (Fig. 1).

Moreover, CD8+ T cells, target vascular smooth muscle cells (comprising blood vessel walls), cancer-responsive immune cells (macrophages), and endothelial cells (lining blood vessels). This deliberate assault exacerbates the existing inflammation, therefore compromising its integrity and heightening the likelihood of plaque rupture. This, in turn, might result in serious cardiovascular complications such as myocardial infarctions or cerebrovascular accidents [4].

Another part of adaptive immunity that plays a crucial role in the formation of atherosclerosis is B-cell. Specifically, B2 subtypes that enhance the stimulation of T helper (Th) cells and the resulting synthesis of interferon-gamma (IFNγ), a cytokine that worsens the development of atherosclerosis (Atherogenesis).

To summarize, Psoriasis, which is the disease that has in its core pathophysiology a systemic inflammation can trigger a progression of Atherosclerosis through activation of inflammation-mediated immunologic cascade.

Not only adaptive immunity involved in progression of atherosclerosis but also an innate immunity. The activation of M1 macrophages lead to production of IL-1β, IL-6, and TNF-α, together with chemokines like CXCL9 and CXCL10 [4]. Therefore, this release resulted in heightened recruitment of immune cells, which subsequently led to increased instability of the plaque [4].

Specific categories of macrophages have distinct functions in the advancement of atherosclerosis. For instance, M4 macrophages are activated by CXCL4, a pro-inflammatory chemokine; M(Hb) macrophages that respond to hemoglobin; and MOX macrophages are triggered by oxidized phospholipids. Specifically, M4 macrophages exhibit pro-inflammatory activities by reducing the expression of CD163, resulting in reduced levels of HMOX 1 (heme oxygenase 1), an enzyme of antioxidant and anti-inflammatory properties [4]. The downregulation of HMOX 1 results in a deficiency of the HIF-1α gene, which then causes an increase in inflammation, ultimately leading to the instability of plaque [4].

One further mechanism by which macrophages exacerbate atherogenesis is their polarization subsequent to the consumption of oxLDL. As previously described, this process transforms macrophages into foam cells, which play an active role in the progression of atherosclerosis [4].

Therefore, macrophages, the characteristic cells of inflammation, have a vital role in initiating, advancing, and worsening atherogenesis through many pathways. Further investigations into the subtypes and functions of macrophages are essential to deepen our understanding and establish their connection to atherosclerosis.

In conclusion, macrophages, B cells, and T cells play indispensable roles in the pathogenesis of atherosclerosis by driving both the immune-inflammatory response and plaque development. Macrophages, through their transformation into foam cells, secrete pro-inflammatory cytokines and contribute to the buildup of lipid-laden plaques, promoting both plaque formation and instability. B cells, particularly through antibody production,

Table 1. Triggers of inflammatory response leading to atherosclerosis [3]

Traditional CV risk factors	low-density lipoprotein cholesterol triglyceride-rich lipoproteins hypertension, smoking, physical inactivity diabetes, obesity
Chronic and acute mental stress	autonomic nervous system
Ageing	bone marrow activation and clonal hematopoiesis
Chronic autoimmune diseases	rheumatoid arthritis, systemic lupus erythematosus, psoriasis, inflammatory bowel disease
Chronic infections	periodontitis, bronchitis
Acute infections	urinary tract infections, endotoxins from gut microbiota
Viral infections	influenza, COVID-19 viruses
Tissue injury	myocardial infarction, non-healing skin ulcers

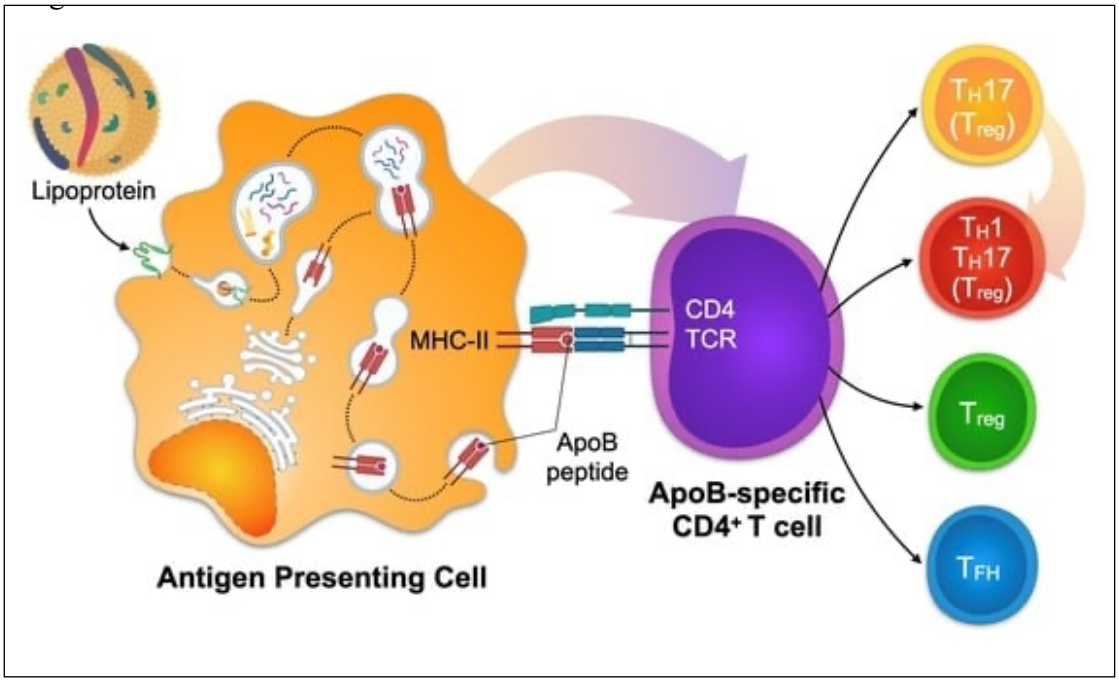

Fig. 1. The role of ApoB peptide component of oxLDL as Antiget Presenting cell [6].

enhance the chronic inflammatory environment, which fosters the progression of atherosclerosis. Meanwhile, CD8+ T cells and other T cell subtypes directly target vascular smooth muscle cells (VSMCs), macrophages, and endothelial cells within plaques, intensifying local inflammation and destabilizing the plaques, thereby increasing the risk of acute cardiovascular events. This concerted action of innate and adaptive immune cells underscores the pivotal role of immune-mediated inflammation in the initiation and progression of atherosclerosis, revealing critical pathways for therapeutic intervention.

NETOSIS AND NETS

NETOSIS AND ATHEROSCLEROSIS

NETosis plays an important role in the development of atherosclerosis. When considering the role of NETs in atherosclerosis, we should consider two distinct but related processes: atherosclerotic plaque development and atherothrombosis. Both are illustrated in Fig. 2 [7]. As we can see there, NETs play an important role as intraluminal, as intralesional. Moreover, the inhibition of PAD4, an enzyme crucial for NET formation, has been shown to prevent NETosis, resulting in a reduction in the size of atherosclerotic lesions and a delay in arterial thrombosis.

NETs components, such as double-stranded DNA, nucleosomes, and myeloperoxidase–DNA complexes, have been shown as biomarkers for the severity of atherosclerosis and the risk of future cardiovascular events. Its elevated levels linked to increased thrombin generation and severe coronary atherosclerosis, including formation of highly calcified plaques. This suggests that measurement of NET components can be beneficial for the atherosclerotic risk stratification [7].

NETS AND ATHEROSCLEROTIC PLAQUE DEVELOPMENT

It is known that endothelial dysfunction is an important element in the pathogenesis of many vascular and cardiovascular diseases [8]. As a powerful damaging factor, NETs can cause endothelial alteration and dysfunction,

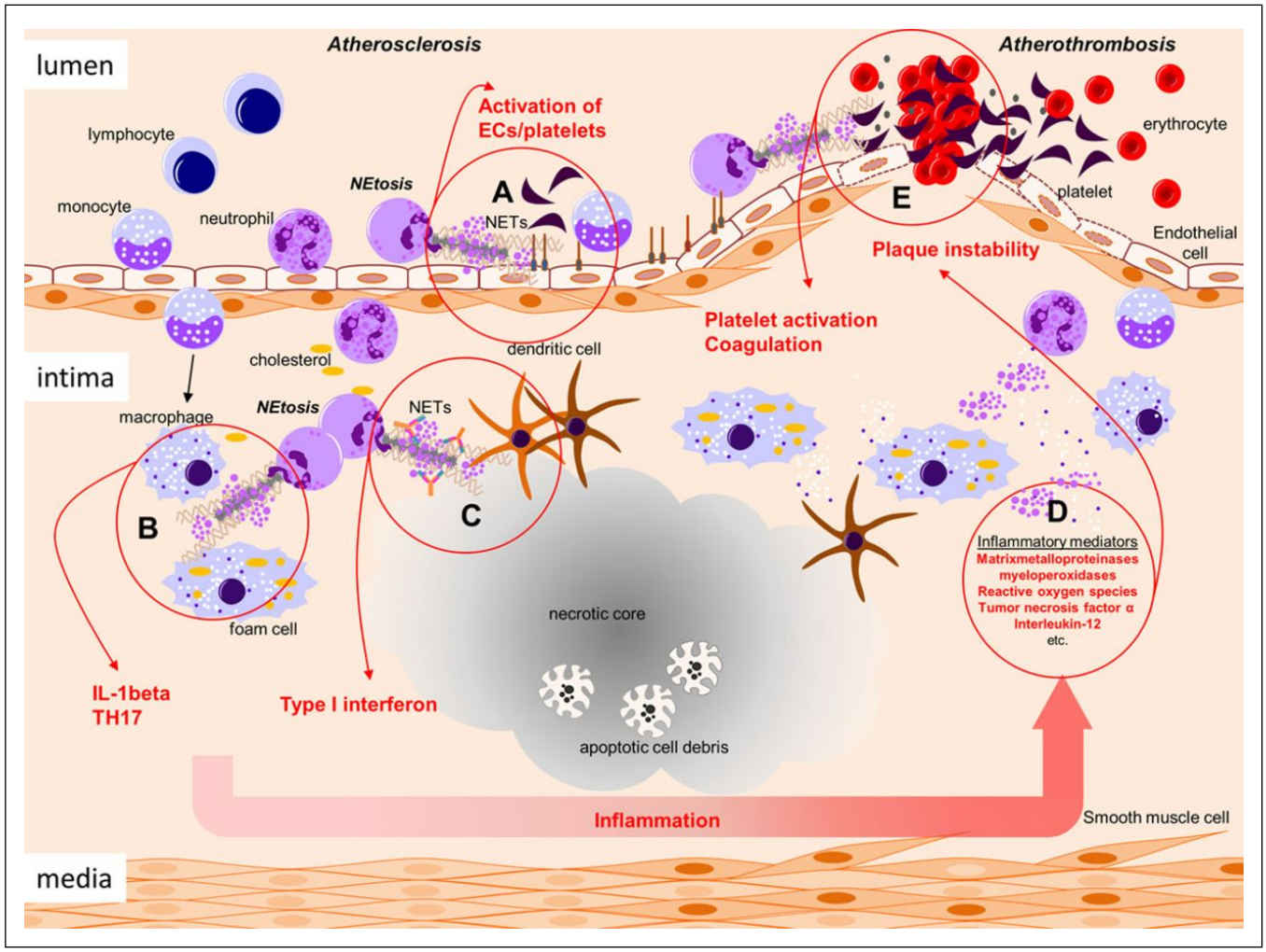

Fig. 2. Emerging roles of NETs in atherosclerosis and atherothrombosis [7].
(A) NETosis in the vascular lumen activates leukocytes, platelets, and endothelial cells, and creates a proinflammatory milieu, resulting in endothelial dysfunction, which is known as the initial trigger for lesion development.
(B/C) Inside the lesions, NETs may start an interleukin-1β/TH17 (T helper 17) and type I interferon response, which further activates the lesional leukocytes, releasing more proinflammatory mediators.
(D/E) NET-induced proinflammatory response creates an inflammatory environment that stimulates plaque destabilization and rupture. In atherothrombosis, NETs can activate the coagulation cascade activation and increase thrombus stability, coordinating arterial occlusion [7].

thus promoting the initial development of atherosclerotic lesions. Being induced, among others, by oxLDL, macrophages and Kupffer cells acquire M4-like proinflammatory phenotype and recruit neutrophils, inducing NETosis [9].

NETs may promote the initial formation of plaques, increase inflammation within existing plaques, and contribute to the formation of thrombi. In the first step, damaged endothelial cells are more likely to allow lipids and inflammatory cells to accumulate in the artery wall, which are early steps in plaque formation. The second step, NETs may promote inflammation in atherosclerotic plaques. Once formed, NETs can activate several types of immune cells, such as plasmacytoid dendritic cells, which then release type I interferons. Additionally, cholesterol crystals in plaques cause NETosis, and NETs trigger macrophages to produce IL-1β, attracting other immune cells to the area. The proinflammatory environment within the atherosclerotic lesions, in turn, makes plaques more likely to rupture. And in the third step, NETs may contribute to the formation of thrombi in the arteries, increasing the risk of heart attack and stroke in people with atherosclerosis. The described mechanism can be confirmed by the fact that NETs are found in the luminal areas of atherosclerotic lesions, both in humans and mice [7].

NETS AND ATHEROTHROMBOSIS

NETs are involved not only in the formation and progression of the atherosclerotic plaque, but in the subsequent thrombosis as well.

In patients with acute coronary syndrome, NETs are found in higher amounts at the sites of ruptured ath-

erosclerotic plaques. NETs have been found in arterial thrombi from people with atherosclerosis and those who have experienced heart attacks. Components of NETs can activate the coagulation cascade, the series of events that leads to the formation of a blood clot, and NETs appear to make clots more stable. Furthermore, there is a correlation between the number of NETs found in the thrombi and the size of the infarct in patients who have had a heart attack. The activity of deoxyribonuclease, an enzyme that degrades NETs, is negatively correlated with the size of the infarct, indicating that NETs contribute to tissue damage following a heart attack. NETs may contribute to thrombus formation following a heart attack by delivering tissue factor to the site of the ruptured plaque as well [7].

THE INTERACTION OF PLATELETS AND NETS

In atherosclerosis, NETs and platelets both promote thrombus formation on ruptured plaques, contributing to heart attacks and strokes [7]. This is the reciprocal process.

On the one hand, NETs recruit platelets. NETs, which include various molecules, create a physical scaffold that traps blood cells, such as platelets and erythrocytes, providing a basis for thrombus growth [7, 11]. NETs themselves can activate platelets, leading to further platelet aggregation and contributing to a prothrombotic state. Platelet activation can occur either by platelet binding to C3b deposits on NETs via complement receptor 1 (CR1) on their surface or directly with histones, specifically H4. On the other hand, platelet activation can trigger NETosis, including in patients with heart attacks. Anyway, activated platelets further stimulate NETosis, creating a positive feedback loop [11].

Activated platelets can stimulate NETosis in neutrophils through either interaction with TLR4 on neutrophils, release of platelet factor 4 (PF4), or via extracellular vesicle-dependent processes [10, 11]. Activated platelets present high-mobility group box 1 (HMGB1) protein to neutrophils, which triggers NETosis, possibly contributing to the plaque rupture.

In turn, NETs promote coagulation via tissue factor (a key protein that initiates the coagulation cascade) [7, 11] and inhibit fibrinolysis, enhancing thrombus stability [11]. NETs can directly activate the intrinsic coagulation cascade by binding and activating Factor XII, which is shown to inhibit fibrinolysis by inhibiting the tissue plasminogen activator (tPA), thus contributing to the stability of the thrombus. Therefore, NETs provide a scaffold for platelet adhesion, activate platelets and the coagulation cascade, and inhibit clot breakdown. They also participate in the inflammatory medium that can start and maintain the atherosclerotic process and are perspective predictors of the severity of atherosclerosis and the probability of future cardiovascular events.

NEW APPROACHES IN THE TREATMENT OF ATHEROSCLEROSIS

Chronic inflammation is a key pathological factor at all stages of atherosclerosis; suppression of the inflammation process is an important therapeutic strategy. As the CIRT study showed, systemic treatment with low doses of the anti-inflammatory drug methotrexate did not lead to a decrease in the level of pro-inflammatory cytokines in blood plasma and did not change the frequency of cardiovascular events in the placebo group [12]. These results, based on the findings of CANTOS and COLCOT studies [13], indicate that direct delivery of anti-inflammatory nanotherapeutic agents to macrophages may be a more effective approach, less systemic treatment, to change combustion while minimizing side effects.

Nanotherapy offers precise targeting, minimizing off-target effects compared to traditional drugs. Studies in mouse models have demonstrated the ability of nanoparticles to inhibit inflammatory processes that drive atherosclerosis [14]. Intravenous nanoparticle administration is a common and effective method, allowing particles to penetrate atherosclerotic plaques and interact with various cell types in the plaque environment, including vascular smooth muscle cells and neutrophils [15]. Nanoparticles in the bloodstream must first penetrate through damaged areas of vascular endothelium or through pores in newly formed vessels to reach subendothelial atherosclerotic plaques. When nanoparticles accumulate in atherosclerotic lesions, they can be taken up by various cell types in the plaque microenvironment, in particular, vascular smooth muscle cells and neutrophils [14].

Macrophages are central to inflammation in atherosclerosis, making them a key target. Unlike traditional therapies, hyaluronan nanoparticles selectively target inflammatory macrophages and reduce inflammation by lowering nitric oxide and TNF production [16]. Wu G. et al. showed that anti-inflammatory cytokines from M2-like macrophages promoted the regression of atherosclerotic plaques in Apoe –/– mice [17].

A 2021 study demonstrated that extracellular vesicles from platelets effectively deliver the NLRP3 inflammasome inhibitor to foamy macrophages in atherosclerotic plaques, reducing inflammation and plaque size [18]. Targeted delivery of methotrexate using polymeric nanoparticles and liposomes improved pharmacokinetics and bioavailability, significantly boosting anti-inflammatory efficacy while minimizing systemic

toxicity [19]. Nakashiro S. et al. delivered pioglitazone using PLGA nanoparticles to convert macrophages to a less inflammatory M2-like subtype in Apoe –/– mice, further reducing inflammation [20].

Flores A.M. et al. found that single-walled carbon nanotubes (SWNTs) accumulate in areas of atherosclerotic lesions as they are taken up by circulating monocytes, which subsequently migrate to plaques [21]. In a mouse study, Leuschner F. et al. showed that a monocyte-targeting approach could prevent their involvement in inflammatory processes by suppressing Ccr2, a gene encoding a chemokine receptor, in proinflammatory monocytes by administration of lipid nanoparticles containing siRNA [22].

In 2023, a study showed that nanoparticles containing IL-10 mRNA targeted M2-like macrophages, triggering IL-10 production in atherosclerotic plaques, which reduced oxidative stress, macrophage apoptosis, and lipid accumulation, ultimately stabilizing plaques [23].

Lameijer M. et al. used HDL nanoparticles to deliver a CD40–TRAF6 inhibitor to Ly6C monocytes, reducing inflammation in plaques and the number of Ly6C monocytes and macrophages in atherosclerosis-prone mice [24]. Long-term administration increased plaque stability by enhancing collagen and reducing plaque size without compromising immunity [25].

Statins, another drug often used in patients with hyperlipidemia, are also already being used in nanotherapy. Nanoparticle delivery of simvastatin, an inhibitor of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase, commonly used to lower LDL-cholesterol in the treatment of atherosclerosis, is able to suppress macrophage growth in affected areas and reduce local inflammation in mice with atherosclerosis (Apoe –/–). Using HDL nanoparticles significantly reduced the number of macrophages in the aorta and reduced plaque size in aortic sinuses in mice [14, 26].

In a study 2020 where Kim H. et al. developed cargo-switching nanoparticles capable of absorbing cholesterol inside cells while simultaneously releasing simvastatin from the core-shell nanoparticles based on methyl-β-cyclodextrin and phospholipid. Such a strategy not only ensured targeted delivery of simvastatin to macrophages at lesion sites, but also reduced plaque cholesterol levels, allowing for a synergistic antiatherogenic effect in Apoe –/– mice with atherosclerosis. However, these nanoparticles did not affect the level of cholesterol in plasma [27]. Therefore, further research on the search for effective drugs against atherosclerotic processes should be continued.

CONCLUSIONS

To date, there is sufficient evidence that atherosclerosis is driven by the inflammatory process. Evidence suggests that atherosclerosis is fundamentally an inflammatory disease, with local systemic inflammation and both being a key drivers of plaque formation and progression. It involves multiple inflammatory mechanisms at all stages, including multiple inflammatory mediators and cytokines (such as IL-1β, IL-6, and TNF-α), CD4+ and CD8+ T-lymphocytes, B-lymphocytes and their subtypes, multiple modes of macrophage activation (including NF-κB-mediated), and neutrophils that undergo NETosis, which exacerbates and builds up inflammation. Inflammatory processes are involved in both plaque formation and plaque destabilization leading to rupture and subsequent thrombosis. Many of these mechanisms have been shown to be applicable as biomarkers of the atherosclerotic process, its severity and its risk level. Currently, a number of antiatherosclerotic therapies targeting inflammation have been developed, but most have not yet been introduced into general medical practice. There is a need to increase awareness of the inflammatory mechanisms underlying atherosclerosis and to apply the knowledge gained in this area to improve the diagnosis, risk stratification and prevention or treatment of this pathology.

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The study was performed as a fragment of the complex scientific project of the Department of Internal Medicine No 4 (Bogomolets National Medical University) «Features metabolism of lipids, proteins, neurotransmitters and hemodynamics in patients with generalized atherosclerosis and secondary prevention of cardiovascular events» (state registration number 0123U105234; term: 2024-2026).

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Andrii A. Kuzminets
Bogomolets National Medical University
13 Taras Shevchenko Blvd, 01601 Kyiv, Ukraine
e-mail: andrewkuzminets@gmail.com

ORCID AND CONTRIBUTIONSHIP
Yuliia O. Bielikova: 0000-0001-6588-9283 A E F
Amir M. Khranovskyi: 0009-0003-9195-0051 A B D
Tetiana M. Motsak: 0000-0002-5545-8583 B D
Andrii A. Kuzminets: 0000-0001-6400-1027 A B D

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

RECEIVED: 10.07.2024
ACCEPTED: 26.10.2024

REVIEW ARTICLE

CONTENTS

Medical genetics as a basis for personalized medicine in contemporary Ukraine

Lyudmila O. Turova, Tatiana A. Vezhnovets, Andrii I. Kurchenko
BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

ABSTRACT

Aim: To systematize and comprehensively analyze scientific sources and research on integrating personalized medicine into the national healthcare system.

Materials and Methods: A comprehensive literature review was conducted using PubMed, Google Scholar, and Scopus, focusing on data from the past 10 years (2014-2024). Articles in both English and Ukrainian were analyzed. Personalized approaches from the authors' own research were also included. The following methods were used: a systematic approach and bibliosemantic analysis.

Conclusions: However, for the implementation of personalized medicine in Ukraine, it is necessary to have awareness of the medical community about the possibilities of using molecular genetic profiling, competence to conduct risk assessment with the participation of several specialists, with the involvement of the patient in decision-making on treatment and diagnostic measures. The main tools for the widespread implementation of personalized medicine in Ukraine are qualified specialists, a wide network of molecular genetic diagnostics and pharmacogenetics laboratories, the development of competencies among healthcare providers and the development of personalized pharmacy. The expected result is the widespread implementation of diagnostic and treatment programs using personalized protocols based on the state of the individual patient's body at a specific time.

KEY WORDS: personalized medicine; omics technologies; health care

Wiad Lek. 2024;77(11):2340-2347. doi: 10.36740/WLek/197123 DOI

INTRODUCTION

Traditional approach to treatment design is based on the universal protocols chosen according to the diagnosed condition. For the reason that disease is an interplay of many variables characteristic of an individual and their environment, the one-fits-all paradigm does not always hold true [1]. The new means of biological data generation and the analysis schemes to derive clinically-relevant insights from the resulting data greatly contributed to precision medicine application in modern medicine. Precision medicine created an avenue for more detailed stratification of patients into the subgroups classified by the potential response to the treatment or a specific drug, which in most cases leads to better treatment outcomes, minimized side-effects and reduced waste of resources. But in practice, simplifying the unique patient case to a set of biological features may be suboptimal to the treatment outcomes. Modern medicine proposes a more comprehensive approach – personalized medicine (PM), which seeks to incorporate not only unique biological characteristics, but also social, psychological, environmental and individual preferences in personalizing the treatment.

Precision and personalized medicine are terms frequently used interchangeably [1]. The common aim of better optimizing clinical decisions for individual cases, along with the phonetic similarity of the terms, has led to confusion in the scientific literature. While precision medicine is data-driven and deeply-rooted in processing large volumes of biological data to obtain the protocols tailored to the specific biological profiles, personalized medicine utilizes a more holistic approach. Personalization attempts to individualize the treatment according to a fully unique case, as opposed to simply segregating patients into groups based on available biomarkers. Personalized schema involves a more interactive relationship between healthcare providers and patients, where treatment plans are co-created based on a combination of scientific evidence and individual circumstances [2].

AIM

The article is aimed at substantiating the implementation and prospects for the development of personalized medicine in Ukraine. Current trends in personalized medicine are outlined, focusing on the improvement of

public health monitoring systems, innovative methods of genetic diagnosis, and disease prevention. The widespread introduction of personalized medicine in Ukraine is supported by the formation of relevant competencies, the establishment of a wide network of molecular genetic diagnostics and pharmacogenetics laboratories, and the involvement of healthcare organizations, which are expected to significantly enhance specialized medical care. The purpose of the work is to systematize and comprehensively analyze scientific sources and research on the integration of the personalized medicine paradigm into the national healthcare system.

MATERIALS AND METHODS

The analysis of literature sources was conducted using the search engines for personalized medicine and biomedical research PubMed and Google Scholar, and the abstract database of scientific literature Scopus. Data for the last 10 years (2014–2024) were analyzed. We searched for articles in English and Ukrainian. Examples of our own research using personalized methods are provided. The following methods were used: systematic approach, bibliosemantic analysis.

REVIEW AND DISCUSSION

RELEVANCE OF PERSONALIZED MEDICINE IN THE WORLD AND IN UKRAINE

The utilization precision as one of the components comprising personalized medicine has long been proven effective [2–4]. Testing for biomarkers is increasingly recognized as a valuable tool, especially in chemotherapy prescription, when recognizing that only a fraction of patients will eventually benefit from a drug [5]. Testing shows means of narrowing the pool of candidate medications and helps avoid trial-and-error approach to the selection, which both improves the prognosis and reduces the costs. Given the rise of medical genetics and the testing.

In Ukraine, the relevance of PM is particularly pronounced given the slowed population growth, aging demographics [6], and the increasing burden of chronic diseases [7]—all of which contribute to escalating need in quality healthcare. The Personalized Prevention of Chronic Diseases (PRECeDI) consortium project underlines thet personalized prevention must be prioritized as a cost-effective measure to relieve the burden of chronic disease [8]. This is critical given the trend of increasing disability years in European countries. By adopting an individualized approach, PM could alleviate these burdens through targeted preventive measures and more effective treatments, thereby improving patient prognoses and reducing long-term healthcare costs.

As advanced PM models become integrated into clinical practice, clinicians will increasingly rely on electronic health records to make informed, real-time decisions, minimizing the need for repeated testing and further optimizing resource utilization. Overall, personalized medicine not only aims to enhance individual health outcomes but also seeks to alleviate financial pressures on healthcare systems through preventive care and efficient resource management [2, 9].

The all-encompassing nature of personalized framework is partially reflected in earlier models. Predictive, preventive and personalised medicine (PPPM), brought forth by European Association for Predictive, Preventive and Personalised Medicine (EPMA) [1], lacks participatory component, included in the P4 public health model fostered by Leroy Hood since the beginning of millenia [10]. Personalization covers all above mentioned points but is not limited to them. Galdo et al. [11] identified three critical aspects missing in the P4 approach and formulated the extended 7P model of medicine, which acknowledges the importance of minimal disruption of patient's life rhythm, parsimonious approach to prescribing medical interventions of any kind as well as incorporation of scientific knowledge into clinical practice.

These aspects collectively (Fig. 1) enhance the personalization of treatment by integrating individual data and preferences into healthcare strategies, ultimately aiming for better health outcomes.

TRANSFORMATION OF 4-5P MEDICINE INTO 7P MEDICINE

The transition to personalized, prognostic, preventive and participatory medicine is driven by significant advances in basic science, digitalization and the development of information and communication technologies, providing a highly dynamic, adaptable and simultaneously personalized approach—anywhere and anytime [12–14].

However, changes in the healthcare system require natural actions and initiatives to improve legal regulations necessary to comply with the new legal environment, moral and ethical principles of a democratic state. [15].

One of the important components of the implementation of this system and the transformation of health care is the need to train modern specialists who are ready to work in the new conditions—both to participate in basic science research and to implement innovative methods in clinical practice, medical and social care, and the educational process [16]. It is the

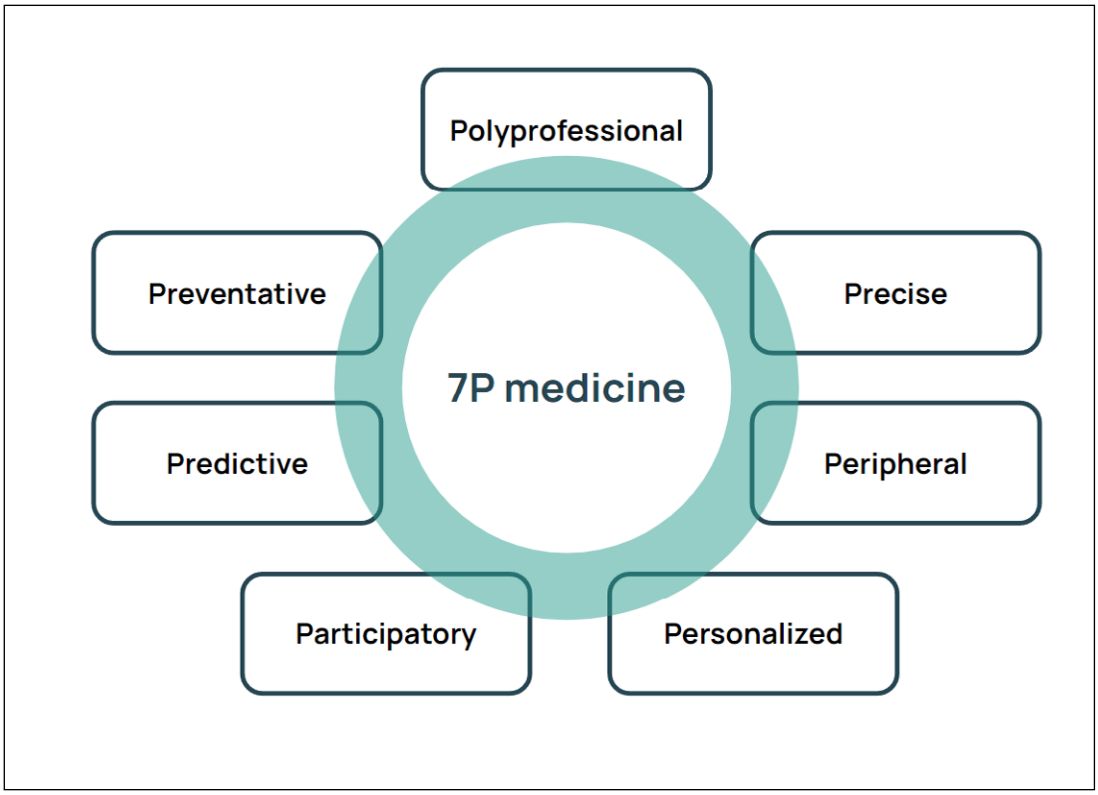

Fig. 1. The components of 7P medicine.

multidisciplinary team that is an absolutely necessary component in the implementation of consistent changes – improvement of the health care model.

PERSONALIZED MEDICINE IN CLINICAL PRACTICE WORLDWIDE

The Personalized Medicine Coalition (PMC) publishes an annual report summarizing the drugs approved by the U.S. Food and Drug Administration (FDA) that are classified as personalized. According to PMC [17], "those therapeutic products for which the label includes reference to specific biological markers identified by diagnostic tools" can be considered personalized. The 2023 report discussed 5 newly approved gene or cell-based therapies reverting rare genetic conditions, 6 new molecular entities found effective in cancer treatment, with some targeting rare forms, personalized therapy for the treatment of Alzheimer's disease and many more, including novel individualized diagnostics and drug combinations. Overall, the 2023 report states that personalized medicines have accounted for at least one-quarter of all new medicines approved for almost the entire decade, making personalization an increasingly sound approach that offers more and more options to those in need.

The diagnostic component of drug prescription personalization is achieved through the means of pharmacogenetic testing. Pharmacogenetics refers to the field of study focusing on the interaction between the individual genetic makeup and a response to a medication, aiming at precise drug selection for the maximization of treatment efficacy and the minimization of adverse reaction probability. For example, individuals with poor metabolizer status for irinotecan derive significant benefit from a genotype-guided dosage strategy, with a reduction in the risk of life-threatening neutropenia and severe diarrhea [18]. Warfarin is another drug with strongly genotype-dependent dosage, varying from 1 to 10 mg a day. The implementation of pharmacogenetic testing has been demonstrated to reduce the dosage adjustment period and to result in a notable decrease in the risk of bleeding or thromboembolism associated with the titration of warfarin.

Acknowledging the role of genetic variation in clinical decision-making, FDA keeps and regularly updates a table titled "Pharmacogenomic Biomarkers in Drug Labeling" [19], listing the personalized drugs and their corresponding biomarkers. Yet another resource called "Table of Pharmacogenetic Associations" [20], also maintained by FDA, keeps the record of drugs for which the evidence of gene-drug interaction is substantial and suggests the optimized approach accordingly. Application of pharmacogenetic testing still remains complicated by the translation of the findings from the lab to clinic. To further aid in the adoption of pharmacogenetics testing, Clinical Pharmacogenetics Implementation Consortium (CPIC) [21] offers standardized,

evidence-based and peer-reviewed drug-gene guidelines. The PharmGKB Genotype Selection Interface (GSI) [22] provides a convenient means of accessing genotype-tailored and up-to-date recommendations, aggregating CPIC and Dutch Pharmacogenetics Working Group (DPWG) guidelines with FDA's approved drug labels and Table of Pharmacogenetic Associations.

Evidence suggests that the application of pharmacogenetic testing can not only enhance treatment outcomes but also offer cost-effective and cost-saving solutions.

Genetic testing may not only guide medication selection, but also offer insight into predisposition to certain conditions and confirm or help establish the diagnosis early [23]. Preconception screening is available on demand and allows assessing the risk of conveying a recessive disorder to the offspring, identifying not manifested variants in otherwise healthy carriers. A more popular option, non-invasive prenatal testing (NIPT), aims to detect chromosomal abnormalities through sequencing cell-free foetal DNA in maternal blood and is available almost worldwide. The application of prenatal screening fulfills supportive function in reproductive autonomy, allowing informed reproductive decisions during pregnancy. With an expanding panel of identifiable conditions NIPT has a potential to inform preventative and therapeutic measures even before birth [9, 23].

Newborn screening allows for the early identification of metabolic and genetic disorders, which can lead to timely interventions. Improved screening processes contribute to better health outcomes and reduced healthcare costs by preventing severe complications from undiagnosed conditions. The Recommended Uniform Screening Panel accepted in the USA covers only a small fraction of genetic disorders, namely 35 out of more than 7500 conditions with therapy options available. For rapid and cost-effective diagnosis, rapid whole-genome sequencing may be a viable option for critically-ill newborns, as explored in Project Baby Bear [24].

Genetic testing has become a cornerstone in the personalized treatment and diagnosis of epilepsy, significantly enhancing clinical outcomes [25]. The approach to genetic testing in neuropathology has evolved from single-gene tests best suited for monogenic disorders to gene panels and whole-exome sequencing, yielding the best diagnosis success rate in pediatric patients. Identifying the specific genetic variant an individual carries comprises a critical foundation for personalized gene therapy, for example in Dravet syndrome (DS), also known as severe myoclonic epilepsy of infancy [26].

The integration of person-centered medicine takes special notice in the education and training of the professionals who will manage the interpretation of the genetic testing and adjust the therapy course accordingly [8, 27]. Providing the clinicians, IT specialists and laboratory technicians with a broad understanding of personalization ensures productive collaboration and implementation of PM [28]. Two personalized multicenter initiatives Genomic Medicine Sweden (GMS) and the Centers for Personalized Medicine (ZPM) initiative in Germany arrange programs that provide exposure to the field of personalized medicine in a multidisciplinary setting [3].

EXAMPLES OF PERSONALIZED MEDICINE IMPLEMENTATION IN CLINICAL PRACTICE

Earlier, we developed, improved and implemented a model of a comprehensive, multidisciplinary, personalized approach to medical and genetic counseling of families to prevent the development of perinatal and congenital pathology in children [29]. Perinatology and pediatrics occupy a special place in the new healthcare model as the most important area of clinical medicine that studies the health of a child in the process of its development and growth. After analyzing all the factors that influence the formation of aspects of a child's health and taking into account the "molecular genetic portrait", we developed health programming paradigms and identified "windows of opportunity" for targeted intervention during these periods.

The principles of personalization were used to predict perinatal pathology based on immunogenetic predictors [30], in children born prematurely, which allowed us to determine the possibilities and potential of targeted stage rehabilitation. The introduction of personalized medicine in today's conditions to prevent a high incidence of perinatal pathology, especially in preterm infants, is of extremely high medical and social importance.

In our other works [30–35], the strategy of personalized medicine was implemented in practice both for children with different nosologies and for adult patients, and with the active participation of both the patient and his or her family in achieving disease control. We studied not only clinical phenotypes, but also molecular and biochemical phenotypes, which, with the help of modern neuroscience programming, allowed us to personalize diagnostic, therapeutic, preventive and rehabilitation measures of medical support.

The personalized and preventive concept of health maintenance and programming of intellectual disabilities based on genetic screening is described in [32, 34, 35]. The study demonstrates broad prospects for the application of molecular genetic screening of children with delayed psycho-neurological development in

clinical practice in order to predict the patient's rehabilitation potential and implement socially adapted development and health of the child, which is of particular importance in martial law conditions for both the health care system and the economic sector of the country.

In addition, in the context of a constant increase in the incidence of allergic diseases, algorithms for determining the molecular genetic profile of sensitization and manifestations of the multimorbid phenotype have been developed and put into practice both at the pre-dosage stage for personalization of preventive measures and for personalized therapy. The predictive role of identifying an individual allergy profile at the prenosological stage for the purpose of preventive measures, as well as the choice of measures for patients with a realized atopy phenotype with an optimal diagnostic algorithm, improving disease control, and programming the prognosis of the patient's further health status has been determined [31, 36].

In conclusion, a detailed and systematic clinical evaluation of big data (patient's metabolic phenotype, proteome, microbiome) is the basis for selecting a genetic test and further application of personalized medicine in practice. To diagnose a clinically heterogeneous phenotype, a multidisciplinary approach should be used, including a clinical geneticist, neurologist, cardiologist, family doctor, or pediatrician. Knowledge about the use of the latest methods of precision genetic diagnosis and interpretation of their results can help diagnose almost all pathological processes, but each patient requires a personalized approach based on a genetic map—a genome study [37].

Highlighting the progress of personalization, we conclude the current state and the examples of personalization by medical field.

ONCOLOGY AND CANCER TREATMENT

Individualized chemotherapeutic drugs containing a dose calculated for a particular patient enable the doctor to achieve the maximum therapeutic effect with minimal side effects in diagnosing tumors, monitoring the effectiveness of radiation and chemotherapy [38].

Orphan diseases. Today, the problem of orphan diseases in Ukraine and the world is extremely relevant. According to the European Committee of Experts for Rare Diseases (EUCERD), the total number of different orphan diseases reaches 8 thousand. Most of them require personalized treatment, which is the only way to save patients with orphan diseases [24, 34].

Hereditary genetic diseases, and congenital defects of the central nervous system, hereditary mental disorders, neurological diseases (epilepsy, migraine, Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, etc.). A combination of non-pharmacological and pharmacological treatments makes up a personalized patient management scheme [39, 40].

Metabolic diseases of metabolism, cardiac diseases, diabetes mellitus, neurodegenerative diseases of the bone and joints, connective tissue diseases, lung diseases, and allergic diseases. A number of markers that help to identify the leading pathogenetic mechanism in each case become a guide to a personalized approach to patient treatment. Personalized medicine is already a reality for gastroenterology and cardiology. Allergen-specific immunotherapy (ASIT) is one of the first personalized treatments in allergology, the essence of which is the administration of allergens to the patient that cause allergic reactions in him [31, 39].

RESPONSE TO IMMUNOPROPHYLAXIS

To date, genetic loci associated with the maintenance of a vaccine-induced immune response have been studied [40].

Another important area of personalized intervention is anti-aging, nutritional genetics, stem cell treatment, regenerative and—another separate area—reproductive medicine. Targeted sequencing embodies the embodiment of the wildest dreams of prenatal medicine representatives: determining the extracellular DNA of the fetus circulating in the mother's blood allows diagnosing chromosomal rearrangements (Down, Edwards, Patau, Turner syndromes) and the likelihood of aneuploidy from the 5th week of pregnancy [29].

CONCLUSIONS

Personalized medicine using scientific and technological progress, achievements in the field of preventive and health-saving technologies, focused on programming and shaping the optimal health trajectory is the basis for preserving the health of the nation.

However, for the implementation of personalized medicine in Ukraine, it is necessary to have awareness of the medical community about the possibilities of using molecular genetic profiling, competence to conduct risk assessment with the participation of several specialists, with the involvement of the patient in decision-making on treatment and diagnostic measures. The main tools for the widespread implementation of personalized medicine in Ukraine are qualified specialists, a wide network of molecular genetic diagnostics and pharmacogenetics laboratories, the development of competencies among healthcare providers and the development of personalized pharmacy.

The expected result is the widespread implementation of diagnostic and treatment programs using personalized protocols based on the state of the individual patient's body at a specific time.

PROSPECTS FOR FURTHER RESEARCH

We are developing a mechanism for implementing personalized medicine for broad integration into healthcare. We are preparing recommendations and effective strategies to overcome the existing barriers and use the potential of personalized medicine to improve health outcomes. An important initiative is to engage all stakeholders, including a multidisciplinary team of healthcare professionals, researchers, scientists, policy makers, and patients, to actively participate in a transformative healthcare paradigm shift. This change will be more precise, predictable, patient-centered, and tailored to individual needs, ultimately leading to better health outcomes and quality of life for citizens.

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This article was performed in the framework of the scientific research works «Assessment of the state of regionalization of perinatal care in the regions in the context of perinatal risk management of newborns, routing of patients according to identified risks. Assessment of material and technical, organizational, analytical, methodical and educational capacity of perinatal centers» (2023–2024, No state registration 0123U103032) and «Study of immunogenetic aspects of diagnosis, treatment and prevention of allergic and other socially significant multifactorial diseases». (2023–2024, No state registration 0124U000023).

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Lyudmila O. Turova

Bogomolets National Medical University

13 Taras Shevchenko Blv., 01601 Kyiv, Ukraine

e-mail: dr.turova@gmail.com

ORCID AND CONTRIBUTIONSHIP
Lyudmila O. Turova 0000-0002-4481-3009 A B D F
Tatiana A. Vezhnovets 0000-0003-1156-8614 B E F
Andrii I. Kurchenko 0000-0001-6858-2025 B E

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

RECEIVED: 21.06.2024
ACCEPTED: 27.10.2024

REVIEW ARTICLE

CONTENTS

Application of i-PRF in dentistry

Wojciech Niemczyk[1], Stanisław Niemczyk[2], Olga Odrzywolska[1], Patrycja Doroz[1], Dawid Hochuł[1], Kaja Zawadzka[3]
[1]FACULTY OF MEDICAL SCIENCES IN ZABRZE, MEDICAL UNIVERSITY OF SILESIA, ZABRZE, POLAND
[2]CITY HOSPITAL NO. 4 IN GLIWICE, GLIWICE, POLAND
[3]PROVINCIAL HOSPITAL NAMED AFTER SAINT LUKE, TARNOW, POLAND

ABSTRACT

Injectable platelet-rich fibrin (i-PRF) is a novel platelet concentrate that has been employed in dentistry with the objective of promoting tissue regeneration and healing. In contrast to platelet-rich plasma (PRP), i-PRF is more straightforward to handle, more cost-effective, and free from anticoagulants, which reduces biochemical alterations. The i-PRF procedure was developed in 2014 by adjusting the centrifugation forces. The resulting product remains in a liquid state for approximately 15 minutes, gradually transforming into a growth factor-rich clot that releases continuously over 10-14 days. It has demonstrated efficacy in a range of dental applications. In periodontology, i-PRF is an effective treatment for periodontitis, enhancing gingival thickness and reducing inflammation, plaque, and recession. Furthermore, it enhances the outcomes of periodontal regenerative surgery and lichen planus treatment. In oral surgery, i-PRF has been shown to facilitate bone regeneration, fistula resolution, and sinus augmentation. Orthodontic studies have indicated that i-PRF accelerates tooth movement and bone remodeling. However, osteomicroperforation remains more effective for some treatments. A limited number of endodontic studies have indicated that i-PRF may support revascularisation and periapical healing. In conclusion, i-PRF's regenerative capabilities, anti-inflammatory and antimicrobial properties offer significant potential in dentistry, and further exploration and application is therefore warranted.

KEY WORDS: dentistry, oral surgery, platelet concentrates, platelet rich fibrin

Wiad Lek. 2024;77(11):2348-2352. doi: 10.36740/WLek/195552 DOI

INTRODUCTION

For over three decades, platelet concentrates have been employed in dentistry as regenerative agents that release growth factors in concentrations exceeding those observed in normal physiological conditions, thereby promoting tissue regeneration from autologous sources [1]. Various types of platelet-rich concentrates, including platelet-rich plasma (PRP) and platelet-rich fibrin (PRF), have been explored in numerous in vitro and in vivo studies for tissue regeneration [2]. However, PRF offers certain benefits over PRP, such as easier handling, lower costs, and the absence of anticoagulants or bovine thrombin, which minimizes biochemical alterations and associated risks [2, 3]. In 2014, injectable platelet-rich fibrin (I-PRF) was introduced, developed by modifying centrifugation forces. By using lower centrifugation speeds and non-glass tubes, a flowable form of PRF, known as I-PRF, is obtained [4]. I-PRF, a new platelet concentrate rich in leukocytes, can stimulate the regeneration of both soft and hard tissues. Its liquid state, which lasts approximately 15 minutes post-application, provides dental practitioners with an alternative, practical form of PRF [5]. Upon application, the liquid fibrinogen in I-PRF gradually forms a PRF clot rich in growth factors, which are released continuously over 10–14 days [6]. Moreover, I-PRF has demonstrated significant anti-inflammatory and antimicrobial properties, which may accelerate tissue regeneration [7].

AIM

The aim of this review was to present the new possibilities offered by the use of i-PRF in the field of dentistry.

REVIEW AND DISCUSSION

PERIODONTOLOGY

In a systematic review, Niemczyk et al. demonstrated that i-PRF is an efficacious intradermal treatment for periodontitis, exhibiting considerable bactericidal effects against *Porphyromonas gingivalis* [36]. Fur-

thermore, the utilisation of i-PRF in patients with thin periodontal phenotypes has been associated with augmented gingival thickness, indicating a prospective non-surgical approach for gingival tissue enhancement [8–10]. Similar conclusions were reached by Żurek et al. in their systematic review on this topic [11]. Pullishery et al. reported that i-PRF, whether used alone or in conjunction with other agents, has a beneficial effect on the reduction of plaque, the alleviation of inflammation, and the stability of gingival tissue, which may contribute to a reduction in gingival recession [12,13]. In a meta-analysis conducted by Kalburgi et al., it was demonstrated that i-PRF has the potential to enhance the outcomes of periodontal regenerative surgery Mahajan et al. observed that the combination of i-PRF and free gingival grafts (FGG) for gingival recession resulted in superior root coverage compared to FGG alone [14]. Similarly, the meta-analysis conducted by Gupta et al. demonstrated that i-PRF is an effective treatment for lichen planus, reducing pain and lesion size and significantly improving patient satisfaction [15]. In a split-mouth study, Saglam et al. demonstrated that i-PRF is as effective as injectable corticosteroids for the treatment of erosive lichen planus [16].

ORAL SURGERY

The utilisation of i-PRF in the context of oral surgery has been demonstrated to facilitate enhanced tissue repair and bone regeneration. In a case report by Giudice et al., i-PRF injections resulted in the complete healing of oro-cutaneous fistulas within 50 days, demonstrating its capability to release growth factors and chemotactic agents that are crucial for tissue repair processes [17]. Melek and Taalab's clinical trial investigated the utilisation of i-PRF in conjunction with guided bone regeneration, demonstrating that i-PRF's elevated growth factor content facilitates more favourable bone formation at graft sites. This combination not only improves the predictability of bone regeneration but also helps to maintain the structural integrity of the alveolar ridge, which is crucial for successful implant placement [18]. In a further study, Dayashankara Rao demonstrated that the combination of i-PRF and autologous iliac crest bone resulted in a significantly enhanced rate of bone formation in alveolar clefts compared to the use of bone grafts alone. This combination also facilitated a reduction in the rate of bone resorption, thereby enhancing periodontal health in the vicinity of the grafted site. This is of particular benefit to patients with cleft lip and palate conditions [19]. Moreover, Gülşen and Dereci demonstrated that i-PRF-soaked collagen plugs used in sinus floor augmentation resulted in substantial new bone formation. The continuous release of growth factors from i-PRF improved the osteogenic environment, thereby facilitating enhanced bone integration at the site of implantation [20]. Furthermore, in vitro studies have demonstrated that i-PRF enhances osteoblast activity when combined with allogenic bone substitutes, indicating the potential for a beneficial impact on bone regeneration in clinical settings [6].

ORTHODONTICS

The effects of i-PRF on orthodontic tooth movement have been examined in several randomised trials [21–23]. The accelerated bone remodelling facilitated by i-PRF allows for a more expeditious tooth movement within the dental arch [24]. Nevertheless, the use of i-PRF alone is not a guaranteed method of preventing root resorption or bone fenestrations [22]. The studies conducted by Alaa and colleagues and Ammar and colleagues demonstrated that i-PRF significantly outperformed PRP in accelerating orthodontic tooth movement, with i-PRF showing more sustained effects [25, 26]. Its application during orthodontic treatment is well tolerated, with minimal discomfort that typically subsides within 24 hours [27]. Furthermore, the retraction of the canines was observed to occur at a rate 1.8 times faster when i-PRF was used, accompanied by an increase in alkaline phosphatase levels shortly after the commencement of treatment [28]. Nevertheless, research conducted by Kushal and Niranjane has indicated that osteomicroperforation may be a more effective technique than i-PRF for accelerating orthodontic tooth movement [29]. Furthermore, animal studies have indicated that i-PRF facilitates cementum and bone formation, increasing the number of cementoblasts, cementoclasts, and new bone in expanded palatal sutures [30, 31].

ENDODONTICS

The number of studies investigating the use of i-PRF in endodontics is limited. Ibrahim et al. demonstrated that i-PRF revascularisation represents a successful regenerative treatment for mature permanent incisors with closed apices [32]. Similar positive outcomes were documented by Mansour and El-Sawy, who observed the healing of periapical lesions with the use of i-PRF [33]. Furthermore, Agrawal et al. documented complete periapical healing and pain resolution when combining i-PRF with mineral trioxide aggregate [34]. It is proposed that liquid PRF may offer enhanced regenerative potential for human dental pulp cells

in comparison to traditional PRP, facilitating odontoblastic differentiation and reparative dentin formation while reducing inflammation [35].

CONCLUSIONS

Injectable platelet-rich fibrin has demonstrated considerable potential in a range of dental applications, largely due to its regenerative and anti-inflammatory properties. Niemczyk et al. emphasised its efficacy in the treatment of periodontitis and its bactericidal activity against *Porphyromonas gingivalis*. i-PRF has been demonstrated to thicken thin gingival phenotypes, potentially offering a non-surgical approach to gingival tissue augmentation. The findings of studies conducted by Pullishery et al. and Kalburgi et al. indicate that i-PRF has the potential to reduce plaque, gingival inflammation and attachment loss, while also enhancing the outcomes of periodontal regenerative surgery. The combination of i-PRF and free gingival grafts demonstrated superior root coverage in the treatment of gingival recession compared to the use of grafts alone. Furthermore, i-PRF has been demonstrated to reduce pain and lesion size in lichen planus, and to be as effective as corticosteroids in treating its erosive form. In the field of oral surgery, i-PRF has been shown to facilitate significant tissue repair, as evidenced by the findings of the study conducted by Giudice et al. on the resolution of oro-cutaneous fistulas. The use of i-PRF with grafts has been demonstrated to enhance bone formation, as evidenced by the findings of Melek and Taalab. Furthermore, the combination of i-PRF with autogenous bone has been shown to improve bone formation in alveolar clefts, as reported by Dayashankara Rao. The study by Gülşen and Dereci demonstrated the efficacy of i-PRF-impregnated collagen plugs in facilitating significant new bone formation in sinus floor augmentation. Furthermore, i-PRF has been demonstrated to enhance osteoblast activity, particularly when employed in conjunction with allogenic bone graft substitutes. In the field of orthodontics, i-PRF has been demonstrated to accelerate bone remodelling and tooth movement, with studies indicating its superiority over PRP. The findings of Alaa et al. and Ammar et al. indicate that i-PRF is a more effective and longer-lasting method than PRP for accelerating orthodontic tooth movement. The procedure is well tolerated and causes minimal discomfort. Nevertheless, osteomicroperforation has been demonstrated to be more efficacious than i-PRF in accelerating tooth movement. The results of animal studies indicate that i-PRF has the potential to enhance cementum and bone formation, as well as blood vessel production. While the evidence is limited, the use of i-PRF in endodontics has yielded promising outcomes. Ibrahim et al. demonstrated the effectiveness of i-PRF in revascularising mature permanent incisors with closed apices. Additionally, case reports by Mansour and El-Sawy and Agrawal et al. illustrated the successful healing of periapical lesions and resolution of pain. In conclusion, the capacity of i-PRF to stimulate tissue regeneration, attenuate inflammation and facilitate healing renders it a valuable instrument in the field of dentistry, with considerable potential for further utilisation and investigation.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Stanisław Niemczyk
City Hospital No4 in Gliwice
20 Zygmunta Starego Str., 44-100 Gliwice, Poland
e-mail: stanislaw.niemczyk98@gmail.com

ORCID AND CONTRIBUTIONSHIP

Wojciech Niemczyk: 0000-0003-0172-0571 A B D
Stanisław Niemczyk: 0000-0001-5236-2298 A B D
Olga Odrzywolska: 0009-0003-2674-8447 A B D
Patrycja Doroz: 0009-0004-9266-0360 A B D
Dawid Hochuł: 0009-0008-5894-4959 A B D
Kaja Zawadzka: 0009-0001-8812-6083 E F

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

RECEIVED: 07.09.2024
ACCEPTED: 04.11.2024

REVIEW ARTICLE

CONTENTS

Correlation between endometriosis and migraine headache – a review of literature

Jacek Kurzeja[1,2], Katarzyna Piotrowicz[3], Emilia Anna Żurowska[1,4], Hubert Piotrowicz[3], Urszula Guderska[1,5]

[1]GRADUATE OF THE FACULTY OF MEDICINE, POZNAN UNIVERSITY OF MEDICAL SCIENCES, POZNAN, POLAND
[2]STUDENT SCIENTIFIC SOCIETY, POZNAN UNIVERSITY OF MEDICAL SCIENCES, POZNAN, POLAND
[3]STUDENT OF THE FACULTY OF MEDICINE, POZNAN UNIVERSITY OF MEDICAL SCIENCES, POZNAN, POLAND
[4]FRANCISZEK RASZEJA MEMORIAL MUNICIPAL, HOSPITAL IN POZNAN, POZNAN, POLAND
[5]UNIVERSITY CLINICAL HOSPITAL IN POZNAN, POZNAN. POLAND

ABSTRACT

Aim: Endometriosis and migraine are common diseases that share many features, and their co-occurrence is the subject of numerous studies. Nevertheless, there is no effective treatment for these diseases and diagnosis is often delayed. This review aims to present correlations between these conditions, which can improve diagnosis and treatment.

Materials and methods: The inclusion criteria were original articles published between 2019 and 2024 describing the correlation between endometriosis and migraine. Analysis of literature. The study describes the connection between endometriosis and migraine, presenting it in a concise and easy to analyze way. The importance of the problem of co-occurrence of endometriosis and migraine was emphasized and the latest articles were analyzed, which differed in the way of determining the correlation between these diseases, but nevertheless confirmed the connection between the two.

Conclusions: Endometriosis and migraine are very important problems, which share many correlations. Appropriate diagnosis and treatment can improve patients' quality of life. All recent publications have confirmed the correlations between these diseases and agree on looking for the features of migraine in patients with endometriosis and looking for endometriosis in patients with migraine.

KEY WORDS: pain, migraine, CGRP, sex hormone, endometriosis (EM)

Wiad Lek. 2024;77(11):2353-2358. doi: 10.36740/WLek/194637 DOI

INTRODUCTION

Endometriosis and migraine are some of the most common diseases in women. Both of these disorders manifest themselves with pain, which is their dominant clinical feature. They have a significant impact on physical and mental health, require attention and proper treatment [1, 2]. Migraine is much more common in patients with endometriosis, which is why these pain disorders have been linked [3].

Endometriosis is a chronic, inflammatory gynecological disease in which the endometrium and stroma proliferate outside the uterus. It is most often located in the pelvic organs, such as the ovaries, fallopian tubes, urinary bladder, intestines or peritoneum [4, 5]. It is much less frequently located in organs outside the pelvis [5]. Endometriosis affects 5-10% of women of reproductive age and 35-50% of women suffering from infertility. It is one of the most common gynecological diseases [6, 7]. It is characterized by heavy periods, infertility, chronic abdominal pain during menstruation, defecation and urination, and painful sexual intercourse. In addition, fatigue, abdominal bloating, nausea may occur, resulting in a significant reduction in quality of life, depressive or anxiety disorders. Because symptoms are often non-specific and may be caused by many other diseases, diagnosis may be delayed, sometimes even for many years [4, 5].

On the other hand, migraine, a serious neurological disorder, affects one in nine adults worldwide. Migraine ranks second among the causes of disability in the general population [8], while in people under 50 years of age it ranks first [9]. About 3.7 million people in Poland suffer from migraine, including about 0.5 million people with chronic migraine [10]. Migraine manifests itself as a unilateral, pulsating headache, ranging from mild to very severe, which may be accompanied by nausea, vomiting, and hypersensitivity to light and noise [11]. Similarly to endometriosis, migraine affects mainly women (2 to 3 times more often than men) of reproductive age [3, 12-14]. In women, attacks are more frequent and more severe, and

Table 1. Clinico-epidemiological and socio-economic similarities of endometriosis and migraine [4, 19-33].

Compared feature	Endometriosis	Migraine
Sex	Almost exclusively in women	In women 2 to 3 times more often than in men
Peak incidence	Reproductive age	Reproductive age
Occurrence	10%	14.7%
Factors associated with increased risk	Early menarche, menorrhagia, shorter menstrual cycle length, hormonal dysregulation, low body weight, taller height, alcohol use, and caffeine intake	Early menarche, menorrhagia, and hormonal dysregulation
The impact of menstruation	Pain intensification	Pain intensification
Risk factor	Diabetes, cardiovascular diseases, autoimmune diseases, chronic liver disease, ovarian cancer, irritable bowel syndrome, depression, obstetric and neonatal complications	Cardiovascular diseases, mental disorders
Diagnostics	Medical interview, clinical (vaginal) examination, TVS, pelvic ultrasonography/MRI, diagnostic laparoscopy, blood tests	Medical interview (ICHD-3 criteria), physical examination, confirmation or exclusion of secondary headaches - neuroimaging, lumbar puncture), blood tests
Treatment	Symptomatic and removal of endometriosis foci: 1) NSAIDs + low dose COCPs 2) Hormonal treatment 3) Surgical techniques	Symptomatic and preventive: 1) paracetamol or NSAIDs (for mild attacks) 2) triptans or 5HT1B/1D receptor agonists (for moderate or severe attacks) 3) CGRP receptor antagonists - gepants and lasmiditan (as effective acute treatments) 4)intramuscularonabotulinumtoxinA (in chronic migraine) 5) monoclonal antibodies targeting CGRP or its receptor (as preventive treatment)
Economic costs per year in the US	$78–119 billion	$78 billion

Abbreviation:
TVS - transvaginal ultrasound imaging
MRI – magnetic resonance imaging
NSAIDs – non-steroidal anti-inflammatory drugs
COCPs - combined oral contraceptive pills
CGRP - calcitonin gene-related peptide

there may be more triggers [15, 16]. The most frequently reported triggers of migraine in women are menstruation [16], and the relationship between menstruation and migraine headache occurs in as many as 20-25% of women [17]. It has been shown that the co-occurrence of migraine in untreated women with endometriosis is associated with deeper gynecological infiltrates and correlates with increased pain intensity [18].

AIM

It is assumed that endometriosis and migraines have a more frequent coincidence and may also have similar clinical symptoms. The aforementioned disease entities are a common focus of many studies examining the correlation between endometriosis and migraine, which may facilitate the understanding of their common etiology and pathogenesis. To our knowledge, this is the first literature review and summary of the common features of endometriosis and migraine headaches based on original publications. The summary of literature data aims to present correlations between endometriosis and migraine, which could ensure faster diagnosis of both diseases, make treatment more effective and, above all, improve the quality of life of patients.

MATERIALS AND METHODS

For this review, we analyzed the Google Scholar and PubMed databases using the keywords: endometriosis, migraine, endometriosis migraine, menstrual migraine, CGRP. Inclusion criteria included original articles published between 2019 and 2024 describing the correlation between endometriosis and migraine. Articles were carefully reviewed and included based on their relevance to our review.

Table 2. Correlation between endometriosis and migraine[3, 21, 22, 18].

Publication's title	Migraine in relation with endometriosis phenotypes: Results from a French case-control study	Shared Molecular Genetic Mechanisms Underlie Endometriosis and Migraine Comorbidity	Plasma calcitonin gene-related peptide (CGRP) in migraine and endometriosis during the menstrual cycle	Correlation between endometriosis and migraine features: Results from a prospective case-control study
Year of publishing	2020	2020	2021	2024
Publication type	Case-control study	Meta-analysis	Observational study	Case-control study
Description	The research team divided the patients into two groups based on histological findings: a control group, consisting of women without visible endometriosis lesions, and a group of women with endometriosis whose lesions were histologically confirmed. The incidence of migraine was studied in relation to three phenotypes of endometriosis: SUP, OMA and DIE	Researchers examined the association between endometriosis and migraine using data from a genome-wide association study (GWAS). A concordance analysis of polymorphism (SNP) effects showed significant concordance of SNP risk effects in GWAS of endometriosis and migraine. A meta-analysis of GWAS data on endometriosis and migraine did not detect any new significant SNPs in the whole genome, and a Mendelian randomization analysis showed a non-causal association between the two traits	Changes in plasma CGRP concentrations during the menstrual cycle were analyzed, examining concentrations during the perimenstrual visit = PM and periovulatory phase = PO of the menstrual cycle. Four groups participated in the study: women with episodic migraine, women with endometriosis, women with endometriosis and migraine, and the control group consisted of healthy women.	A group of women with endometriosis with coexisting migraine (EM-MG) was included in the study, while women with endometriosis alone (EM-O) and women with migraine alone (MG-O) were considered two different control groups. The diagnosis of endometriosis was confirmed based on combined clinical symptoms and transvaginal ultrasound (TVS). The International Classification of Headache Syndromes 3rd Edition (ICHD-3) was used to confirm migraine
Clinical significance	Patients with migraine should undergo diagnostic tests to look for endometriosis, especially ovarian endometriosis (OMA) and deeply infiltrating endometriosis (DIE)	A noncausal relationship between the diseases has been suggested. These diseases may have common genetic pathomechanisms. Common genes and biological pathways have been identified that may serve as potential future therapeutic targets	Abnormal regulation of CGRP peptides may be related to the pathophysiology of these comorbidities.	The co-occurrence of endometriosis and migraine intensifies basic symptoms of both endometriosis and migraines.This may be due to the presence of more CGRP-positive nerve fibers, causing inflammation. Treatment with drugs targeting CGRP in patients with comorbid migraine and endometriosis may also have a positive effect on the symptoms of endometriosis

REVIEW AND DISCUSSION

Table 1. contains summary of common clinical-epidemiological and socio-economic features of endometriosis and migraine.

Table 2. is a summary table containing information about the original articles of our review on the correlation between endometriosis and migraine.

The exact mechanisms of endometriosis and migraine are not fully understood and remain the subject of many studies. This makes understanding the pathogenesis of the relationship between endometriosis and migraine even more difficult and incomplete [3]. It has been known for years that the neuropeptide CGRP plays an important role in migraine attacks. This resulted in the development of highly effective anti-CGRP monoclonal antibodies that block the CGRP receptor [34]. But is the neuropeptide CGRP also involved in endometriosis? According to a 2021 case-control study conducted by Raffaelli et al, the neuropeptide CGRP (calcitonin gene-related peptide) probably plays a role in both of these diseases [22]. CGRP is released from nerve cells into the blood and is a potent vasodilator. Its synthesis is increased in tissues affected by inflammation [35].

The study by Raffaelli et al looked at changes in plasma CGRP concentrations during the menstrual cycle, examining concentrations at the perimenstrual

visit = PM and at the periovulatory phase = PO of the menstrual cycle. Four research groups took part in the study: women with episodic migraine, women with endometriosis, women with endometriosis and migraine, and the control group consisted of healthy women. CGRP concentrations during menstruation were similar in all groups, but there were significant differences in CGRP concentrations during PO. Women with comorbidities had higher CGRP concentrations in the PM, while healthy women from the control group had higher CGRP concentrations in the PO. This study showed that abnormal regulation of the CGRP peptide occurring in patients suffering from both endometriosis and migraine may play a role in the pathophysiology of these comorbidities [22].

Adewuyi et al. confirmed the co-occurrence of endometriosis and migraine and pointed to genetic disorders as a possible common pathological mechanism. Previous studies have suggested common genetic determinants of these diseases, but no causative gene has been identified [36]. Only a genome-wide association study identified two previously unknown loci in protein-coding genes (TRIM32 and SLC35G6). These were previously unknown and may represent new genes and susceptibility loci for these two diseases. Common genetically controlled biological mechanisms may be responsible for the abnormal CGRP release detected in endometriosis and migraine patients by Raffaelli et al, but this hypothesis requires further investigation [21, 22].

CONCLUSIONS

Endometriosis and migraine are different diseases that share many similarities. All the studies we had analyzed have confirmed the correlation between migraine and endometriosis and have emphasized the importance of simultaneous gynecological and ultrasound examination in women with migraine, as well as performing comprehensive diagnostics for migraine in patients with endometriosis and headaches. These two comorbidities need to be treated by the appropriate medical team, which can improve patients' quality of life. Determining the exact cause of the co-occurrence of these two diseases is difficult to understand due to many uncertainties related to the pathogenesis of endometriosis and migraine. However, medicine is a dynamically developing science and hopefully future research will shed more light on this issue, which could certainly improve the quality of life of many patients.

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ORCID AND CONTRIBUTIONSHIP

Jacek Kurzeja: 0009-0004-4075-7208 A B D E F
Katarzyna Piotrowicz: 0009-0009-3888-3885 B D F
Emilia Anna Żurowska: 0009-0006-5952-1064 E
Hubert Piotrowicz: 0009-0008-2453-3561 E
Urszula Guderska: 0009-0000-9287-6672 E

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Jacek Kurzeja
Graduate of the Faculty of Medicine,
Poznan University of Medical Sciences, Poland
e-mail: jacekkurzeja96@gmail.com

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

RECEIVED: 08.07.2024
ACCEPTED: 15.10.2024

CASE STUDY

CONTENTS

The impact of acquired immunodeficiency in a patient with b-cell chronic lymphocytic leukemia on the course of coronavirus disease (COVID-19): a case report

Oksana M. Kostiukevych, Oleksandr V. Naumenko, Lesia K. Benkovska, Mariia A. Trypilka
STATE INSTITUTION OF SCIENCE «CENTER OF INNOVATIVE HEALTHCARE TECHNOLOGIES» STATE ADMINISTRATIVE DEPARTMENT, KYIV, UKRAINE

ABSTRACT

Chronic lymphocytic leukemia (CLL) is a lymphoproliferative disorder that affects the bone marrow, lymph nodes, and spleen, and is associated with certain complications. One of the complications of CLL is acquired immunodeficiency, leading to a predisposition for prolonged respiratory viral and bacterial infections. This publication presents a clinical case of a prolonged and severe course of COVID-19 in a patient with CLL, who was successfully treated with immunoglobulin replacement therapy.

KEY WORDS: chronic lymphocytic leukemia, COVID-19, immunodeficiency, immunoglobulin replacement therapy

Wiad Lek. 2024;77(11):2359-2363. doi: 10.36740/WLek/197124 DOI

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is a malignant disorder characterized by increased production and accumulation of monoclonal mature B-lymphocytes. It is the most common leukemia among adults in Europe and North America, accounting for 25-30% of all leukemia cases. The annual incidence in these regions is 20,7 per 100 000 population [1]. CLL is primarily considered a disease of the elderly, with about 70% of patients being diagnosed between the ages of 50 and 70 [2]. However, this diagnosis can also be made at a younger age (e.g., between 15 and 39 years). Men are more frequently affected than women, even in younger age groups [3].

At the time of diagnosis, 70% to 80% of CLL patients are asymptomatic. However, "B" symptoms (fever, profuse night sweats, unintentional weight loss, marked fatigue), significant or progressive lymphadenopathy, and/or hepatosplenomegaly may be present. A complete blood count typically shows at least 5×10^9/L monoclonal $CD5^+$ B-cells. Anemia and/or thrombocytopenia may also be observed [4].

CLL is characterized by immune dysfunction, leading to increased susceptibility to infections and higher morbidity rates [5]. The cause of this is a complex multi-component immune dysregulation. First, up to 85% of such patients have hypogammaglobulinemia – deficiencies in immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM) – which is associated with defective T-cell functioning and non-clonal $CD5^-$ B-cells [6]. Second, there is T-cell dysfunction, characterized by increased expression of inhibitory receptors PD-1, CD160, and CD244 on $CD4^+$ and $CD8^+$ cells, indicating their exhaustion [7]. Third, B-cell dysfunction is also present, as regulatory B-cells (Breg) acquire inhibitory ligands (PD-1, PD-L1), which suppress their anti-tumor functions [8]. Additionally, CLL cells and regulatory T-cells (Treg) produce pro-inflammatory cytokines IL-10 and TGF-b, creating an immunosuppressive environment [7].

As a result, severe infectious complications develop in nearly 80% of patients with CLL. To reduce the incidence and severity of infection-related complications, immunization against common pathogens is recommended. However, due to the immunodeficient status of such patients, issues arise in achieving an adequate humoral and cellular immune response to vaccination [5].

A meta-analysis of 64 studies involving 8,546 adults with hematologic malignancies showed a serological response among patients with CLL of 44% and 16% in patients with prior treatment with anti-CD20 monoclonal antibodies [9].

Thus, patients with CLL represent a vulnerable population for the COVID-19 virus, and the protective effect of vaccination against COVID-19 is less effective compared to individuals without CLL [10].

This article presents a clinical case of COVID-19 in a fully vaccinated patient with CLL who experienced a severe and prolonged course of the disease.

CASE REPORT

Patient K., 73 years old, was diagnosed with B-cell CLL, stage A (I) according to Rai-Binnet, in 2013, with a stable course. Given the indolent nature of the disease, the absence of significant lymphadenopathy, splenomegaly, and normal levels of hemoglobin and platelets, specific cytostatic therapy was not administered. A "watch and wait" strategy was chosen for managing the patient.

The patient also had comorbidities: coronary heart disease (CHD) with stable angina, functional class II; the history of myocardial infarction (September 2004); heart failure stage B; and degree 2 hypertension. He was regularly taking perindopril, bisoprolol, and acetylsalicylic acid.

As of July 2021, he was fully vaccinated (received 2 doses) with the mRNA vaccine against SARS-CoV-2.

On October 6, 2021, the patient consulted a physician with complaints of a temperature increase to 38°C, dry cough, general weakness, sweating, and shortness of breath, which had been troubling him for the past three days. He had been self-treating at home with vitamins and paracetamol. On October 5, 2021, he had a PCR test for SARS-CoV-2, which returned positive.

Upon examination, the patient's condition was of moderate severity. Breath sounds were harsh, and no wheezing was noted. The mucous membranes of the oral cavity were hyperemic. Oxygen saturation was 95%. Respiratory rate was 17 breaths per minute. Temperature was 37,7°C. Blood pressure was 115/70 mmHg. Heart rate was 81 beats per minute. The complete blood count on October 6, 2021, showed: white blood cell (WBC) – 16,6 x 10^9/L; red blood cells (RBC) – 4,54 x 10^{12}/L; hemoglobin (HGB) – 138 g/L; hematocrit (HT) – 41%; mean corpuscular volume (MCV) – 92,3 fl; mean corpuscular hemoglobin (MCH) – 30 pg/cell; erythrocyte sedimentation rate (ESR) – 28 mm/hr; platelets (PLT) – 210 x 10^9/L; lymphocytes (LYM) – 65% (absolute lymphocyte count 10,92 × 10^9/L); band neutrophils – 0%; segmented neutrophils – 30%; eosinophils – 0%; basophils – 1%; monocytes – 4%. A chest CT scan revealed bilateral polysegmental viral lung involvement, with 10% involvement on the left side and 16% on the right. The diagnosis of COVID-19 was confirmed, classified as moderate severity. The patient was prescribed azithromycin, paracetamol, warm fluids, and regular monitoring of temperature and saturation.

The patient was treated at home and reported improvement in his condition. His temperature gradually decreased to 37,0 – 37,2°C, and oxygen saturation remained stable at 95-96%.

On October 11, 2021, the patient experienced a temperature increase to 38,9°C, with worsening cough and shortness of breath, and oxygen saturation decreased to 90%. He was hospitalized on the 8th day of illness. A repeat PCR test for SARS-CoV-2 was conducted, which returned positive. A follow-up chest CT scan showed bilateral polysegmental lung involvement of viral origin, with 45% involvement in the right lung and 35% in the left. Oxygen saturation was at 90%.

Upon admission to the hospital, the complete blood count showed: WBC – 15,31 x 10^9/L; RBC – 3,75 x 10^{12}/L; HGB – 116 g/L; HT – 34,4%; PLT – 180 x 10^9/L; ESR – 34 mm/hr; LYM – 45,3%; neutrophils – 53,1%; monocytes – 1,6%; eosinophils – 0%; basophils – 0%.

C-reactive protein (CRP) was 89,35 mg/L. Ferritin - 621 ng/ml. Interleukin-6 (IL-6) – 68,7 pg/ml (normal up to 7 pg/ml). D-dimer – 2,89 mcg/ml. Procalcitonin - 0,6 ng/ml.

The patient was prescribed cefixime, methylprednisolone 32 mg per day, and enoxaparin. Due to the positive PCR test result for SARS-CoV-2, the antiviral medication remdesivir was initiated at a dose of 200 mg on the first day, followed by 100 mg daily for 5 days.

On October 15, 2021, the patient experienced a repeat temperature increase to 38,6°C, with complaints of severe shortness of breath at rest, and oxygen saturation dropped to 82%. A follow-up chest CT scan showed negative dynamics, with an increase in the area of lung involvement to 60% (Fig. 1). The patient was transferred to CPAP (Continuous Positive Airway Pressure), and saturation improved to 96%. Respiratory exercises and patient mobilization were prescribed. Cefixime was replaced with meropenem, and a pulse therapy of methylprednisolone was initiated at 250 mg per day for 3 days, followed by a gradual reduction in dose to 48 mg per day. Enoxaparin was continued.

A blood test for antibodies to the spike (S) protein of SARS-CoV-2 showed an IgG level of 3 BAU/ml (normal is above 25 BAU/ml). A procalcitonin test revealed a level of 1,4 ng/ml (normal is below 0,5 ng/ml), and the serum immunoglobulin G (IgG) level was 2 g/L (normal is 6,5-16 g/L).

Due to the low IgG level, human immunoglobulin was administered at a dose of 40 g (0,4 g/kg/day) for 4 days. The IgG level was 12 g/L 24 hours after administration and 8.6 g/L one week later.

The patient's condition gradually began to improve. On November 5, 2021, he was discharged from the hospital under the supervision of a pulmonologist, hematologist, and district therapist, with rehabilitation therapy conducted in an outpatient setting. The duration

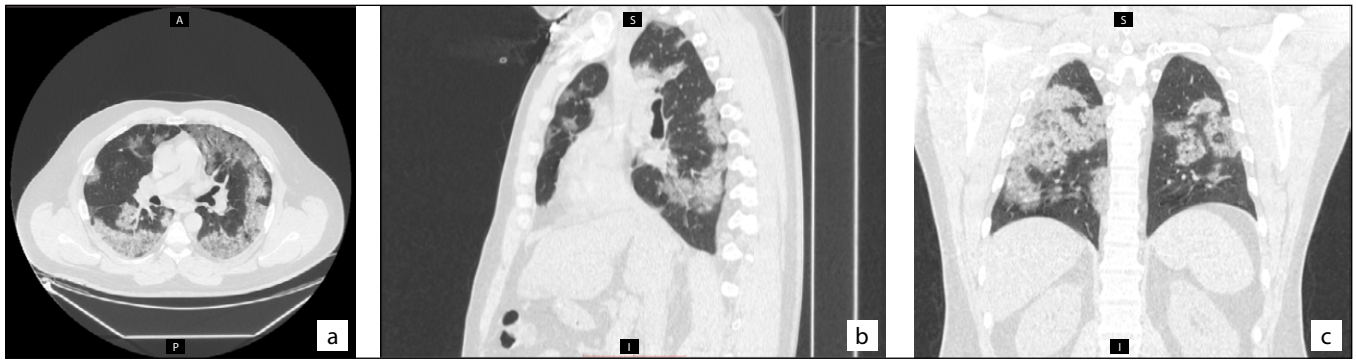

Fig. 1. Lung CT scan of the patient K. (day 9 of illness), demonstrating the signs of viral bilateral pneumonia, with involvement of 60% of the lung parenchyma. (a) axial plane; (b) sagittal plane; (c) coronal plane. A – anterior; P – posterior; S – superior; I – inferior.

of hospitalization was 25 days. After discharge, oxygen saturation remained at 93-95% while breathing oxygen.

In December 2021, the patient continued to complain of shortness of breath during significant physical exertion, with saturation levels at 93-97%. A chest CT scan revealed diffuse polygonal-shaped consolidations in the lung parenchyma, with ground-glass opacities and elements of coalescent fibrosis, with multiple nodules measuring up to 15,3 mm in maximum size.

Considering the signs of lung fibrosis, the pulmonologist prescribed nintedanib at a dose of 150 mg twice a day. The patient continues to be monitored by the hematologist. After experiencing an extremely severe course of COVID-19, there has been no progression of CLL.

DISCUSSION

The reduced adequate antibody production after vaccination is characteristic of patients with CLL. One study demonstrated that among patients with CLL vaccinated with the BNT162b2 mRNA COVID-19 vaccine, antibody response to the vaccine was present in only 39,5% of patients, compared to 100% in control subjects of the same age and gender. Furthermore, CLL patients with a serological response to vaccination also exhibited lower antibody titers than their healthy counterparts. Patients undergoing active therapy for CLL (including Bruton's tyrosine kinase inhibitors (BTKis), B-cell lymphoma-2 (BCL-2) antagonists, or anti-CD20 monoclonal antibodies) showed an even lower response to the COVID-19 vaccination [9].

It is known that COVID-19 can present more severe forms and higher mortality rates in comorbid patients [11, 12]. Consequently, patients with concurrent oncological and hematological diseases face greater risks of prolonged treatment and recovery periods. This is particularly true for patients with conditions associated with secondary immunodeficiency, of which CLL is a classic example. One potential approach to correcting treatment in these patients is the use of immunoglobulins.

The rationale for the mandatory use of human immunoglobulins in treating severe COVID-19 remains controversial. One study that analyzed COVID-19 cases in 59 patients with moderate to severe illness found that their use expedited recovery, especially in patients requiring mechanical ventilation. The study noted that immunoglobulin therapy should be initiated as early as possible to achieve faster recovery [13].

Another study analyzed data from 84 patients with severe COVID-19: 52 were administered immunoglobulins at a dose of 400 mg/kg per day for three days, while 32 patients were in the control group. No significant differences were found in hospitalization duration, length of stay in the intensive care unit, or duration of mechanical ventilation between the two groups [14].

However, cases of COVID-19 in patients with CLL should be viewed from a different perspective. Hypogammaglobulinemia is a significant factor contributing to the immunodeficiency status in these patients and has a substantial impact on the risk of secondary infections, their severity, and duration. For patients with CLL and secondary immunodeficiency, it is recommended to conduct immunoglobulin replacement therapy (IgRT) when the IgG level is below 4 g/L [15].

Regarding the appropriateness of using immunoglobulins in the treatment of secondary infections in patients with CLL, several studies have demonstrated the high efficacy of IgRT in such patients. One study analyzed data from 1086 patients with hematological malignancies across 86 centers, of which 490 had CLL and 596 had multiple myeloma (MM). Among these patients, 34,8% developed IgG deficiency during treatment (35,5% in CLL and 34,2% in MM). IgRT was administered to 23,5% of patients with CLL and 14,4% of patients with MM. The study confirmed that the use of IgRT correlated with a lower risk of infection in patients with CLL and MM who had secondary immunodeficiency [15].

In the case of patient K., the confirmed secondary hypogammaglobulinemia developed against the background of the underlying disease (CLL), which most likely

led to severe viral and subsequently bacterial lung infections. Even with appropriately selected antibiotic therapy, the condition continued to progressively worsen.

The implementation of IgRT contributed to the onset of a positive dynamic in the disease course.

CONCLUSIONS

CLL is well-known for increasing susceptibility to various infections, with COVID-19 being no exception. A significant contributing factor to these infections is the secondary immunodeficiency, particularly hypogammaglobulinemia. To reduce the incidence of infectious diseases, preventive measures such as vaccination are rational; however, it is important to note that some patients with hematological malignancies may not produce sufficient antibody levels for adequate protection against pathogens. This is especially true for patients undergoing active therapy with BTKis, BCL-2 antagonists, or anti-CD20 monoclonal antibodies. The use of immunoglobulins may alleviate the course of COVID-19 in oncological patients with confirmed hypogammaglobulinemia, shorten their hospital stay, and reduce the time needed for post-COVID rehabilitation.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Oksana M. Kostiukevych
State Institution of Science
«Center of innovative healthcare technologies»
State Administrative Department
5 Verkhnia St., 01014 Kyiv, Ukraine
e-mail: oksakost@gmail.com

ORCID AND CONTRIBUTIONSHIP
Oksana M. Kostiukevych: 0000-0003-3499-080X A D E F
Oleksandr V. Naumenko: 0009-0007-7113-7361 A D E
Lesia K. Benkovska: 0000-0003-3436-3128 A B D
Mariia A. Trypilka: 0009-0005-8504-0240 B D

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

RECEIVED: 07.07.2024
ACCEPTED: 29.10.2024