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ORIGINAL ARTICLE

Postpartum infections and antimicrobial resistance of responsible pathogens in Ukraine: results a multicenter study (2020-2022)

Aidyn G. Salmanov[1,2], Andrii O. Shchedrov[3], Andrii P. Prishchepa[4], Volodymyr Artyomenko[5], Svitlana M. Korniyenko[5], Victor O. Rud[6], Anna V. Kolesnik[7]

[1]SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE
[2]INSTITUTE OF PEDIATRICS, OBSTETRICS AND GYNECOLOGY OF THE NATIONAL ACADEMY OF MEDICAL SCIENCES OF UKRAINE, KYIV, UKRAINE
[3]SCHOOL OF MEDICINE OF V. N. KARAZIN KHARKIV NATIONAL UNIVERSITY, KHARKIV, UKRAINE
[4]KYIV CITY MATERNITY HOSPITAL №3, KYIV, UKRAINE
[5]ODESA NATIONAL MEDICAL UNIVERSITY, ODESA, UKRAINE
[6]NATIONAL PIROGOV MEMORIAL MEDICAL UNIVERSITY, VINNYTSIA, UKRAINE
[7]LUTSK ACADEMY OF RECREATION TECHNOLOGIES AND LAW, LUTSK, UKRAINE

ABSTRACT

Aim: To determine the current prevalence of postpartum infections and antimicrobial resistance and antimicrobial resistance of responsible pathogens in Ukraine.

Materials and Methods: Multicenter prospective cohort study was conducted from January 2020 to December 2022 in fifteen hospitals from twelve regions of Ukraine. Definitions of healthcare- associated postpartum infection were adapted from the Centers for Disease Control and Prevention's National Healthcare Safety Network. Antibiotic susceptibility was done by the disc diffusion test as recommended by EUCAST.

Results: Among 21,968 women, 6,175 (28.1%) postpartum infections were observed. Of all postpartum infection cases, 83.1% were detected after hospital discharge. The postpartum infection rates were 17.3% after cesarean section and 10.8% after vaginal delivery. The most common postpartum infection types were endometritis (17.3%), followed by urinary tract Infection (3.5%), mastitis (3.4%), surgical site infection (excluding endometritis) (2.4%), and episiotomy site infection (1.5%). The predominant postpartum infection pathogens in Ukraine were: *Escherichia coli* (10.4%), *Enterococcus* spp. (9.6%), *Staphylococcus aureus* (6.7%), *Pseudomonas aeruginosa* (5.8%), *Enterobacter* spp. (5.8%). In our study pathogens of postpartum infection had differently levels of resistance to antibiotics.

Conclusions: Our results indicate that postpartum infections requiring medical attention are common in Ukraine and that most postpartum infections occur after hospital discharge, so that use of routine inpatient surveillance methods alone will lead to underestimation of postpartum infection rates. Optimizing the antibiotic prophylaxis may reduce the burden of postpartum infection, but prevention is the key element.

KEY WORDS: Cesarean section, vaginal delivery, postpartum infection, responsible pathogens, antibiotic prophylaxis, antimicrobial resistance, Ukraine

INTRODUCTION

Maternal morbidity and mortality are global socio-economic and healthcare burdens, and postpartum infections account for a significant, and often preventable, portion of that burden. Postpartum infections, primarily caused by bacterial pathogens, leading to poor reproductive perfor mance. Approximately five million cases of pregnancy-related infection occur every year globally, and approximately 75,000 results in death [1]. Postpartum infections are a major cause of prolonged hospital stay and comprise a large burden to our health care system.

The postpartum period is traditionally defined as the six weeks following delivery, and infections are relatively common, affecting an estimated 5 to 7% of women during this time. Postpartum infection is an important preventable cause of maternal morbidity and mortality, with pregnancy-related sepsis accounting for approximately 11% of maternal deaths globally [2]. Recent 2017 Global Burden of Disease data estimate 12.1 million incident cases of maternal sepsis and other maternal infections, including mastitis [3]. Infection also contributes significantly to deaths from other causes and leads to serious consequences, including chronic

pelvic inflammatory disease, ectopic pregnancy, and infertility [4, 5]. One study attributed costs of an additional $3700 for wound infection and an additional $4000 for endometritis (in 2008 US dollars, corresponding to $4200 and $4500 today, respectively) [6].

According to literature, the risk factors include poor intrapartum hygiene, low socioeconomic status, primiparity, prolonged rupture of membranes, prolonged labor, and having vaginal exams intrapartum. Most study on postpartum infections has occurred in high resource countries. However, in low-resource settings, risk factors for postpartum infection may differ from high-resource settings due to patient, environmental and healthcare system factors. In addition, most published studies do not include microbiological confirmation of infection or infectious outcomes.

Improved understanding of postpartum maternal infection is key to achieving the sustainable development goals and executing the strategies toward ending preventable maternal and neonatal mortality. These infections are also the most common cause of death following spontaneous or induced abortions. The medical burden of these infections is compounded by the alarmingly rapid increase in bacterial resistance to commonly used antibiotics. Currently, prevalence of postpartum infection in women and the bacteria responsible for these infections have not been adequately studied, nor has the antibiotic susceptibility of the causative bacteria been frequently tested in Ukraine.

AIM

The aim this study to determine the current prevalence of postpartum infections and antimicrobial resistance and antimicrobial resistance of responsible pathogens in Ukraine.

MATERIALS AND METHODS

STUDY DESIGN, SETTING AND POPULATION
We performed a multi-centre prospective cohort study was based on surveillance data for postpartum infection. The study population consisted of all women who had a vaginal delivery or cesarean section from January 1, 2020, to December 31, 2022, and who received postpartum care at fifteen hospitals from twelve regions of Ukraine, which are similar in terms of medical equipment, personnel, and laboratory facilities.

DEFINITION
Postpartum period in this study defined as the six

weeks following delivery. An HAPI was defined as an infection arising >48 h after delivery and not present or incubating on admission. Puerperal sepsis is defined as an infection of the genital tract occurring at any time between rupture of membrane or labour and 42 days postpartum in which two or more of the following are present; pelvic pain, fever (temperature ≥38.5°C on any occasion), abnormal vaginal discharge, delay in the rate of uterine involution. The criteria for specific HAI site were adapted from the Centers for Disease Control and Prevention's (CDC) and National Healthcare Safety Network's (NHSN) case definitions [7]. An incident postpartum infection was defined by microbiologically confirmed CDC/NHSN HAI epidemiological case definitions. In this study antimicrobial treatment by a physician was not considered to be sufficient for diagnosis of a postpartum infection because of widespread use of empiric antimicrobial therapy in Ukrainian hospitals. The criteria for determining postpartum infection were the presence of at least 2 clinical symptoms, i.e., abnormal vaginal discharge, pyrexia (oral temperature measurement more than 38.5°C), abnormal smell/foul odour discharge, delay in uterine involution (less than 2 cm per day during the first 8 days after delivery), and pelvic pain, assessed by the trained clinicians.

DATA COLLECTION
In this study endometritis, urinary tract Infection (UTI), mastitis, surgical site infection (excluding endometritis) (SSI), and episiotomy site infection. Clinical data were collected on 21,968 women who delivered at local hospitals. Participants providing written informed consent were followed by research nurses who measured vital signs including heart rate, blood pressure, respiratory rate and temperature approximately every 6 h starting immediately after delivery. All clinical signs and relevant history regarding postpartum problems were also documented. In hospital all women were informed of the symptoms of postpartum infection and advised to notify the observer right away after seeing the first infection symptom for a month. The discharged patients were advised for ongoing follow-up care for a month after delivery in the outpatient department. Information regarding the postpartum period following discharge was obtained from the outpatient records and from records documenting postpartum follow-up by referring gynecologists. Questionnaires and laboratory results were entered into a study database. Data were analyzed to document and classify all type of postpartum infection rates. Antibiotic prophylaxis was identified as a systemic or oral antibiotic was prescribed for

Table 1. Distribution of 6,175 postpartum infections in Ukraine, 2020-2022

Type of delivery	Mastitis n (%)	UTI n (%)	SSI (excluding endometritis) n (%)	Endometritis n (%)	Episiotomy site infection n (%)
Cesarean section	286 (1,3)	329 (1,5)	528 (2,4)	2659 (12,1)	0
Vaginal delivery	461 (2,1)	440 (2,0)	0	1142 (5,2)	330 (1,5)
Total	747 (3,4)	769 (3,5)	528 (2,4)	3801 (17,3)	330 (1,5)

Table 2. Demographics and obstetric history of study participants in Ukraine, 2020-2022

Characteristic	Postpartum infection		P-value
	No n (%)	Yes n (%)	
All women	15,793 (71.9)	6,175 (28.1)	<0.001
Age (years)			<0.001
≤20	1,896 (12.1)	1,482 (23.8)	
21–34	12,475 (79)	4,507 (73.1)	
≥35	1,422 (8.9)	186 (3.1)	
Total number of pregnancies			<0.001
1	5,697 (36.1)	3,402 (55.1)	
2–4	8,067 (51.1)	2,186 (35.4)	
≥5	2,029 (12.8)	587 (9.5)	
Gestational age at delivery			0.45
Preterm (< 37 weeks)	1,617 (10.2)	772 (12.5)	
Term (37–42 weeks)	12,861 (81.4)	4,839 (78.4)	
Post-term (> 42 weeks)	1,315 (8.4)	464 (9.1)	
Peri-Cesarean antibiotic prophylaxis received	15,647 (99.1)	6,144 (99.5)	0.85

women with normal vaginal delivery for an indication to prevent postpartum infections and documented in hospital medical records. Antibiotic prescribing for women after delivery at discharge was also identified as antibiotic prophylaxis.

MICROBIOLOGICAL METHODS

Pathogen strains were identified by an automated microbial identification system. Bacterial isolation, identification, and antimicrobial susceptibility test (AST) were performed. Antibiotic breakpoints were interpreted according to the European Committee on AST (EUCAST) guidelines, version 9.0 [8]. *E. coli* (ATCC-25922), *S. aureus* (ATCC-25923), and *P. aeruginosa* (ATCC 27853) were included as reference strains to assure the quality of antibiotic discs. The quality of the culture media, staining and biochemical test reagents, and antibiotic disc performance was assured by including international standard control strains, such as *E. coli* (ATCC 25922) for Gram-negative bacteria, *S. aureus* (ATCC 25923) for Gram-positive bacteria, throughout all assays.

ETHICS

The study received ethical approval from the Institutional Research Ethics Committee of Shupyk National Healthcare University of Ukraine (Kyiv, Ukraine). All procedures and methods adhered to the relevant guidelines and regulations. Written informed consent was also obtained from the participating women in the study. All data from participants in this study were anonymized prior to analysis.

STATISTICAL ANALYSIS

Infection rates for the entire study population of postpartum women were extrapolated by standard methods from the estimated infection rates for the sample of individual medical records reviewed. The data was entered into a spreadsheet in Microsoft Excel and coded for statistical analysis. All statistical analyses were conducted using Stata 14.2 statistical software (Stata Corp LLC, 4905 Lakeway Drive, College Station, Texas). The prevalence of postpartum infection was calculated as the number of all events or cases of postpartum infection during the study period divided by the total number of women in the population at risk at the beginning

Table 3. Types of microorganisms isolated from postpartum infections in Ukraine, 2020-2022

Microorganisms	All infections	
	n	%
Gram-positive cocci	4,316	23,4
Staphylococcus aureus	1,230	6,7
Enterococcus spp.	1,780	9,6
Coagulase-negative staphylococci	902	4,9
Streptococcus spp.	292	1,6
Other *gram-positive cocci*	112	0.6
Gram-negative bacilli	13,846	75,0
Enterobacteriales	5,019	27,2
Citrobacter spp.	267	1,4
Enterobacter spp.	1,075	5,8
Klebsiella spp.	470	2,5
Proteus spp.	590	3,2
Escherichia coli	1,917	10,4
Serratia spp.	405	2,2
Other *Enterobacteriales*	295	1,6
Non-fermenting gram-negative bacteria	1,904	10,3
Acinetobacter baumannii	595	3,2
Pseudomonas aeruginosa	1,072	5,8
Stenotrophomonas maltophilia	177	1,0
Other *Pseudomonadaceae*	60	0,3
Fungi	295	1,6
Candida spp.	295	1,6
Total no. of isolates	18,457	100

of the study. Descriptive statistics were used to express the frequency and proportion of postpartum infections and bacteria isolated from infected women, as well as the proportion of isolated bacteria that developed resistance to tested antimicrobials. The chi-square ($\chi 2$) test was used to determine the association of postpartum infection with the considered risk factors. Associations were deemed statistically significant if the calculated p-value was below 0.05.

RESULTS

PREVALENCE AND INCIDENCE OF POSTPARTUM INFECTIONS

The study population consisted of 21,968 women who underwent 17,768 vaginal deliveries and 4,200 cesarean sections. During study period 6,175 cases of postpartum infections were observed. Of all postpartum infection cases, 83.1% (5,132/6,175) were detected after hospital discharge. For these post-discharge infections, 35% of patients did not return to the hospital where they delivered for evaluation or treatment. The prevalence of postpartum infection in Ukraine was 28.1% (95% confidence interval [CI] 27.8-28.4). The postpartum infection rates were 17.3% after cesarean section (95% CI 16.7-17.9%) and 10.8% (95% CI 10,6-11.0%) after vaginal delivery. The most common postpartum infection types were Endometritis, followed by Urinary Tract Infection (UTI), Mastitis, Surgical Site Infection (SSI) (excluding endometritis), and Episiotomy site infection. The most frequent specific types of postpartum infection are reported in Table 1.

Among women undergoing cesarean section, the sitespecific infection rates (number of infections/100 deliveries) were mastitis 1.3% (95% CI 1.1-1.5%), urinary tract infection 1.5% (95% CI 1.3- 1.7%), surgical site infection (excluding endometritis) 2.4% (95% CI 2.2-2.6%), and endometritis 12.1% (95% CI 11.6- 12.6%). Following vaginal delivery, the infection rates were mastitis 2.1% (95% CI 2.0-2.2%), urinary tract infection 2.0% (95% CI 1.9- 2.1%), episiotomy site infection 1.5% (95% CI 1.4-1.6%), and endometritis 5.2% (95% CI 5.0-5.4%) (Table 1). The demographics and obstetric history of study participants in Ukraine are shown in Table 2.

RESPONSIBLE PATHOGENS AND ANTIMICROBIAL RESISTANCE

A total 18,457 microorganisms isolated from 6,175 postpartum infections in Ukraine, 2020-2022. By type of pathogen, 4316 strains were Gram-positive bacteria (23.4%), 13846 were Gram-negative bacteria (75%) and 295 were fungi (1.6%). The predominant postpartum infection pathogens in Ukraine were: *Escherichia coli* (10.4%), *Enterococcus* spp. (9.6%), *Staphylococcus aureus* (6.7%), *Pseudomonas aeruginosa* (5.8%), *Enterobacter* spp. (5.8%). Distribution of pathogens isolated from women with postpartum infection are presented in Table 3.

Antimicrobial susceptibility testing data were available for all pathogens causing postpartum infection. Meticillin resistance was found in 11.3% of *S. aureus* (MRSA), and vancomycin resistance was found in 4.8% of enterococci. Antimicrobial resistance to third-generation cephalosporins was detected in 12.5% of all Enterobacterales, and was most common among *K. pneumoniae* (27.4%) and *E. coli* (17.3%). Carbapenem resistance was found in 11.7% of Enterobacterales. Antimicrobial resistance to carbapenems was detected in 42.5% of all non-fermentative, Gram-negative bacteria, and was most common among *A. baumannii* (46.7%), and *P. aeruginosa* (37.4%).

DISCUSSION

The results presented in this study are based on multi-centre prospective surveillance data for prevalence of postpartum infection and antimicrobial resistance of responsible pathogens in Ukraine. This study expands upon the previous reports [9-14] and is the first study to publish frequent postpartum infections and/or characterization of the phenotypic mechanisms of responsible pathogens in Ukraine.

The epidemiology of postpartum infections has not been well characterized. In part this is because of the limitations of surveillance systems, which usually monitor infections that are recognized during hospitalization [15]. Hulton et al. used physician questionnaires for postdischarge surveillance of patients undergoing cesarean section. With only inpatient surveillance, 59% of postpartum infections they ultimately detected would not have been identified. The overall infection rate after postdischarge surveillance was implemented was fourfold higher than the previous rate (6.3% vs. 1.6%) [15]. Our study showed that of all postpartum infection cases, 83.1% were detected after hospital discharge. Studying rates of postpartum infections and their effects is difficult, as most of these infections occur following maternal hospital discharge, and decreasing hospital stay following childbirth further inhibits the detection of postpartum complications, including infection [17]. Therefore, several methods for postdischarge surveillance of postpartum infections have been evaluated. In this study, we used the inpatient and outpatient data to identify postpartum infections and describe the epidemiology of these infections [15]. All of these postpartum infections have been reported to predominantly present after discharge from hospital [18, 19]. The prevalence of infections has therefore been reported to be at various levels, depending on health care systems, the availability of health care, mode of surveillance and different definitions. Surveillance of the prevalence of specific infection after childbirth in Ukraine is difficult, since the obstetric care providers do not routinely follow up regarding infections.

According to the literature, during the puerperium, women have an increased risk of infection. The most common infections associated with childbirth are endometritis, infections in perineal or cesarean wounds, UTI and mastitis [20]. A postpartum infection was related to whether pregnancy was full-term, differences in the mode of delivery, and the labor process.

Infections occurring during pregnancy, childbirth and the puerperium are associated with considerable maternal and perinatal morbidity and mortality. However, global data on the incidence of maternal infection morbidity are scarce. The World Health Organization (WHO) estimates the global incidence of puerperal infections at 4.4% among live births, representing over 5.7 million cases a year [21]. Important variations exist between regions, with higher incidence in low- and middle-income countries (up to 7%) compared to high-income countries where the estimated incidence is lower (1% to 2%) [22]. Surgical site infections (SSI) are relatively common following birth, complicating 2-7% of cesarean deliveries [6]. Postpartum infections are more common in women who underwent cesarean section as opposed to vaginal delivery, and the risk is further increased for women who underwent labor before the cesarean section [12, 23]. Our study identified a high prevalence of postpartum infection (28.1%). The most common postpartum infections in Ukraine include endometritis, urinary tract infections, surgical site infections (excluding endometritis), and wound infections (episiotomy site infection). The postpartum infection rates were 17.3% after cesarean section and 10.8% after vaginal delivery. The most common postpartum infection types were endometritis (17.3%), followed by urinary tract Infection (3.5%), mastitis (3.4%), surgical site infection (excluding endometritis) (2.4%), and episiotomy site infection (1.5%).

According to the literature, bacterial infections around the time of childbirth are generally polymicrobial, including aerobic and anaerobic bacteria, and reflect vaginal colonization [9-14]. In our study by type of pathogen, 23.4% strains were Gram-positive bacteria, 75% were Gram-negative bacteria and 1.6% were fungi The predominant postpartum infection pathogens in Ukraine were: *E. coli, Enterococcus* spp., *S. aureus, P. aeruginosa,* and *Enterobacter* spp.

Knowledge of the microbiological profiles of different postpartum infections, local resistance patterns, and the severity of the patient's illness should guide antibiotic choices. As a general rule, antibiotic regimens, particularly in very sick patients, should have a broad-spectrum of antimicrobial coverage at the beginning and should be narrowed as more clinical evidence from cultures or pathology specimens becomes available. The medical burden of these infections is compounded by the alarmingly rapid increase in bacterial resistance to commonly used antibiotics [17]. In present study pathogens of postpartum infection had differently levels of resistance to antibiotics.

Infectious morbidities contribute to considerable maternal and perinatal morbidity and mortality, including women at no apparent increased risk of infection. To reduce the incidence of infections, antibiotics are often administered to women after uncomplicated childbirth, particularly in settings where women are at higher risk of puerperal infectious morbidities [24].

In Ukraine antibiotic prophylaxis is characterised by the use of broad-spectrum antibiotics (e.g. ampicillin, cephalosporin, a combination of antibiotics) effective against the micro-organism most likely to cause infections, to be given before, during or immediately after the procedure and for a short period of time (single dose or for less than 24 hours), and in the absence of any sign of infection. In this study the incidence of postpartum infection was similar in both antibiotic prophylaxis and unprophylaxis women. The results of present study limits conclusions on the benefits of antibiotic intake.

Antibiotic prophylaxis given after normal birth has the potential to further decrease infection risk, particularly in settings where appropriate hygiene, infection-control measures and sanitation during labour, childbirth and the postpartum period are not ensured, or where early detection of puerperal infections and laboratory investigations is limited [24]. The goal of antibiotic prophylaxis is to prevent infection by reaching therapeutic tissue levels at the time microbial contamination is most likely to occur.

Antibiotic prophylaxis after normal vaginal birth could help to prevent maternal infections by ensuring adequate antimicrobial serum and tissue concentrations during the postpartum period. To be effective, such antibiotics have to be active against the predominant organisms that cause postpartum infections and administered for the shortest period to minimise side effects and the impact of its routine use on emerging antimicrobial resistance. Indeed, exposure to antibiotics in the postpartum might cause adverse effects to the mother or the breast-fed neonate, including disruption of the normal flora, increased risk of resistant bacterial infections, allergic reactions as well as increased healthcare costs [25]. There are also concerns about rising resistance to antibiotics at the facility and community level [26]. This might further complicate the choice of suitable prophylac-

tic antibiotics, as generally broad-spectrum antibiotics are required to cover common pathogens.

There are increasing public health concerns about emerging antibiotic resistance following misuse or overuse of antibiotics [26]. This is also applicable to the obstetric populations and the possibility of inadequate response to treatment of puerperal infections due to early exposure to undereffective antibiotic prophylactic regimens. Given the large proportion of women experiencing uncomplicated vaginal birth, a universal application of antibiotics to such women has the potential to lead to substantial clinical benefits in terms of reducing infection risk, but could also lead to direct harm for the woman and indirect harm to the general public with increasing resistance to antibiotics. The increasing trend observed in facility-based births may increase the risk of hospital-acquired infections after normal vaginal birth if not accompanied by improvements in the quality of hygiene and infection-control measures [27]. However, the evidence is unclear about the added effect of antibiotic prophylaxis on the prevention of postpartum infections after an uncomplicated vaginal birth [24].

Our study show that the scarcity of data and inherent limitations observed in studies limit interpretation of the evidence for routine use of antibiotics in cases of normal vaginal births. The available evidence is insufficient to support the use of routine antibiotic prophylaxis in women who had normal vaginal births. More evidence is needed to inform practice. In the interim, a balance among health provider experience, settings, participant characteristics and eventual cost in cases of uncomplicated births, including considerations of the contribution of indiscriminate use of antibiotics to raising antimicrobial resistance, needs to be considered when making routine prescriptions of antibiotic prophylaxis. Lack of evidence on the effect of routine antibiotic prescription for prevention of other postpartum infections, antimicrobial resistance and maternal satisfaction with treatment calls for further research [24].

STRENGTHS AND LIMITATIONS

Strengths of our study include the prospective study design, large sample size, near-complete enrollment of eligible women seeking care at hospitals during the study period, and an in-depth clinical and microbiological evaluation of participants with suspected postpartum infection.

Limitations: Due to resource constraints, we were unable to perform clinical or microbiological testing of participants with chorioamnionitis in labour, puerperal sepsis, and thrombotic phlebitis and thus unable to determine the incidence of these infections. Prolonged rupture of membranes is a known risk factor for postpartum infection but was not directly measured in this study. We collected participant-reported duration of labor as one measurement of prolonged

labor but we did not measure duration of membrane rupture directly. We documented whether a woman was prescribed antibiotics on the same day as her cesarean section procedure, but we were unable to confirm whether these were given, nor determine the timing of the prescription relative to the procedure. Future research should address postpartum infections occurring after hospital discharge, incident in-hospital and post-discharge surgical site infection, and the impact of prophylactic antibiotics on incident infection and development of antimicrobial resistance.

CONCLUSIONS

Our results indicate that postpartum infections requiring medical attention are common in Ukraine and that most postpartum infections occur after hospital discharge, so that use of routine inpatient surveillance methods alone will lead to underestimation of postpartum infection rates. Use of information routinely collected by infection control personnel allows efficient identification of women who are very likely to have postpartum infections that are not detected by conventional surveillance. Information resulting from more complete surveillance could be used to identify settings with unusually high or low infection rates to identify practices associated with lower infection rates [15]. This information could then be used to focus, motivate, and assess the effectiveness of infection control practice changes aimed at improving infection rates in all settings.

The most of postpartum infections result from physiologic and iatrogenic trauma to the abdominal wall and reproductive, genital, and urinary tracts that occur during childbirth or abortion, which allows for the introduction of bacteria into these normally sterile environments [17]. The study found that a significant proportion of study population were affected by postpartum infections caused by bacteria developed resistance to several antimicrobials. Therefore, responsible use of antimicrobials is necessary to prevent the emergence of antimicrobial resistance, and further research is needed to understand the mechanisms of antimicrobial resistance. Optimizing the antibiotic prophylaxis may reduce the burden of postpartum infection, but prevention is the key element.

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CORRESPONDING AUTHOR
Aidyn G. Salmanov
Shupyk National Healthcare University of Ukraine,
9 Dorohozhytska St, 04112, Kyiv, Ukraine
e-mail: mozsago@gmail.com

ORCID AND CONTRIBUTIONSHIP
Aidyn G. Salmanov: 0000-0002-4673-1154 Ⓐ Ⓒ Ⓓ Ⓔ Ⓕ
Andrii O. Shchedrov: 0000-0002-1737-9171 Ⓑ Ⓒ Ⓓ Ⓕ
Andrii P. Prishchepa: 0009-0008-0246-581X Ⓑ Ⓒ Ⓓ Ⓕ
Volodymyr Artyomenko: 0000-0003-2490-375X Ⓑ Ⓒ Ⓓ Ⓕ
Svitlana M. Korniyenko: 0000-0003-3743-426X Ⓑ Ⓒ Ⓓ Ⓕ
Victor O. Rud: 0000-0002-0768-6477 Ⓑ Ⓒ Ⓓ Ⓕ
Anna V. Kolesnik: 0000-0001-5046-862X Ⓑ Ⓒ Ⓓ Ⓕ

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

ORIGINAL ARTICLE

Characteristics of brain lesions found using MRI imaging in patients with post-COVID with signs of cognitive decline

Artur V. Kurakh, Vladyslava I. Ahii, Ivan V. Chopey, Mykhailo M. Hechko, Kseniya I. Chubirko

UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

Aim: To describe and evaluate abnormalities of the brain in post-COVID patients with neurologic symptoms and cognitive deficits using MRI imaging of the brain.

Materials and Methods: We included 21 patients with a previous positive PCR testing for SARS-CoV-2 and one or more of the following symptoms: memory and cognitive decline, dizziness, anxiety, depression, chronic headaches. All patients had MRI imaging done at onset of symptoms, but after at least 1 year after positive testing for COVID-19 based on the patient's previous medical history.

Results: All of the patients complained of lack of concentration, forgetfulness, hard to process information. 15 patients suffered from confusion, 10 from anxiety. Of the 21 patients 14 had isolated chronic headaches, 3 had isolated dizziness, 4 patients had both symptoms upon inclusion. All patients underwent MRI imaging as a part of the diagnostic workup and had varying degrees of neurodegeneration.

Conclusions: Our data correlates with existing research and shows tendency for cognitive decline in post-COVID patients. This provides groundwork for further research to determine correlation between acceleration of neurodegeneration and post-COVID.

KEY WORDS: cognitive decline, brain fog, long-COVID, leukoaraiosis

INTRODUCTION

At the start of the COVID-19 pandemic most of the research was guided towards acute signs and symptoms, diagnostic methods of the disease in early stages and available treatment options, especially for patients with moderate to severe cases. Due to this fact, the acute phase of the COVID-19 infection was thoroughly mapped, even though the symptoms vary and include: fevers/chills, cough, shortness of breath, fatigue, muscle and body aches, new loss of taste/smell, sore throat, congestion, runny nose, nausea/vomiting and diarrhea. And it is noted that this may not be the full range of symptoms [1]. First instances of persisting symptoms after an acute disease started to arise in the beginning of summer of 2020, when medical providers started noticing that even after patients tested negative for Sars-Cov-2 during PCR testing, they still had one or several lingering COVID-19 symptoms. One of the first studies to evaluate the post-acute phase of COVID-19 (now known as long COVID) by Angelo Carfi et al. evaluated potential patients a mean of 60 days after onset of the first COVID-19 symptom and that had at least 2 negative PCR tests in 3 consecutive days before inclusion [2,3]. They included 143 patients and found that only 18 (12.6%) were fully free of any COVID-related

symptoms, 1-2 symptoms were observed by 32% of patients and 55% had 3 or more. The highest reported symptoms were fatigue, dyspnea, joint and chest pain. Another study by Hannah E. Davis et al. evaluated data of a higher range of surveyed patients with suspected and confirmed COVID-19. The surveys were distributed via support groups for long-COVID patients. A total of 6504 patients with confirmed or suspected COVID-19 were surveyed, which had long-COVID symptoms that lasted 28 days or more. For most respondents (>91%) the time of recovery from the acute disease was exceeded 35 weeks. After 6 months, the most prevalent symptoms were fatigue, malaise after exertion and cognitive disfunctions. These symptoms greatly impacted patients' quality of life and work level. Although this study had it`s limitations, it and many others made it clear, that COVID-19 may linger through the acute phase and greatly influence patients' quality of life [4].

While self-reported data was being gathered and evaluated, other research was conducted on physical changes in long-COVID patients. Clinicians reported physical and functional changes in the lungs though imaging and functional testing. Patients of different age groups had abnormalities in the lungs during imaging, abnormal pulmonary function, disturbances in heart
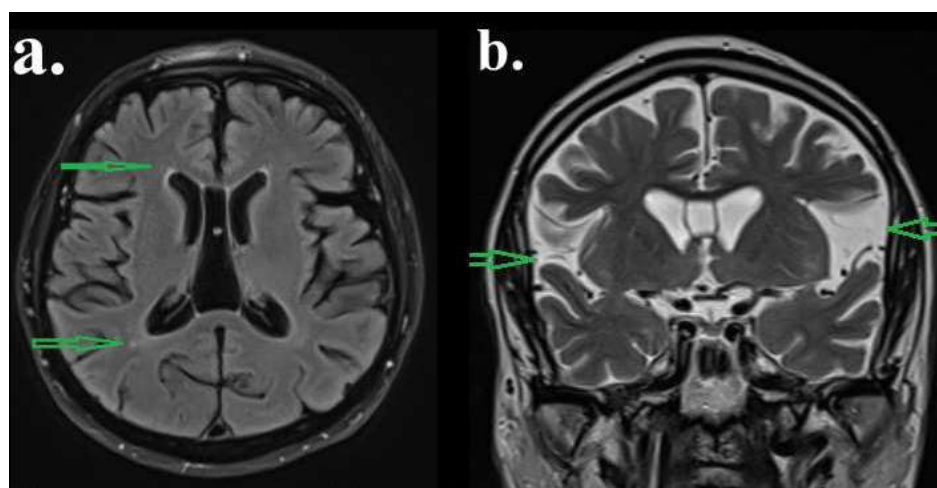
Fig. 1. Patient, 71 years old with clinical signs of neurodegeneration and COVID-19 infection 2 years prior. a. FLAIR sequence, periventricular areas of leukoaraiosis and gliosis focal points. b. T2WI sequence, atrophic changes of the brain, more prominent on the frontal and temporal parts, Sylvian fissure is widened.
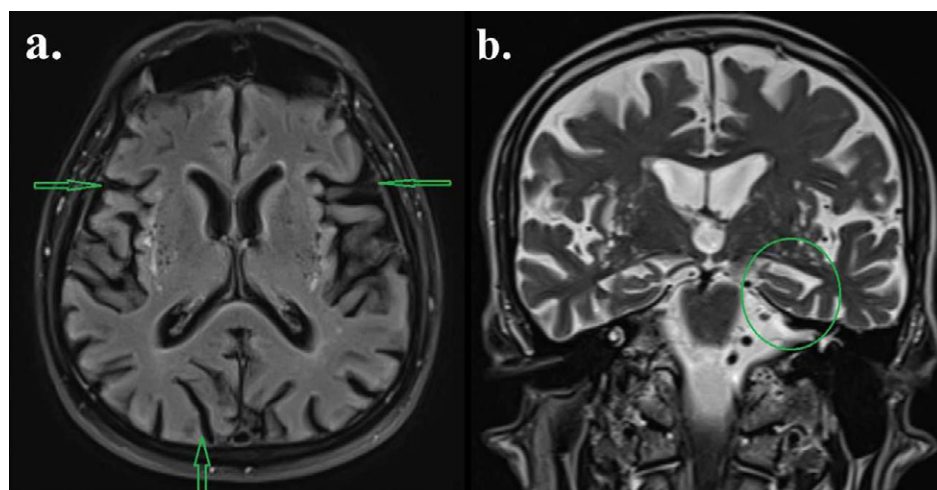
Fig. 2. Patient, 79 years old with clinical signs of neurodegeneration and COVID-19 infection 3 years prior. a. FLAIR sequence, widened subarachnoid space due to atrophic changes in the brain, focal gliotic changes of the white matter. b. T2WI sequence, widened lateral ventricles and atrophic changes of the hippocampus.

rhythm/palpitations. It should be noted that even in children, post-COVID resulted in functional limitations and decreased exercise capacity. Of 127,568 children and adolescents included in a meta-analysis by Catherine Campos et al. 20% of participants had decreased exercise capacity and 48% had functional limitations. Abnormalities in lung imaging were found in 10% of patients [5].

There is doubt that post-COVID is a continued manifestation of acute SARS-CoV-2 infections. In interesting syndrome, that post-COVID patients might experience is the so called "brain fog" – a lay term that describes mild memory loss inappropriate for patients' age, that manifests as forgetfulness, confusion, loss of concentration, difficulty to process information. It is associated with menopause and hormonal changes, sleep deprivation, usage of certain drugs and other causes [6-8]. And now, post-COVID started causing this symptom as well, some studies even suggesting that it may affect up to 46% of US post-COVID patients 2 months after testing positive for COVID-19 [9]. Despite the fact that research data on symptomatic and organic changes in the lungs of post-COVID patients are abundant, data regarding changes in the central nervous system that may cause the brain fog are still not fully researched.

AIM

To describe and evaluate abnormalities of the brain in post-COVID patients with neurologic symptoms and cognitive deficits using MRI imaging of the brain.

MATERIALS AND METHODS

We included 21 patients with a previous positive PCR testing for SARS-CoV-2 and one or more of the following symptoms: memory and cognitive decline, dizziness, anxiety, depression, chronic headaches. The mean age was 68+-8, 7 female and 14 male patients. All patients had MRI imaging done at onset of symptoms, but after at least 1 year after positive testing for COVID-19 based on the patient's previous medical history. To determine the scale of white matter loss we used the Fazekas scale [10].

RESULTS

All of the patients complained of lack of concentration, forgetfulness, hard to process information. 15 patients suffered from confusion, 10 from anxiety. Of the 21 patients 14 had isolated chronic headaches, 3 had isolated dizziness, 4 patients had both symptoms upon
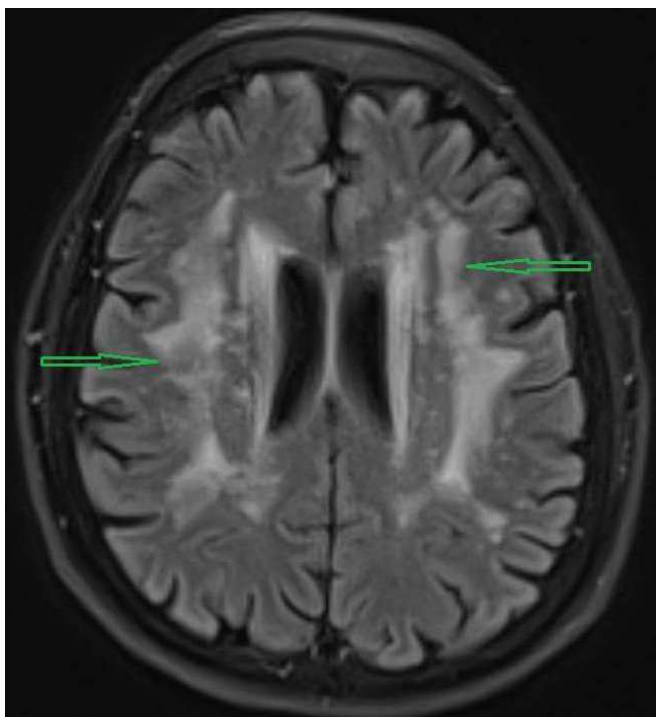
Fig. 3. Patient, 76 years old with clinical signs of neurodegeneration and COVID-19 infection 4 years prior. FLAIR sequence, multiple focal gliotic changes with a tendency for periventricular confluence.

inclusion. All patients underwent MRI imaging as a part of the diagnostic workup and had varying degrees of neurodegeneration.

The group of 13 patients with Fazekas I type changes had isolated mild changes in the brain. Patients had signs of thin lesions along the periventricular lines or a limited number of focal lesions as seen in Fig. 1.

Fazekas II was determined in 5 patients. Patients had widened subarachnoid spaces and/or confluence of focal lesions of the white matter. Widened lateral ventricles were observed in this group of patients, as well as atrophic changes in the hippocampus (Fig. 2).

Only 3 patients had Fazekas III changes, where degenerative processes were most severe. This group had multiple focal points with a tendency for confluence and widened subarachnoid spaces (Fig. 3).

DISCUSSION

The aim of the study was to determine causality between cognitive decline and MRI abnormalities in the brain in the post-COVID period. Several causes were proposed as potential pathogenic candidates for brain fog symptoms in post-COVID patients. Anosmia in early COVID-19 cases led researchers to believe that SARS-CoV-2 virus may invade and damage the olfactory nerve and disrupt the epithelial lining which is a potential gateway for the virus to invoke lesions in other parts of the central nervous system [11,12]. And taking into account that earlier mutations of SARS-CoV-2 virus strains had higher rates of loss of taste/smell symptoms compared to following strains, this may indicate that earlier cases may have a higher rate of post-COVID cognitive decline [13].

Another association was made between cerebral injury and dysregulated innate and adaptive immune responses. Immune biomarkers were determined to be severity-dependent and elevated levels persisted up to 4 months after the acute phase of the disease with a steady decline until 6 months. This along with coagulopathy and formation of microthrombi in blood vessels of the brain may lead to acute and lingering cerebral lesions that can contribute to brain fog symptoms and cognitive deficit [14,15].

Another link between COVID-19 severity, post-COVID syndrome and cognitive decline is hypoxia. It is well established that cerebral hypoxia leads to brain injury, and adequate lung function is essential for brain oxygenation. During moderate and severe COVID-19 cases lung function is disrupted, which leads to hypoxia and can be fatal without appropriate treatment. Prolonged hypoxia may also lead to lasting ischemic changes in the brain that can contribute to new or worsened degenerative processes and cause cognitive decline in surviving post-COVID patients [16,17].

CONCLUSIONS

Our data correlates with existing research and shows tendency for cognitive decline in post-COVID patients. This provides groundwork for further research to determine correlation between acceleration of neurodegeneration and post-COVID. Dynamic observation which should include follow-up MRI imaging, evaluation of severity and comorbidities of these patients can provide a pattern for adequate diagnosis and treatment which will lead to increased survival and better quality of life.

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CORRESPONDING AUTHOR

Artur V. Kurakh
Uzhhorod National University
71 Mynaiska st, 88000 Uzhhorod, Ukraine
e-mail: Artur.kurakh@uzhnu.edu.ua

ORCID AND CONTRIBUTION

Artur V. Kurakh: 0000-0002-2763-2935 A B C D
Vladyslava I. Ahii: 0000-0003-0677-7278 A B C F
Ivan V. Chopey: 0000-0003-4626-0855 A B D E F
Mykhailo M. Hechko: 0000-0003-2793-5044 A B D E F
Kseniya I. Chubirko: 0000-0002-4379-0538 A 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

ORIGINAL ARTICLE

Dynamics of indicators of functional state and physical development of students in the process of high-intensity interval training

Anatolii A. Rebryna[1], Andrii A. Rebryna[2], Halyna A. Kolomoiets[3], Vasyl F. Antonets[1], Viktor V. Flerchuk[1], Nataliia Liakhova[4], Natalia P. Kasich[5]

[1]KHMELNYTSKYI NATIONAL UNIVERSITY, KHMELNYTSKYI, UKRAINE

[2]NATIONAL UNIVERSITY OF PHYSICAL EDUCATION AND SPORTS OF UKRAINE, KYIV, UKRAINE

[3]STATE SCIENTIFIC INSTITUTION "INSTITUTE FOR MODERNIZATION OF THE CONTENT OF EDUCATION", KYIV, UKRAINE

[4] POLTAVA STATE MEDICAL UNIVERSITY, POLTAVA, UKRAINE

[5]DRAGOMANOV UKRAINIAN STATE UNIVERSITY, KYIV, UKRAINE

ABSTRACT

Aim: To investigate the effect of high-intensity interval training (HIIT) based on the CrossFit system on the level of students' functional state and physical development.

Materials and Methods: The research involved 36 male students who were engaged in HIIT during their studies. Indicators of students' functional state and physical development after 1 and 3 years of HIIT were studied. The functional state was assessed by indicators of the cardiovascular and respiratory systems, and physical development – by physical education tests.

Results: It has been found that HIIT has a positive effect on the indicators of the functional state of the cardiovascular and respiratory systems of the body and the physical development of students. After three years of HIIT sessions, students significantly improved their heart rate, vital capacity of the lungs, Stange test, Genchi test, duration of recovery heart rate, as well as the level of development of speed qualities, strength qualities, endurance and flexibility.

Conclusions: It has been established that the sports-oriented form of organization of physical education training sessions with the use of HIIT based on the CrossFit system is quite effective in improving the indicators of the functional state and physical development of students. A high level of these indicators will help to improve students' health, improve their well-being, and increase the effectiveness of their educational and, in the future, professional activities.

KEY WORDS: functional state, physical development, physical education, HIIT, students

INTRODUCTION

The works of many scientists [1, 2] note that today the state of health and physical fitness of students in higher educational institutions (HEIs) of Ukraine is critically low. The reasons that led to this state of affairs include: the Covid-19 pandemic and the full-scale war with the russian aggressor, which caused students to switch to distance learning during the forced quarantine in 2019-2023 and, accordingly, led to a significant restriction of their motor activity; low level of physical development and health of high schoolers and applicants to higher educational institutions; decreased interest and motivation of students in the traditional form of organizing physical education training sessions; complication of the conditions of study for students in modern higher educational institutions, which provide for an increase in the number of hours of study resulting in nervous

and emotional exhaustion and a further decrease in their motor activity [3, 4]. All of this, as well as the fact that most modern students do not adhere to a healthy lifestyle, does not allow to ensure the required level of functional state and physical development of students in HEIs [5].

One of the most effective ways to solve this problem is to improve the existing system of physical education in HEIs, which should be a reliable basis for a high level of health, and intellectual development of students in the learning process, promote their involvement in systematic physical exercises and sports, and form the need for physical development to ensure their healthy life-sustaining activities. Instead, the works of scientists [6] note that the current system of physical education in Ukrainian HEIs is not effective enough to solve these problems. At the same time, according to many scien-

tists [7, 8], a promising way to improve the situation with students' health is to introduce a sports-oriented form of organizing physical education training sessions in HEIs, taking into account the interests and motivation of students, which influence their choice of motor activity. This form of organization of physical education is quite common in HEIs of developed countries of Europe and the world. Among the modern types of motor activity, which are often chosen by students for physical education in HEIs, is CrossFit, which is based on high-intensity interval training (HIIT) [9, 10]. Cross-Fit is a system of training that is characterized by high intensity and constant change of exercises. The main goal of CrossFit training is to improve physical fitness, cardiovascular and respiratory systems, and to teach the body to quickly adapt to changes in loads [11]. The essence of HIIT based on the CrossFit system is to perform simple and accessible physical exercises with high intensity to develop different muscle groups and improve the functional state and physical development of those involved in circuit training [12]. According to experts [13], HIIT is a short series of intense exercises alternating with low-intensity recovery periods. A positive characteristic of HIIT is its versatility, which indicates that, unlike other types of motor activity, this type of training is the most adapted to real-life situations. According to scientists [14], engagement in this type of motor activity significantly develops physical qualities and improves the functional state of the cardiovascular and respiratory systems. At the same time, there is insufficient research on the impact of HIIT on the level of students' functional state and physical development in Ukrainian HEIs.

AIM

The aim is to investigate the effect of high-intensity interval training (HIIT) based on the CrossFit system on the level of students' functional state and physical development.

MATERIALS AND METHODS

This research was conducted during 2020-2023 at the Department of Theory and Methods of Physical Education and Sport of Khmelnytskyi National University (Ukraine). The research involved 36 male students who independently chose HIIT based on the CrossFit system for physical education training sessions, which was conducted in a sports-oriented form in the HEI. The research was conducted in 3 stages: stage 1 – determining the initial level of the studied indicators of students (beginning of the 1st instructional year); stage 2 – after

one year of HIIT (beginning of the 2nd instructional year); stage 3 – after three years of HIIT (end of the 3rd instructional year).

Research methods: analysis and generalization of literary sources, medical and biological methods, testing, statistical analysis. The method of analysis and generalization of literary sources contributed to the study of the literature on the topic of the research (18 sources from the scientometric databases PubMed, Scopus, Web of Sciences Core Collections, Index Copernicus and others were investigated). Medical and biological methods were used to study indicators of -functional state of students.

We determined the following indicators: resting heart rate, blood pressure (systolic and diastolic), vital capacity of the lungs, timed inspiratory (Stange test) and expiratory (Genchi test) capacity, cardiovascular system response after breath-holding test (Rosenthal test), duration of heart rate recovery after standard exercise (Martine-Kushelevsky test).

Testing was used to assess the physical development of students based on the results of the following tests: 100 m run (speed qualities), 3 km run (endurance), pull-ups (strength qualities), and leaning torso forward (flexibility).

Statistical analysis was applied to correctly process the data and identify the difference between the indicators under study. The compliance of the data distribution with the Gauss' law was assessed using the Shapiro-Wilk W-test. The significance of the difference in the results of the students was determined during the studying based on the Student's t-test. The significance for all statistical tests was set at $p < .05$. All statistical analyses were performed with the SPSS software, version 11.0. This research followed the regulations of the World Medical Association Declaration of Helsinki and ethical principles for medical research involving human subjects and was approved by the Academic Council of Khmelnytskyi National University (Protocol No. 17 dated 29.08.2020). Informed consent was received from all students who took part in this research.

RESULTS

The results of studying the functional state and physical development of students in the process of HIIT are presented in Table 1 and Table 2, respectively. The study of cardiovascular and respiratory systems functioning indicators showed their improvement in the process of HIIT sessions. Most of the studied indicators significantly ($p \leq .05\text{-}.001$) improved during the period of study at the HEI. Thus, the analysis of the dynamics of resting heart rate showed that after one year of training, stu-

Table 1. The dynamics of students' indicators of functional state in the process of HIIT (n=36), Mean±m

Indicators of functional state	Stages of research			Student's t-test		
	1st	2nd	3rd	1-2	2-3	1-3
Resting heart rate, beats/min	70.4±0.81	69.8±0.81	65.9±0.78	t=0.52	t=3.47	t=4.00
Systolic blood pressure, mm Hg	120.2±1.10	119.7±1.06	118.4±1.02	t=0.33	t=0.88	t=1.20
Diastolic blood pressure, mm Hg	72.3±0.89	71.7±0.85	70.8±0.81	t=0.49	t=0.77	t=1.25
Vital capacity of the lungs, ml	4160.3±78.1	4278.3±77.6	4671.5±74.2	t=1.06	t=3.66	t=4.75
Timed inspiratory capacity, s	66.7±1.92	74.2±1.85	86.9±1.77	t=2.81	t=4.96	t=7.74
Timed expiratory capacity, s	43.5±1.17	48.3±1.14	54.7±1.08	t=3.06	t=4.08	t=7.03
Duration of heart rate recovery, s	102.5±3.34	97.6±3.24	86.9±3.15	t=1.05	t=2.37	t=3.40

Note: Mean - arithmetical average; m - standard deviation error; 1-2 (2-3; 1-3) - the significance of the difference between the indicators of students at the 1st and 2nd (2nd and 3rd; 1st and 3rd) stages of research.

Table 2. The dynamics of students' indicators of physical development in the process of HIIT (n=36), Mean±m

Indicators of physical development	Stages of research			Student's t-test		
	1st	2nd	3rd	1-2	2-3	1-3
100 m run, s	13.8±0.12	13.6±0.10	13.3±0.09	t=1.28	t=2.23	t=3.33
Pull-ups, times	14.3±0.48	16.4±0.45	20.1±0.41	t=3.19	t=6.08	t=9.19
3 km run, s	789.6±10.03	761.1±9.37	733.8±2.08	t=2.55	t=2.07	t=4.09
Leaning torso forward, cm	9.8±0.90	11.9±0.86	15.6±0.81	t=1.69	t=3.13	t=4.79

Note: Mean - arithmetical average; m - standard deviation error; 1-2 (2-3; 1-3) - the significance of the difference between the indicators of students at the 1st and 2nd (2nd and 3rd; 1st and 3rd) stages of research.

dents' indicators improved by 0.6 beats/min (p > .05), and after three years of training – by 4.5 beats/min (p ≤ .001). At the same time, the difference between the indicators of the 2nd and 3rd stages of the study was also significant (p ≤ .01) and amounted to 3.9 beats/min, which confirmed the effectiveness of HIIT in improving students' cardiovascular system. At the same time, all heart rate values of students, both after one and three years of training, were in the range of 55-76 beats/min, which indicated the absence of signs of tachycardia. On the contrary, the presence in the majority of students after three years of training of economic activity of the cardiovascular system testified to signs of bradycardia, which was characteristic for representatives of sports with the predominant development of endurance, in which training activity was carried out in the aerobic mode of energy supply.

Students' systolic and diastolic blood pressure also tended to improve in the course of HIIT based on the CrossFit system: the difference between the 1st and 3rd stages was 1.8 and 1.5 mm Hg, respectively. At the same time, there was no significant difference between the baseline and final data of the study (p > .05). Indicators of the vital capacity of the lungs (VCL) characterize the maximum amount of air exhaled by athletes after the deepest breath and the state of the external respiratory apparatus. The analysis of the vital capacity of the lungs showed that the students' indicators increased by

118 ml after a year of training in HIIT. Still, the difference between the indicators was not significant (p > .05). After three years of exercise in HIIT, the indicators of the vital capacity of the lungs significantly improved compared to both the baseline and the values after one year of training by 511.2 ml (p ≤ .001) and 393.2 ml (p ≤ .01), respectively.

The Rosenthal test included a 5-fold check of VCL after 15 seconds. If the volume of exhaled air decreased after the 5th time, it indicated a deterioration in the functional capabilities of the respiratory system (excessive fatigue, overstrain, overtraining, or illness). If the value of the VCL remained unchanged after the test, this indicated a satisfactory condition of the respiratory system of the body. If the VCL increased, the endurance of the respiratory muscles was rated as "excellent", and the performance of the system improved. The analysis of the Rosenthal test indicators showed that 80.5 % of students after three years of HIIT had 2-5 % better VCL indicators than at the beginning of the test. At the same time, after one year of HIIT sessions, 47.2 % of such cases were detected, which indicated an increase in respiratory muscle endurance in students due to HIIT. This allows us to assert the effectiveness of HIIT sessions in improving the functioning of the respiratory system of students. We used a functional test referred to as timed inspiratory capacity (Stange test) to assess students' respiratory function. It is believed that if an untrained

student can hold his or her breath for more than 60 s during inhalation, his or her respiratory function corresponds to a high level. Thus, the analysis of the duration of students' breath holding during inhalation showed that at all stages of the research, the indicators of the Stange test corresponded to a high level. At the same time, in the process of HIIT sessions, there was a significant improvement in the indicators of the Stange test by 7.5 s after one year of training sessions (p ≤ .05) and by 20.2 s after three years of training sessions (p ≤ .001). A similar trend was observed in the indicators of timed expiratory capacity (Genchi test), which characterize the external respiratory system of students. The time of breath holding during exhalation in healthy untrained men ranges from 25-40 s, and in trained men – up to 50-60 s. The high level of the Genchi test corresponds to the indicators of breath holding over 40 s. In our research, it was found that in students who were engaged in HIIT, the indicators of the Genchi test at all stages corresponded to a high level and significantly improved during the instructional period. The difference between the indicators of the 1st and 2nd stages was 4.8 s (p ≤ .01), and between the indicators of the 2nd and 3rd stages – 11.2 s (p ≤ .001), and between the indicators of the 1st and 3rd stages – 11.2 s (p ≤ .001). The analysis of the duration of heart rate recovery to the initial level after a standard load (20 squats for 30 s) testified that in the course of HIIT sessions this indicator improved: after one year of training by 4.9 s (p > .05), and after three years – by 15.6 s (p ≤ .01). The conducted analysis of indicators of students' functional state in the process of HIIT sessions showed a positive effect of this type of motor activity on the functional capabilities of cardiovascular and respiratory systems of students.

The study of physical development indicators of students based on the results of their physical education tests (Table 2) showed that in the process of HIIT sessions, there was a significant (p ≤ .01-.001) improvement in the results in all the studied exercises. However, the most pronounced effect of HIIT was revealed on the development of students' strength qualities and their endurance.

The analysis of students' results in the 100-meter run showed that after one year of training the development of speed qualities improved by 0.2 s, but the changes were not significant (p > .05). After three years of HIIT the results significantly (p ≤ .001) improved by 0.5 s, which indicated a positive influence of HIIT on the development of students' speed qualities. The most pronounced changes in physical development indicators occurred in students' results in pull-ups. Already after one year of HIIT the results significantly improved by 2.1 times (p ≤ .01), and after three years – by 5.8 times (p ≤ .001).

The results of students in pull-ups demonstrated after three years of training (20.1 times) indicated a high level of development of students' strength qualities. Similar changes occurred in the indicators of students' endurance development in the process of HIIT: after one year of training, the results in the 3 km run significantly improved by 28.5 s (p ≤ .05), and after three years – by 55.8 s (p ≤ .001). Moreover, the results of students in the 3 km run after three years of training in the HIIT were 12 min 13.8 s and corresponded to a high level of students' endurance development. Students' results in leaning their torso forward from a sitting position also tended to improve. Still, after one year of HIIT the changes (2.1 cm) were not significant (p > .05), and they turned out to be significant (p ≤ .001) after three years of training (5.8 cm). In general, the analysis of the level of development of students' physical qualities indicated a positive impact of HIIT on their physical development.

DISCUSSION

According to scientists [10, 15], harmony of physical development, versatile physical training, and ability to perform any task as effectively as possible – is the ideology of life of the XXI century, in which CrossFit takes a significant place. CrossFit training usually consists of several rounds, each of which includes 3-5 exercises. The main task is to complete the maximum number of rounds in the allotted time or to complete a certain number of rounds in the minimum time. Exercises are performed in sets, i. e. without a rest interval. CrossFit uses three types of exercises: cardio (running, rowing, cycling, etc.); gymnastic exercises (pull-ups, push-ups, squats, etc.); and weight exercises (barbell, kettlebells, dumbbells). New combinations of exercises are used at almost every training session. CrossFit experts note that the specificity of CrossFit lies in the rejection of any specialization. The combination of weightlifting, gymnastics, running, kettlebell lifting, and bodyweight exercises provides a wide range of different workouts for every day, which allows you to add variety and make the training process much more interesting and effective [13, 15].

The peculiarity of HIIT based on the CrossFit system is the variability of exercises, mainly strength and endurance development. The advantages are no material costs for equipment (most exercises are performed with the weight of one's own body, significant opportunities to use improvised means and natural conditions); the possibility of using in conditions of time shortage and limited space, in any conditions (in the gym, in open areas, at home); exclusion of the possibility of adaptation of the body to loads due to variability of means;

accessibility for students with different levels of physical fitness, different genders [9, 16]. Scientists [17] argue that the largest number of muscle fibers are used during high-intensity exercises. Low-intensity exercises involve only 10-20 % of muscle fibers. High-intensity exercises allow you to use muscle fibers to the maximum while increasing the metabolic rate and reducing adipose tissue. Authors [18] prove that you can do any sport and achieve high results after CrossFit training sessions, because HIIT builds basic physical fitness, promotes the development of endurance, and muscle strength, and improves the cardiovascular and respiratory systems. Our research has confirmed the results of many scientists on the positive impact of HIIT based on the CrossFit system on the level of functional state and physical development of students. However, when planning training programs, it is necessary to pay special attention to the rational dosage of the load, taking into account the age, gender, and level of fitness of students.

CONCLUSIONS

It has been found that HIIT has a positive effect on the indicators of the functional state of the cardiovascular and respiratory systems of the body and the physical development of students. After three years of HIIT sessions, students significantly ($p \leq .01$-.001) improved their heart rate (by 4.5 beats/min), vital capacity of the lungs (by 511.2 ml), Stange test (by 20.2 s), Genchi test (by 11.2 s), duration of recovery heart rate (by 15.6 s), as well as the level of development of speed qualities (in 100 m run – by 0.5 s), strength qualities (in pull-ups – by 5.8 times), endurance (in 3 km run – by 55.8 s) and flexibility (in leaning torso forward – by 5.8 cm).

It has been established that the sports-oriented form of organization of physical education training sessions in HEIs with the use of HIIT based on the CrossFit system is quite effective in improving the indicators of the functional state and physical development of students. A high level of these indicators will help to improve students' health, improve their well-being, and increase the effectiveness of their educational and, in the future, professional activities.

PROSPECTS FOR FURTHER RESEARCH

We plan to investigate the influence of HIIT based on the CrossFit system on the level of functional state and physical development of female students of Ukrainian HEIs.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Nataliia Liakhova
Poltava State Medical University
23 Shevchenko st., 36000 Poltava, Ukraine
e-mail: NataNew2017@ukr.net

ORCID AND CONTRIBUTIONSHIP
Anatolii A. Rebryna: 0000-0001-7707-5324 Ⓐ Ⓓ
Andrii A. Rebryna: 0000-0002-5108-2793 Ⓓ
Halyna A. Kolomoiets: 0000-0002-4315-3977 Ⓒ
Vasyl F. Antonets: 0000-0002-1116-820X Ⓑ
Viktor V. Flerchuk: 0000-0001-8667-5487 Ⓑ
Nataliia Liakhova: 0000-0003-0503-9935 Ⓔ
Natalia P. Kasich: 0000-0002-8756-8218 Ⓕ

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

ORIGINAL ARTICLE

Multivariate analysis and mathematical modeling of the informativeness of patients cases data in chronic pancreatitis associated with concomitant pathology

Olesya M. Horlenko, Lyubomyra B. Prylypko, Mykola V. Rishko, Galyna M. Beley, Fedir V. Horlenko, Bohdan M. Halay, Lyubov A. Halay
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

Aim: To investigate and analyze homeostatic disorders in patients with a combination of Chronic Pancreatitis(CP) and Arterial Hypertension (AH) and to develop correcting ways of the detected changes.

Materials and Methods: General clinical, laboratory-instrumental examination of 121 patients, who were undergoing inpatient treatment with a diagnosis of Chronic Pancreatitis in combination with Arterial Hypertension of the II stage during 2021–2022.

Results: In the majority of cases of patients signs the increasing in IL-1,6 and Cortisol levels were found. A decrease in Ca to the lower limit of the norm was observed (2.18 ± 0.26 mmol/l to the data of control group patients   (2.32 ± 0.12 mmol/l, p= 0.01), the levels of trace elements Zn and Se were determined within the reference values. The Atherogenic Index was increased 1.8 times and was significantly different from the control group date. During the FE-1 study, a decrease in the level of this indicator was revealed by 151.71 ± 13.91 mg/g of feces, both to the values of reference values and a significant difference to the data of the control group (241.28 ± 29.17 mg/g of feces, p<0 .05)

Conclusions: Based on the multivariate linear regression analysis of the obtained data, formulas have been developed that can be used to predict the dynamics of the dependent variable (FE-1, IL-1, Selenium level, Glutathione Peroxidase, blood pressure) according to changes in the studied influencing factors.

KEY WORDS: Chronic pancreatitis, Hypertension, associated pathology of the Digestive tract (Non-alcoholic Fatty Liver disease, Gastroesophageal Reflux disease), inflammatory response, patient

INTRODUCTION

In the structure of diseases of the gastrointestinal tract (GIT), CP is from 5.1 to 9% per 100,000 population, and in general clinical practice - from 0.2 to 0.6% [1]. In modern studies, CP is considered not only as a combination of local lesions due to the influence of pathochemical inflammatory mechanisms, but as a systemic gastroenterological disease, in the development of which hereditary-constitutional, immunological, psychosocial and psychosomatic mechanisms are involved. At the same time, a cohort of patients in whom a clear relationship between the genesis, the onset of the disease, the phase of the disease and the features of the premorbid condition can be traced is quite common [2, 3]. The situation is exacerbated by the frequent combination of CP not only with diseases of the digestive organs, but also with CVD, among which hypertension is considered the unchanging leader, since the combination of CP and Arterial Hypertension (AH) is pathogenetically determined, in particular, as a result of generalized systemic damage to the vascular bed, which is the basis for the formation of ischemic changes, activation local inflammatory component of diseases and triggering of systemic inflammatory response, metabolic disorders [4, 5].

The etiological factors of Chronic Pancreatitis (CP) include various types of pathology of organs that are anatomically and physiologically related to Pancreas. According to the literature, in 35-56% of cases, pathology of the biliary tract is recognized as a factor that leads to the development and exacerbation of CP. Pancreatitis develops much faster in patients with gallstone disease, which confirms the role of gallstone disease in the development of CP, therefore, patients suffering from gallstone disease are a group at risk of developing CP [6, 7].

Peculiarities of the etiology and pathogenesis of CP combined with hypertension should be taken into account in clinical practice when examining and treating this cohort of patients. At the same time, management
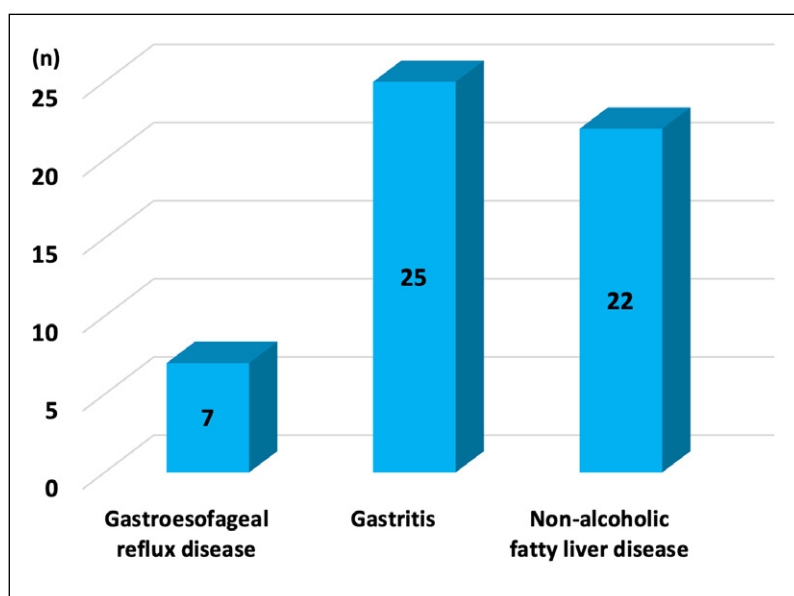
Fig. 1. Associated pathology of the digestive tract in patients with Chronic Pancreatits and Arterial Hypertension.

of patients with comorbidities still remains a methodologically complex task. The variety of reasons for the formation of CP combined with hypertension motivates researchers to study in-depth the causes and features of the development of this combined pathology.

AIM

The aim is to investigate and analyze homeostatic disorders in patients with a combination of chronic pancreatitis and hypertension and to develop ways of correcting the detected changes.

MATERIALS AND METHODS

A general clinical, laboratory-instrumental examination of 121 patients (average age of patients 48.6 ± 8.9 years) who were undergoing inpatient treatment in the therapeutic department of the Khust Central Regional Hospital with a diagnosis of chronic pancreatitis (CP) in combination with hypertension of the II stage during 2021–2022. Control group include 25 healthy person identified the studied parameters. The diagnostic algorithm for CP and associated pathology was carried out in accordance with the clinical protocols of the Ministry of Health of Ukraine and guidelines.

RESULTS

To assess the features of the clinical course of the combined pathology, we performed a comprehensive analysis of the results of objective and subjective examinations of the studied selection of patients. The diagnosis of hypertension was also confirmed in all patients with chronic pancreatitis (121/100%). At the level of the esophago-gastro-duodenal segment of the digestive system, pathological changes were established, which were finally confirmed thanks to endoscopic esophagogastroduodenoscopy. Gastritis (n=25; 24.5%) was most often registered, and Gastroesophageal Reflux disease was registered in 7 (6.9%) of the examined. Non-alcoholic Fatty Liver disease was established in 22 (21.6%) patients examined using steatometry (Fig. 1.).

From the group of patients with fixed signs of fatty inclusions in the Liver parenchyma, grade S1 was determined in 11 (50 %), S2 – 9 (41 %), and S3 – in 2 patients (9 %) (Fig. 2)

The complex of laboratory examinations included the study of the links of patients' homeostasis and the identification of imbalances. The first stage was a study of biochemical blood analysis (Table 1).

The following changes were noted in the results of the biochemical study, in particular, the level of total protein had a direction towards the lower limit of reference values and was significantly different from the indicators of the control group (68.51 ± 3.66 to 75.01 ± 2.77 g/l, $p<0.05$) due to the low value of albumin (39.56 ± 2.91 g/l against the data of the control group 45.12 ± 2.04 $p< 0.05$). A slight increase in the levels of transaminases ALT (0.97 ± 0.49 mmol/l) and AST (0.97 ± 0.49 mmol/l) was also observed and had significant differences from the data of the control group (0.65 ± 0.12 mmol /l, $p< 0.002$ and 0.42 ± 0.11 mmol/l, $p< 0.05$, respectively). Increasing levels of the last two indicators show the influence of pathological changes at the level of the pancreas on the functioning of the liver, which confirms the presence of associated pathology. Biochemical blood analysis indicated the presence of cytolytic syndrome (increased levels of transaminases), a decrease in the concentration of Total Protein, due to a low level of Albumin.
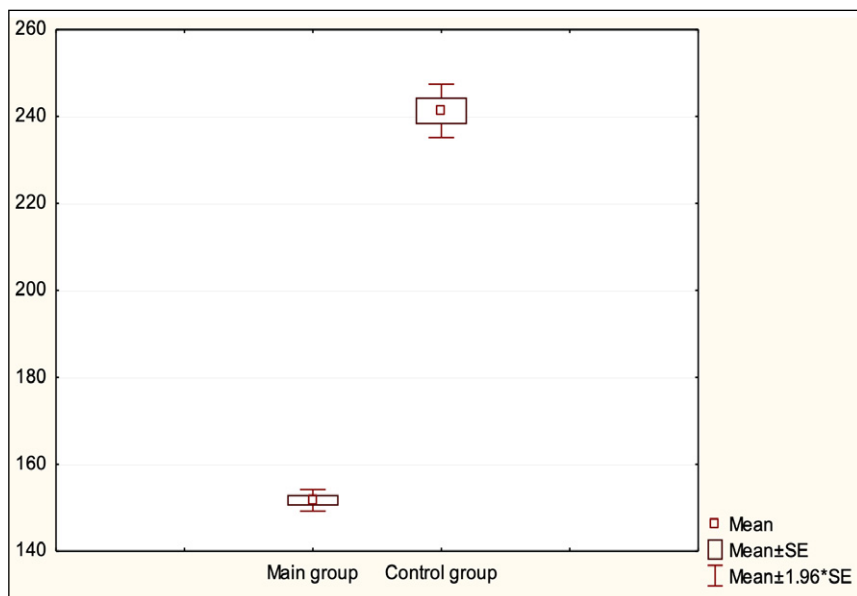
Fig. 2. Percentage value of steatometry results in patients.
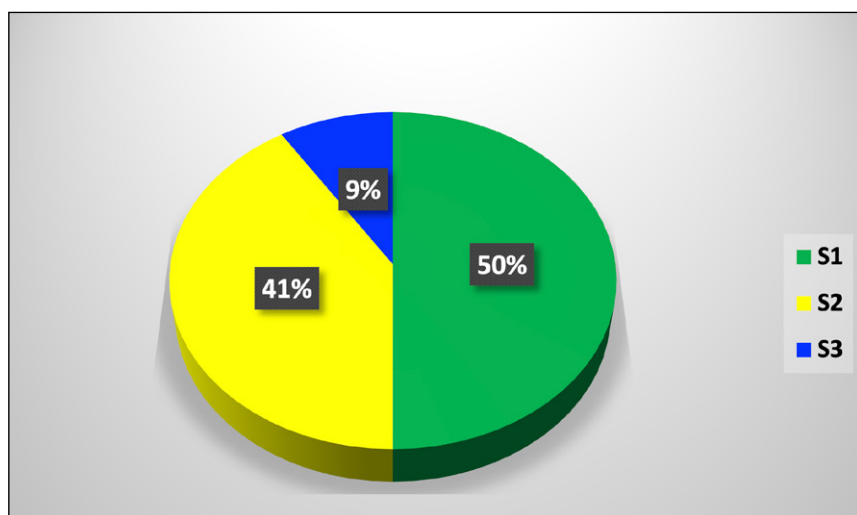
Fig. 3. Comparing levels diagram of Fecal Elastase-1 between the main group and the control group.

The state of inflammatory markers in CP patients with comorbid pathology (Table 2) is bringing to your attention.

Analyzing the obtained data, all the studied indicators are significantly different from the corresponding parameters of patients from the control observation group ($p<0.05$). Specifying individual indicators, it was found that the level of leukocytes (8.95 ± 2.95 G/l is significantly different from the data of the control group, 6.12 ± 1.77 G/l), but was near the upper limit of the physiological norm. The levels of ESR and fibrinogen are also within the reference values and may be a sign of insufficient immunological reactivity of the studied contingent. There was a slight increase in the level of pro-inflammatory IL-6 (11.99 ± 1.64 pg/ml and 6.94 ± 1.79 pg/ml in comparison with the control group), and a reliable variation of IL-4 within the reference values with the data of the control group. One can think about the presence of an inflammatory reaction of the body in conditions of persistence of the inflammatory process.

The level of cortisol in the blood of patients showed an increase in values (972.61 ± 220.08 nmol/l) by 1.5 times, which indicates the initiation of activation of anti-inflammatory mediators and inhibition of the synthesis of pro-inflammatory ones. Values of Glutathione Peroxidase(GPO), as a representative of the enzymatic link of Antioxidant protection, show an approach to the lower limit of normal, which indicates insufficient activity of GPO in the conditions of oxidative stress (OS) launch in patients with associated pathology. So, the results indicate that in most patients, during the initial examination, signs of persistence of the inflammatory reaction due to the increase in the levels of IL-1,6 and Cortisol were found. Other indicators of biochemical blood analysis in the examined patients were less indicative. The levels of the Leukocyte pool and ESR varied within the reference values, but with statistical probability with the indicators of the control group.

Violation of mineral metabolism in diseases of the digestive tract is important (Table 3).

Table 1. Biochemical indicators of blood serum in the studied contingent

Parameters	Main group (n = 121)	Control group (n = 25)	Statistical significance of differences (p)
Total protein (65-85, g/l)	68,51 ± 3,66	75,01 ± 2,77	< 0,05
Albumin (38-51, g/l)	39,56 ± 2,91	45,12 ± 2,04	< 0,05
Total bilirubin (3,4-20,05, μmol/l)	14,36 ± 11,89	10,31 ± 3,22	0,09
Alanine aminotransferase (0,1-0,45, mmol/l)	0,97 ± 0,49	0,65 ± 0,12	0,002
Aspartate aminotransferase 0,1-0,68, mmol/l	0,52 ± 0,25	0,42 ± 0,11	0,05

Note: p- statistical significance between the indicators of the main and control groups.

Table 2. Analysis of inflammatory markers in CP patients with comorbid pathology

Indexes	Main group (n = 121)	Control group (n = 25)	Statistical significance of differences (p)
Leukocytes (4.0-9.0, G/l)	8,95 ± 2,95	6,12 ± 1,77	< 0,05
ESR (2-15, mm/h)	9,93 ± 5,72	5,42 ± 2,93	< 0,05
Fibrinogen (1.8-3.5, g/l)	2,94 ± 0,54	2,44 ± 0,36	< 0,05
Cortisol (in the morning, 190-690, nmol/l)	972,61 ± 220,08	495,62 ± 159,54	< 0,05
IL-1 (0-11, pg/ml)	23,77 ± 8,61	2,57 ± 1,02	< 0,05
IL-4 (0-4, pg/ml)	1,94 ± 0,21	2,39 ± 0,62	< 0,05
IL-6 (0-10, pg/ml)	11,99 ± 1,64	6,94 ± 1,79	< 0,05
Glutathione peroxidase (12.5-200, ng/ml)	45,55 ± 10,98	65,28 ± 9,47	< 0,05

Note: p- statistical significance between the indicators of the main and control groups.

According to the data in Table III, the concentrations of Na, K, Cl in both studied groups were not significantly different from the results of the control group, which indicates the absence of changes in the metabolism of these elements in the combined pathology of CP. However, our results indicate a tendency to decrease Ca content to the lower limit of normal (2.18 ± 0.26 mmol/l in comparison with the data of control group patients (2.32 ± 0.12 mmol/l, p=0.01) The balance of Ca plays an important role in the synthesis and excretion of Pancreas enzymes, as well as in the regulation of vascular tone [9].

According to our data, the levels of trace elements Zn and Se are determined within the reference values, but with statistically significant differences from the data of patients of the control group: Se (63.74 ± 18.03 μg/l vs. 87.58 ± 14.03 μg/l, p<0 ,01); Zn (743.33 ± 206.01 μg/l vs. 901.23 ± 168.57 μg/l (p=0.05)). In our opinion, the lower content of Se and Zn in patients with associated pathology is caused by functional disorders of the liver and, as a result, disorders of mineral homeostasis and disruption of microelement assimilation processes due to enzymatic dysfunction of the liver.

The components of lipid metabolism in the pathology under study in our patients (Table 4) was considered.

Evaluating the studied indicators, it can be concluded that in patients with associated pathology, a statistically significant difference was established in all parameters of the Lipidogram compared to the data of the indicators of patients in the control group (p<0.05). In particular, slight Hypercholesterolemia (5.13 ± 1.07 mmol/l against the data of the control group 4.21 ± 0.45 mmol/l, p < 0.05) and Hypertriglyceridemia (1.79 ± 0.45 mmol/l against 1.29 ± 0.22 mmol/l, p < 0.05), a reduced level of HDL Cholesterol in the blood serum of patients (0.85 ± 0.17 mmol/l, against 1.45 ± 0.32 mmol/l, p < 0, 05),

Table 3. Indicators of mineral metabolism in patients

Parameters	Main group (n = 121)	Control group (n = 25)	Statistical significance of differences (p)
K (3,6-5,5, mmol/l)	4,39 ± 0,37	4,48 ± 0,36	0,27
Na (135-155, mmol/l)	143,19 ± 3,20	142,71 ± 3,22	0,49
Ca (2,1-2,6, mmol/l)	2,18 ± 0,26	2,32 ± 0,12	0,01
Cl (95-108, mmol/l)	101,88 ± 3,14	103,17 ± 2,48	0,06
Se (23-190, µg/l)	63,74 ± 18,03	87,58 ± 14,03	< 0,05
Zn (543-1130, µg/l)	743,33 ± 206,01	901,23 ±168,57	< 0,05

Note: p- statistical significance between the indicators of the main and control groups.

Table 4. Lipidogram indicators of the studied contingent

Parameters	Main group (n = 121)	Control group (n = 25)	Statistical significance of differences (p)
Total cholesterol (< 5.0 mmol/l)	5,13 ± 1,07	4,21 ± 0,45	< 0,05
Triglycerides (< 1.7, mmol/l)	1,79 ± 0,45	1,29 ± 0,22	< 0,05
HDL (> 1.0, mmol/l)	0,85 ± 0,17	1,45 ± 0,32	< 0,05
LDL (< 3.0, mmol/l)	3,46 ± 1,04	2,37 ± 0,55	< 0,05
VLDL (0.26-1.04, mmol/l)	0,82 ± 0,20	0,65 ± 0,09	< 0,05
IA (≤3)	5,43 ± 2,24	2,38 ± 1,01	< 0,05

Note: p- statistical significance between the indicators of the main and control groups.

increased levels of LDL Cholesterol (3.46 ± 1.04 vs. 2.37 ± 0.55 mmol/l, p < 0.05). Levels of VLDL Cholesterol (0.82 ± 0.20 mmol/l and 0.8 ± 0.22 mmol/l vs. 0.65 ± 0.09 mmol/l, p < 0.05) varied within the reference limits. The Atherogenic Index was increased in 1.8 times and significantly differed from the values of the control group (5.43 ± 2.24 vs. 2.38 ± 1.01, p < 0.05).

Identified Dyslipidemic disorders have pathological effects on the state of functioning of the Pancreas and, along with this, the vascular component of the body, which can be considered as a risk factor for the development of pathology of the Cardiovascular system and Digestive tract.

Monitoring of pressure values showed the following changes (Table 5).

Average Blood pressure(BP) levels in patients corresponded to the parameters of Arterial Hypertension of the 1st-2nd degree. Statistically significant differences were observed between the groups of patients with associated pathology in the levels of SBP (153.47 ± 9.42 mmHg) and DBP (94.75 ± 8.61 mmHg)) with indicators in the control group (p<0.05).

To determine the exocrine capacity of the parenchymal organ, we measured the concentration of elastase-1 in feces (Table 6).

The level of fecal elastase-1 plays an important role in the diagnosis of Pancreatic Exocrine insufficiency (PEI), as a specific indicator of the exocrine function of the pancreas. During the FE-1 study, a decrease in the level of this indicator was revealed by 151.71±13.91 mg/g, both to the values of reference values and a significant difference to the data of the control group (241.28±29.17 mg/g of feces, p<0 .05), which can be regarded as a mild degree of PEI [10].

To confirm and optimally interpret the indicators of the studied contingent with a diagnosis of CP in association with concomitant pathology, a multivariate linear regression analysis of the obtained data was conducted. According to the calculated regression formula, it is possible to predict the dynamics of the dependent variable

Table 5. The value of blood pressure monitoring in patients

Parameters	Main group (n = 121)	Control group (n = 25)	Statistical significance of differences (p)
Systolic blood pressure (mm Hg)	153,47 ± 9,42	125,73 ± 6,38	< 0,05
Diastolic blood pressure (mm Hg)	94,75 ± 8,61	72,41 ± 5,92	< 0,05

Note: p- statistical significance between the indicators of the main and control groups.

Table 6. Value of fecal elastase level in patients

Indexes	Main group (n = 121)	Control group (n = 25)	Statistical significance of differences (p)
Fecal elastase-1 (> 200, μg/g stool)	151,71 ± 13,91	241,28 ± 29,17	< 0,05

Note: p- statistical significance between the indicators of the main and control groups.

according to the changes in the studied influencing factors [11]. The created mathematical models of the obtained data are presented in formulas. Credible levels of indicators were used to create mathematical models.

1. Fecal elastase-1 = 167.29 – 0.22 * Total Bilirubin + 2.30 * Total Cholesterol – 5.91 * Fibrinogen. (formula 1)
EXPLANATION:
- with an increase in the level of Total Bilirubin by 1 μmol/l, the value of FE-1 will decrease by 0.22 μg/g of feces;
- with an increase in the level of Total Cholesterol by 1 mmol/l, the concentration of FE-1 will increase by 2.30 μg/g of feces;
- with an increase in the level of Fibrinogen by 1 g/l, the FE-1 value will decrease by 5.91 μg/g of feces.

According to the data of formula 1, the highest level of influence of bilirubin values is observed, which indicates the biliary component of the development of pancreatitis. There is also a significant influence of fibrinogen values on the reduction of FE-1 level and increase of PEI. Since Fibrinogen belongs to acute phase Proteins and immunoinflammatory modulators, it can be assumed that the leading role of the inflammatory factor in the development of diseases of the studied contingent. The lowest influence in the development of CP is the level of Total Cholesterol.

2. IL-1 = 22.55 – 9.27 * HDL + 0.20 * Glutathione Peroxidase. (formula 2)
EXPLANATION:
- an increase in the concentration of HDL by 1 mmol/l will cause a decrease in the level of IL-1 by 9.27 pg/ml;
- with an increase in the level of Glutathione Peroxidase by 1 ng/ml, the level of IL-1 will increase by 0.20 pg/ml.

According to formula 2, there is a high value of the level of the HDL component on the reduction of the pro-inflammatory cytokine IL-1 and a slight Antioxidant protection due to glutathione peroxidase.

3. Se = 62, 94 + 1.08 * Atherogenicity Index – 0.69 * Na + 12.32 * Ca + 0.03 * Zn + 0.86 * Glutathione Peroxidase + 0.009 * cortisol. (formula 3)
EXPLANATION:
- if the value of Atherogenicity Index increases by 1 Unit, the concentration of Se will increase by 1.08 μg/l;
- when the level of Na increases by 1 mmol/l, the concentration of Se will decrease by 0.69 μg/l;
- when the concentration of Ca increases by 1 mmol/l, the concentration of Se will increase by 12.32 μg/l;
- if the concentration of Zn increases by 1 μg/l, the level of Se will increase by 0.03 μg/l;
- when the Glutathione Peroxidase concentration increases by 1 ng/ml, the Se concentration will increase by 0.86 μg/l;
- with an increase in the level of Cortisol by 1 nmol/l, the level of Se will increase by 0.009 μg/l.

According to the obtained data of formula 3, the level of Selenium will increase due to the concentration of Ca, the value of Atherogenicity Index, and with the minimum values of the concentration of GPO, Zn, and Cortisol. The level of Na helps to reduce the content of Selenium

4. Glutathione peroxidase = -10.66 +10.64 * HDL Cholesterol + 0.48 * Se + 0.28 * ESR – 0.82 * IL-6 + 0.16 * systolic blood pressure. (formula 4)
EXPLANATION:
- an increase in the concentration of HDL Cholesterol by 1 mmol/l will cause an increase in the level of GPO by 10.64 ng/ml;

- when the concentration of Se increases by 1 µg/l, the concentration of GPO will increase by 0.48 ng/ml;
- when the ESR concentration increases by 1 mm/h, the GPO concentration will increase by 0.28 ng/ml;
- when the level of IL-6 increases by 1 pg/ml, the concentration of GPO will decrease by 0.82 ng/ml;
- with an increase in SBP value by 1 mm. Hg the GPO concentration will increase by 0.16 ng/ml.

Based on the components of formula 4, it can be noted that with an increase in the concentration of HDL Cholesterol, an increase in the level of GPO is observed and, as a result, an increase in the body's antioxidant capacity in CP with Arterial Hypertension. Concentrations of Se, ESR, IL-6, and SBP are characterized by minimal contributions to the maintenance of the body's Antioxidant protection.

5. Systolic blood pressure = 225.93 – 0.57 * Total Protein - 0.12 * Se – 1.09 * Leukocytes – 1.36 * IL-6. (formula 5)
- when the Total Protein concentration increases by 1 g/l, the SBP level will decrease by 0.57 mm Hg;
- with an increase in the concentration of Se by 1 µg/l, the SBP level will decrease by 0.12 mm Hg;
- with an increase in the concentration of leukocytes by 1 G/l, the SBP level will decrease by 1.09 mm Hg;
- with an increase in the concentration of IL-6 by 1 pg/ml, the SBP level will decrease by 1.36 mm Hg.

According to formula 5, the category of systolic pressure increases due to the increase in the concentration of IL-6, Leukocytes and with minor effects of the levels of the concentration of Total Protein, the concentration of Se. That is, inflammatory factors of influence are decisive.

DISCUSSION

Usually, the patient has a comorbid pathology, which is based on common risk factors, pathogenetic mechanisms and requires a systematic approach to solving patient management tactics, which is significant in the choice of treatment and monitoring

CP is often determined by its combination with other diseases in the conditions of polymorbidity of the modern patient, which is an indication for expanding therapy [5,12]. Since CP is known to be associated with an increased risk of cardiovascular disease (CVD), D. De la Iglesia et al. (2019) interpret the risk of cardiovascular events in CP patients with pancreatic exocrine insufficiency [9]. Dysfunctional motor disorders of the upper parts of the alimentary canal, processes of stomach accommodation form the pathophysiological basis for associated pathologies in CP and non-erosive GERD. Numerous data on the most frequent risk factors that prevent the development and progression of chronic

pancreatitis and the high probability of combining chronic pancreatitis with diseases of the gastroduodenal and biliary zones and as a result - a violation of the regular course of the disease and changes in clinical presentations have been presented in scientific research [13]. According to our data, hypercholesterolemia and hypertriglyceridemia, significant hyperproduction of pro-inflammatory cytokines (IL-1.6), increased cortisol levels, imbalance of macro- and microelements were identified as common components. Chronic oxidative stress, hyperproduction of proinflammatory cytokines, and hyperlipidemia are essential factors in the pathogenesis of both CVD and CP. Dyslipidemia acts as a common mechanism for the formation of fatty infiltration of the liver, which contributes to the formation of steatopancreatitis, non-alcoholic fatty liver disease and the development of vascular atherosclerosis in the comorbid course of CP with hypertension. At the same time, the nature and depth of lipid metabolism disorders in patients with a combined course of CP and hypertension have not yet been definitively investigated.

Associated diseases should be considered as an interaction of the patient, risk factors, triggers and basic protective compensatory mechanisms that underlie the development of a disease state with certain characteristic clinical manifestations. It is very important for the doctor to recognize them early in a single disease process, to prevent the generalization of homeostasis and stimulation disorders, or to model compensatory and adaptive mechanisms [14].

CONCLUSIONS

1. Biochemical blood analysis indicated the presence of cytolytic syndrome (increased levels of transaminases), a decrease in the concentration of Total Protein, due to a low level of Albumin.
2. Therefore, the results indicate that in most patients during the initial examination signs of persistence of the inflammatory reaction due to the increase in the levels of IL-1,6 and Cortisol were found. Other indicators of biochemical blood analysis in the examined patients were less indicative. The levels of the Leukocyte pool and ESR varied within the reference values, but with statistical probability with the indicators of the control group.
3. Our results indicate a tendency to decrease the content of Ca to the lower limit of normal (2.18 ± 0.26 mmol/l) in comparison with the data of patients of the control group (2.32 ± 0.12 mmol/l, p=0.01). According to our data, the levels of trace elements Zn and Se are determined within the reference values, but with statistically significant differences from the data of patients of

the control group. In our opinion, the lower content of Se and Zn in patients with associated pathology is caused by functional disorders of the liver and as a result of mineral disorders homeostasis and disruption of processes of assimilation of microelements due to Pancreatic enzyme dysfunction.

4. Evaluating the studied indicators, slight Hypercholesterolemia and Hypertriglyceridemia, a reduced level of HDL Cholesterol, in the blood serum of patients, and an increase in the level of LDL Cholesterol were found. Levels of HDL cholesterol (0.82 ± 0.20mmol/l and 0.8 ± 0.22mmol/l vs. 0.65 ± 0.09mmol/l, $p<0.05$) varied within the reference limits. The Atherogenicity Index was increased 1.8 times and significantly differed from the values of the control group.

5. During the FE-1 study, a decrease in the level of this indicator was revealed by 151.71 ± 13.91 mg/g of feces, both to the values of the reference values and a significant difference to the data of the control group (241.28 ± 29.17 mg/g of feces, $p<0, 05$), which can be considered as a mild degree of PEI.

6. We have the next result, according to mathematical formulas,:

- formulas 1 - the highest level of influence of bilirubin values on the FE-1 level is observed, which indicates the biliary component of pancreatitis development. There is also a significant influence of Fibrinogen values on the reduction of the FE-1 level and the increase of PEI;

- formula 2 - a high value of the HDL Cholesterol level is observed for the reduction of the pro-inflammatory cytokine IL-1 and a slight Antioxidant protection due to Glutathione Peroxidase.

- formula 3 - the level of Selenium will increase due to the concentration of Ca, the value of AI, and with the minimum values of the concentration of GPO, Zn, Cortisol. The level of Na helps to reduce the content of Selenium

- formula 4 - it can be noted that with an increase in the concentration of HDL, an increase in the level of GPO is observed and, as a result, an increase in the body's Antioxidant capacity in CP with Arterial Hypertension. Concentrations of Se, ESR, IL-6, and SBP are characterized by minimal contributions to the maintenance of the organism's Antioxidant protection.

- formula 5 - the category of systolic blood pressure increases due to the increase in the concentration of IL-6, Leukocytes and with minor effects of the concentration levels of the Total Protein, Se. That is, inflammatory factors of influence are decisive.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Olesya M. Horlenko
Uzhhorod National University
1 Narodna squ., 88000 Uzhhorod, Ukraine
e-mail: ohorlenko@gmail.com

ORCID AND CONTRIBUTIONSHIP
Olesya M. Horlenko: 0000-0002-2210-5503 Ⓐ Ⓓ Ⓕ
Lyubomyra B. Prylypko: 0000-0002-4131-5450 Ⓒ Ⓓ
Mykola V. Rishko: 0000-0002-9624-432X Ⓔ Ⓕ
Galyna M. Beley: 0000-0002-7715-2948 Ⓓ Ⓔ
Fedir V. Horlenko: 0000-0002-0496-2069 Ⓔ Ⓕ
Bohdan M. Halay: 0000-0002-7566-4982 Ⓑ Ⓔ
Lyubov A. Halay: 0000-0003-2833-5577 Ⓑ Ⓔ

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

ORIGINAL ARTICLE

A differentiated approach to the diagnosis of overweight and obesity in children based on bioimpedance analysis of body composition

Olga S. Palamarchuk, Myroslav M. Leshko, Vladyslav O. Klushyn, Volodymyr P. Feketa
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

Aim: The current study introduces a novel diagnostic algorithm employing bioimpedance analysis to comprehensively evaluate body composition in children, assessing fat content, skeletal muscle content, and fat distribution.

Materials and Methods: Bioelectrical impedance measurements were obtained using the TANITA MC-780 MA analyzer. Indicators such as body weight, BMI, total fat content, absolute limb muscle mass, skeletal muscle strength, and waist-to-hip ratio (WHR) were assessed. A sample of 101 children aged 9 to 14 were studied using the proposed algorithm, refining BMI-based classifications.

Results: The algorithm comprises three steps, categorizing children based on fat content, presence of sarcopenia, and central fat distribution. It identified diverse somatotypes within the groups classified by BMI. Notably, it revealed prognostically unfavorable somatotypes, such as sarcopenic obesity with central fat distribution, highlighting potential health risks. Current BMI-centric diagnoses may misclassify cardiometabolic risks, making early detection challenging. The algorithm enables a detailed evaluation, unmasking metabolically unfavorable conditions like sarcopenic obesity. The incorporation of functional tests, such as a standardized hand-grip test, enhances diagnostic accuracy. The proposed WHR indicator for characterizing fat distribution provides a practical method for determining somatotypes in children.

Conclusions: This comprehensive algorithm offers an alternative to BMI-based classifications, enabling early detection of obesity and associated risks. Further validation through large-scale epidemiological studies is essential to establish correlations between somatotypes and cardiometabolic risks, fostering a more nuanced and individualized approach to pediatric obesity management.

KEY WORDS: childhood obesity, body composition analysis, sarcopenic obesity, somatotypes, cardiometabolic risk

INTRODUCTION

Over the past several decades, the prevalence of obesity has been constantly increasing in most countries of the world, including Ukraine. Obesity has become an epidemic and is one of the main causes of morbidity and mortality in this century [1]. Moreover, this epidemic affected not only the adults, but also children [2]. The main reasons for this growth are considered to be overeating, hypodynamia, the negative impact of environmental and social factors, and an increase in the number of stressful situations characteristic of modern civilization [3]. Obesity is a risk factor for morbidity and mortality due to its association with such pathological conditions as type 2 diabetes, cardiovascular disease, non-alcoholic fatty liver disease, dyslipidemia, non-alcoholic fatty liver disease, stroke, bone and joint disorders, obstructive sleep apnea syndrome, sleep apnea syndrome and certain types of cancer [4, 5]. Childhood obesity is also a persistent, epidemic, international

problem, as obesity in childhood is a predictor of future serious disorders in adulthood [6].

Although obesity is defined as an excess of adipose tissue, the most used clinical tool for diagnosing obesity in children, adolescents, and adults is the body mass index (BMI), since direct measurement of body fat content is rarely available in routine clinical practice. However, in children and adolescents, it is impossible to establish absolute ranges of BMI, as it is done in the diagnosis of overweight and obesity in adults, because the distribution of BMI in children changes significantly with age and sex [7,8]. Therefore, according to the latest methodological recommendations of the American Academy of Pediatrics [9], children and adolescents aged 2 to 18 years are diagnosed with overweight if the BMI is ≥ 85th percentile and < 95th percentile of this indicator ranked by age and sex, and obesity - if BMI is ≥ 95th percentile. At the same time, epidemiological studies show that the use of BMI to diagnose obesi-

ty in the pediatric population misses approximately 25% of obese children and misclassifies a significant proportion of children as overweight and obese due to increased skeletal muscle and other components of lean body mass [10-13]. All this indicates that BMI cannot be considered a reliable marker of overweight and obesity, as it does not provide information on the content and distribution of fat and skeletal muscles in the body. Precisely this information makes it possible to reliably identify the presence of obesity in the child's body and establish its type, in particular - sarcopenic obesity. Sarcopenic obesity occurs at the background of reduced content of skeletal muscles even with a normal body weight [14]. The gold standard for body composition analysis is dual energy X-ray absorptiometry (DXA) [15]. However, it was not widely used in clinical practice due to its high cost and low availability for primary care physicians. This problem can be solved by the widespread use and introduction into clinical practice of a relatively affordable bioimpedance method of assessing body composition, which allows determining not only the content of fat and fat-free mass, but also of the content of skeletal muscles, intra- and extracellular water, bone tissue and visceral fat level. The latest generations of impedance bioanalyzers are even capable of evaluating the component composition of individual regions of the body, in particular of the trunk, upper, and lower extremities. But the information value of individual indicators of body composition and their combinations in the diagnosis of various types of obesity remains insufficiently elucidated. The determination of obesity-associated cardiometabolic risk is also poorly understood.

AIM

The purpose of this study is to justify a new algorithm for diagnosing obesity in children based on a comprehensive analysis of the component composition of the body, obtained by bioimpedance examination.

MATERIALS AND METHODS

The primary indicators necessary for the implementation of the presented algorithm were obtained by the method of impedance measurement using a bioelectrical impedance analyzer "TANITA MC-780 MA" (Japan). Measurements were carried out according to the standard protocol in the standing position of the patient. The children were dressed in sports suits, the weight of which was measured in advance and entered into the device in order to correct the measured body weight. 8 electrodes were used (4 circular and 4 manual electrodes). To obtain accurate results, the examinees stood with their bare feet on the four circular electrodes of the device and held the manual electrodes in their palms with their arms down.

The following indicators were determined: body weight (M, kg), body mass index (BMI, kg/m^2), total fat content (BF, %), absolute limb muscle mass (AMSM, kg), which was obtained by summing the muscle content of all 4 limbs. To correct ASMM according to anthropometric data, the percentage of the sum of the absolute muscle mass of all limbs to body weight was calculated using the formula ASMM%=AMSM/M x 100. Height (L, m) was measured using a GIMA (Italy) height meter. As an additional parameter for sarcopenia detection, skeletal muscle strength was measured using a standardized hand isometric test using a Handexer Grip Strength Tester digital hand dynamometer (USA). Grip strength (F, kg) was determined in a sitting position for the leading arm, while the humerus was located on the side of the body, and the elbow was bent at 90 degrees [16]. For each test, the study participants were asked to squeeze the dynamometer with maximum effort for two to three seconds. Participants performed three consecutive sets with a few seconds of rest between each trial. The grip strength of the leading hand was measured in three attempts, and the best result of these three attempts was recorded.

To determine the type of fat distribution in the body of the examinees, the ratio of waist circumference (W) to hip circumference (H), known as WHR (waist to hip ratio), was determined [17]. Children's waist and hip circumferences were measured in centimeters using a non-stretchable anthropometric tape in a standing position with arms spread out to the sides and legs close together. Waist circumference was measured midway between the lower border of the chest and the upper border of the iliac crest at the end of normal expiration. Thigh circumference was measured at the widest part of the thighs, at the level of the greater trochanter.

To test the diagnostic algorithm, 101 children aged 9 to 14 (54 girls and 47 boys) who underwent 14-day rehabilitation at the Transcarpathian Regional Children's Sanatorium "Malyatko" were involved in the study. All children at the time of examination were healthy according to clinical examination and physical examination. Exclusion criteria were the presence of genetic syndromes associated with obesity, endocrine disorders leading to obesity, medical obesity, and neuromuscular pathology affecting muscle quality and content. The study was conducted in compliance with the basic bioethical norms of the Helsinki Declaration adopted by the General Assembly of the World Medical Association on human rights, the International Code of Medical Ethics and the laws of Ukraine.
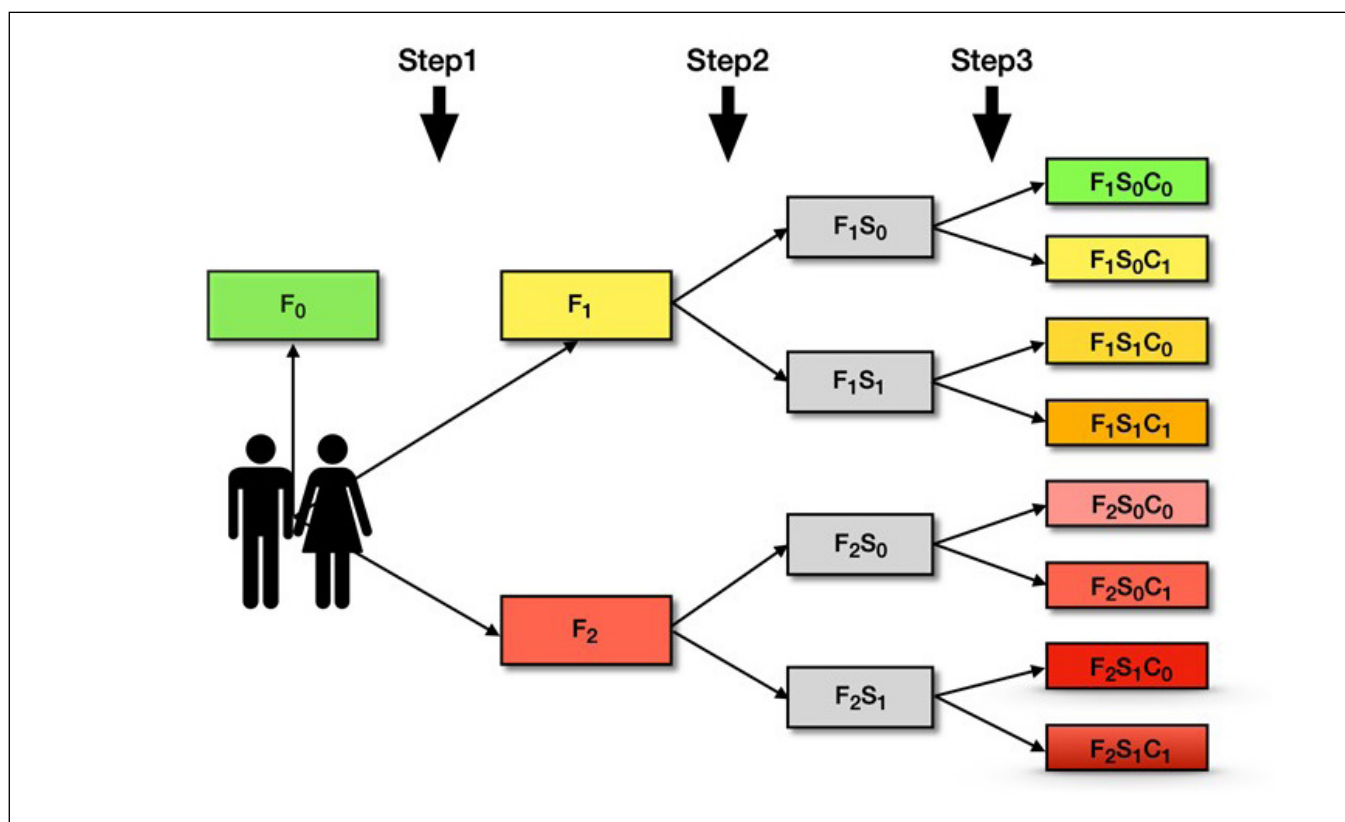
Fig.1. The diagram illustrating the algorithm for determining the somatotype according to the FSC system.
Somatotypes with the hypothetically highest risk of comorbid pathology are marked red on the diagram, those with a moderate risk are marked yellow and those with minimal risk are marked green. The shade of each color indicates the intensity of the risk.

RESULTS

1. The algorithm for diagnosing overweight and obesity.

The diagnostic algorithm presented by us includes 3 steps:

1st step: The distribution of children into 3 groups according to the BF% indicator:

F_0 - with normal fat content;

F_1 - with increased fat content;

F_2 - with obesity.

The criterion for assigning children to these groups is the percentile distribution of fat content considering the age and gender of children according to the National Health Statistics Reports [18]. The F_0 group included children with BF% in the 15-85th percentile range; the F_1 group included children with BF% in the 85-95 percentile range; The F_2 group included children with a BF% above the 95th percentile.

2nd step: The distribution of children of each of these groups into 2 sub-groups according to the ASMM% criterion:

S_0 - absence of sarcopenia;

S_1 - presence of sarcopenia;

The criterion for assigning children to subgroup S_0 was the simultaneous finding of the ASMM% indicator above the 20th percentile in the reference curve of the percentile distribution of this indicator, considering the age and gender of the children according to McCarthy et al. [19], and the F indicator above the 10th percentile according to the reference curve of the percentile distribution of this indicator, considering the age and gender of children according to Dodds et al. [20]. Subgroup S_1 included children with ASMM% and F indicators that were below the 20th and 10th percentile in the respective reference curves at the same time.

3rd step: The distribution of children of each of the subgroups selected at the previous stage into 2 subgroups according to the WHR indicator:

C_0 - lack of the central type of fat distribution;

C_1 - the presence of the central type of fat distribution;

The criterion for assigning children to subgroup C_0 was finding the WHR indicator below the 90th percentile in the reference curve of the percentile distribution of this indicator, considering the age and gender of the children according to Kułagay et al. [21], and C_1 is above the 90th percentile of this distribution.

The block diagram of the proposed diagnostic algorithm is presented in Fig. 1.

2. Evaluation of the diagnostic algorithm on a sample of examined children.

The entire sample of examined girls and boys was

Table 1. Distribution of the examined subjects into somatotypes according to the proposed algorithm

Girls (n=54)			
Group 1 (n=28) Excessive body weight		Group 2 (n=26) Obesity	
Somatotype	Quantity	Somatotype	Quantity
$F_1S_0C_0$	6	$F_1S_0C_1$	8
$F_1S_0C_1$	4	$F_2S_0C_0$	7
$F_1S_1C_0$	6	$F_2S_0C_1$	6
$F_1S_1C_1$	5	$F_2S_1C_0$	4
$F_2S_1C_0$	4	$F_2S_1C_1$	1
$F_0S_0C_1$	3		
Boys (n=47)			
Group 1 (n=25) Excessive body weight		Group 2 (n=22) Obesity	
Somatotype	Quantity	Somatotype	Quantity
$F_1S_0C_0$	14	$F_1S_1C_1$	10
$F_1S_0C_1$	5	$F_2S_0C_0$	5
$F_1S_1C_0$	3	$F_2S_0C_1$	4
$F_1S_1C_1$	1	$F_2S_1C_0$	2
$F_2S_1C_0$	2	$F_2S_1C_1$	1

divided into two groups according to BMI. The first group included 28 girls and 25 boys with a BMI that met the criteria of overweight, and the second group included 26 girls and 22 boys with a BMI that met the criteria of obesity according to the American Academy of Pediatrics [9]. In each of these groups, subgroups with a different combination of BF%, ASMM% and WHR indicators were determined according to the algorithm presented above. The distribution of children in these subgroups is shown in Table 1.

It was notable that significant heterogeneity of somatotypes identified using our proposed algorithm was observed in all groups of examined girls and boys formed according to the criteria of overweight and obesity based on BMI. The largest number of somatotypes (6) was found in the group of overweight girls. 5 different somatotypes were detected in all other groups of examinees. In group 1, out of 28 girls with excess body weight, only 21 girls had excessive fat content according to the BF% indicator; 4 of them were diagnosed with obesity, and in 3 girls this indicator was within the normal range. In the second group of 26 girls, obesity according to the BF% indicator was confirmed only in 18 girls. The rest were diagnosed only with excessive fat content, and their high BMI was explained by a relatively high skeletal muscle content. In boys from group 1, excessive fat content was confirmed in 23 out of 25 subjects. Two boys from this group had the value of BF%, which corresponds to the diagnosis of obesity, which led to only a moderate increase in

BMI on the background of sarcopenia. In 10 boys of the second group, excessive fat content was found, despite high BMI values. This is probably explained by their relatively high skeletal muscle content. The remaining 12 boys of this group had real obesity according to the BF% indicator, and in 3 of them the obesity was on the background of sarcopenia.

It should be emphasized that thanks to our algorithm, it was possible to identify prognostically unfavorable somatotypes characterized by sarcopenia with a central type of fat distribution. These are somatotypes $F_1S_1C_1$ and $F_2S_1C_1$. A total of 8 subjects (7.92% of the sample) had these somatotypes.

DISCUSSION

Although the research into all aspects of childhood obesity has made significant progress in recent decades, BMI is still the main criterion for diagnosing overweight and obesity in children and adults. At the same time, many authors express doubts about the validity of the BMI as a diagnostic tool. Epidemiological studies analyzing the degree of misdiagnosis of overweight and obesity in the pediatric population using BMI suggest that the use of this indicator increases the likelihood of underestimating cardiometabolic risk in children and adolescents [19, 20]. Zapata, J.K et al. [21] report that 7% of children and adolescents classified according to BMI as having a normal body weight and 62% of children classified as having an excessive body

weight were diagnosed with obesity according to the total fat content (BF%). On the other hand, 2% and 4% of children and adolescents with BF% in the normal or excess fat range, respectively, were misclassified as obese by the BMI criterion. According to Javed A. et al [18], the sensitivity of BMI for detecting severe obesity is only 73–82%. This suggests that more than a quarter of children who are not classified as obese by BMI may actually be obese.

The results of our study are fully consistent with the above-mentioned publications. We demonstrated that BMI masks metabolically unfavorable sarcopenic obesity in children with excessive body weight and, conversely, falsely diagnoses obesity in children with increased skeletal muscle mass. Our results indicate a significant risk of misdiagnosing overweight and obesity in routine clinical practice using BMI-based classifications. This, in turn, does not allow the timely detection of children with increased cardio-metabolic risk. We see a solution to this problem in the widespread use and introduction into clinical practice of a relatively affordable bioimpedance method for determining body composition with a mandatory assessment of not only fat content, but also skeletal muscle content. The latter is especially relevant in the context of a new, previously unknown pediatric problem - sarcopenic obesity. While previously sarcopenia was considered to reflect the age-related reduction of striated muscle tissue in the elderly, according to recent studies, it is also relevant for children [14]. In both adults and children with sarcopenic obesity, BMI masks the cardiometabolic risks associated with this condition and makes early diagnosis difficult. When assessing the muscle content from the point of view of the presence of sarcopenia, we consider it necessary to supplement the patient's examination with functional tests, particularly with a standardized hand-grip test. The diagnosis of sarcopenia should be made only in case the low index of appendicular muscle mass is confirmed by the low indicators of this test.

Finally, the third key parameter that we consider in the presented algorithm for the diagnosis of somatotypes is the distribution of fat in the body. It is well known that the concentration of fat in the upper half of the body (so-called central obesity) is more dangerous for health than fat accumulation in the subcutaneous tissue of the lower half. A particularly dangerous component of central fat is visceral fat, which causes a much greater pathogenetic effect on metabolic processes than subcutaneous fat [23]. The simplest, but at the same time sufficiently informative indicators of the central type of fat distribution are waist circumference and its relation to hip circumference (waist to hip ratio or WHR). They have been shown to correlate well with obesity-related morbidity and mortality [24]. We propose to use precisely the WHR to characterize the distribution of fat in the body, which determines the somatotype. Literature contains many sources with reference data regarding this indicator for all age categories, which can be used to determine the somatotype according to our method [25].

CONCLUSIONS

In our opinion, a comprehensive approach to determining the somatotype of children with simultaneous consideration of all three most important factors of its formation - fat content, skeletal muscle content, and fat distribution in the body - allows to detect obesity in the early stages, to give a scientifically-based forecast regarding the risks associated with comorbid pathology, and to develop individual programs for correcting the patient's somatotype. The presented diagnostic algorithm needs to be validated by establishing the relationship between each somatotype we selected and cardiometabolic risk. This is possible only with large-scale epidemiological studies in cooperation with specialists of various medical specialties. We hope that our approach will be received positively by them.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Olga S. Palamarchuk
Uzhhorod National University
1 Narodna sq., 88000 Uzhhorod, Ukraine
e-mail: olga.palamarchuk@uzhnu.edu.ua

ORCID AND CONTRIBUTIONSHIP
Olga S. Palamarchuk: 0000-0002-9742-1906 ⒷⒹⒺⒻ
Myroslav M. Leshko: 0009-0005-5441-6292 Ⓑ
Vladyslav O. Klushyn: 0009-0001-8145-8528 Ⓑ
Volodymyr P. Feketa: 0000-0002-4951-4040 ⒶⒸⒺ

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

ORIGINAL ARTICLE

Necrotizing enterocolitis in premature infants at different gestation ages

Kateryna Doikova[1], Michael Jerdev[1], Larysa Koval[2], Dmytro Valantsevych[3]
[1]BARUCH PADEH MEDICAL CENTER, PORIA, ISRAEL
[2]ODESA NATIONAL MEDICAL UNIVERSITY, ODESA, UKRAINE
[3]COMMUNAL NONPROFIT ENTERPRISE "CITY CLINICAL HOSPITAL №11" ODESA CITY COUNCIL, ODESA, UKRAINE

ABSTRACT

Aim: To compare X-ray signs in different gestational and body weight groups of patients with NEC.

Materials and Methods: We conducted a retrospective study, enrolling 52 preterm newborns with symptoms of NEC regardless of onset time, who underwent treatment at Neonatal Intensive Care Units in Municipal Non-commercial enterprise "City Children Hospital №2", Odesa. The patients were split into 3 clinical groups: very preterm newborns (VPN), moderately preterm newborns (MPN), and moderately preterm newborns with intrauterine growth restriction (MPN+IUGR).

Results: In the VPN group NEC was diagnosed at stage II (58,82±12,30) % and III (41,18±12,30) % by Bell MJ, p>0,05. In the group MPN+IUGR, NEC stage II (33,33±14,21) % and stage III (66,66 ±14,21) %, p>0,05, were equally observed. In the MPN group, NEC was diagnosed at stage I (41,67±10,28) % and II (58,33±10,28) %, p>0,05, without prevalence of any. Also only localized forms were observed. In VPN, we observed localized forms in most cases, while diffuse forms were diagnosed in (11,76±8,05) % cases, p<0,05. In the MPN+IUGR group, we found diffuse form of the NEC in half of the cases – (50,00±15,08) %. In the VPN and MPN+IUGR groups, NEC developed at 13,23±0,39 and 14,33±1,19 days, respectively. However, in MPN without IUGR, NEC developed at 17,75±0,55 days, significantly later than in the MPN+IUGR group, p<0,05.

Conclusions: We have described distinct features of NEC in MPN with IUGR. Compared to MPN without IUGR, NEC had more severe course and earlier manifestation in such neonates.

KEY WORDS: preterm infants, abdominal radiographs, low birth body mass

INTRODUCTION

Necrotizing enterocolitis (NEC) is one of the life-threatening conditions in newborn infancy. NEC treatment is one of the most complex and crucial for better outcome in Neonatal Intensive Care Units (NICU) [1,2].

Incidence rate of NEC in preterm infants population is significantly higher compared to full-term [3]. It is typical in NEC that the lower gestational age and birth body mass, the latter is the expected onset, higher complications rates and worse outcome [4]. Reviewed literature provides that gastrointestinal perforations incidence rate in newborns is at 20-30% [5]. Mortality rate and spread of this disease appear to be increasing in the last decades, mostly due to increase of underweight preterm babies in the population [6]. Incidence rate is reported at the level of 1-5% in all NICU patients and 5-10% for extremely low birth weight newborns [7]. Clinical onset often appears asymptomatic [8]. The mortality rate of NEC in newborns varies and is estimated to be 10-60% [9-12].

The problem of estimating probability of NEC development, early and timely diagnosis became even more significant with introduction of governmental program for protection and care of newborns with very low and extremely low birth weight in Ukraine [13].

The distribution of NEC incidence rate in the world is mostly even and is about 2-13% in newborns with very low and extremely low birth weight [4]. For instance, multicentric studies in US and Canada reported average incidence of 7% in newborns with birth weight 500-1500 g [12]. This significant variation can be attributed as much to nutritional protocols in different countries as to different survival rates of newborns from this group across centers within the same country.

NEC can be divided into following categories by triggering mechanisms:
- Posthemotransfusion – onset is typically within 48 hours after erythrocytic mass transfusion.
- Triggered by lactose or cow protein intolerance in bottle-feeding newborns.
- Triggered by bacteria [14].
- Triggered by viral infection.
- NEC because of hypoxia or ischemia longer than 72 hours.

Fig. 1. Chest and abdominal X-ray, upright. Localized gastric substage of NEC. Neonate 11d, birth body weight 1600 g. 30 weeks gestation, VPN group (Oxygen dependent. Endotracheal tube, Nasogastric tube).
Right upper lobe atelectasis. Endotracheal tube at carina level. Bilateral hyperventilation. Flattening of right hemidiaphragm, right lateral sinus partially opaque. Left lateral sinus deepened due to slightly elevated left hemidiaphragm. Nasogastric tube is touching gastric wall at major curvature. Gastric wall at fundus is noticeably thickened with intramural gas shown as a linear translucency (arrows). Intestinal loops are located atypically, intermittent pneumatosis and reduced pneumatization. Liver somewhat rounded with inferolateral margin round.

– NEC resulting from cold stress in preterm babies with body weight less than 2 kg.
– NEC in twins.
– Triggered by gastroschisis.
– Related to congenital heart diseases and anomalies.
– Related to other congenital diseases and anomalies.
We would also like to emphasize that NEC is not a single disease but a collective term covering multiple pathomorphologically similar diseases with different pathogenesis [5].

In general, all factors that cause blood circulation centralization and/or disruption of blood flow in superior mesenteric artery system regardless of development mechanisms favor the NEC development, such as:
– Intrauterine fetal stress and asphyxia.
– Maternal drug addiction (especially cocaine).
– Congenital immunodeficiency in newborns.
– Gramm-negative bacteria translocation through mucinous membrane of intestines, resulting in ischemia and intestinal wall necrosis.

In clinical setting, when estimating probability of NEC, it is determining to consider gestational under-development of preterm intestines, circulation centralization (not necessarily leading to mesenteric steal syndrome), intestinal wall oxygenation pathologies, which may also be caused by preserving intrauterine features of intestinal circulation (immature circulation) [4, 5].

Timely medical imaging is a significant factor for improvement clinical course prognosis since radiological symptoms appear earlier than clinical. In turn, this allows for early feeding adjustments and timely therapy start [8].

Everything stated above transform the task of setting a timely and validated diagnosis of NEC in the NICU setting into a multiparametric problem. It requires a systemic and interdisciplinary team approach. In the case of our hospital, collaboration of neonatologists, pediatricians and radiologists was essential.

AIM
The aim of this study is to compare X-ray signs in different gestational and body weight groups of patients with NEC.

MATERIALS AND METHODS
We conducted a retrospective study, enrolling 52 preterm newborns with symptoms of NEC regardless of onset time, who underwent treatment at NICU in Municipal Non-commercial enterprise "City Children Hospital №2", Odesa in the period 2014-2022 years. The included 52 patients (males (48,08±6,93) %, females (51,92±6,93) %) were split into 3 groups (Table 1). Entry criteria: Gestational age 29-36 weeks (dated by antenatal ultrasound or clinically). We excluded the patients with radiological signs suspicious of NEC without further clinical confirmation or development from this work. Other exclusion criteria include: lethal congenital anomaly, twin-twin transfusion, significant multi-organ failure prior to trial entry, symmetric IUGR.

Imaging performed using X-ray machine Multimobile 2.5 (Siemens LTD, India, Germany), mobile X-ray system ULTRA 200A (EcoRay, Seoul, South Korea). In order to ensure proper patient positioning, reduce patient

Table 1. Clinical groups

Clinical group	Number of patients (n, %)
Very preterm newborns (VPN) (29-32 weeks) with very low birth body mass (<1500 g)	16, (32,08±6,41) %
Moderately preterm newborns (MPN) (33-36 weeks) with low birth body mass (<2500 g)	24, (45,28±6,84) %
Moderately preterm newborns (33-36 weeks) with intrauterine growth restriction (MPN+IUGR)	12, (22,64±5,75) %

Table 2. Clinical characteristic of patients

Measures Groups	Gestation, weeks (M±m)	Weight, g (M±m)	Onset of NEC, days (M±m)
VPN, (n=16)	30,52±0,23	1358,82±16,10[1]	13,23±0,39[1]
MPN, (n=24)	33,91±0,190	2010,01±56,44[1,2]	17,75±0,55[1,2]
MPN+IUGR, (n=12)	34,16±2,84	1471,16±122,63[2]	14,33±1,19[2]

[1] – the significance of difference between groups VPN and MPN: $p<0,001$, [2] – the significance of difference between groups MPN and MPN+IUGR: $p<0,01$.

Table 3. Radiological changes structure in preterm neonates with NEC

Stage by Bell MJ	Substage		Number of patients (n, %)
Neonates from the VPN group			
Stage II	Localized	Gastral	3 (17,65±9,53) %
		Intestinal	5 (29,41±11,39) %
		Multiple segments	2 (11,76±8,05) %
Stage III	Localized	Gastral	4 (23,53±10,60) %
		Intestinal	1 (5,88±5,86) %
	Diffuse		2 (11,76±8,05) %
Neonates from the MPN group			
Stage I	Localized	Intestinal	10 (41,67±10,28) %
Stage II	Localized	Intestinal	12 (50,00±10,43) %
		Multiple segments	2 (8,33±5,76) %
Neonates from the MPN+IUGR group			
Stage II	Localized	Multiple segments	4 (33,33±14,21) %
Stage III	Localized	Multiple segments	2 (16,67±11,24) %
	Diffuse		6 (50,00±15,08) %

dose, protect staff from radiation, and enhance X-ray unit productivity, we utilized the Oniko (ONIKO Ltd, Ukraine) X-ray patient stand, equipped with a special holder for children up to 1 year old. This table facilitates the upright suspension of children without causing any strain on the body. Additionally, we designed a custom plastic insert to enhance limb security, improved skin protection with a soft cotton surface, and included additional spine and body supports. The custom plastic base is then placed in foam rubber-filled holders on a metal base with adjustable radiation protection covers. This device enables safe upright diagnostic imaging for children under 1-2 years old, weighing less than 15 kg, with all body parts securely positioned and fixed without harm to the patient.

All newborns also underwent abdominal ultrasound to evaluate mesenterial circulation, and suspected in-testinal ischemia, infiltrative changes, ascites, pneumatosis portalis, which was not reflected in this paper. For image reporting, we used modified NEC classification by Bell MJ [15].

All newborns admitted to NICU had a chest and abdominal X-ray performed during the first 24 hours. The imaging was repeated at least after 6 hours from the initial imaging for those newborns with indications. In the case with erythrocyte mass transfusion, all patients underwent an abdominal X-ray after 24 hours regardless of symptoms.

Other cases when abdominal X-ray was performed:

- in cases when one of the twins/triplets had symptoms of NEC, imaging of apparently healthy child (children) was obligatory
- in patients with congenital heart pathology during clinical worsening or deterioration
- in bottle-fed newborns preventively
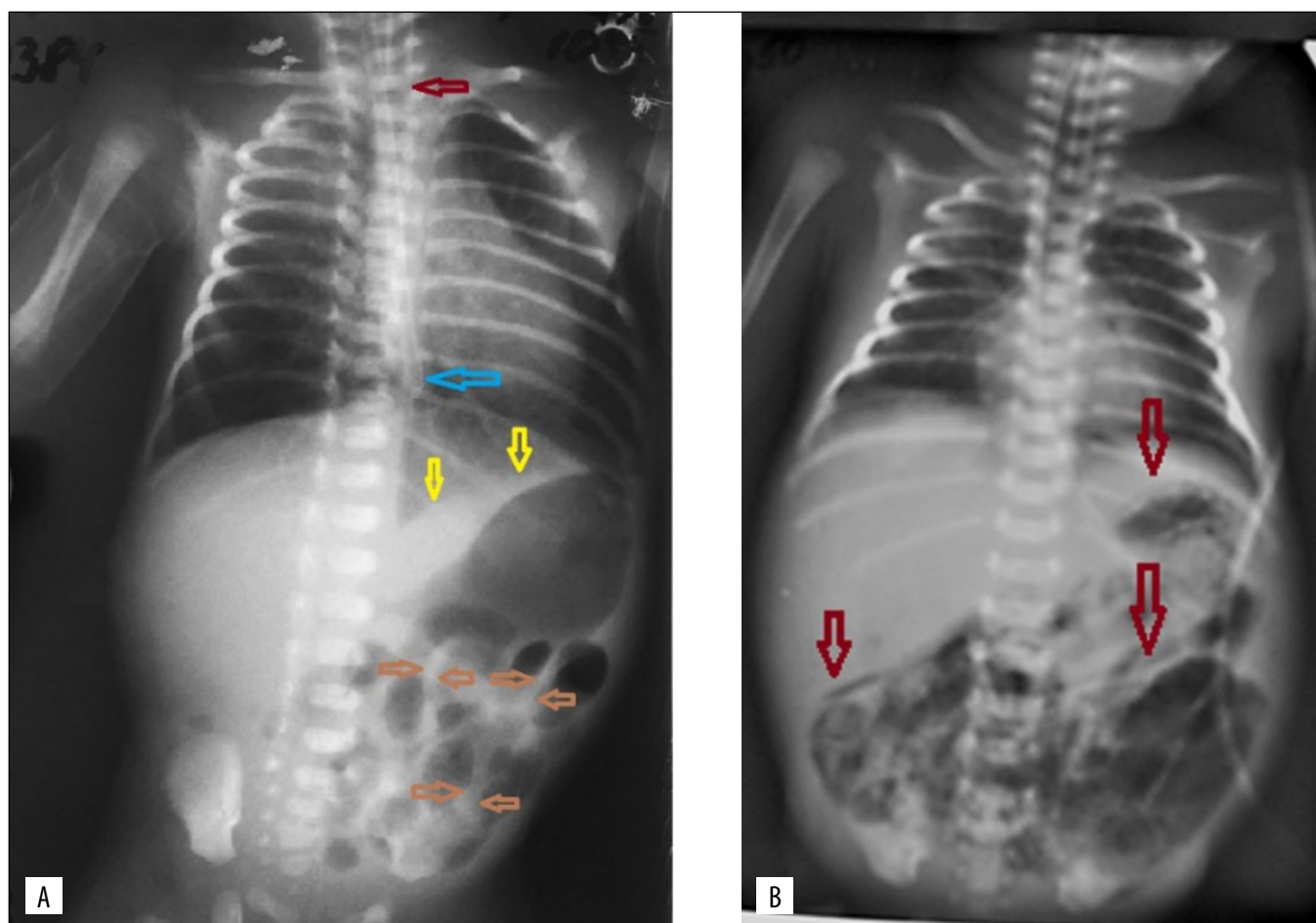
Fig. 2. A. Chest and abdominal X-ray, upright, slight rotation to the left. Suspected NEC Neonate 15d. Suspected NEC. Neonate 15d, body birth weight 1350 g, 32 weeks gestation. VPN group.

Endotracheal tube (red arrow) is located distally. Bilateral hyperventilation: posterior-basal margin of left lung (yellow arrows) is moved caudal, causing not enlarged spleen to visualize significantly lower. Tip of nasogastric tube (blue arrow) is located above cardia in esophagus. Gastrointestinal hyperpneumatosis. Some spaces between loops are widened. Intestinal walls are thickened on significant duration of intestine (brown arrows). The liver has a rounded shape, slightly enlarged.

On cardiac ultrasound: perimembranous ventricular septal defect, open oval window, open arterial duct (not shown). Clinical presentation: NEC symptoms, remittent fever, diffuse intravascular coagulation, bloody stool. No lab results abnormalities.

B. Chest and abdominal X-ray, upright. Diffuse form of NEC. (Same patient as in figure 2A) 35 days (as image F-G). VPN group.

Endotracheal tube, Nasogastric tube. Diffuse hyperpneumatosis of the stomach and intestines. Linear radiolucencies are caused by intramural gas (red arrows). The liver is enlarged and has a rounded shape.

- in patients with umbilical catheterization longer than 3 days, within the first 24 hours after its removal regardless of symptoms.

To compare patient characteristics, we determined distribution types and applied appropriate descriptive statistics methods. We determined the arithmetic mean (M), the arithmetic mean error (m), and parametric Student test with a p-value <0.05 was considered statistically significant. The statistical analyze was performed with GNU Project (2015) (GNU PSPP (Version 0.8.5) [Computer Software]. Free Software Foundation. Boston, MA).

This study was conducted in accordance with the principles of the Helsinki Declaration of the World Medical Association "Ethical principles for medical research involving human subjects". The informed consent was taken from parents and guardians of all children involved in the study.

RESULTS

Results evaluation revealed a significant discrepancy in clinical course of NEC in neonates from the MPN+IUGR group. Compared to the neonates without IUGR, children from this group had a much earlier onset, similar to the VPN group (Table 2).

Further analysis of radiological signs of NEC in different clinical groups revealed several features. In the VPN group, radiological picture was of the stage II (58,82±12,30) % (Fig. 1., Fig. 2. a, b) and III
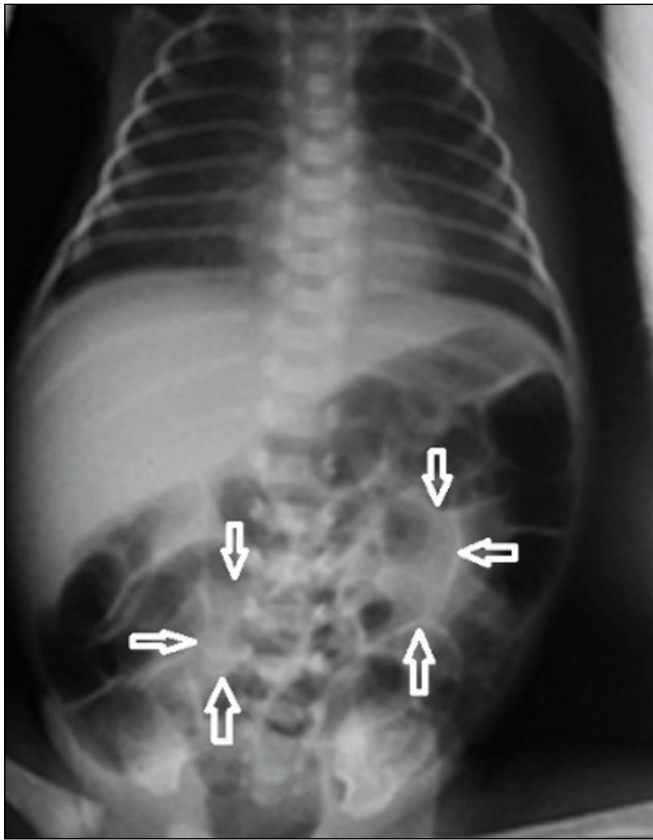
Fig. 3. Chest and abdominal X-ray, upright. Suspected NEC. Neonate 14d, birth body weight 1350 g, 33w gestation age. First of the triplets. MPN+IUGR group
Intestinal pneumatosis. Local widening of interloop spaces caused by fluid collection (arrows). No signs of hepatosplenomegaly.

(41,18±12,30) % by Bell, p>0,05. Moreover, localized substages with isolated gastric (Fig. 1.) and intestinal involvement were observed in a similar number of patients (Table 3).

In the MPN group, imaging symptoms typical for stage I and II were observed equally (41,67±10,28) % and (58,33±10,28) %, respectively p>0,05, with predominantly localized intestinal substage. Multiple segments involvement was observed only in 2 cases (Table 3).

As with the VPN group, children from MPN+IUGR group (Figure 3) also had prevalence of stages II and III by Bell, although type III was observed more often (66,66 ±14,21) % compared to stage II (33,33±14,21) %, p >0,05. From distinct features, we found that localized substage of NEC manifested with multiple segments involvement only (Table 3).

Another distinctive feature in the MPN+IUGR group was that half of the patients (50,00±15,08) % had a diffuse stage III clinical presentation (Fig. 4. a-d), which was significantly more often than in the VPN group (11,76±8,05) %, p<0,05, (Fig. 2. a, b). In the MPN group similar radiological presentation was not observed (Table 3).

DISCUSSION

Our study focused on researching NEC presentation with IUGR. Majority of authors, who studied NEC, considered IUGR as a risk factor for NEC in preterm neonates [15]. In our study however, we stress the distinct features of NEC clinical course and radiological features in neonates from this group.

Our colleagues research shows that most NEC cases in preterm neonates were observed at stage III by Bell, considering all gestational age groups, extremely low birth weight included [16]. In our study we separately observed children with NEC and different grades of prematurity. A distinct feature of NEC clinical manifestations in the MPN group is absence of cases with stage III by Bell, while in children with the same gestational age and IUGR, such patients represented half of the cases (50,00±15,08) %. To some extent our research is supported by paper by Hassan et al., who concluded that low birth weight in preterm neonates is a risk factor for NEC [17]. The clinical onset we found is similar to that from referenced sources. In particular it was reported to be on 14.44 (4.75–21.25) day with average gestation age 33.06 (30.25–36.14) [18]. However, different median of 22 days [16] with gestation age median at 33 weeks [19] was also reported. Overall, onset estimation in different sources is characterized by a marked variability and typically consider all preterm neonates regardless of the prematurity grade. Our research has shown that depending on gestation ages, there is a difference in NEC onset time as much as clinical symptoms. Considerations for IUGR also have demonstrated that NEC clinical manifestations were different from a similar patient group without IUGR as well.

The methodological limitations of the current study include relatively small size of the clinical groups. Thus, we were unable to consider neonates with congenital heart anomalies separately.

As a future perspective we consider studying radiological features of NEC in full-term neonates with normal birth weight and IUGR.

CONCLUSIONS

1. NEC in children from VPN and MPN+IUGR groups manifested in a similar timeline at 13,23±0,39 and 14,33±1,19, p >0,05 days, respectively.
2. In VPN and MPN+IUGR groups, radiological findings were represented exclusively by stages II and III by Bell, while in the MPN group – by stages I and II.
3. In MPN group, NEC manifested with only localized substage. In MPN+IUGR group, a diffuse stage III cases were observed more often than in the VPN group.
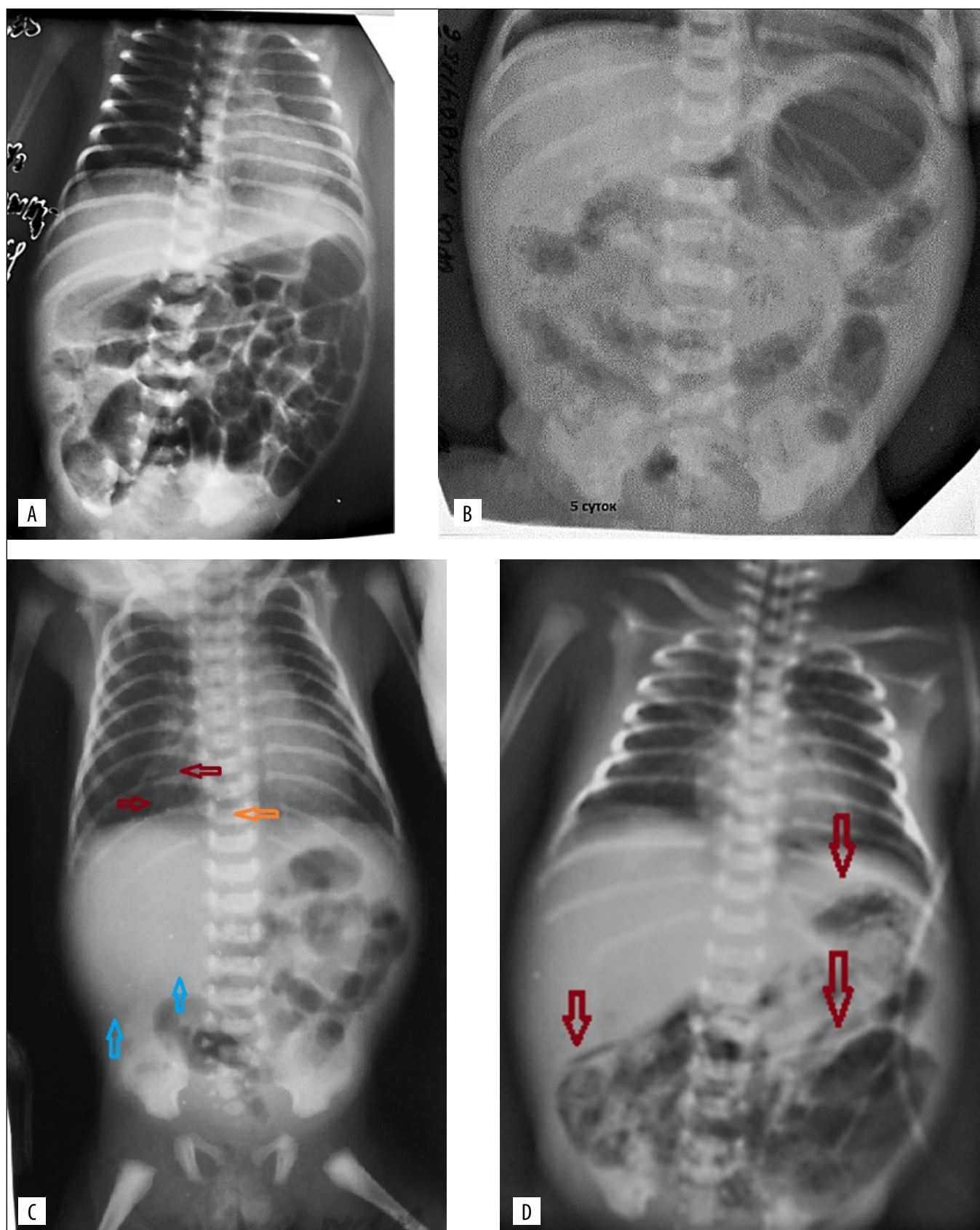
Fig. 4. A. Chest and abdominal X-ray, upright. Suspected NEC. Neonate 7d, birth body weight 1120 g, 33w gestation age, first of the twins. Received parenteral feeding. MPN+IUGR group.

Intestinal hyperpneumatosis. Small and large bowel proportion is changed. Colon may not be followed in all parts; certain levels of displacement and haustration can be seen. B). Abdominal X-ray, upright. Diffuse form of NEC, Pre-perforation phase. Neonate, 15 d (Same patient as in figure 3A). MPN+IUGR group.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Larysa Koval
Odesa National Medical University
2 Valikhovsky Lane, 65000 Odesa, Ukraine
e-mail: larikuk87@gmail.com

ORCID AND CONTRIBUTIONSHIP

Kateryna Doikova: 0000-0001-9289-394X Ⓐ Ⓑ Ⓓ Ⓕ
Michael Jerdev: 0009-0001-6968-9622 Ⓐ Ⓔ Ⓕ
Larysa Koval: 0000-0003-4050-5954 Ⓒ Ⓓ
Dmytro Valantsevych: 0000-0002-3882-4529 Ⓒ Ⓔ

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

ORIGINAL ARTICLE

Effectiveness of bruxism treatment in young adults

Oksana V. Klitynska[1], Yurii M. Martyts[2], Viacheslav R. Gurando[1], Nataliya V. Layoch[1]
[1]UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE
[2]TERNOPIL NATIONAL MEDICAL UNIVERSITY NAMED AFTER I.YA. GORBACHEVSKIY, TERNOPIL, UKRAINE

ABSTRACT

Aim: This study aimed to assess the effectiveness of the developed algorithm for treatment and prevention measures aimed at eliminating clinical manifestations of bruxism in young people.

Materials and Methods: A cohort of 377 individuals aged 25 to 44 years underwent examination. Based on identified etiological factors, three distinct groups were delineated. Treatment and preventive strategies were then implemented and evaluated. Tailored treatment algorithms were devised for each group: Group one received selective grinding of supracontacts (up to 0.5-0.75 mm) and treatment for orthodontic issues utilizing removable and fixed orthodontic structures. Group two underwent finger self-massage of masticatory muscles, fabrication of biting dental splints, and anti-inflammatory drug therapy. Group three received sedative drug therapy in conjunction with psychiatric consultation, based on indications. Treatment efficacy was assessed 12 months post-initiation. Statistical analyses were conducted using Statistica 10.0 (StatSoft, Inc., USA) and Microsoft Office Excel 2010.

Results: In cases where orthodontic pathology and supracontacts predominated (r=0.99, p<0.05), employing selective grinding and orthodontic treatment according to specific indications yielded significant efficacy. This approach resulted in a notable reduction in bruxism severity, corroborated by occludogram results 12 months post-treatment initiation. Notably, 90.0% of occludogram indicators fell within the 90-100% range, accompanied by a decrease in the BruxChecker abrasion facets area (p<0.05). Further, there was a substantial enhancement in occlusal contacts (Ck=0.68, Ck =0.71, Ck =0.93). In instances where TMJ pathology predominated with high reliability (r=0.98, p<0.05), effective normalization of masticatory muscle tone and alleviation of temporomandibular joint issues were observed. After 12 months, palpation revealed minimal tenderness in specific muscle areas and normalization of electromyography readings from initial indicators (p<0.05). Moreover, when the psycho-emotional factor primarily contributed to bruxism etiology (r=0.97, p<0.05), medical intervention proved effective. This approach led to bruxism disappearance and normalization of the psycho-emotional state within 12 months (p<0.05).

Conclusions: The conducted studies provide high-confidence evidence of the effectiveness of bruxism treatment when diagnosing the prevailing etiological factor and targeting its specific impact, leading to the normalization of all other factors, a reduction in bruxism intensity, and its complete disappearance.

KEY WORDS: bruxism, young patients, occludogram, bioelectropotential of masticatory muscles, psychoemotional state

INTRODUCTION

Bruxism, a dental pathology characterized by teeth grinding during involuntary jaw clenching, stems from the involuntary contraction of masticatory muscles. Its prevalence ranges from 5% to 90% in adults and 10% to 50% in children, with observed decreases in individuals over 60 years old. Such discrepancies are explained by imperfect diagnosis, since the verification of the diagnosis is based on the subjective data of the questionnaire of states during sleep as the first symptoms of the disease. Often a manifestation of systemic disorders, bruxism results from psychological stress. As a rule, the first person to diagnose this pathology is a dentist. Locally, it disrupts natural teeth alignment, complicating dentition restoration. Scientific evidence underscores psycho-emotional instability as the primary factor triggering bruxism, alongside factors such as genetics, alcohol, tobacco, drug use, and specific medications [1-3].

Dental theories attribute bruxism to various dental structure and function abnormalities, encompassing bite pathologies, dentition anomalies, inadequately selected orthopedic and orthodontic structures, TMJ arthritis, dysfunctions, and post-traumatic maxillofacial changes. Diagnostic methods for bruxism include abrasion presence, BruxChecker assessments, occludograms, and evaluating masticatory muscle condition and functionality. Despite these methods, a lack of a defined examination and treatment algorithm results in ineffective interventions and pathology progression [4-6].

The appearance of this pathology is influenced by a number of reasons, therefore this disease is studied not only within dentistry, but also in psychology, neurology, otorhinolaryngology, gastroenterology [6].

Given the absence of a singular etiological origin for bruxism, a clear diagnostic and treatment algorithm remains elusive, underscoring the significance of this research.

AIM

This study aims to assess the effectiveness of a developed algorithm for treatment and preventive measures targeting the elimination of clinical manifestations of bruxism in young individuals.

MATERIALS AND METHODS

A total of 377 patients aged 25 to 44 years participated in this study, recruited from the Department of Therapeutic and Pediatric Dentistry at Ternopil State Medical University, Ministry of Health of Ukraine. Standard clinical dental examinations were conducted using a dental kit, and data were meticulously recorded in the "Dental Patient Examination Card" (form 043).

Clinical assessments involved the identification of pathological tooth abrasion types following Butan MG's methodology (2010). Additionally, examinations included palpation of masticatory muscles and TMJ, occludogram determination, and evaluation of occlusal contacts utilizing methods proposed by Flis P.S. (2007) and Khvatova V.A. (2005 [7] . Bruxism diagnosis employed BruxChecker, developed by Professor Sadao Sato (2005), allowing assessment of occlusal contact topography. The severity of bruxism, measured quantitatively, relied on the determination of abrasion facet areas following the methodology by D. V. Shershneva and M. I. Soicher (2015) [8]. Bioelectropotential analysis of masticatory muscles utilized the Bio EMG III electric myograph, enabling recording of craniofacial muscle activity during rest and function. This diagnostic tool aids in identifying maxillofacial muscle disorders and monitoring therapy effectiveness [9]. Functional analysis of the masticatory apparatus involved Slavicek R.'s occlusal index determination [9]. Assessment of patients' psycho-emotional states was conducted through administering the Eysenck questionnaire and J.Taylor's questionnaire (1953) of the Minnesota Multidimensional Personality Inventory focusing on anxiety scales. [10]

The study employed statistical analysis and cluster assessments to establish examination and treatment algorithms based on prevailing etiological factors within clinical groups. Treatment methods varied accordingly: the first group underwent selective grinding of supracontacts (0.5-0.75 mm) and received orthodontic treatment with removable and fixed structures as needed. The second group underwent finger self-massage of masticatory muscles, fabrication of biting dental

splints, and anti-inflammatory drug therapy. The third group received sedative drug therapy in conjunction with psychiatric consultation. Treatment effectiveness was assessed 12 months post-initiation, considering the lengthy rehabilitation period of the masticatory and maxillofacial apparatus. Evaluation criteria encompassed occludogram and wax template assessments for supracontacts, BruxChecker evaluations, palpation, electromyography of masticatory muscles, and levels of personal anxiety [10].

The statistical probability coefficient was calculated using Student's table, considering the t values and the number of compared criteria. To align with recommended standards for medical research, the minimum acceptable probability was set at p<0.05, ensuring a 95% or higher likelihood of prediction accuracy. Calculations were executed using MS Excel 7 for the Windows XP operating system, alongside standard statistical software packages "Statistica 6.0" and "SPSS 14" (StatSoft Inc.) as indicated by Mintser OP, Voronenko YV, Vlasov VV. [11]

The reliability of research findings was assessed via statistical processing of obtained data, employing widely accepted methods of variation statistics, both parametric and nonparametric. The analysis utilized programs "StatSoft Statistica 10 Enterprise" and "Microsoft Office Excel 2007".

RESULTS

Normalization of occlusal contacts in patients of clinical groups was confirmed by the results of occludograms 12 months after the start of treatment (Table 1).

Analyzing the results of occludograms 12 months after the start of treatment, significantly better indicators of occlusal contact of teeth in all clinical groups were found, the value of OCG up to 80% was not observed at all, 80-90% in 10.0% - 3 patients of the first group, 4% - 1 patient of the second group and 6.7% - 2 patients of the second group.

Indicators in the range of 90-100% were observed in 90.0% - 27 patients of the first group, 96.0% - 24 patients of the second group, 93.3% - 28 patients of the third group. Such indicators can be explained by the long period of complete orthodontic treatment and retention period in the first group, the difficulty of normalizing the functioning of the masticatory muscles in the second group, and the prevalence of psycho-emotional etiological factors in the third group. This was confirmed by the results of the Brux Checker study, which revealed a decrease in the area of abrasion facets in all patients (Table 2).

The results of palpation of the masticatory muscles after 12 months are presented in Table 6.3: 3.1R - shoulder

Table 1. Occludogram results in patients of clinical groups after 12 months

Clinical groups	Ck-correlation	The first group (n = 30)		The second group (n =25)		The third group (n=30)	
Parameters	p-reliability	abs.	%	abs.	%	abs.	%
<80%	Ck=0.93, p<0.05	-	-	-	-	-	-
80 - 90%	Ck = 0.68, p = 0.03	3	10,0	1	4,0	2	6,7
90-100%	-	27	90,0	24	96,0	28	93,3

Table 2. Brux Checker study results in patients of clinical groups

Clinical groups	Ck-correlation	The first group (n = 30)	The second group (n =25)	The third group (n=30)
Tooth	P-value			
1.5	Ck = 0.71, p = 0.19	-	-	0,02±0,01
3.4	Ck=0.93, p<0.05	0,01±0,01	0,01±0,01	0,07±0,01
3.1	-	0,01±0,01	-	0,03±0,01
2.4	-	-	0,02±0,01	0,01±0,01
3.3	Ck = 0.68, p = 0.03	0,03±0,01	-	0,08±0,02

Table 3. Results of palpation of the masticatory muscles and TMJ after 12 months

Clinical groups	Ck-correlation	The first group (n=30)	The second group (n=25)	The third group (n = 30)
Parameters	P-value			
3.1R	Ck=0.76, p=0.10	0,01±0,01	-	0,05±0,02
3.3bL	Ck=0.86, p<0.05	0,01±0,01	-	0,01±0,01
3.3cL	-	-	-	0,02±0,02
3.3cR	-	-	0,03±0,01	0,06±0,04
3.4bR	-	-	0,04±0,02	0,06±0,01
3.15aL	-	-	0,01±0,01	0,08±0,04

and neck muscles on the right, 3.3bL - middle bundles of the temporalis muscle on the left, 3.3cL - posterior bundles of the temporalis muscle on the left, 3.3cR - posterior fascicles of the temporalis muscle on the right, 3.4bR - deep palpation of the masseter muscle on the right, 3.15aL - lateral pole of the left temporomandibular joint (Table 3).

A significant improvement in the condition of occlusal contacts was found in patients of the first group, where the main etiological factor of bruxism was the presence of supracontacts and orthodontic pathology (Ck=0.68, Ck=0.71, Ck=0.93), which, after their elimination, led to the disappearance of clinical manifestations of bruxism.

In patients of the second group, a significant improvement in the state of the occlusal relationship of the jaws was noted, but some features of closure are associated with prolonged restructuring of the muscular apparatus. In patients of the third clinical group, a significant improvement in the state of occlusal contacts was noted, but abrasion facets remained on all the studied teeth, which indicates other mechanisms of bruxism.

When analyzing the palpation of the masticatory muscles after 12 months, a significant decrease in the soreness of all muscles was noted in patients of all clinical groups, which substantiates the correctness of the treatment in all clinical groups. Patients in the first group were diagnosed with mild soreness of the shoulders and neck (Ck=0.76, p=0.10), in the second group - soreness of the posterior bundles of the temporalis muscle on the right, and the lateral pole of the left temporomandibular joint and at deep palpation of the masseter muscle on the right (Ck=0.86, p<0.05). In the third group, a significant decrease in the intensity of pain was noted, but its complete disappearance was not observed. There was a significant difference in the results of palpation between the clinical groups (p<0.05). The results of palpation were confirmed by electromyography (Table 4).

In all clinical groups, 12 months after the start of treatment, significant differences in the indicators of electromyography of the masticatory muscles from the initial indicators (p<0.05) were recorded, close to normal, namely: latency of the M-response of the masticatory muscle on the left (3.45±1.02;

Table 4. EMG Study Results in Clinical Groups after 12 Months

Groups Parameters to be monitored	Norma	Group 1 (n=30)	Group 2 (n=25)	Group 3 (n=30)
The latency of the M-response itself chewing muscle on the left	3,56 ±0,65	3,45 ±1,02	3,13 ±1,73	3,09 ±1,37
The average amplitude of the interference electromyograms of m. masseter dextra	511,19 ±42,19	504,64 ±18,43	501,14 ±27,34	506,23 ±11,15
The average amplitude of the interference electromyograms of m. masseter sinistra	508,78 ±43,84	508,14 ±21,37	502,23 ±17,81	501,14 ±24,35
The average amplitude of the interference electromyograms of m. temporalis dextra	358,61 ±26,47	350,82 ±19,11	351,25 ±14,23	343,83 ±12,87
The average amplitude of the interference electromyograms of m. temporalis sinistra	393,99 ±28,61	393,17 ±19,83	385,74 ±14,25	380,22 ±17,73
The average frequency of interference electromyograms of m. masseter dextra	340,39 ±15,01	337,51 ±21,07	334,43 ±13,59	328,53 ±27,51
The average frequency of interference electromyograms of m. masseter sinistra	358,08 ±19,07	358,01 ±21,92	354,16 ±19,81	354,22 ±16,72
The average frequency of interference electromyograms of m. temporalis sinistra	363,65 ±19,45	363,43 ±23,44	360,65 ±14,23	359,78 ±27,52
The duration of a late response right flicker reflex (ms)	25,30 ±1,45	25,20 ±2,34	26,20 ±1,64	26,51 ± 2,45
The duration of a late response left flicker reflex (ms)	24,50 ±1,87	24,73 ±3,12	24,40 ±1,23	25,50 ±1,01

Table 5. The level of personal manifestations of anxiety in clinical groups after 12 months (according to the J. Taylor questionnaire, 1953)

Clinical groups	Cc - correlation	The first group (n= 30)		The second group (n= 25)		The third group (n= 30)	
Factors.	p-reliability	abs.	%	abs.	%	abs.	%
A	-	4	13,3	1	4,0	2	6,7
B	-	2	6,7	3	10,0	6	20,0
C	Ck=0.56, p=0.10	1	3,3	1	4,0	2	6,7
D	Ck=0.86, p<0.05	-	-	-	-	1	3,3

3.13±1.73; 3.09±1.37; 3 . 56±0.65; p>0.05); average amplitude of the interference electromyogram of m. masseter dextra (504.64±18.43, 501.14±27.34, 506.23±11.15, 511.19±42.19; p>0.05); average amplitude of interference electromyogram (EMG) m. masseter sinistra (508,14±21,37; 502,23±17,81; 501,14±24,35; 508.78±43.84; p>0.05); average amplitude of interference electromyogram m. temporalis dextra (350.82±19.11; 351.25±14.23; 343.83±12.87; 358.61±26.47; p>0.05); mean amplitude of interference EMG of m. temporalis sinistra (393.17±19.83; 385.74±14.25; 380.22±17.73; 393.99±28.61; p>0.05); mean interferential EMG frequency of m. masseter dextra (337.51±21.07, 334.43±13.59, 328.53±27.51, 340.39±15.01; p>0.05); mean interferential EMG frequency m. masseter sinistra (358.01±21.92; 354.16±19.81; 354.22±16.72; 358.08±19.07; p>0,05); average frequency inter-ference EMG m. temporalis sinistra (363.43±23.44; 360.65±14.23; 359.78±27.52; 363.65±19.45; p>0.05);

duration of the late response of the blink reflex on the right (25.20±2.34, 26.20±1.64, 26.51±2.45, 25.30±1.45 p>0.05); and a significant increase in the duration of the late of the blink reflex response on the left (24.73±3.12; 24.40±1.23; 25.50±1.01; 24,50±1,87; p>0,05).

According to the J.Taylor questionnaire, a repeated survey was conducted 12 months later to assess the personality scale of anxiety (Table 5). Factor values: A - chronic fear associated with anxiety, sensitivity, and self-doubt; factor B - lability of the autonomic nervous system in threatening situations; factor C - sleep disorders associated with general internal tension; factor B - feelings of inferiority (Table 5).

After evaluating the personal manifestations of anxiety 12 months post-initial visit, a notable decrease across all clinical groups was observed (p<0.05).

A minority of patients received diagnoses related to nervous system lability: chronic fear associated with anxiety, sensitivity, and self-doubt (13.3% - 4 patients

in the first group, 4.0% - 1 patient in the second group, 6.7% - 2 patients in the third group); autonomic nervous system lability in threatening situations (6.7% - 2 patients in the first group, 10.0% - 3 patients in the second group, 20.0% - 6 patients in the third group); sleep disorders due to general internal stress (3.3% - 1 patient in the first group, 4.0% - 1 patient in the second group, 6.7% - 2 patients in the third group); and feelings of inferiority (3.3% - 1 patient in the third group).

The normalization of psychoemotional states across all patient groups can be attributed to the effectiveness of treatments administered, including those in the third group, where the primary factor contributing to bruxism onset was identified as psychoemotional lability.

DISCUSSION

Bruxism, characterized by teeth grinding due to involuntary contraction of the masticatory muscles, exhibits a prevalence ranging from 5% to 90% in adults and 10% to 50% in children, with a decrease observed in individuals over 60 years old [4-6]. As a dental pathology often associated with various somatic disorders, bruxism is frequently linked to psychological stress. Locally, it disrupts the natural occlusion of teeth, significantly complicating the restoration of proper dentition alignment. [4, 6, 9]. Studies have consistently identified psycho-emotional instability, frequent stress, genetics, alcohol, smoking, drug abuse, and certain medications as influential factors in bruxism's onset [6, 7].

According to dental theory, bruxism stems from various abnormalities in dentition structure and function, including occlusion pathologies, dentition anomalies, poorly selected orthopedic and orthodontic structures, TMJ arthritis, arthrosis, dysfunction, and post-traumatic changes in the maxillofacial region [3, 6, 8].

Diagnosing bruxism in dentistry relies on several methods (e.g., abrasion detection, bruxchecker, occludogram, assessment of masticatory muscle condition), but the lack of a clear examination algorithm for this patient population results in ineffective treatment and disease progression [2, 3, 5, 8]

However, due to the absence of a singularly established etiological point for bruxism onset, a clear diagnostic algorithm and optimal treatment approach are yet to be defined. This knowledge gap underscores the significance of this study. The study categorized patients into clinical groups based on the predominant etiological factor of bruxism and subsequently devised treatment algorithms. Specifically, 30 patients in the first group, exhibiting orthodontic pathology predominance, received treatment involving selective grinding of supracontacts (0.5-0.75 mm) and orthodontic pathol-

ogy treatment with removable and fixed orthodontic structures as deemed necessary. The second group comprising 25 patients, primarily characterized by TMJ pathology and masticatory muscle spasm, underwent finger self-massage of masticatory muscles, utilization of biting dental splints, and anti-inflammatory drug therapy. Lastly, the third group of 30 patients, where the predominant etiological factor was psycho-emotional, received sedative drug therapy as indicated (in collaboration with a psychiatrist).

The normalization of occlusal contacts was confirmed through occludogram results obtained 12 months after treatment initiation. Substantial improvement in teeth occlusal contact was evident across all clinical groups. Notably, an OCG value of up to 80% was entirely absent, with 10.0% (3 patients) in the first group, 4.0% (1 patient) in the second group, and 6.7% (2 patients) in the third group falling within the 80-90% range. Impressively, 90.0% (27 patients) in the first group, 96.0% (24 patients) in the second group, and 93.3% (28 patients) in the third group exhibited OCG indicators ranging between 90-100%.

Corroborating these findings, the Brux Checker study highlighted a reduction in the area of abrasion facets in all patients. Notably, the first group, primarily characterized by the presence of supracontacts and orthodontic pathology, exhibited significant improvement in occlusal contact conditions (Ck=0.68, Ck=0.71, Ck=0.93). The elimination of these factors led to the disappearance of clinical manifestations of bruxism in this group. The variations in these indicators could be attributed to the comprehensive orthodontic treatment and retention period in the first group, challenges in normalizing masticatory muscle function in the second group, and the prevalence of psycho-emotional etiological factors in the third group.

Evaluation of masticatory muscle palpation after 12 months showed a considerable reduction in muscle soreness across all clinical groups, validating the efficacy of treatments. The first group exhibited residual mild soreness in the shoulders and neck (Ck=0.76, p=0.10), while the second group displayed soreness in specific areas. In the third group, a notable decrease in pain intensity was observed, although complete disappearance was not attained. Significant differences in palpation results between clinical groups were noted (p<0.05).

Across all clinical groups, significant differences in electromyography (EMG) indicators of masticatory muscles were observed 12 months after treatment initiation compared to the initial readings (p<0.05). These measurements approached normal values, including the latency of the M-response of the left masticatory muscle

(3.45±1.02; 3.13±1.73; 3.09±1.37; 3.56±0.65; p>0.05); average amplitude of interference EMG for m. masseter dextra (504.64±18.43; 501.14±27.34; 506.23±11.15; 511.19±42.19; p>0.05); mean amplitude of interference EMG of m. masseter sinistra (508.14±21.37; 502.23±17.81; 501.14±24.35; 508.78±43.84; p>0.05); mean amplitude of interference EMG of m. temporalis dextra (350.82±19.11; 351.25±14.23; 343.83±12.87; 358.61±26.47; p>0.05); mean amplitude of interference EMG of m. temporalis sinistra (393.17±19.83; 385.74±14.25; 380.22±17.73; 393.99±28.61; p>0.05); average frequency of interference EMG of m. masseter dextra (337.51±21.07; 334.43±13.59; 328.53±27.51; 340.39±15.01; p>0.05); average frequency of interference EMG of m. masseter sinistra (358.01±21.92; 354.16±19.81; 354.22±16.72; 358.08±19.07; p>0.05); average frequency of interference EMG of m. temporalis sinistra (363.43±23.44; 360.65±14.23; 359.78±27.52; 363.65±19.45; p>0.05); duration of late reflex response on the right (25.20±2.34; 26.20±1.64; 26.51±2.45; 25.30±1.45, p>0.05); and a significant increase in the duration of late reflex response on the left (24.73±3.12; 24.40±1.23; 25.50±1.01; 24.50±1.87, p>0.05).

After the 12-month reassessment of personal anxiety manifestations, a notable decrease across all clinical groups was observed (p<0.05). A minor percentage of diagnoses reflecting nervous system lability included chronic fear associated with anxiety, sensitivity, self-doubt (13.3% - 4 patients in the first group, 4.0% - 1 patient in the second group, 6.7% - 2 patients in the third group); lability of the autonomic nervous system in threatening situations (6.7% - 2 patients in the first group, 10.0% - 3 patients in the second group, 20.0% - 6 patients in the third group); sleep disorders linked to general internal stress (3.3% - 1 patient in the first group, 4.0% - 1 patient in the second group, 6.7% - 2 patients in the third group); feelings of inferiority in 3.3% - 1 patient in the third group.

Addressing the leading etiological factor resulted in the normalization of other causes and a decrease in the intensity of bruxism manifestations, sometimes leading to their complete disappearance.

CONCLUSIONS

The evaluation of treatment effectiveness over 12 months revealed that targeting the primary etiological factor not only normalized other contributing causes but also notably reduced the intensity of bruxism manifestations, sometimes resulting in their complete disappearance. This conclusion is supported by the comprehensive assessment involving occludograms, BruxChecker study, palpation, electromyography of the masticatory muscles, and psychoemotional state analysis. The findings indicated that in cases where orthodontic pathology and supracontacts were predominant (r=0.99, p<0.05), interventions involving selective grinding and orthodontic treatment as needed were highly effective. This approach resulted in a reduction in bruxism severity, evidenced by occludogram indicators within the range of 90-100% in 90.0% of cases, decreased BruxChecker abrasion facets (p<0.05), and significant enhancement in occlusal contacts (Ск=0.68, Ск=0.71, Ск=0.93). For cases predominantly related to TMJ pathology (r=0.98, p<0.05), interventions focusing on normalizing masticatory muscle tone and addressing temporomandibular joint issues proved effective. This was confirmed by the 12-month palpation results, indicating slight tenderness in specific muscle areas, and normalization of electromyography readings from the initial indicators (p<0.05). In instances where the psycho-emotional factor was prominent in bruxism etiology (r=0.97, p<0.05), medical corrections effectively led to bruxism disappearance and normalization of the psycho-emotional state within 12 months (p<0.05).

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CONFLICT OF INTEREST

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CORRESPONDING AUTHOR

Oksana V. Klitynska
Uzhhorod National University
3 Narodna Square, 88000 Uzhhorod, Ukraine
e-mail: oksana.klitynska@uzhnu.edu.ua

ORCID AND CONTRIBUTIONSHIP

Oksana V. Klitynska: 0000-0001-9969-2833 Ⓐ Ⓓ
Yurii M. Martyts: 0000-0002-9222-5017 Ⓑ Ⓔ
Viacheslav R. Gurando: 0000-0001-6303-3799 Ⓕ
Nataliya V. Layoch: 0000-0003-4741-1731 Ⓒ

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

ORIGINAL ARTICLE

Coronary artery calcium score as independent risk factor of obstructive coronary artery disease

Vitalina V. Ivachevska, Rostyslava R. Supyk, Mykhailo M. Ivachevskyi, Mykhailo M. Hechko, Antonina V. Varvarynets

UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

Aim: To prove an independence of CAC score comparatively to conventional risk factors such as age, and dyslipidemia especially in patients under forty years of age.

Materials and Methods: Thirty-four asymptomatic adult patients with no prior established atherosclerotic cardiovascular disease, diabetes mellitus or severe comorbidities, except of complex clinical examination, underwent CT scan with evaluation of coronary artery calcium score.

Results: The average total cholesterol level in the group was (5.62±1.02) mmol/l, indicating the presence of dyslipidemia. The average HDL level was (1.26±0.24) mmol/l, suggesting an average risk of atherosclerosis. The average LDL levels were within the borderline range at (3.63±1.01) mmol/l. The average triglyceride level was within the safe range at (1.93±1.08) mmol/l. The atherogenicity coefficient indicated a moderate risk of atherosclerosis with an average value of 3.64±1.31. The average coronary artery calcium score was 56.71±143.85, indicating minor plaques and a moderate risk of coronary artery disease. Correlation analysis revealed no significant correlation between age and the CAC score (r=0.1, p>0.05). However, reliable direct correlation of weak strength was found between the CAC score and LDL level (r=0.35, p<0.05). Direct correlations of weak strength were also observed between age and the levels of total cholesterol, LDL and the atherogenicity coefficient (r=0.43, 0.49, 0.42 respectively, p<0.05).

Conclusions: Coronary artery calcium score is a valuable screening tool for identifying potential obstructive coronary artery disease, not only for individuals aged forty and above, but also for younger asymptomatic patients.

KEY WORDS: obstructive coronary artery disease, coronary artery calcium score

INTRODUCTION

Coronary artery atherosclerosis typically presents as coronary artery disease (CAD). It is presented by a variety of symptoms such as chest discomfort during physical activity caused by decreased coronary flow reserve, or development of acute coronary syndrome even as the first manifestation of CAD. Coronary artery disease is one of the most common causes of global morbidity and mortality [1]. Consequently, it becomes crucial to identify individuals at risk of CAD, especially in the absence of symptoms, to effectively guide decisions regarding primary prevention strategies.

The traditional approach to stratify the risk of coronary events in individuals without prior symptoms of CAD has been to use medical risk models such as the Framingham risk score. These risk models classify individuals into low, intermediate, or high-risk categories, helping determine the appropriate course of action for prevention and management of CAD [2]. Although these tools provide a convenient and affordable way to assess the risk of potential health issues, it is acknowledged that a significant number of patients are not identified as high risk before experiencing their initial coronary event [3,4].

Non-contrast computed tomography (CT) assessment of coronary artery calcium (CAC) score in asymptomatic patients offers an alternative strategy to enhance risk prediction beyond the Framingham risk score (Fig. 1). By incorporating CAC score, clinicians can obtain additional information on the presence and extent of coronary artery calcification, which has been shown to correlate with the risk of future cardiovascular events. This approach provides a more comprehensive evaluation of an individual's risk profile, enabling more accurate risk stratification and potentially guiding preventive interventions [5].

According to the 2019 ESC guidelines on chronic coronary syndromes, the CAC score can be used in non-acute settings for patients presenting with angina and/or dyspnea. It's use can help to augment the
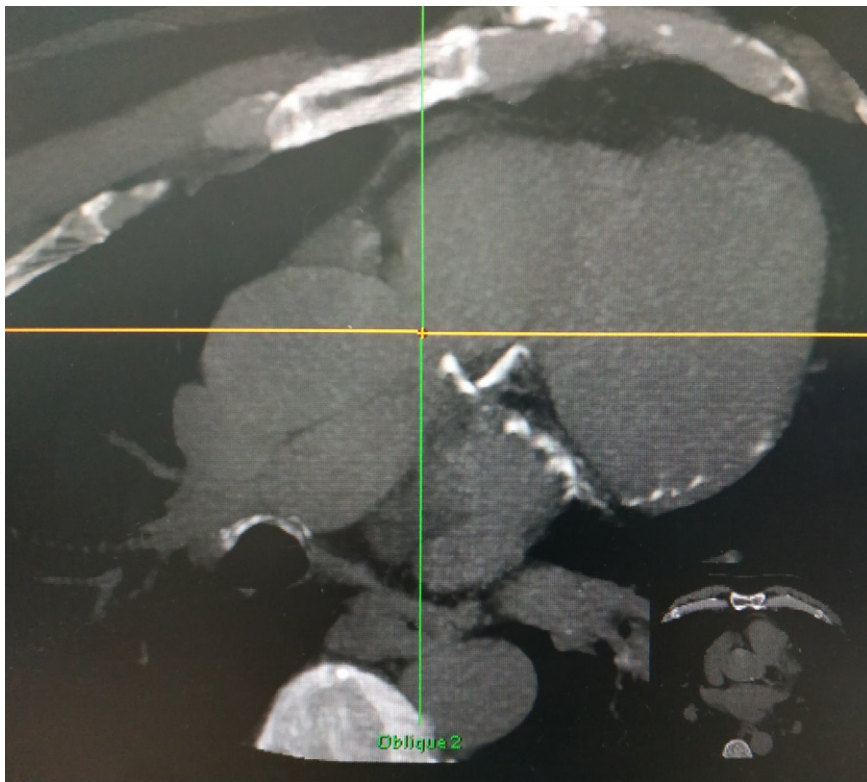
Fig. 1. Example of CAC score using non-contrast CT.

clinical likelihood of CAD if the CAC score is above 0, or practically exclude the possibility of obstructive CAD if the CAC score is 0 [6, 7]. Unlike coronary CT angiography, which is primarily performed for symptomatic patients with suspected obstructive CAD, calcium scoring does not involve the administration of contrast but does not provide an assessment of luminal stenosis [8].

However, there is limited information available regarding the use of coronary artery calcium score for risk stratification in asymptomatic individuals. Moreover, risk assessment models to estimate the 10-year risk of cardiovascular disease in Europe such as SCORE2 estimates an individual's 10-year risk of fatal and non-fatal CVD events (myocardial infarction, stroke) in apparently healthy people starting from the age of 40 [9]. So if there is a need to evaluate CVD risk in younger persons score2 can't be used.

AIM

To prove an independence of CAC score comparatively to conventional risk factors such as age and dyslipidemia especially in patients under 40 years of age.

MATERIALS AND METHODS

Thirty-four asymptomatic adult patients with no prior established atherosclerotic cardiovascular disease, diabetes mellitus or severe comorbidities were included into the study. Exclusion criteria for the study were pregnancy, organic heart pathology, arrhythmias, and familial hypercholesterolemia. All participants underwent a thorough clinical examination, included the collection of demographic and anthropometric data, physical examination and laboratory tests including lipid profile, as well as performing a chest X-ray, electrocardiogram and echocardiogram. Among the examined patients 55.9% (19/34) were men, while 44.1% (15/34) were women.. The average age of the patients was 51.26 ± 13.48 years. The percentage of examined patients under the age of 40 was 32.4% (11/34).

All CT scans were performed using a GE LightSpeed 64 with ECG synchronization. All investigations were done by the same physician throughout the study. CT scans of the heart were conducted with a slice thickness of 3 mm. The calculation of the CAC value was done according to the Agatston method which involves the multiplication of the density score by the area of calcified plaque. The entire procedure was typically completed within 10 minutes, and the radiation dose was kept low at around 1 mSv. Interpretation of the results of CT scan by Agatston method is presented in the table 1.

The obtained results were statistically analyzed using Office Excel 2010 and Statsoft Statistica 12.0 software package on a personal computer. The discrepancy was considered probable if the probability value was 95% or higher ($p < 0.05$). We used variational statistics to analyze the data, considering average values and average error

Table 1. Clinical interpretation of CT scan by Agatston method

Coronary artery calcium score	Diagnosis	Clinical interpretation
0	There are no atherosclerotic plaques, a very low level of CAD.	The negative predictive value is more than 90-95% for the absence of significant CAD.
1-10	Minimal atherosclerotic plaques. Low level of CAD.	Significant CAD is highly doubtful
11-100	Minor atherosclerotic plaques. Moderate risk of CAD.	Minimal or minor stenoses of the coronary arteries are possible.
101-400	Moderate atherosclerotic plaques. High level of CAD.	Hemodynamically significant stenoses are possible in some localizations.
>400	Pronounced atherosclerotic plaques. Very high level of CAD.	High probability of significant stenosis.

Table 2. Parameters of lipid profile and CAC score of of examined persons (M ± m)

Parameters	Group of examined persons (n=34)
Total cholesterol, mmol/l	5.18±1.06
HDL, mmol/l	1.23±0.35
LDL, mmol/l	3.12±0.50
Triglycerides, mmol/l	3.80±1.45
Atherogenicity coefficient	3.64±1.31
CAC score	56.71±143.85

Table 3. Correlation between age and indicators of lipid profile and CAC score of examined patients

Parameters	Age	
CAC score	r=0.1	p>0.05
Total cholesterol	r=0.43*	p<0.05
HDL	r=-0.21	p>0.05
LDL	r=0.49*	p<0.05
Triglycerides	r=0.23	p>0.05
Atherogenicity coefficient	r=0.42*	p<0.05

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

(M±m). The reliability of the values was assessed using the Student's t-criterion, and the correlation coefficient was determined using the paired Pearson method.

RESULTS

Although the study group included asymptomatic patients of various ages, from 31 to 71 years, the average value of the total cholesterol level ((5.62±1.02) mmol/l) indicates the presence of dyslipidemia. Thus, the average value of the HDL level in the examined patients was (1.26±0.24) mmol/l, which indicated an average risk of atherosclerosis; the average values of LDL were within the borderline level and amounted to (3.63±1.01) mmol/l. The average value of the triglycerides of the examined persons was within the safe level - (1.93±1.08) mmol/l. Due to the reduced level of HDL and the borderline level of LDL, a moderate risk of atherosclerosis was observed according to the indicator of the atherogenicity coefficient. The average value of the atherogenicity coefficient was 3.64±1.31, which

corresponds to a moderate risk of atherosclerosis. Average value of the CAC score was 56.71±143.85 corresponding to a minor atherosclerotic plaques and indicates moderate risk of CAD (Table 2). It is worth noting that among the examined asymptomatic persons the high probability of significant stenosis with Agatston score >400 was revealed in 5.9% of patients. Such a low rate of possible CAD among the examined persons was expected, since all patients were asymptomatic and had no history of prior established atherosclerotic cardiovascular disease.

According to the results of the correlation analysis, it was found that there is no correlation between age and the coronary calcium score (r=0.1, p>0.05), but a reliable direct correlation of weak strength was confirmed between coronary calcium score and the level of LDL (r=0.35, p<0.05). While between the levels of total cholesterol, LDL, atherogenicity coefficient and age was found direct correlation of weak strength (r=0.43; 0.49; 0.42 respectively, p<0.05) (table 3). It is noteworthy that the percentage of patients with CAC score above 0 among the examined

persons under the age of 40 was 36.4% (4/11). The obtained results indicate the independence of CAC score from other conventional risk factors of cardiovascular diseases.

DISCUSSION

Early diagnosis and treatment of coronary artery disease is both cost-effective and linked to better outcomes in terms of illness and death rates [10]. It is crucial to identify asymptomatic individuals who are at higher risk of future cardiovascular events. It is essential for implementing cost-effective preventive measures [11].

Budoff MJ, Young R, Burke G, et al. in their study demonstrated positive correlation between CAC strata and risk of CAD despite the age, sex or ethnicity [12]. There are studies which confirm that the younger age occurs CAC score is >0 the more pronounced the clinical manifestations. Thus, Carr JJ, Jacobs DR Jr, Terry JG, et al. showed that among persons aged 32 to 46 years, a calcium score of 100 or more was associated with premature death [13]. Silverman et al. found that there is a strong correlation between the extent of coronary artery calcification and the need for revascularization. Their study showed that as the amount of plaque or the number of affected vessels increases, the likelihood of requiring revascularization also increases [14]. The moment when CAC score become more than 0 is very important as the progress after that is exponential. Therefore, depending on value of CAC score management of patients can be revised [15-17].

It is important to note that currently there are no pharmacological agents, which can lead to regression of coronary artery calcification. Even with the use of statins, there is an increase in the value of CAC score over time. This phenomenon is due to changes in plaque features, such as an increase in plaque calcium content and is associated with the process of «stabilization» of the atherosclerotic plaque. Although statins are still effective in reducing the risk of major adverse cardiovascular events by decreasing the percent atheroma volume [18-21].

CONCLUSIONS

Coronary artery calcium score can be considered as an independent screening indicator of probable obstructive coronary artery disease in asymptomatic patients not only starting from the age of forty years but in younger persons too.

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CORRESPONDING AUTHOR
Vitalina V. Ivachevska
Uzhhgorod National University
148 Sobranetska st, 88015 Uzhhgorod, Ukraine
e-mail: vitalina.ivachevska@uzhnu.edu.ua

ORCID AND CONTRIBUTIONSHIP
Vitalina V. Ivachevska: 0000-0002-2036-3568 Ⓐ Ⓑ Ⓒ Ⓓ
Rostyslava R. Supyk: 0009-0008-4323-7012 Ⓐ Ⓓ
Mykhailo M. Ivachevskyi: 0000-0002-9254-7163 Ⓐ Ⓑ
Mykhailo M. Hechko: 0000-0003-2793-5044 Ⓒ Ⓓ Ⓔ Ⓕ
Antonina V. Varvarynets: 0000-0001-5859-1040 Ⓔ Ⓕ

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

ORIGINAL ARTICLE

Cytological and microbiological investigations of professional hygiene efficiency in patients with generalized periodontitis

Olha Ripetska[1], Volodymyr Hrynovets[1], Ihor Deneha[1], Ihor Hrynovets[1], Anatoliy Potapchuk[2], Vasyl Almashi[2]

[1]DANYLO HALYTSKYI LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE

[2]UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

Aim: The purpose of this study is to assess the impact of occupational hygiene procedures for microbiological and cytological contents of periodontal pockets.

Material and Methods: Cytological and microbiological content of the periodontal pockets before treatment and after professional hygiene procedures including scaling with hand instruments and root cementum polishing have been investigated in patients with periodontitis.

Results: According to obtained data it can be resumed that in periodontitis patients with the depth of pockets 3-5,5 mm before professional hygiene all the pockets contain great number of *Cocci, Spirochetes, Candida Albicans, Flagellated* rods and *Protozoa* species. It was proved by revealing of small amount of Polymorphonuclear leukocytes with active phagocytosis. After scaling and planing of the roots, a decrease in the number of *Protozoa* and *Candida Albicans* was observed in 97% and 72% of the investigated cells, respectively.

Conclusions: Cytological and microbiological content of periodontal pockets before treatment and after professional hygiene procedures including scaling and root planning testify to the level of local protective mechanisms, especially process of phagocytosis and virulence of microbial species in periodontal pockets.

KEY WORDS: periodontitis, scaling, microbiological, cytological investigation

INTRODUCTION

Professional oral hygiene is an important part of treatment of periodontitis patients. It includes the removal of dental calculus – scaling, as well as leveling and polishing the surface – root planning [1]. The procedure for leveling and grinding the enamel and planning of tooth root cement improves the treatment results, as pathologically altered layers are removed from the surface of the root of tooth, which contains the remains of the gram-negative microflora. The surface of the root of tooth is aligned and smoothed out after the manipulation procedures by dental scaling, which makes the plane of the dental root surface smooth up to the mirroring effect [2, 3]. Medical practice proves that after performing the instrumental procedure of dental scaling, the degree of smoothness of the root part of tooth is not as important criterion as the reduction of the critical number of gram-negative microflorae [4-6]. Main purpose of professional hygiene in these patients is reduction of total number and partial inactivation of microbiota in periodontal pockets.

Pathogenesis of periodontal diseases is usually associated with microorganisms, mostly anaerobes, of the subgingival dental plaque. In advanced periodontitis, gram-negative anaerobic flora is prevailing: bacteriodes, fusobacteria etc. Developed periodontitis is characterized by the presence of great numbers of spirochetes and different specific microorganisms. Microflora of the oral cavity may be of different kinds and variable localization [7, 8].

The presence of periodontal pathogens and their metabolic by-products in the mouth may in fact modulate the immune response beyond the oral cavity, thus promoting the development of systemic conditions.

The process of tissue destruction results from the elaboration of bacterial substances that directly or indirectly cause degradation of the periodontal tissues [9] (Fig. 1).

The production of immunoglobulin-degrading proteases by (*P. gingivalis, P.intermedia, P.melaninogenica, Capnocytophaga sp.*) may counteract these host defenses. Some bacteria produce substances suppressing the activity of polymorphonuclear leucocytes and lymphocytes. *A. actinomycetemcomitans* produces leukotoxin inhibiting the function of PMNs and killing of mature B and T cells, *P. gingivalis* inhibites of superoxide production by PMNs [9, 10].

Pathogenic bacteria also produce enzymes capable of degrading host tissues: collagenase, trypsin-like enzyme, keratinase, arylsulfatase, neuraminidase, fi-
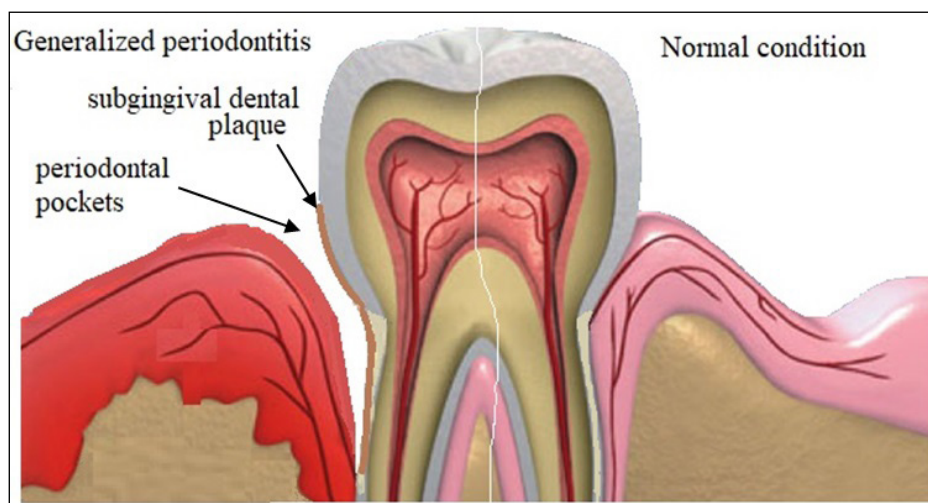
Fig. 1. Clinical situation before the operation teeth 1.4 and 1.5.

Fig. 2. Slowly progressing not active periodontitis, II-nd stage of heaviness in patient B., 43-year-old male. The depth of a periodontal pocket at the medial surface of 33 tooth is 6mm.

Fig. 3. Chronic generalized periodontitis, II-nd stage of heaviness, bleeding on probing of 1st degree, chronic periodontal abscess in the region of 41 and 42 teeth.

Fig. 4. Exacerbation of chronic generalized periodontitis, II-nd stage of heaviness, bleeding on probing of the 3-rd degree.
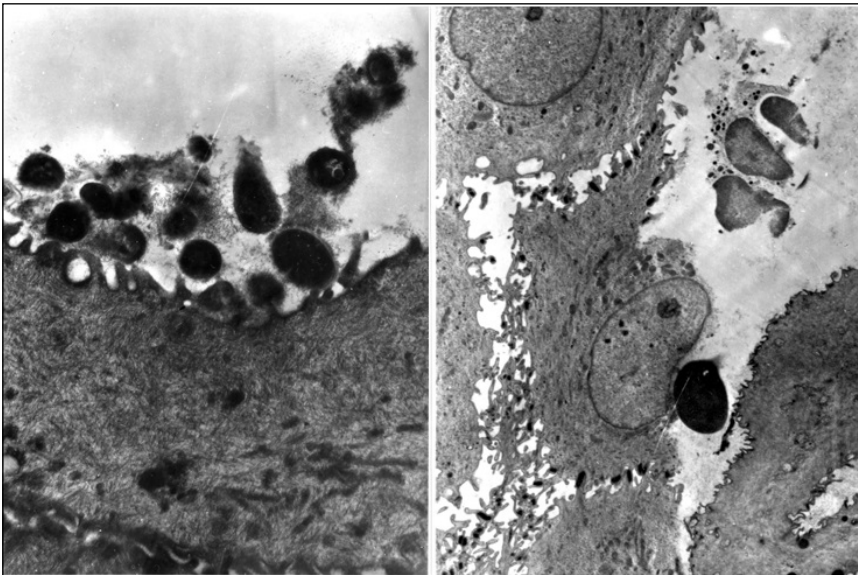
Fig. 5. Ultrastructural organization of the surface layers of human gums with dystrophic periodontal damage (periodontitis):
a) accumulation of bacterial bodies on the surface of the epithelium. Coll. x 5,000.
b) stratified cells of the granular layer, in the spaces between which elements of destroyed platelets and microbes were found. Coll. x 5.
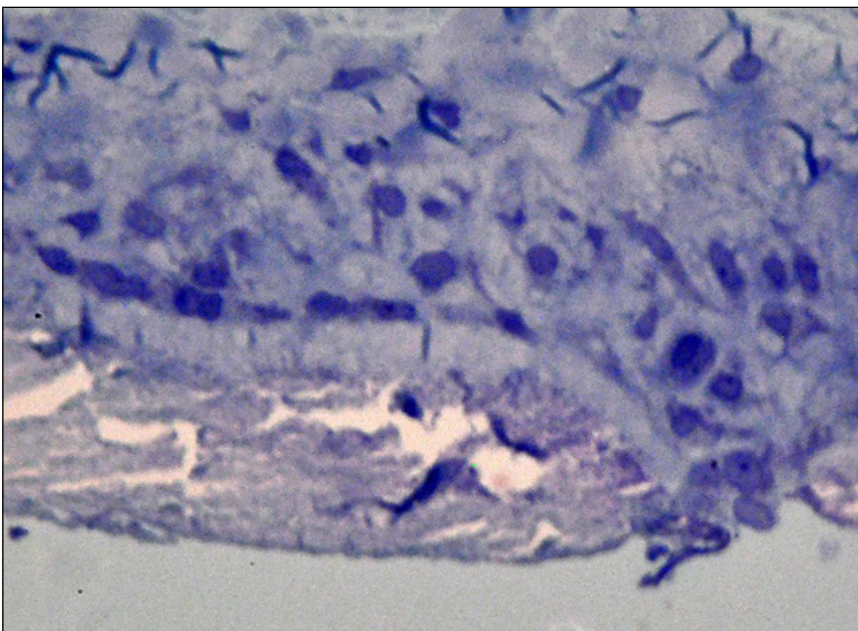
Fig. 6. A fragment of the multilayered squamous epithelium of the gums with moderately pronounced dystrophic changes. Semi-thin cut. Stained with toluidine blue. Magnification 400. (The photos were taken on a Nikon E200 microscope with a Nikon D5000 camera).

Table 1. Cytological content of periodontal pockets in patients with generalized periodontitis (periodontal pockets 3-5,5 mm)

Time of examination	Cells (M±m)					
	Polymorphonuclear leukocytes			Macrophages	Lymphocytes	Epithelial cells
	without changes	without changes	without changes			
Before the treatment (1)	27,7±0,7	11,7±0,5	12,3±0,5	0-1	0-1	1-2
After the treatment (2)	5,2±0,2	1,7±0,2	2,8±0,2	1-2	1-2	1-2
P 1,2	< 0,01	< 0,01	< 0,01	-	-	-

Table 2. Distribution of periodontal pockets according to the content of *Flagellated rods* and *Protozoa* in patients with Generalized periodontitis (periodontal pockets 3-5,5 mm)

Periodontal pockets being explored		Estimation of microbiota *			
		Flagellated rods		Protozoa	
		-; ±	+; ++	-; ±	+; ++
Before the treatment	absolute number	63	237	233	67
	%	21,0±2,3	79,0+2,3'	77,8±2,3	22,2±2,3
After the treatment	absolute number	152	108	251	9
	%	58,3±3,0	41,7±3,0	96,5±1,1	3,5±1,1
t		9,8		7,5	
P		< 0,01		< 0,01	

* - not fined, ± - small amount, + - moderate amount, ++ - many.

Table 3. Distribution of periodontal pockets according to the content of *Candida Albicans, Cocci* and *Spirochetes* in patients with Generalized periodontitis (periodontal pockets 3-5,5mm)

Periodontal pockets being explored		Estimation of microbiota *					
		Candida		Cocci		Spirochetes	
		±	+;++	-;±	+;++	-;±	+;++
Before the treatment	absolute number	103	197	69	231	90	210
	%	34,3±2,7	65,7±2,7	23,1 ±2,4	76,9±2,4	30,1±2,6	69,9±2,6
After the treatment	absolute number	189	71	141	119	125	135
	%	72,7±2,8	27,3±2,8	54,4±3,1	45,6±3,1	48,1±3,1	51,9±3,1
t		9,8		8,0		4,5	
P		<0,01		<0,01		<0,01	

* - not fined, + - small amount, + - moderate amount, ++ - many.

bronectin- degrading enzyme, phospholipase A [10]. Some bacterial products inhibit the growth or alter the metabolism of host tissue cells, these are: ammonia, volatile sulfur compounds, fatty acids, peptides, indole.

Thus, virulent properties can be broadly categorized into factors that enable a bacterial species to colonize and invade host tissues, and factors that enable a bacterial species to directly or indirectly cause host damage [11, 12]. Bacterial species that have been identified as capable of tissue invasion are strongly associated with diseased sites. The ability to invade has been proposed by Loesche W.J. [11, 13] as a key factor distinguishing pathogenic from non-pathogenic gram-negative species. For bacteria to survive in the periodontal environment, they must evade the host mechanisms involved in bacterial clearing.

Macrophages, monocytes exposed to bacterial endo-toxin (lipopolysaccharide) release interleukin-1, tumor necrosis factor and prostaglandins. These host- derived cytokines stimulate a bone resorption, activate or inhibit other host immune cells [12, 13].

Neutrophils can be seen in relatively high numbers in both the gingival connective tissue and the sulcus. It is common to see them migrating through the sulcular and junctional epithelium. These cells perform a protective role by phagocytizing bacteria and other foreign substances. They contain lysosomes which in turn contain a variety of hydrolytic enzymes that kill bacteria after phagocytosis. When neutrophils die these enzymes are released and may contribute to tissue destruction.
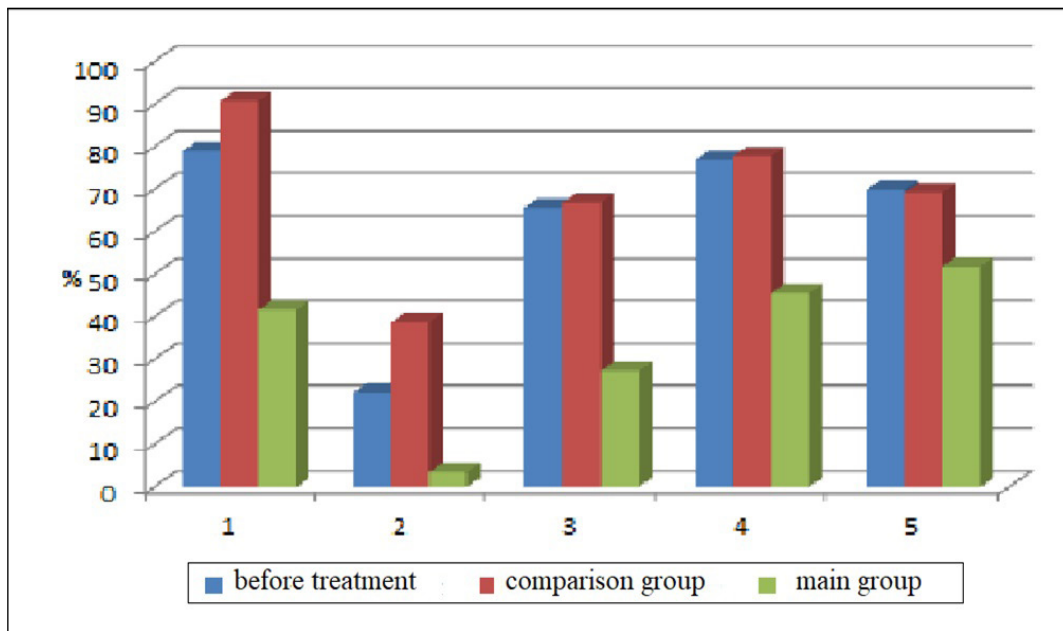
Fig. 7. Characteristics of the microflora of periodontal pockets in mild periodontitis before and after treatment. Horizontally: 1 – *Flagellated rods*; 2 – *Protozoa*; 3 – *Candida*; 4 – *Cocci*; 5 - *Spirochetes*. Vertically: the number of periodontal areas (%).

Neutrophils are a major component of the innate host response against bacterial challenge, and under homeostatic conditions, their microbicidal functions typically protect the host against periodontitis. However, a number of periodontal pathogens developed survival strategies to evade neutrophil microbicidal functions while promoting inflammation, which provides a source of nutrients for bacterial growth [13-15].

Neutrophils play a key role in periodontal health and disease. In their absence or when they are functionally inferior, as is the case with some congenital disorders, patients develop severe forms of periodontitis at an early age. These observations make it possible to state that the presence of immunocompetent neutrophils is important for homeostasis. However, the presence of excess or hypersensitive neutrophils due to systemic priming or innate immune conditioning results in an imbalance of the host-microbe interaction in the periodontium, leading to dysbacteriosis and inflammatory tissue breakdown [16-18].

In a rat model of periodontal disease using bacterial species incriminated in the pathology of human periodontitis (*Aggregatibacter actinomycetemcomitans (A.a), Fusobacterium nucleatum (F.n) and Streptococcus oralis (S.o)*) neutrophilic infiltrate in the periodontal tissue, and periodontal lysis was identified [19, 20].

That is why it is important to estimate not only microbiota but also cytological spectrum of periodontal pockets to evaluate host cells respond to bacteria invasion.

AIM

The aim of present study is estimation of the influence of professional hygiene procedures on the microbiological and cytological content of periodontal pockets.

MATERIALS AND METHODS

During the study, we examined 47 patients (20-40 years old) with generalized periodontitis. All the patients were thoroughly motivated for the prophylactic dental examination and the following treatment in the case of necessity. Periodontal condition of all teeth has been conducted (including the estimation of pockets depth and presence of bleeding on probing) during the examination) (Fig. 2, Fig. 3).

In each patient with generalized periodontitis minimum 20 teeth were preserved and not less than 10-12 true periodontal pockets (3-5,5 mm) and radiographic symptoms of bone destruction have been revealed. All the patients were not reported serious general pathology.

Mechanical plaque and calculus cleaning and tooth surface polishing was conducted in each periodontal pocket being combined with antiseptic – 0,2% Solution of Chlorhexidinum bigluconat.

The level of inflammation in periodontal tissues have been estimated with bleeding index [2, 6]. Gingival bleeding varies in severity, duration and the ease with which it is provoked. The severity of the bleeding depends upon the intensity of the inflammation (Fig.4).

Smears for research were taken from periodontal pockets after rinsing the oral cavity with physiological solution and saliva secretions. When making smears, Romanovsky staining was used.

RESULTS

Conducted investigations, considering clinical data, allow to some extent the estimation of pathological process in periodontal pockets.

According to cytological analyses (Table 1), before professional hygiene all smears show great number of unchanged Polymorphonuclear leukocytes (PMN), in average 27,7±0,7; relatively small amount of PMN with active phagocytosis and great number of destroyed PMN.

Almost all samples show some Lymphocytes and Epithelial cells, but macrophages are absent. Great number of destroyed Polymorphonuclear leukocytes should be noted (12,3±0,5). This type of cytological picture testifies to insufficient functional activity of local protective factors, Polymorphonuclear leukocytes.

All sections were prepared from the obtained tissue blocks using a UMTP-3M ultramicrotome and contrasted in solutions of uranyl acetate and lead citrate. Contrasted ultrathin sections were studied and photographed using an electron microscope UEMV - 100K (accelerating voltage 75 kV) (Fig. 5, Fig. 6).

After the debridement and root planing procedures analyzed smears from periodontal pockets show substantial decrease in number of Polymorphonuclear leukocytes (5,2±0,2). Considerable lowering of Polymorphonuclear leukocytes with active phagocytosis (1,7±0,2) has been noted. Especially important and positive change after professional hygiene tends to be substantial decrease (2,8±0,2) in number of destroyed PMN. Almost all samples show solitary Lymphocytes and Epithelial cells, with some macrophages, being completely absent before the treatment. These changes testify to positive course of pathological process in the periodontium.

Investigation of microbial spectrum followed cytological analysis of periodontal pockets (Table 2, Table 3). *Cocci, Spirochetes, Candida Albicans, Flagellated rods and Protozoa* have been revealed. Bacteria were in different stages of phagocytosis. In 79% of investigated smears from periodontal pockets before treatment prevailed flagellated rods, in 22% Protozoa were in great amounts. *Candida Albicans* was present in big quantities in 66% of examined smears, *Cocci* - in 77% of examined samples and Spirochetes - in 70% of cases.

All investigated pockets were much less contaminated after scaling and root planing. 58% of investigated samples showed small amount or absence of *Flagellated rods* and there were almost 97% of periodontal pockets with very small amounts or not revealed Protozoa species. It should be mentioned that in 42% of pockets *Flagellated rods* were still in big quantities (Table 2).

In 72% of pockets after treatment *Candida* was not defined, while *Cocci* and *Spirochets* were in big quantities in 45% and 52% of pockets accordingly (Table 3).

According to obtained data it can be resumed that in periodontitis patients with the depth of pockets 3-5,5 mm before professional hygiene all the pockets contain great number of *Cocci, Spirochetes, Candida Albicans, Flagellated rods and Protozoa* species. Functional activity of local defence mechanisms in examined samples, especially Polymorphonuclear leukocytes, was insufficient before the treatment. It was proved by revealing of small amount of Polymorphonuclear leukocytes with active phagocytosis, great number of destroyed olymorphonuclear leukocytes and absence of macrophages in examined smears.

After scaling and root planing positive changes in the number of microbial species and cytological spectrum of periodontal pockets have been observed. Considerable decrease in number of *Protozoa* and *Candida Albicans* was present in 97% and 72% of investigated pockets accordingly (Fig.7).

Almost half of investigated samples were characterized by low level of *Cocci* and *Spirochets*. These changes were accompanied by positive modification of periodontal pockets cytology: considerable lowering of Polymorphonuclear leukocytes with active phagocytosis substantial decrease in number of destroyed PMN.

DISCUSSION

Almost all specimens show single lymphocytes and epithelial cells with some macrophages present. It can be assumed that phagocytosis, as a reaction to the presence of pathogenic microbes in periodontal pockets, can be expressed to a certain extent. In a good or satisfactory condition of the organism phagocytosis leads to the total elimination of microbes, while in lowered resistance of the organism or very high virulence of microbes in smears were revealed neutrophils filled with great number of microbes. The presence in smears from periodontal pockets of great numbers of microbes in combination with absence or weak phagocytic reaction of neutrophils is considered as a bad prognostic sign for periodontal pocket [5, 6].

Superficial and intermediate cell values were significantly greater in patients with AP than in patients with CP or the control group. Histiocyte number was higher in patients with CP than in those with AP and differed significantly in both types of periodontitis compared to the control group. There were significant differences in polymorphonuclear neutrophil leukocytes when both types of periodontitis were compared to the control group. Microbial flora was statistically higher in patients with CP, and there were differences between patients with periodontitis and the control group [10, 12].

A significant decrease in clinical and biochemical parameters after two months of treatment in the curcumin group (P<0.05). Almost the same pattern for the chlorhexidine group (P<0.05), with minor differences from

baseline for albumin (P>0.05). A reduction in clinical parameters (P<0.05) and an increase in CRP, ALP and TP levels were observed after scaling and root planing in the SRP group. There were no significant differences between the three main groups in terms of clinical parameters (P>0.05), except for gingival index and biochemical parameters (P<0.05) [8, 11, 13].

Oral exfoliative cytology includes the study and interpretation of the features cells exfoliated from the oral mucosa. The aim of this study was to analyze cytological changes in the periodontal pocket of patients with different clinical stages of aggressive periodontitis (AP) and chronic periodontitis (CP). Superficial and intermediate cell values were significantly greater in patients with AP than in patients with CP or the control group. Histiocyte number was higher in patients with CP than in those with AP, and differed significantly in both types of periodontitis compared to the control group. There were significant differences in polymorphonuclear neutrophil leukocytes when both types of periodontitis were compared to the control group. Microbial flora was statistically higher in patients with CP, and there were differences between patients with periodontitis and the control group [12, 14-20].

CONCLUSIONS

Cytological and microbiological content of periodontal pockets before treatment and after professional hygiene procedures including scaling and root planning testify to the level of local protective mechanisms, especially process of phagocytosis and virulence of microbial species in periodontal pockets.

The presence in smears from periodontal pockets of great numbers of microbes in combination with absence or weak phagocytic reaction of neutrophils is considered as a bad prognostic sign for periodontal pocket.

Cytological and microbiological investigations of periodontal pockets help in estimation of treatment efficiency and prognosis in patients with periodontitis.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Anatoliy Potapchuk
Uzhhorod National University
16a University St, 88000 Uzhhorod, Ukraine
e-mail: anatoliy.potapchuk@uzhnu.edu.ua

ORCID AND CONTRIBUTIONSHIP
Olha Ripetska: 0000-0001-8989-0799 Ⓐ Ⓑ Ⓓ Ⓔ
Volodymyr Hrynovets: 0000-0002-6359-3793 Ⓐ Ⓑ Ⓒ Ⓓ
Ihor Deneha: 0000-0001-8610-0771 Ⓐ Ⓑ Ⓓ Ⓔ
Ihor Hrynovets: 0000-0003-1727-3526 Ⓐ Ⓑ Ⓓ Ⓔ
Anatoliy Potapchuk: 0000-0001-9857-1407 Ⓓ Ⓕ
Vasyl Almashi: 0000-0002-2943-4844 Ⓓ Ⓕ

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

ORIGINAL ARTICLE

Assessment of antimicrobial drugs employed at government-operated hospitals in central and northern provinces of Iraq

Mahmood Jawad[1], Shathel Khalaf Noman[2], Mohammed Rasool[3]
[1]DEPARTMENT OF PHARMACY, AL-ZAHRAWI UNIVERSITY COLLEGE, KARBALA, IRAQ
[2]DEPARTMENT OF PHARMACOLOGY, COLLEGE OF PHARMACY, TIKRIT UNIVERSITY, TIKRIT, IRAQ
[3]DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY, COLLEGE OF PHARMACY, UNIVERSITY OF KARBALA, KARBALA, IRAQ

ABSTRACT

Aim: To document the clinical patterns of antibiotic prescriptions in government hospitals, where the majority of physicians possess a degree-based training.

Materials and Methods: A Retrospective cross section study carried out between 1/7/2022 and April 2023 that enrolling 300 patients from governmental hospitals from different provinces of Central and northern Iraq. The research form contained 15 fields divided into three sections. The first section contains social information such as age, gender, field of work, Residence and education. The second part consists of diagnosis and lab. Finding. The third part related to antibiotic uses: Number of AB prescribed, duration of using, type of use, route of administration, AB interaction, dose administration of AB, indication of Ab, and Class of AB.

Results: A total of 300 eligible patients, 165 patients (55.0%) were male and 135 (45.0%) were female, patients were <20 years ages were 117 (39.0%), 25 (8.3%) from the 20–29 years age group, 40-49 years ages were 28 (9.3%) and >50 years ages were 105 (35.0%) were which belong to the pediatric population. The 198 patients (66.0%) were used cephalosporins and 106 (53.5%) of them used alone. A 13-19% percentage of patients had used penicillin, carbapenem, anti-fungal, and aminoglycoside in combination form.

Conclusions: The implementation of clinical guidelines, the provision of direct instruction, and the regular dissemination of antibiogram data have the potential to encourage a more judicious consumption of antibiotics.

KEY WORDS: antibiotics, governmental hospitals, duration of using, and antibiotic resistance

INTRODUCTION

Antibiotics are a class of chemotherapeutic drugs that exhibit selective toxicity towards microbes and cancer cells, while exerting minimal impact on the host organism. Historically, the term "antibiotic" referred to a substance that was generated by an organism and had harmful effects on another organism. However, in contemporary usage, substances that possess antibiotic properties are commonly referred to as antibiotics, regardless of whether they are derived from biological, synthetic, or semi-synthetic sources [1]. Antimicrobials refer to a class of chemical substances that possess the ability to either eradicate or impede the proliferation of microorganisms, encompassing viruses among their targets. Pharmaceutical substances are commonly classified as antibacterial if they possess the ability to eradicate or impede the growth of bacteria, antifungal if they exhibit efficacy against fungi, antiparasitic if they demonstrate activity against parasites (specifically antiprotozoals if they target protozoa, and antihelmintics if they combat helminths), and antivirals if they manifest

effectiveness against viruses [2]. The emergence of antibiotic resistance poses a significant and escalating challenge to worldwide public health. According to data collected from 71 nations, the global consumption of antibiotics experienced a growth rate above 50% during the period from 2000 to 2010 [3]. The primary consequence of the overconsumption of antibiotics is the emergence and spread of antibiotic resistance. The primary manifestations of the overutilization and misuse of antibiotics encompass non-indicated administration, excessive dosing, and the concurrent use of multiple medicines, all of which contravene the fundamental principles governing antibiotic usage [4].

China is a significant consumer of antibiotics globally, ranking second in terms of consumption. The prescription rate of antibiotics in China is twice as high as the suggested guidelines set by the World Health Organisation (WHO). Moreover, rural regions within China exhibit greater rates of antibiotic prescription compared to metropolitan areas [5]. A significant proportion of physicians in this regions do not have special training,

although they are authorised to prescribe antibiotics listed in the national registry as a result of personnel shortages. Prior research has documented a significant prevalence of inappropriate antibiotic prescription practises among primary care providers [6]. Moreover, studies conducted in Italy and Germany investigating antibiotic prescribing practises of primary care physicians revealed a significant prevalence of improper antibiotic utilisation [7, 8]. In Iraq, antibiotics are exclusively classified as prescription medications; nonetheless, it is prevalent for community pharmacies to distribute them without requiring a prescription. In alternative terms, the oversight of antibiotic dispensation in community pharmacies exhibits inadequate regulation. The regulation of antibiotic prescription and distribution in the public sector is more effectively managed through formal protocols, routine assessments of drug inventory and documentation conducted by government inspectors, and the inclination of pharmacists to safeguard themselves against any legal liabilities. Prior research has indicated that the practise of prescribing antibiotics without a prescription is prevalent in community pharmacies, not only in low-income nations but also in developed nation [9, 10].

The unrestricted over-the-counter availability of antibiotics results in the selection of incorrect antibiotic options and contributes to a higher incidence of non-compliance with prescribed antibiotic regimens. The Iraqi population exhibits a tendency to employ antibiotics as a treatment for various respiratory diseases, including those of viral origin, such as influenza. Despite the fact that antibiotics are typically classified as prescription-only medications, individuals can readily acquire them without obtaining a prescription. A significant proportion of community chemists deviate from adhering to the established legal protocols governing the administration of antibiotics, hence facilitating their availability to patients without the requisite prescription [11].

AIM

The aim of the study is to explore the clinical patterns of antibiotic prescriptions in government hospitals, with a specific focus on physicians who have primarily received degree-based training.

MATERIALS AND METHODS

PATIENTS AND METHOD

The retrospective cross-section study, carried out between July 1, 2022 and April 2023, that enrolling 300 patients from different government hospitals (obstetrics, children, internal medicine, and surgery) of different provinces in central and northern Iraq (Baghdad, Babylon, Karbala, Anbar, Salah l-Din, Najaf, Al_Muthanna, and Diwaniya) with prescribed antibiotics. The research form contained 15 fields divided into three sections. The first section contains social information such as age, gender, field of work, residence and education. The second part consists of results of diagnostics and laboratory tests. The third part related to antibiotic (AB) uses: number of AB prescribed, duration of using, type of use, route of administration, AB interaction, dose administration of AB, indication of AB, and class of AB. After that, we collect the obtained data in Microsoft Excel program tables and then statistically analyzed it as shown in the results using IBM SPSS Statistics version 26.

STATISTICAL ANALYSIS

The statistical package for social sciences (SPSS® version 26, IBM Inc., Chicago, IL, USA) was used to analyses the data. The standard deviation was used to express the numerical variables. When the Kolmogorov-Smirnov test failed to reveal a normal distribution, the variables were examined using the nonparametric Kruskal-Wallis's test to evaluate the mean differences between groups. Bivariate correlation analysis was also carried out to evaluate the relationship between parameters. Statistics were deemed significant at $P < 0.05$.

RESULTS

DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

During the study period, a cohort of 300 eligible patients was admitted, with 165 patients (55.0%) were identified as males and 135 patients (45.0%) were identified as females. In the sample population, it was observed that 117 patients (39.0%) were below the age of 20, 25 patients (8.3%) belonged to the age group of 20-29 years, 28 patients (9.3%) were between the ages of 30-39 and the same was in group of 40-49 years, and 105 patients (35.0%) were over the age of 50. The average age of the patients who were enrolled in the study was 31.1 years, with a standard deviation of 1.77. Among the enrolled patients 0.7% were had medical jobs and 99.3% had others jobs. A significant proportion of the patient population (69.3%) hailed from an urban setting and 30.7% from countryside. Nearly 45% of patients were received one to two antibiotics during

Table 1. The demographic and clinical characteristics of the patients who were included in the point prevalence survey of antibiotic consumption conducted at hospitals

		Frequency	Percent	Std. Deviation	Sig.
Age	<20	117	39.0	1.773	0.000
	20-29	25	8.3		
	30-39	25	8.3		
	40-49	28	9.3		
	>50	105	35.0		
	Total	**300**	**100.0**		
Gender	Male	165	55.0	0.498	0.083
	Female	135	45.0		
	Total	**300**	**100.0**		
Health worker	Yes	2	0.7	0.082	0.000
	No	298	99.3		
	Total	300	100.0		
Residence	City	208	69.3	0.462	0.000
	Countryside	92	30.7		
	Total	**300**	**100.0**		
Amount of antibiotic prescribed	One	124	41.3	0.684	0.000
	Two	144	48.0		
	Three	30	10.0		
	More than three	2	0.7		
	Total	**300**	**100.0**		
Duration of using	3 days	154	51.3	1.240	0.000
	5 days	56	18.7		
	7 days	18	6.0		
	>7 days	72	24.0		
	Total	300	100.0		
Type of use	Without culture	298	99.3	0.082	0.000
	Empirical	2	0.7		
	Total	**300**	**100.0**		
Route of administration	Intravenous	269	89.7	0.588	0.000
	Oral	3	1.0		
	Intravenous follow by oral	28	9.3		
	Total	**300**	**100.0**		
Dose administration of antibiotic	Regular dose	267	89.0	0.627	0.000
	High dose	33	11.0		
	Total	**300**	**100.0**		
Indication of antibiotic	Proper	290	96.7	0.180	0.000
	Improper	10	3.3		
	Total	**300**	**100.0**		

they admission in hospital. 154 (51.3%) of patients were used antibiotics for 3 days while 72 (24.0%) of them used antibiotics for >7 days. Nearly ≥ 90% of patients used antibiotics without culture, IV, in regular dose, and with proper see (Table 1).

ANTIMICROBIAL USAGE AT HOSPITALS

During the duration of the study, a cohort of 300 patients was admitted, with 198 patients (66.0%) receiving treatment with cephalosporins which considered a significant (p<0.05) when compared with

Table 2. Number and percentage of antimicrobial usage at hospitals

	N	%	P value
Antibiotic used			
cephalosporin	198	66.0	0.000*
penicillin	49	16.3	0.000#
aminoglycoside	40	13.3	0.000#
carbapenem	52	17.3	0.000#
glycopeptide	28	9.3	
fluoroquinolone	22	7.3	
anti-fungal	58	19.3	0.000#
others	43	14.3	0.000#
Amount of antibiotic used			
one drug	129	43.0	0.000*
two drugs	139	46.3	0.000*
three drugs	32	10.7	
Total	300	100.0	
* vs others types; # vs fluoroquinolone			

other antimicrobials. Also, penicillin, carbapenem, anti-fungal, and aminoglycoside had high percentage of used (13-19%) as compared with fluoroquinolone (7.3%). On other hands, 129 (43.0%) and 139 (46.3%) patients were used one and two combined antimicrobial drugs which considered significant (p<0.05) as compared with those that used three types (Table 2, Fig.1).

COMPARISON OF ANTIMICROBIAL COMBINATION USED AT HOSPITAL

Our results showed that cephalosporins alone were used by 106 (53.5%), which was considered significant (p<0.05) compared with their use in combination with other antimicrobials in two or three combinations by 82 (41.4%), 10 (5.1%) respectively. Penicillin, aminoglycoside, carbapenem, glycopeptide, fluoroquinolone and antifungal combination are used in two combinations, while other types are used in less than 14%, which is considered significant (p < 0.05) (Table 3, Fig. 2).

DURATION OF ANTIBIOTIC USE

144 (48.0%) patients used two antibiotics, of which 80 (55.6%) were prescribed for a period of 3 days and about 38 (21.8%) used it for more than 7 days. During survey period and rest period, 124 (41.3%) patients received one prescribed antibiotic, on the other hand, 30 (10.0%) and 2 (0.7%) prescribed antibiotics were a combination of three and more than three antibiotics, respectively (Table 4, Fig. 3).

DISCUSSION

The present study examined the utilisation of antibiotics in surgical and gynaecological and obstetric departments located in the central and southern regions of Iraq. It is important to remember that a significant percentage of patients received antibiotics were less than 20 years old and more than 50 years that indicated most of infectious agents affected these range of ages and nearly equally between male and female. This hypothesis could be formulated based on the over utilisation of antibiotics in the paediatric population. Initially, it is plausible for physicians to posit that paediatric populations exhibit heightened vulnerability to infections, with hospitals serving as reservoirs for medically significant infections. Furthermore, as a result of inadequate comprehension about antimicrobials and antibiotics, parents may express a desire or insist upon the administration of novel or costly antibiotics for their offspring [12]. The survey revealed a discrepancy in the utilisation of Watch antibiotics, as opposed to the aim set by the World Health Organisation (WHO) for the administration of Access group antibiotics in 60% of patients. Despite the presence of laboratory capacities in all the study hospitals, the implementation of test-based directed antibiotic usage was found to be restricted. The temporal demands associated with the cultivation of biological specimens may impede the timely administration of targeted therapies within healthcare facilities. The considerable prevalence of antibiotic utilisation also suggests a deficiency in the adoption of guidelines inside the hospitals under research, as a majority of these hospitals lack internal guidelines. Furthermore, the current national guidelines primarily concentrate on a limited number of specific conditions [13]. During the survey, it was observed that patients received two or more antibiotics while they were

Table 3. Number and percentage of antimicrobial combination used at hospital

Antimicrobial	Combination			P value
	Amount of antibiotics used	No	% of Total	
Cephalosporin combination	Single	106	53.5%	0.000
	Two	82	41.4%	
	Three	10	5.1%	
	Total	**198**	**100.0%**	
Penicillin combination	Single	7	14.3%	0.000
	Two	38	77.6%	
	Three	4	8.2%	
	Total	**49**	**100.0%**	
Aminoglycoside combination	Two	36	90.0%	0.000
	Three	4	10.0%	
	Total	40	100.0%	
Carbapenem combination	Single	7	13.5%	0.000
	Two	25	48.1%	
	Three	18	34.6%	
	More than three	2	3.8%	
	Total	**52**	100.0%	
Glycopeptide combination	Single	1	3.6%	0.000
	Two	20	71.4%	
	Three	5	17.9%	
	More than three	2	7.1%	
	Total	28	100.0%	
Fluoroquinolone combination	Two	17	77.3%	0.000
	Three	5	22.7%	
	Total	**22**	**100.0%**	
Antifungal combination	Two	33	56.9%	0.000
	Three	23	39.7%	
	More than three	2	3.4%	
	Total	**58**	**100.0%**	
Others combination	Two	26	60.5%	0.000
	Three	17	39.5%	
	Total	**43**	**100.0%**	

hospitalised. The utilisation of several antibiotics during a single hospitalisation should be regarded as a significant matter of concern. The study showed a significant burden associated with the usage of antibiotics. In this hospital, antibiotics are dispensed without the international guidelines, and without the instructions approved by the hospital. Where the dispensing of antibiotics is based on the experiences of doctors in dealing with disease cases, which generates some errors in that dispensation and the consequent side effects according to this case. Observing the results in the table above. There is no relation to the standards adopted in age, gender, work, education and areas of residence. As it was not completely or definitively affected by the misuse of antibiotics, these standards may be included in the field of the private sector

more than in the context of work, prescribing treatment in Iraqi hospitals. The study yielded findings pertaining to the use of two distinct categories of antibiotics, constituting the predominant portion. This reason is due to covering the bacterial infection by more than one mechanism. In these hospitals, it is not dependent on dispensing treatment according to the most correct method, as the table showed that the percentage of antibiotic use according to the blood culture is very small 2 patients, and randomly 298 patients out of 300 patients. The reason for that may be the delay in the results of laboratory tests although the culture sensitivity tests not performed even if the patients stay for a long time in the hospitals. The results also showed that the use of treatment was inappropriate in 10 patients out of 300 patients
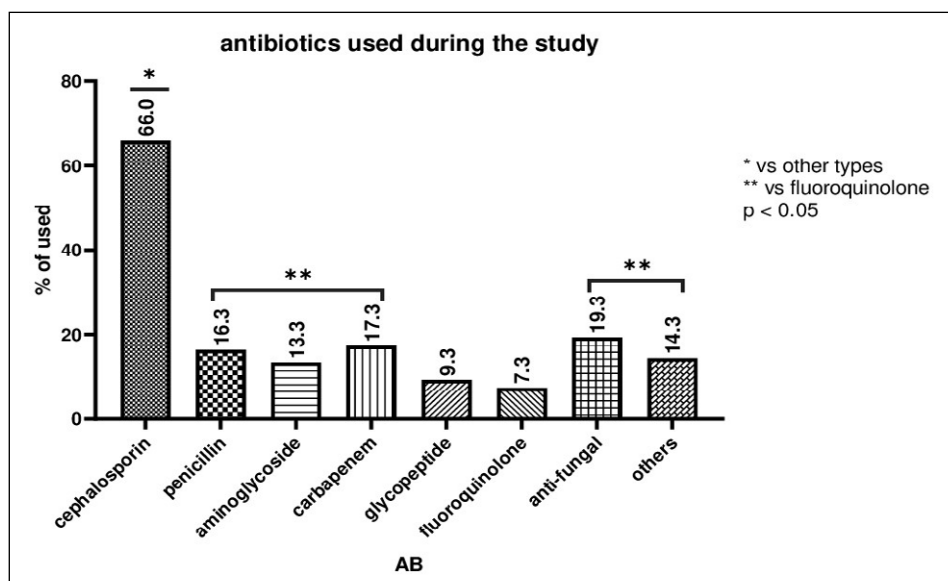
Fig. 1. Number and percentage of antimicrobial usage at hospitals.
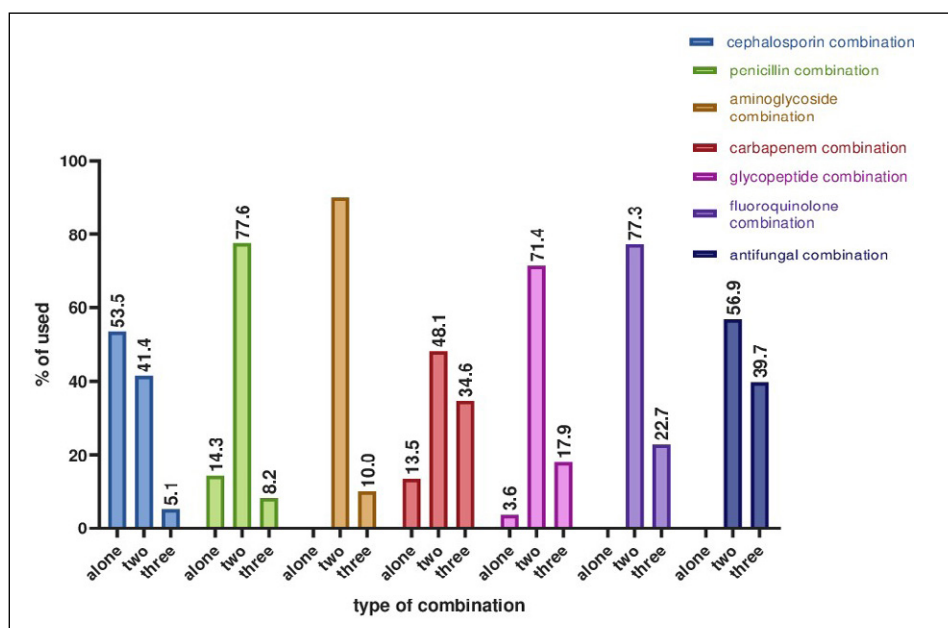
Fig. 2. Number and percentage antimicrobial combination used at hospital.
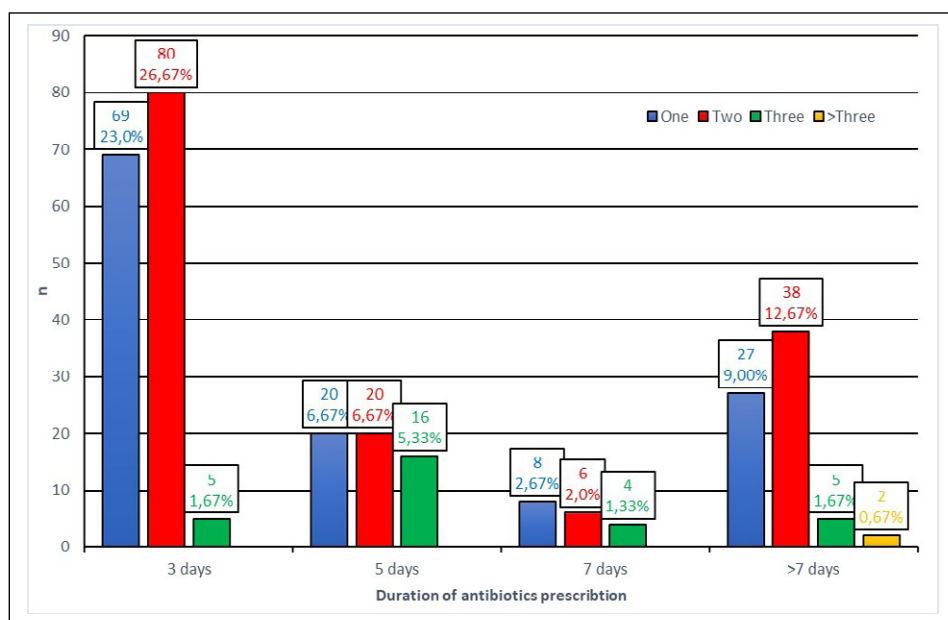
Fig. 3. Duration of using prescribed antibiotics.

Table 4. Duration of using prescribed antibiotics

			No. of antibiotics prescribed				Total	Chi-esquire	Sig.
			One	Two	Three	More than hree			
Duration of using	3 days	N	69	80	5	0	154		
		% antibiotics prescribed	55.6%	55.6%	16.7%	0.0%	51.3%		
		% of Total	23.0%	26.7%	1.7%	0.0%	51.3%		
	5 days	N	20	20	16	0	56		
		% antibiotics prescribed	16.1%	13.9%	53.3%	0.0%	18.7%		
		% of Total	6.7%	6.7%	5.3%	0.0%	18.7%		
	7 days	N	8	6	4	0	18	40.617	0.000
		% antibiotics prescribed	6.5%	4.2%	13.3%	0.0%	6.0%		
		% of Total	2.7%	2.0%	1.3%	0.0%	6.0%		
	>7 days	N	27	38	5	2	72		
		% antibiotics prescribed	21.8%	26.4%	16.7%	100%	24.0%		
		% of Total	9.0%	12.7%	1.7%	0.7%	24.0%		
Total		N	124	144	30	2	300		
		% antibiotics prescribed	100%	100.0%	100%	100%	100.0%		
		% of Total	41.3%	48.0%	10.0%	0.7%	100.0%		

due to misdiagnosis and the use of antibiotics as a form of prevention for secondary infections related, for example, in viral infections. High doses appeared in infants and preterm infants. Doctors considered obligatory in using high doses to avoid aggravation of bacterial infection as a precaution. On the other hand, in the elderly, the doses are appropriate. German specialist associations, such as the German Society for Pediatrics and Infectious Diseases, stress that sick children should not take antibiotics unless absolutely necessary, such as if the disease is bacterial rather than viral. This applies to adult patients as well, because antibiotics can do nothing against viruses, which often cause coughing and runny nose. US researchers also warn that over-giving antibiotics to growing children can lead to health problems, such as allergies and obesity. It is very important that such long-term side effects be closely researched by Caproni et al. [14, 15]. It is possible to conduct a test called "CRP" to see if the cause of the disease is bacteria or viruses. Doctors say it is important to differentiate between virus and bacteria. The virus may or may not have a vaccination, and bacteria often do not have a vaccination, but they do have a cure. It is important not to make a mistake in differentiating between them, and not to give antibiotics to the patient except when the disease is bacterial, because viruses do not respond to antibiotics [8].

CONCLUSIONS
The issue of antimicrobial resistance in bacterial pathogens is a substantial challenge, resulting in significant morbidity and mortality. The treatment of multidrug resistance patterns in both Gram-positive and Gram-negative bacteria poses significant challenges and may potentially render standard antibiotic therapies ineffective. The implementation of clinical guidelines, provision of direct education, and regular dissemination of antibiogram data have the potential to foster a more judicious utilisation of antibiotics.

RECOMMENDATIONS
The use of antibiotics should be according to the results of the culture sensitivity tests and not randomly. And that the doses be according to the correct scientific standards.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Mahmood Jawad
Department of Pharmacy,
Al-Zahrawi University College,
G499+JPR Karbala, Iraq
e-mail: mahmood@g.alzahu.edu.iq

ORCID AND CONTRIBUTIONSHIP
Mahmood Jawad: 0000-0001-6542-7230 Ⓐ Ⓕ
Shathel Khalaf Noman: 0000-0002-7065-6924 Ⓑ Ⓒ Ⓓ
Mohammed Rasool: 0000-0003-0992-320X Ⓓ Ⓔ

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

ORIGINAL ARTICLE

CONTENTS

Ocular lesions in patients with *ulcerative colitis*

Antonina V. Varvarynets, Valerii D. Beliayev, Mykhailo M. Hechko, Artur V. Kurakh
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT
Aim: To analyze the data and evaluate the prevalence of ocular lesions in patients with moderate ulcerative colitis.
Materials and Methods: We observed 112 patients aged 18-75 years old with clinically, endoscopically and histologically confirmed moderate ulcerative colitis which lasted at least 6 months. An ophthalmologic exam was performed to determine the presence of ocular symptoms.
Results: Of the 112 patients with moderate ulcerative colitis, 21 (18,75%) had the following ocular lesions: episcleritis - 7 patients (6,25%), keratopathy - 5 patients (4,46%), uveitis - 5 patients (4,46%), *cataract* - 2 (1,78%) and *scleritis* - 2 (1.78%).
Conclusions: Because ocular symptoms in patients with UC are often nonspecific, it may be beneficial to perform ophthalmologic examinations as a routine follow-up component of in such patients.

KEY WORDS: ulcerative colitis, scleritis, blepharitis, uveitis, episcleritis

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory, immune-mediated disease, which manifests as inflammation of the gastrointestinal tract. The treatment consists of both local and systemic anti-inflammatory drugs, biological drugs, antibiotics and immunomodulators [1,2]. More than a third of the patients with UC experience extraintestinal manifestations, the pathogenesis of which is not fully studied however there is an opinion, that this is also an immune-mediated process, because treatment of the main disease with immunomodulators often has a positive effect on extraintestinal symptoms [3,4].

Keratopathy is a lesion of the cornea of the eye, which manifests itself as changes in the anatomical structures of the cornea and, possibly, a concomitant inflammatory process, due to damage to the corneal epithelium and Bowman's membrane, which act as a natural biological barrier. The quality and quantity of tears plays a great role in the development of keratopathy, and in patients with dry eye disease the risk of keratopathy occurring is much higher [5]. Diagnosis of keratopathies is conducted with the help of fluorescein dye, which allows to highlight the lesions [6]. The clinical manifestations are feelings of discomfort and pain in the eyes, irritation, sensation of a foreign object in the eye and blurry or low vision. Many research results suggest that patients with UC have a higher risk of keratopathy when compared with healthy patients of the same

age group [7,8]. Others suggest that UC patients more often develop dry eye after 5-aminosalicylic treatment. Mild keratopathy can be initially treated with artificial tears, but if symptoms progress, an ophthalmological examination is necessary.

Episcleritis is a fairly common disease of the cornea, manifested by a burning sensation, irritation, pain and redness of the eyes. The episclera is the surface layer of fibrous tissue located between the sclera and the conjunctiva. Episcleritis should be clinically differentiated from scleritis, since the latter is a more severe and deep manifestation of inflammation, the signs of which are severe pain and discomfort in the eyes. In severe cases, scleritis can be accompanied by thinning of the sclera and the risk of perforation, which requires urgent ophthalmological evaluation to avoid permanent vision loss [9].

The disease course of episcleritis correlates with the aggravation of the main condition, which is UC. Less severe cases respond well to topical treatment with steroids and NSAIDs.

Scleritis, although a rare manifestation in patients with UC, can cause significant visual impairment and be the cause of loss of the eye, if not adequately treated. Unlike episcleritis, scleritis is not always associated with active intestinal inflammation in patients with UC. Although, despite thus, it is important to reach a long-term remission of the main disease, to prevent a relapse and visual impairment. Repeated episodes of

scleritis often lead to the perforation the sclera. Treatment usually begins using local hormonal drugs and local and systemic NSAIDs; but they should be used with caution in patients with UC. With a poor response to treatment, systemic steroids and immunosuppressive therapy is used. In unresponsive cases use of such biologic medications like infliximab, adalimumab was found to be more effective which successfully replace more toxic alkalizing agents [10].

Uveitis (inflammation of the vascular layer of the eye) is one of the less common but potentially more serious ocular manifestations of UC. The vascular layer is a complex layer of the eye which consists of the iris, ciliary body and choroid. Usually uveitis is divided by location: anterior uveitis (iritis) affects the iris, intermediate uveitis (cyclitis) affects the ciliary body, and posterior uveitis (choroiditis) affects the choroid. When all of the vascular layers are affected, it's called panuveitis. As is the case with scleritis, uveitis can cause severe complications of the eye that can lead to blindness. Acute anterior uveitis or iritis are observed most often in patients with UC, however cases of posterior uveitis or panuveitis may be observed as well [11].

Patients with uveitis complain of pain in the eyeballs, blurred vision up to loss of vision, photophobia and headache. The diagnosis is verified using a slit-lamp examination, which allows you to differentiate between anterior and posterior uveitis. In anterior uveitis cases change in the color of the iris, precipitates on the back surface of the cornea and pathological deposits in the anterior chamber (fibrin, hypopyon) are observed during examination. In posterior uveitis we see clouding of the vitreous and changes in the fundus. In some cases, uveitis can precede the diagnosis of UC and occur regardless of the activity of the inflammatory process in the intestine. If uveitis occurs in cases when UC is already established, it can be bilateral, have a long disease course, and an unexpected onset. Like scleritis, uveitis requires immediate ophthalmologic treatment with local and systemic corticosteroids and mydriatics, to prevent complications, such as glaucoma, cataract, fibrosis of the vitreous and retinal detachment. In severe or resistant to treatment cases of uveitis with concurrent complications (cataract or glaucoma) systemic immunosuppressive and symptomatic (hypotensive) treatment may be required [12]. Since steroid eye drops do not fully reach the posterior part of the eyeball with a high enough concentration, intermediate, posterior uveitis and panuveitis are treated with systemic steroids, periocular injections of steroids and consecutive immunotherapy.

In biologic therapy, adalimumab is widely used for treatment of UC, as well as ocular symptoms and is the only medication approved by the U.S. Food and Drug Administration (FDA) for treatment of uveitis [13]. Infliximab is also quite effective in uveitis cases and is approved by the FDA for UC treatment. Calcineurin inhibitors, such as ciclosporin are effective for treatment of both intestinal and ocular manifestations of UC, but have more side effects. Modern medications for UC treatment and the potential for uveitis treatment are janus kinase inhibitors, such as tofacitinib [14,15].

AIM
To analyze the data and evaluate the prevalence of ocular lesions in patients with moderate UC.

MATERIALS AND METHODS
We observed 112 patients with clinically, endoscopically and histologically confirmed moderate ulcerative. Patients were aged 18 to 75 years old, with a clinical course of UC of at least 6 months. All patients complained of loss of vision, dryness, redness and pain of the eye and underwent an ophthalmologic examination. Visual acuity was evaluated using the Snellen chart, intraocular pressure (IOP) was measured with the iCare tonometer and a slit-lamp exam was used to examine the anterior part of the eyeball. If IOP was in normal range a binocular indirect ophthalmoscopy examination was conducted under drug-induced mydriasis.

RESULTS
This study was conducted at the therapy department of the municipal non-commercial enterprise "Uzhhorod city multidisciplinary clinical hospital". 112 patients with endoscopically verified UC were included in the study, average patient age was 39,2 +/-12,6 years (ranged from 18 to 75). Of the 112 patients with UC, 21 (18,75%) had ophthalmologic symptoms.

The most common ocular lesions were as follows: episcleritis - 7 patients (6,25%), keratopathy - 5 patients (4,46%), uveitis - 5 patients (4,46%), cataract - 2 (1,78%) and scleritis - 2 (1.78%).

All 7 episcleritis cases were unilateral, 2 of which manifested prior to intestinal lesions. All cases were treated with local NSAIDs, 3 cases required topical steroids during the treatment course. 2 of these patients received biologic therapy for the main disease (Fig. 1).

All cases of scleritis were unilateral, manifested after UC was diagnosed and were treated with topical steroids. All patients received immune suppression therapy for the main disease.
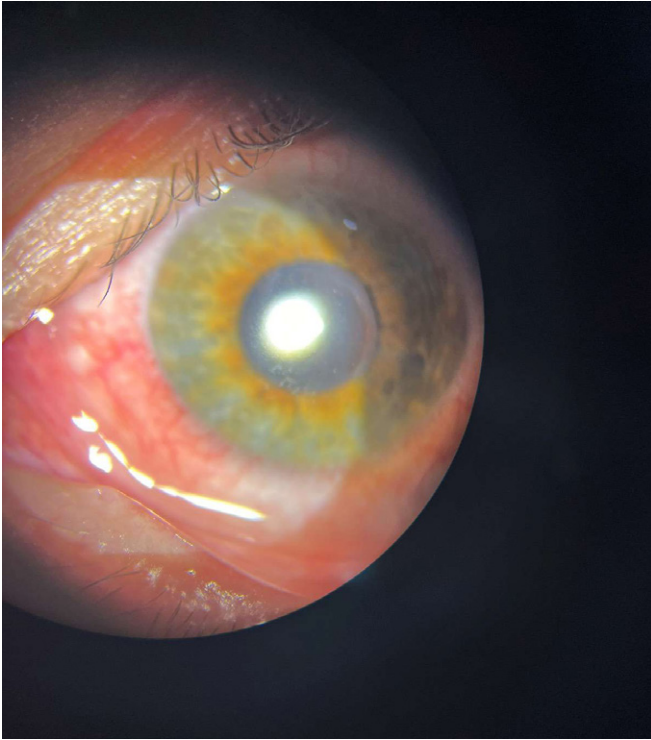
Fig. 1. Episcleritis in a patient with moderate UC.
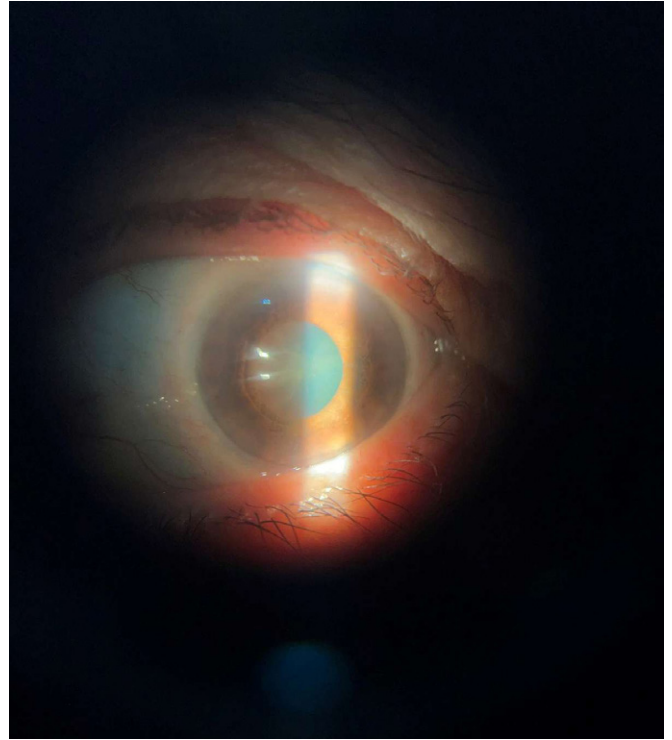
Fig. 2. Uveitis in a patient with moderate UC.
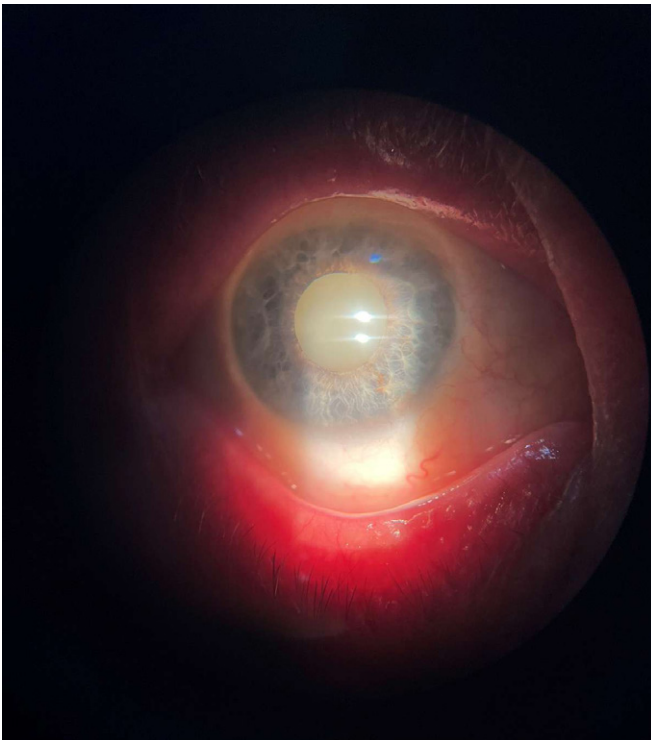
Fig. 3. Cataract in a patient with moderate UC.
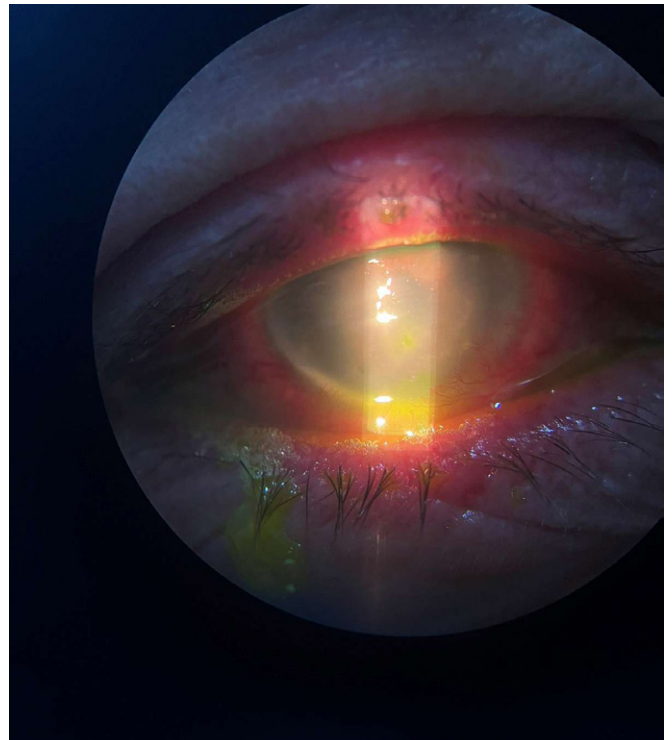
Fig. 4. Keratopathy in a patient with moderate UC.

Of the 5 uveitis cases: 2 had anterior uveitis, 1 – panuveitis, 2 – posterior uveitis. Patients receive topical steroid treatment and mydriatics, and therapy for the main disease. 4 patients received tofacitinib due to acute intestinal inflammation (Fig. 2).

Only 2 patients had cataract, ocular symptoms manifested prior to UC, but did not seek adequate treatment (Fig. 3).

All cases of keratopathy were observed after UC diagnosis and manifested in mild form. These patients used artificial tears and received treatment for the main disease (Fig. 4).

DISCUSSION

Understanding the occurrence of ocular signs in patients with UC is a difficult process, because the pathogenesis of this extraintestinal manifestation is still not fully researched. Most of the ocular symptoms that we observed were in patients with established moderate UC, only a few of them had ocular symptoms prior to UC diagnosis. A lot of research is devoted to studying the relationship between extraintestinal manifestations in patients with inflammatory bowel disease (IBD) for better treatment options.

Our results are similar to the study by Safiye Yilmaz et al. where they explored the prevalence of ocular lesions in patients with IBD, which included 116 patients: 20 patients with Crohn's disease and 96 patients with UC. All patients were examined for signs of ocular lesions. They concluded that in the group of patients with the Chron`s disease, 12 (60%) had ocular lesions, and of the UC group – 22 (22.92%). The most prevelant were conjunctivitis (8.62%, blepharitis (6.9%), uveitis (5.17%), cataract (5.17%) and episcleritis (3.45%) [15].

Another study showed that among 1432 patients that were included with diagnosed IBD (both Crohn's disease and UC as the criteria), 5% had ocular signs. Patients were monitored for the period from 2013 to 2020. Patients were divided into 2 groups: group I consisted of 53 (3.7%) patients with ocular lesions that were related to IBD, and group II – 34 (2.4%) were patients with ocular lesions, that were determined to be not related to IBD. Among these, the most prevalent ocular lesions were dry eye (17.2%), blepharitis (10.3%), uveitis (14.9%), episcleritis (14.9%) and scleritis (2.3%) [16].

It should also be mentioned that in this study, most of the patients were in the active stage of the intestinal disease, so additional follow-up ophthalmologic evaluation during intestinal remission allowed to better establish the connection between the range and prevalence of ocular lesions in UC patients.

CONCLUSIONS

UC is a systemic chronic disease, which often has various extraintestinal manifestations that need timely evaluation for successful treatment. Because ocular symptoms in patients with UC are often nonspecific, it may be beneficial to perform ophthalmologic examinations as a routine follow-up component of in such patients.

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CONFLICT OF INTEREST

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CORRESPONDING AUTHOR

Antonina V. Varvarynets
Uzhhorod National University
71 Mynaiska st, 88000 Uzhhorod, Ukraine
e-mail: antonina.varvarynec@uzhnu.edu.ua

ORCID AND CONTRIBUTIONSHIP

Antonina V. Varvarynets: 0000-0001-5859-1040 Ⓐ Ⓑ Ⓓ Ⓔ Ⓕ
Valerii D. Beliayev: 0009-0003-1641-9076 Ⓐ Ⓑ Ⓒ Ⓓ Ⓕ
Mykhailo M. Hechko: 0000-0003-2793-5044 Ⓐ Ⓑ Ⓓ Ⓔ
Artur V. Kurakh: 0000-0002-2763-2935 Ⓓ Ⓔ Ⓕ

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

ORIGINAL ARTICLE   CONTENTS ↗

Pseudomembranous colitis as a complication in Covid-19

Stepan S. Filip, Rudolf M. Slivka, Andrii M. Bratasiuk, Yuriy P. Skrypynets, Anatoly I. Shitev
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT
Aim: To improve the results of treatment of patients with *pseudomembranous colitis* against the background of coronavirus infection.
Materials and Methods: The study presents the results of a retrospective analysis of 96 patients with *pseudomembranous colitis*, who were treated in the infectious Covid department at the base of the Uzhhorod City Clinical Hospital since 2020 to 2022. The average age of patients was 55.2 years, there were 38 (39.5%) men and 58 (60.5%) women. Diagnosis of complications – *pseudomembranous colitis (PMC)* – was based on clinical data, ultrasound and CT of the abdominal organs, fibrocolonoscopy, laparoscopy.
Results: The frequency of PMC from the total number of patients who were in hospital treatment (8205 patients) due to COVID-19 was 1.17%, and this indicator was 0.62% in 2020, and 2.28% in 2021. Indications for operative treatment were: colon perforation – 9.4% of patients; peritonitis (diffuse, widespread) without obvious perforation of the colon wall – 85.5% of patients; mesenteric thrombosis – 4.1% of patients. In the case of perforation of the colon, resection of the colon was performed with the formation of a proximal colostomy and ileostomy. In case of mesenteric thrombosis, resection of the affected part of the small intestine was performed. In case of peritonitis without clear intraoperative detection of perforation of the colon wall, intraoperative lavage was performed.
Conclusions: 1) The frequency of detection of PMC in patients with COVID-19 in 2020 was 0.62%, and in 2021 - 2.28%. 2) The sensitivity of CT in the diagnosis of surgical complications of PMC was 72%, and the specificity was 58%. 3) Conservative treatment was effective in patients with PMC in 88.8% of cases, 21.2% had complications that required emergency surgical interventions. 4) The total mortality in patients with PMC was 11.36%, although this indicator was significantly higher in the event of surgical complications and operative treatment (22.4%).

KEY WORDS: SARS-CoV-2 virus, intestinal microbiome, intestinal perforation, colostomy, peritonitis

INTRODUCTION

The 2019 coronavirus disease (COVID-19) pandemic has brought significant changes to the course of many chronic diseases. In addition to damage to the lungs, various complications may occur from other organs and systems [1,2].

One of the frequent complications is a violation of the digestive system - a change in the intestinal microbiome due to the impact of the SARS-CoV-2 virus. This is due to the significant impact of the virus on the microbiota and the permeability of mucous barriers, as well as changes in the barrier function of the digestive system in the development of local and systemic immune response. Most often, against the background of COVID-19, nonspecific symptoms from the digestive system develop — nausea, vomiting, diarrhea, and abdominal pain [3]. Diarrhea occurs in every second patient with COVID-19 [4]. Studies of the long-term consequences of SARS-CoV-2 infection indicate that after the end of the acute respiratory form of COVID-19, changes in the gut persist in patients [5,6]. Against the background of existing intestinal

diseases, the course of COVID-19 is more difficult, in addition, the transferred SARS-CoV-2 infection changes and complicates the course of chronic intestinal pathology and can cause the appearance of a new intestinal disease [7].

The working group of the Ukrainian Gastroenterological Association developed a classification of post-covid intestinal lesions, which was presented at the association's congress in September 2021 [7]. According to this classification, the condition is distinguished: Post-covid antibiotic-associated diarrhea - *pseudomembranous colitis* with confirmed Clostridium difficile infection. *Pseudomembranous colitis (PMC)* is a diarrheal disease with an acute course, characterized by the presence of gray-yellow plaques (pseudomembranes) on the surface of the mucous membrane of the large intestine. It is characterized by frequent diarrhea, spastic abdominal pain, and dehydration.

Etiological factor: toxins A and B, secreted by the anaerobic gram-positive bacillus *C. Difficile*, multiplying excessively in the intestine as a result of the use of
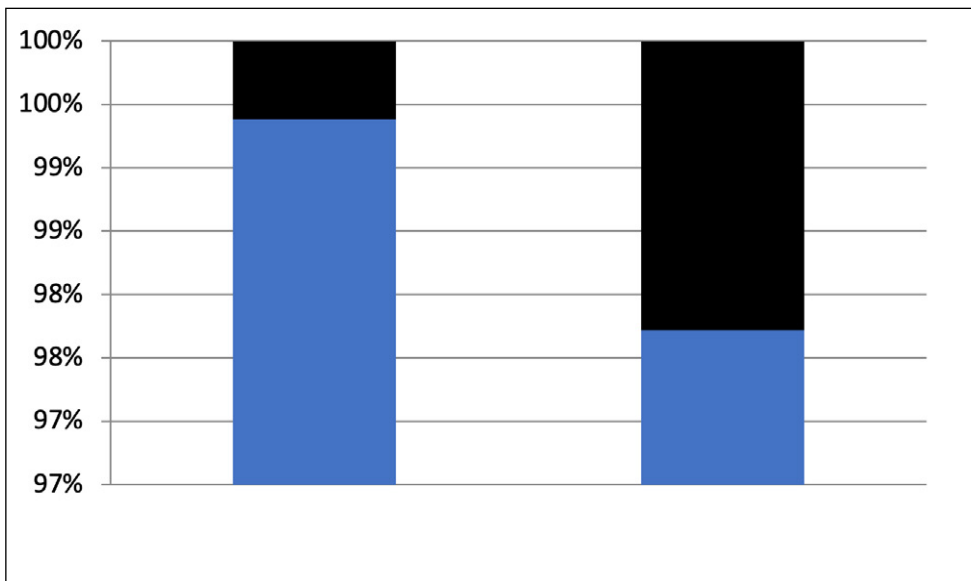
antibiotics with a broad spectrum of antibacterial action. Very often, patients interpret this condition as a dysbacteriosis that occurs after antibiotic therapy [9,10,11].

Against the background of the COVID-19 pandemic, the number of patients with PMC has increased significantly. On the one hand, this can be explained, because among the risk factors for the occurrence of clostridial colitis are the uncontrolled use of antibiotics in large doses, advanced age, and inpatient treatment — all conditions accompanying the modern pandemic [12]. On the other hand, recently there have been publications about an increase in the frequency of severe PMC without previous diarrhea with the development of toxic megacolon, sepsis, multiple organ failure, as well as works describing the phenomena of PMC, confirmed by an endoscopic picture and a negative test for the presence of clostridial toxin in feces of convalescents with COVID-19.

The course of COVID 19 is aggravated by comorbid pathology. Despite the fact that the development of acute complications occurs with the same frequency in patients both before and after 50 years of age, the presence of comorbid pathology, in particular cardiovascular diseases, diabetes, oncological diseases, and CKD significantly increase the probability of death from this disease [13]. Death in such patients occurred more often with a combination of three or more concomitant diseases, due to the development of acute left ventricular failure and thrombotic complications [14,15].

The problems of treating PMC remain relevant in connection with the tendency to increase the irrational use of antibiotics and the low level of prenatal diagnosis of this pathology, as well as the development of surgical complications from the abdominal organs.

AIM

To improve the results of treatment of patients with *pseudomembranous colitis* against the background of coronavirus infection.

MATERIALS AND METHODS

The study presents the results of a retrospective analysis of 96 patients with PMC, who were treated in the infectious covid department at the base of the Uzhhorod City Clinical Hospital since 2020 to 2022. The average age of patients was 55.2 years, there were 38 (39.5%) men and 58 (60.5%) women. A mild form of COVID-19 was established in 3 (3.2%), a moderate form in 71 (73.9%), and a severe form of the disease in 22 (22.9%).

The diagnosis of coronavirus disease is established on the basis of the epidemiological history, clinical manifestations, PCR results, presence of IgM, computer tomography (CT) of the chest organs. Diagnosis of complications — *pseudomembranous colitis (PMC)* — was based on clinical data, ultrasound and CT of the abdominal organs, fibrocolonoscopy, laparoscopy.

Statistical processing of the obtained data was carried out using the licensed package of application programs «IBM SPSS Statistics» using non-parametric methods.

RESULTS

The frequency of PMC from the total number of patients who were in hospital treatment (8205 patients) due to
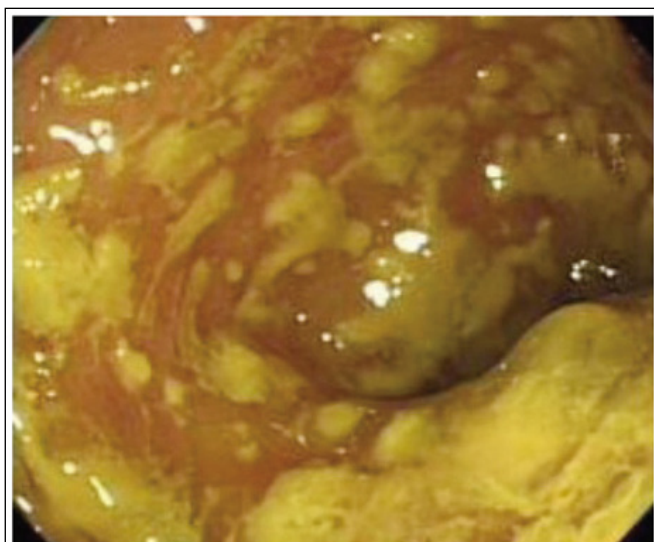
Fig. 2. Endoscopic changes in PMC.

COVID-19 was 1.17%, and this indicator was 0.62% in 2020, and 2.28% in 2021 (Fig. 1).

PMC was diagnosed during the outbreak of a new wave of coronavirus infection in 79 (82.3%) patients, after stabilization of the general condition, elimination of the cytokine «storm» - in 8 patients, 7 days after discharge from the hospital - in 4 patients, after 14 days — 3 patients and 3-4 weeks after discharge from the infectious covid hospital — in 2 patients.

Clinical symptoms characteristic of PMC — diarrhea up to 10-15 times a day, fever, abdominal pain, sudden deterioration of the patient's condition against the background of the treatment of COVID-19, were detected in 84.5% of patients.

Symptoms such as abdominal distension, nausea, and vomiting were relatively rare. A sharp reduction in the number of stools with pain and abdominal distension may indicate the beginning of the development of acute toxic dilatation of the colon or other serious complications of the PMC (perforation, peritonitis).

When examining feces for *C.difficile* toxins, toxin A was detected in 7.4%, toxin B in 55.5%, and both toxins A and B in 37.03%. As you know, toxin B (cytotoxin) is 1000 times stronger than toxin A and its presence is mandatory for the development of PMC.

Computed tomography (CT) is another method of examination for PMK in the arsenal of clinicians. Although CT does not reveal specific signs of PMC, it helps in assessing the state of the colon wall, the state of the mucous membrane, the diameter of the large intestine, and provides some additional information in terms of confirming the diagnosis. Similar data were obtained with ultrasound of the large intestine, although the informativeness of this method was lower than that of CT (sensitivity, specificity — 72% and 58%, respectively).
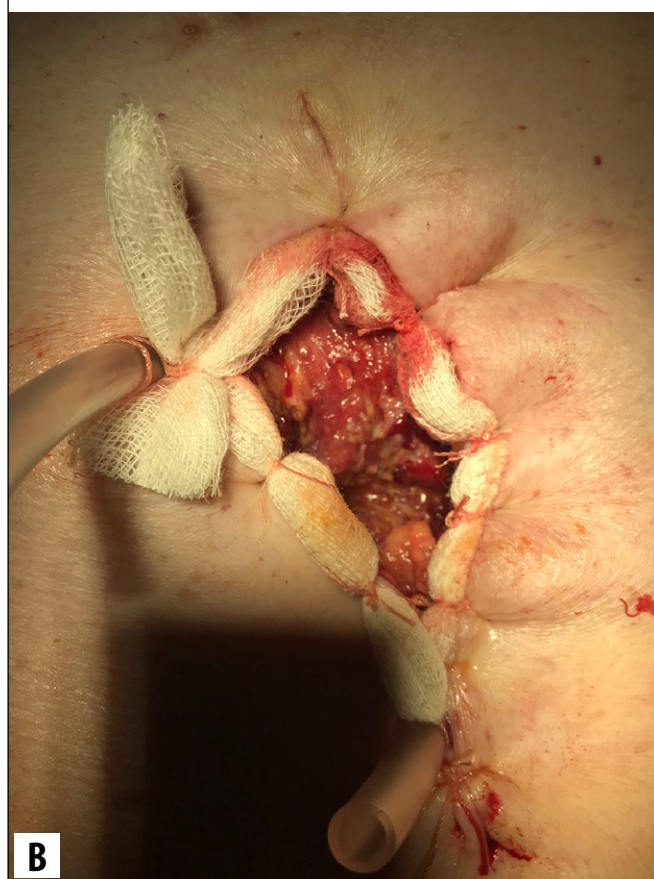
Fig. 3. Intraoperative photo of the affected part of the colon (a). View of colostomy (b).

Table 1. The volume of operative interventions in case of complications and postoperative mortality

Volume of surgical intervention	Number (% of the total number of transactions)	The number of deaths (postoperative mortality)
Resection of the affected intestinal segment due to mesenteric thrombosis	1	1
Ileo/colostomy	3	0
Colon resection	2	1
Laparoscopic peritoneal lavage	18	2
In total	24 (100%)	4 (16,7%)

However, with the development of PMC complications, CT was the most reliable and accurate method of investigation (perforation, peritonitis, toxic megacolon). Thus, in PMC patients with surgical complications, out of 16 cases, the diagnosis after CT scan was confirmed intraoperatively in all cases (Fig. 2).

32 patients (57.14%) had intestinal disorders of varying intensity: the patients noted the presence of blood and mucus in the feces. A colonoscopy was performed on these patients, according to the results of which PMC was endoscopically diagnosed in all examined patients, which was confirmed by a laboratory study and a significant excess of the maximum permissible limits of *Clostridium difficile* was found. It should be noted that in 5 patients endoscopic lesions were localized only in the left part of the colon, while in other patients there was a total lesion of the colon.

Laparoscopy was performed in 36 patients with suspicion of acute surgical complications, when fluid accumulation was detected according to CT scan, ultrasound in order to rule out other acute surgical diseases of the abdominal organs. Diagnostic laparoscopy made it possible to rule out «false» acute abdomen in 19 cases and to confirm complications of PMC in 17 patients (47.2%).

All patients with diagnosed PMC received antibiotics, most often - cephalosporins (84.69%), fluoroquinolones (26.3%), semi-synthetic penicillins (28.9%), azithromycin (43.32%), and, moreover, 44.12% — 2 or 3 antibiotics (27.03% and 17.59%, respectively), and a repeat course of antibacterial therapy was carried out in 14.8% of patients with COVID-19. To continue the treatment of pneumonia in patients with SZV symptoms, 81 patients with PMC (84.37%) received prophylactic doses of probiotics simultaneously with antibacterial therapy, all these patients were treated with glucocorticoids (most often methylprednisolone, prednisone, less often - hydrocortisone). 17 (17.7%) patients were in the intensive care unit on mechanical ventilation before or during the diagnosis of PMC.

In the vast majority of patients, conservative treatment was effective in 88.8% of cases and included the use of metronidazole - 500 mg 3 times a day orally or vancomycin - 125 mg 4 times a day, in severe cases - vancomycin 250-500 mg 4 times a day. In mild, moderately severe forms, oral fluid intake was not restricted, and in severe forms, infusion of 5% glucose and Ringer's solutions was performed at the rate of 30-40 ml/kg.

The term of conservative treatment was within 10 days in 48 (50.0%) patients, up to 14 days in 34 (35.3%) and more than 14 days in 14 (14.7%) patients.

Indications for operative treatment were the development of clinical manifestations of the following complications:
• colon perforation — 9.4% of patients;
• peritonitis (diffuse, widespread) without obvious perforation of the colon wall — 85.5% of patients;
• mesenteric thrombosis — 4.1% of patients.

The volume of operative interventions in case of complications and postoperative mortality are shown in Table 1.

In the case of perforation of the colon, resection of the colon was performed with the formation of a proximal colostomy and ileostomy. The results of the operations were disappointing, the mortality rate after resection operations and the imposition of stomas reached 37.5-66.6%, the latter were forced interventions in terminal patients against the background of a severe course of COVID-19. In case of mesenteric thrombosis, resection of the affected part of the small intestine was performed (Fig. 3).

In case of peritonitis without clear intraoperative detection of perforation of the colon wall, intraoperative lavage was performed (washing with 3-4 liters of physiological saline and antiseptic solutions), sanitation and drainage of the abdominal cavity, drains were installed in the side canals and small pelvis. Postoperative mortality in this group of patients was 11%, the cause of death was abdominal sepsis, multiple organ failure.

The total postoperative mortality was 16.7%.

The total frequency of postoperative surgical complications was 66.7%, including cavity (abdominal) — 14.5%, systemic, mainly abdominal sepsis, were found in 15 (20.8%) patients. In addition to surgical complications from the side of the abdominal wall and cavity, there were severe respiratory complications (pneumonia) in 18 (75.0%) patients, venous thrombosis and pulmonary embolism in 2 (8.3%) and 3 (12.5%) in accordance.

In general, the total mortality rate at PMC was 11.45%, while it is possible to note an increase in mortality in 2021

compared to 2020, which is associated with a more severe course of the COVID-19 strain (delta).

DISCUSSION

As the effects of the coronavirus on the human body are studied, more and more data are emerging that vascular changes are a leading factor in the pathogenesis of the disease. Damage to the endothelium followed by disseminated microvascular thrombosis can serve as the basis of ischemia of the intestinal wall, which leads to the development of PMC.

Complications are observed in 18.9% of patients with COVID-19, and according to other authors, in a quarter of patients with COVID-19, including in 0.5% - with acute inflammation of various parts of the large intestine [5].

It is known that, according to PCR data, the coronavirus in the respiratory tract is detected, on average, within 16.7 days from the onset of the disease, and in feces - 27.9 days, the frequency of detection of RNA virus in feces is about 50% of patients with COVID-19 [5, 7].

C.difficile is diagnosed on the basis of characteristic symptoms and the detection of *C.difficile* toxins or bacteria in the stools of patients. During colonoscopy, focal yellow-green or yellowish deposits tightly fused to the underlying mucous membrane are detected, between which there are areas with hyperemic mucous membrane, bleeding occurs during the attempt to remove them, and as the process progresses, they merge with each other, completely cover the surface of the mucous membrane of the large intestine, undergoing necrosis [9-11].

Typical manifestations of PMC are watery stool, abdominal pain, low-grade fever, often leukocytosis, hypoalbuminemia, with the frequency of diarrhea reaching 10 times a day, in severe cases - up to 20-30 times, in the absence of treatment, fluid and electrolyte disturbances develop rapidly. With a severe course, systemic manifestations come to the fore: confused consciousness, septic fever, respiratory disorders, pronounced leukocytosis, significant electrolyte disturbances, up to the development of toxic dilatation of the colon in the absence of diarrhea.

For the treatment of PMC, metronidazole and vancomycin are used, and as one of the treatment options, microbiota transplantation is proposed, especially for recurrent *C.difficile*-associated disease refractory to antibacterial drugs.

The question of the scope of emergency surgical intervention is decided individually, based on the changes detected intraoperatively, in some cases, surgical intervention is limited to the formation of an ileostomy, followed by the introduction of intraluminal antibiotics in the disconnected parts of the intestine, and in the case of severe toxic dilatation with diastatic damage or perforation of the intestinal wall, there is a need to perform colectomy followed by a stoma. Indications for colectomy in complicated course of the disease: perforation of the large intestine, development of a systemic inflammatory reaction, lack of effect from conservative therapy for 5 days, development of toxic megacolon, intestinal obstruction, symptoms of «acute» abdomen. The level of serum lactate (> 5 mmol/l) can be used as a marker of the severity of the disease. Despite the results of treatment of complications from the digestive system, the issues of surgical treatment remain unsolved.

CONCLUSIONS

1) The frequency of detection of PMC in patients with COVID-19 in 2020 was 0.62%, and in 2021 - 2.28%, which indicates a more aggressive course of the delta strain and the development of antibiotic resistance, which requires the use of reserve antibiotics and the combination of several antibacterial agents .
2) The sensitivity of CT in the diagnosis of surgical complications of PMC was 72%, and the specificity was 58%.
3) Conservative treatment was effective in patients with PMC in 88.8% of cases, 21.2% had complications that required emergency surgical interventions.
4) The total mortality in patients with PMC was 11.36%, although this indicator was significantly higher in the event of surgical complications and operative treatment (22.4%).

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CORRESPONDING AUTHOR
Stepan S. Filip
Uzhhgorod National University
71 Minayska St, 88000 Uzhhgorod, Ukraine
e-mail: filip.uz@i.ua

ORCID AND CONTRIBUTIONSHIP
Stepan S. Filip: 0000-0002-6549-3892 Ⓐ Ⓑ Ⓒ
Rudolf M. Slyvka: 0000-0002-0187-2711 Ⓐ Ⓒ Ⓓ
Yuriy P. Skripinets: 0000-0002-9741-4175 Ⓑ Ⓕ
Andriy M. Bratasyuk: 0000-0003-4390-2357 Ⓔ Ⓕ
Anatoliy I. Shitev: 0000-0002-1034-2405 Ⓐ Ⓒ Ⓔ Ⓕ

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

ORIGINAL ARTICLE   CONTENTS 🔗

Dynamic comparison the lower extremities length in students education in higher institutions

Svitlana Yu. Karatieieva[1], Oleksandr M. Slobodian[1], Yuriy Yu. Moseychuk[2], Natalya Ya. Muzyka[1], Kseniya V. Slobodian[1], Olena O. Moroz[2]

[1]BUKOVINIAN STATE MEDICAL UNIVERSITY, CHERNIVTSI, UKRAINE
[2]YURIY FEDKOVYCH CHERNIVTSI NATIONAL UNIVERSITY, CHERNIVTSI, UKRAINE

ABSTRACT

Aim: Dynamic comparison of the lower limbs length, depending on the type of sport, followed by the construction of a mathematical model for predicting sports abilities.

Materials and Methods: The comparison of the lower limbs length in dynamics was carried out on 132 students of higher education institutions of Bukovyna. While the primary study was carried out during September-October 2021, next study of these same students was conducted in September-October 2022. The main group consists of 92 (69.7%) students, the control group - 40 (30.30%) students aged 16 to 18 years. All students underwent an anthropometric study (determination of the length of the lower limbs) according to the method of P.P. Shaparenka. The comparecement of anthropometric parameters in the main group depending on the type of sport used the Kruskel-Wallis test (non-parametric analysis of variance) in order to identify a reliable difference in the average indicators of the respondents depending on the type of sport (the median of the distribution was considered as a measure of central tendency). In order to establish which pairs of age groups had a statistical difference in the medians, the Conover-Iman test was used. A paired t-test (t-test of paired samples) was performed to compare the length of the respondents' lower limbs during the first measurement and again one year later. Statistical analysis of the obtained data was performed using the licensed RStudio program.

Results: The distribution of the length of the right lower limb of the respondents of the main group by measurement shows that the average value of the length of the right lower limb has changed: a significant difference in the length of the right lower limb was found between the first (M = 88.812, SD = 5.287) and the second (M = 89.377, SD = 5.347) measurements; t (68) = -5.223, p < 0.001. The distribution of the length of the left lower limb shows that the average value of the length of the left lower limb has changed also: a significant difference in the length of the left lower limb was found between the first (M = 88.667, SD = 5.266) and the second (M = 89.435, SD = 5.309) measurements; t (68) = -8.289, p < 0.001.

Conclusions: In order to dynamically compare the length of the lower limbs for comprehensive control and selection of promising students in football, volleyball, handball and basketball, a mathematical model was derived for predicting the length of the lower limbs, in corresponding sports: right lower limb y = 0.506 x and left y = 0.507x, where y – is the length of the left lower limb, x – is the height. The coefficient of determination is 99.8%. A significant predictor for the length of both lower limbs is the height.

   KEY WORDS: anatomy, students, sport, length of lower limbs, mathematical model

INTRODUCTION

According to many scientists conclusions, the continuous growth of sports achievements and taking into account the fact that there is competition in the field of sports, the question arises of finding new adequate methods of training athletes, as well as the correct selection for the type of sport. So, the issue of sports selection is the main one for coaches. [1-5]. It should also be noted that individual differences of a person are manifested in various types of his activities, including sports [6]. So, everything can be done in a huge amount large quantities, better and easier, if to perform some work in accordance with personal natural abilities" [7].

K.M. Gurevich, a modern psychologist, wrote: "In principle, every person can have any profession (or almost any), but the whole point is how much effort and time it will take. So, forecasting of professional suitability and the ways of its establishing formation will never lose its relevance" [8].

All these quotes indicate that in order to achieve success in sports, it is necessary to select those athletes which are suitable specifically for a certain type of sport [9-11].

One cannot but agree with the opinion of experts who claim that selection is a long process, and it is also impossible to instantly assess sports fitness, no matter

how perfect the assessment methods are. If we take into account the dynamic nature of eligibility criteria and also the variability of many of the most important characteristics of an athlete for sports activities, it becomes clear about the need for a systematic addition and revision of the primary assessment. In fact, the selection is carried out throughout the entire sports career, so it makes sense to talk about its various stages [12-14]. A number of scientists believe that carrying out the sports selection, it is necessary to ensure the complexity of the assessment of prospects using morpho-functional, socio-psychological and other criteria [15-19].

So, in our opinion, a dynamic comparison of the length of the lower limbs as an anatomical criterion for recommendations for sports selection for specific sports is relevant and requires further study.

AIM

To find out dynamic comparison of the lower limbs length, depending on the type of sport, followed by the construction of a mathematical model for predicting sports abilities.

MATERIALS AND METHODS

The comparison of the lower limbs length in dynamics was researched 132 students of Bukovyna. While the primary study was during 2021 year, and a next study of the same students was during 2022 year. The main group consists of 92 (69.7%) students of the Faculty of Physical Culture and Human Health of the Yuri Fedkovich Chernivtsi National University, and the control group - 40 (30.30%) students of the medical and dental faculties of the Bukovyna State Medical University. The age from 16 to 18 years. In the main group, 65 (70.66%) are young boys and 27 (29.34%) are young girls. In the control group are 21 (52.50%) young boys and 19 (47.50%) young girls, correspondingly.

Students of the main group during the year in addition, they visited sports sections by type of sport, under the supervision of a coach. Training of medium intensity, 2-3 in a week (90 minutes each). Some students have sports category. University experience is from two to three years.

So, they were doing in the following sports: football - 46 (50.00%) students, of which 38 (41.30%) were young boys and 8 (8.70 %) young girls; volleyball generally – 19 (20.65%), were 10 (10.86%) young boys and 9 (9.78%) young girls; handball generally – 14 (22.58%), were 8 (8.69%) young boys and 6 (6.52%) young girls; basketball generally – 13 (14.13%), were 9 (9.78%) young boys and 4 (4.34%) young girls. Students of the control group

were loaded with hours of physical education, according to the programs of their specialty, and additionally did not play sports.

The students were anthropometrically indicators according to the method of P.P. Shaparenka The length of the lower limbs was determined between two points: the upper point is located along the crest of the wing of the iliac bone and corresponds to the iliac-crest highest point, the lower one corresponds to the lower medial point, which is located at the lowest point of the medial bone [20].

The comparesement of anthropometric parameters in the main group depending on the type of sport used the Kruskel-Wallis test (non-parametric analysis of variance) in order to identify a reliable difference in the average indicators of the respondents depending on the type of sport (the median of the distribution was considered as a measure of central tendency). In order to establish which pairs of age groups had a statistical difference in the medians, the Conover-Iman test was used. A paired t-test (t-test of paired samples) was performed to compare the length of the respondents' lower limbs during the first measurement and again one year later.

The comparesement of anthropometric parameters in the main group depending on the type of sport used the Kruskel-Wallis test (non-parametric analysis of variance) in order to identify a reliable difference in the average indicators of the respondents depending on the type of sport (the median of the distribution was considered as a measure of central tendency). Since the average values (as a measure of central tendency trends) was compared in more than 2 groups, to test the hypothesis of a statistically significant difference in mean values, analysis of variance (ANOVA) is used, however, the ANOVA test is based on the assumption of a normal distribution of the sample in each group. The conducted Shapiro-Wilk test showed that the data in the "football" group are not normally distributed (W=0.901, p=0.002). For the other groups, there is insufficient evidence to reject the null hypothesis of a normal sampling distribution (all p>0.05). Since the assumption of normality of samples in each group by sport is violated, the ANOVA test cannot be used to test the hypothesis of equality of mean values of the lower limbs length in the main group by sport [21].

In our case, this assumption is violated, so we used the non-parametric Kruskel-Wallis test, which tests the hypothesis of equality of medians in each group. Here, the median is used as a measure of central tendency because it is more robust to random outliers than the mean. The Conover-Iman test was used in aim to establish for which pairs of age groups there is a statistical difference in the medians [22]. A paired t-test (paired-samples t-test)

Table 1. Dynamic comparison of the lower limbs length of respondents by sport

lower limbs	basketball		control		football		volleyball		handball	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
right	89,42 ±2,0 cm	90,73 ±2,0 cm	85,90 ±2,0 cm	85,92 ±2,0 cm	90,02 ±2,0 cm	91,36 ±2,0 cm	91,72 ±2,0 cm	92,88 ±2,0 cm	89,80 ±2,0 cm	90,84 ±2,0 cm
left	89,57 ±2,0 cm	90,80 ±2,0 cm	85,72 ±2,0 cm	85,75 ±2,0 cm	89,72 ±2,0 cm	90,89 ±2,0 cm	90,38 ±2,0 cm	91,95 ±2,0 cm	89,40 ±2,0 cm	90,50 ±2,0 cm
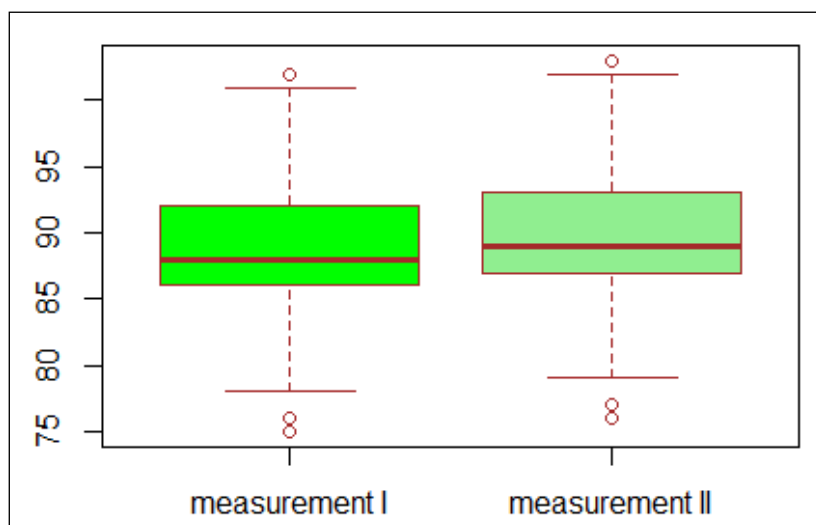
Fig. 1. Distribution of the length of the right lower limb of respondents of the main group by measurement.

was performed to compare the respondents' indicators during the first measurement and the second one a year later. Statistical analysis of the obtained data was carried out using the licensed program RStudio.

RESULTS

According to the results of the study, the average difference in the length of the lower limbs between the subjects of the main and control groups (Table 1) draws attention to the fact that the representatives of the main group in all sports have a length indicator of both limbs higher than the representatives of the control group.

And also according to the comparison of the length of the lower limbs in the dynamics after a year, in student-athletes of the main group, who had intense physical activity and professionally engaged in relevant sports, there is an obvious difference in the increase in the length of the lower limbs in 2022, compared to 2021 (±1.0- 1.5 cm). By comparison, there is almost no obvious difference in the dynamics of the respondents of the control group who did not have physical exertion.

The results of the divide of the length of the right lower limb of the representatives of the main group show that the average value at the first measurement is: [1] 88.81159 (Fig. 1).

Standard deviation at the first measurement is: [1] 5.286707. The results of the divide of the length of the right lower limb of the representatives of the main

group show that the average value at the second measurement is: [1] 89.37681.

Standard deviation at the second measurement is: [1] 5.347076. The distribution of the length of the right lower limb of the respondents of the main group by measurement shows that the average value of the length of the right lower limb has changed significantly: a significant difference in the length of the right lower limb was found between the first (M = 88.812, SD = 5.287) and the second (M = 89.377, SD = 5.347) measurements; $t(68) = -5.223$, $p < 0.001$.

When testing the hypothesis that the average value of the length of the right lower limb significantly increased, a significant increase in the length of the right lower limb was observed between the first (M = 88.812, SD = 5.287) and the second (M = 89.377, SD = 5.347) measurements; $t(68) = -5.223$, $p < 0.001$.

For the purpose of dynamic comparison of the length of the lower limbs for comprehensive control and selection of promising athletes in professional football, volleyball, handball and basketball, a mathematical model was derived for predicting the length of the right lower limb, of the corresponding sports.

The regression equation has the form: $y = 0.506\,x$, where y – is the length of the right lower limb, x – is the height. The coefficient of determination is 99.8%. Height is a significant predictor (Fig. 2).

The results of the divide of the length of the left lower limb of the representatives of the main group shows that the average value at the first measurement is: [1] 88.66667.
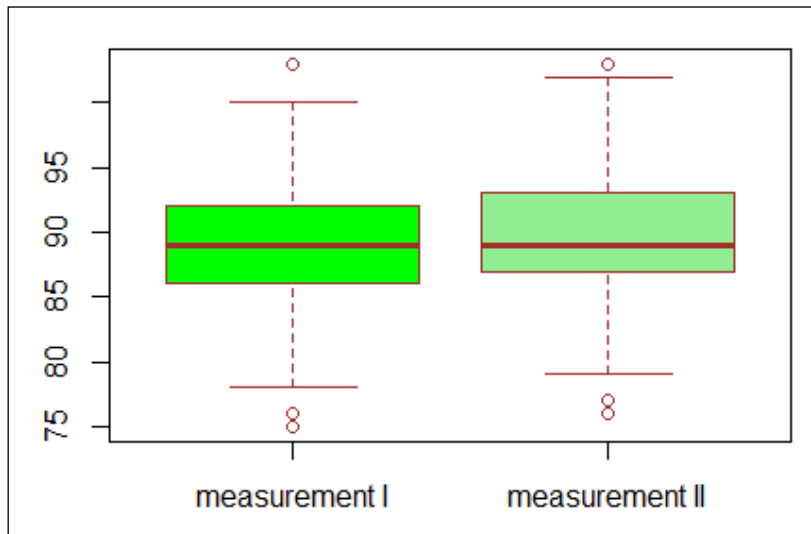
Fig. 2. Distribution of the length of the left lower limb of respondents of the main group by measurement.

Standard deviation at the first measurement is: [1] 5.2655. The results of the divide of the length of the left lower limb of the representatives of the main group shows that the average value at the second measurement is: [1] 89.43478. Standard deviation at the second measurement is: [1] 5.309476.

When testing the hypothesis that the average value of the length of the left lower limb has changed significantly, a significant difference in the length of the left lower limb was found between the first (M = 88.667, SD = 5.266) and the second (M = 89.435, SD = 5.309) measurements; t (68) = -8.289, p < 0.001.

For the purpose of dynamic comparison of the length of the lower limbs for comprehensive control and selection of promising athletes in professional football, volleyball, handball and basketball, a mathematical model was derived for predicting the length of the left lower limb, the corresponding sports: The regression equation has the form: y = 0.507x, where y – is the length of the left lower limb, x – is the height. The coefficient of determination is 99.8%. Growth is a significant predictor.

DISCUSSIONS

The search for works devoted to the comparison of the length of the lower limbs in the dynamics of specific sports with the subsequent construction of a model for predicting their length showed poor result or were almost absent.

Scientists who studied the selection of children in sports came to the conclusion that the basis of the selection of talented children in sports is based on both psychological and pedagogical and morphofunctional characteristics. The effectiveness of the first stage of the selection involves focusing on stable, little changed over the course of age development, as well as those that are slightly susceptible to the influence of training - hereditary morphological characteristics.

Sara Jane Cullen et al. (2022) [1], examining anthropometric profiles of elite athletes, concluded that quantification of body composition is central for checking the performance and training of athletes, but there is extremely limited anthropometric data for certain sports assessed using a standardized method.

So, analyzing the average length of both lower limbs at the initial study, as well as after a year in the dynamics, it is noteworthy that the average difference in the length of the lower limbs between the respondents of the groups shows that representatives of the main group in all types of sports have a length indicator of both limbs higher than representatives of the control group (±4.5-5.0 cm).

The creation of a model based on anthropometric indicators (the length of the lower limbs is reflected in this work) will allow for sports selection (recommended) regarding the selection of a certain type of sport in order to achieve high results.

Also, according to the comparison of the length of the lower limbs in dynamics after a year, student-athletes of the main group, who had intense physical activity and professionally engaged in relevant sports, have an obvious difference in the increase in the length of the lower limbs in 2022, compared to 2021 (±1.0-1.5 cm). By comparison, there is almost no obvious difference in the dynamics of the respondents of the control group who did not have physical exertion.

The results of the study of the length of the lower limbs of student-athletes in 2021 and a year later in 2022 show that volleyball and football players have the longest lower limbs (91.72±2.0 cm, 90.02±2.0 cm), slightly smaller - basketball and handball players (89.55±2.0 cm, 89.00±2.0 cm).

The significant difference in the average length of both lower limbs of young girls and young boys is determined (main group: young girls - 87.48±2.0 cm, young boys - 90.96±2, 0 cm; control group: young girls – 83.42±2.0 cm, young boys – 88.14±2.0 cm).

The average difference in the length of the lower limbs between subjects of the main and control groups also draws attention to the fact that the representatives of the main group in all sports and the total length of both limbs are higher than the representatives of the control group (the main group - 90.07±3.0 cm, the control group – 85.81±2.0 cm). The conducted regression analysis shows that height is a significant factor for the length of both lower limbs.

So, for the purpose of dynamic comparison of the length of the lower limbs for comprehensive control and selection of promising athletes in professional football, volleyball, handball and basketball, a mathematical model was derived for predicting the length of the right and left lower limbs, corresponding to sports. The regression equation for the right lower limb is: $y = 0.506 x$, where y – is the length of the right lower limb, x – is height. The coefficient of determination is 99.8%. The regression equation for the left lower limb is: $y = 0.507x$, where y – is the length of the left lower limb, x – is height. The coefficient of determination is 99.8%.

So, the results of the comparison of the length of the lower limbs in dynamics after a year of the study, as a anatomical index in student of higher educational institutions, for the purpose recommendations of sports selection for specific types of sports, are relevant and require further research.

CONCLUSIONS

1. Comparison the length of the lower limbs in student-athletes of the main group in dynamics after a year, had determined an obvious difference in increase of the length of the lower limbs in 2022, compared to 2021 (±1.0-1.5 cm). There is almost no obvious difference in the dynamics of respondents of the control group.

2. Comparison the length of the lower limbs in student-athletes in dynamics shows that the longest length of the lower limbs is inherent for volleyball and football players (91.72±2.0 cm, 90.02±2.0 cm) correspondingly, basketball and handball players have a slightly smaller length (89, 55±2.0 cm, 89.00±2.0 cm) correspondingly.

3. The average indicator of lower limbs length in the dynamics of representatives of the main group in all sports and the overall indicator is higher than the representatives of the control group (the main group - 90.07±2.0 cm, the control group - 85.81±2.0 cm).

4. Height is a significant predictor for the length of both upper limbs.

5. A model was derived for predicting the length of the right lower limb $y = 0.506 x$ and the left $y = 0.507x$, where y – is the length of the left lower limb, x – is the height. The coefficient of determination is 99.8%.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Svitlana Yu. Karatieieva
Bukovinian State Medical University
2 Theatralna st, 58000 Chernivtsi, Ukraine
e-mail: Karatsveta@gmail.com

ORCID AND CONTRIBUTIONSHIP
Svitlana Yu. Karatieieva: 0000-0003-1836-8375 Ⓐ Ⓑ
Oleksandr M. Slobodian: 0000-0002-0444-8457 Ⓒ Ⓕ
Yurii Yu. Moseychuk: 0000-0002-2457-6552 Ⓕ
Natalya Ya. Muzyka: 0000-0002-3381-1163 Ⓓ
Kseniya V. Slobodian: 0000-0001-7872-6731 Ⓔ
Olena O. Moroz: 0000-0003-4142-2712 Ⓓ

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

ORIGINAL ARTICLE

The influence of immediate intraoperative loading with a splinting component on supporting tissues during a one-stage implant

Anatoliy Potapchuk[1], Vasyl Almashi[1], Yevhen Onipko[1], Csaba Hegedűs[2]
[1]UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE
[2]UNIVERSITY OF DEBRECEN, DEBRECEN, HUNGARY

ABSTRACT
Aim: To study the specifics of the impact of immediate intraoperative loading with a splinting component on supporting tissues during a one-stage implantation protocol.
Materials and Methods: In the course of the study, orthopedic treatment was carried out for 55 patients aged 29 to 60 years. The following were performed: cone-beam computed tomography, software planning and intraoral scanning with an optical scanner, one-stage implantation protocol, assessment of implant stability with the Osstell ISQ device, microcirculation study in the peri-implant area using laser Doppler flowmetry (LDF).
Results: It was established that around loaded implants there is an increase in blood flow and vasomotor activity of the microcirculatory channel of the supporting tissues, an increase in the volume of bone tissue and an increase in torque, which is the optimal forecast for the acceleration of the pace of osseointegration.
Conclusions: The use of a splinting component during immediate intraoperative functional masticatory load accelerates the dynamics of bone tissue remodeling processes around the implant, which is an optimal prediction of osseointegration rates in various dental implantation protocols and is consistent with high values of the implant stability coefficient.

KEY WORDS: one-stage dental implantation protocol, immediate intraoperative load, splinting component, LDF (laser Doppler flowmetry), stability indicator

INTRODUCTION

Based on the results of monitoring and analysis of clinical and experimental studies on the success of dental implantation in the early and long-term follow-up periods, scientists were able to justify the feasibility of introducing into clinical practice not only classical, but also modified implantation protocols with different periods of follow-up load [1,2]. The systematization of modern views on the differentiation of the results of immediate loading and immediate implantation and their interpretation from the point of view of the modern understanding of the mechanisms of bone remodeling expands the opportunities for discussion and argumentation of the prognosis of various dental implantation protocols, taking into account the initial conditions of the clinical situation [3-6]. As the analysis of scientific sources on dental implantation shows, specialists are increasingly turning to one-stage implantation with the use of immediate loading [5, 7-9]. Today, there are discussions about the features of the formation and the level of reduction of peri-implant bone tissue in various conditions, in particular, under load and micromobility [10].

Specialists in practical dentistry do not have a single opinion regarding the terms of application of immediate masticatory load. Most of the publications are devoted to determining the indications and evaluating the clinical results of prosthetics before the use of immediate loading, diagnostics and anatomical and topographical features of bone tissue in the areas of implant installation with complete and partial adentity [11-13]. The influence of personalized dental implantation protocols on the processes of osseointegration and analysis of the results is also being studied prosthetics with the use of immediate loading [14-16]. The very term immediate load also causes controversy among clinicians. Foreign researchers define a period of up to 48 - 72 hours between the implantation operation and the installation of a temporary prosthesis, as the period of the beginning of the impact of immediate load on the implants [17].

In clinical practice today, there is a tendency to shorten the terms of predicted and successful orthopedic rehabilitation. Dental implantation with immediate intraoperative functional chewing load allows to reduce the duration of treatment and the volume of surgical intervention and to obtain a high functional and aesthetic result. Such a modified surgical protocol can be performed only by specialists with high manual capabilities and extensive clinical experience, if certain clinical conditions are met and maintained [16-18].

In this regard, especially in recent years, the commitment of patients and dental specialists to the use of immediate masticatory loading with implant-supported dentures in the treatment of patients with partial and complete edentation of the jaws has significantly increased. The capabilities of computer tomography (CT) and computer programs (3d modeling), which are used in the planning of dental implant technologies and dental prosthesis designs, have expanded [18-20].

The success of treatment is usually associated with individual protocols, which include the use of temporary splinting prostheses at almost all clinical stages, starting from the moment of installation of implants and covering the period of their osseointegration, up to the manufacture of permanent dental prostheses [19,21,22].

To date, precise objective hardware assessment of osseointegration is not possible. The stability of implants can be interpreted as the absence of their clinical mobility, which is also one of the criteria for osseointegration. Therefore, achieving and maintaining the stability of implants is the main condition for a successful long-term clinical result of implantation [22, 23].

Nevertheless, clinically stable implants also have micromobility when they are loaded. When a lateral load is applied to an osseointegrated implant, the implant is displaced, but returns to its original position immediately after its removal, depending on the type of peri-implant bone. A stable implant may have varying degrees of stability (ie, varying degrees of displacement or resistance to loading) depending on factors related to bone quality, surgical technique, and implant design. Therefore, the result of the assessment of implant stability largely depends on the assessment method. It has been established that around loaded implants there is an increase in blood flow and vasomotor activity of the microcirculatory bed of the supporting tissues, an increase in the volume of bone tissue and an increase in torque, which is an optimal prediction of the acceleration of the rate of osseointegration [23].

Fig. 1. Clinical situation before the operation.

In modern implantology, standard examination methods do not allow detecting disorders that occur in the peri-implant mucosal zone during dental implantation. Therefore, the use of highly informative methods of assessing hemodynamics and metabolism reveals hidden mechanisms of disease development. To date, the most objective method of assessing the state of microcirculation is laser diagnostics, in particular laser Doppler flowmetry [24, 25], the use of such a technique allows for the analysis of capillary hemodynamics on a real-time scale and allows to determine changes in microcirculation during various protocols of dental implantation and functional load [26-28].

AIM
To study the specifics of the impact of immediate intraoperative loading with a splinting component on supporting tissues during a one-stage implantation protocol.

MATERIALS AND METHODS
The clinical study was conducted on the basis of the Department of Post-Graduate Education Dentistry of the Uzhhorod National University, the dental clinic «Art dentistry» (Zaporizhia, Ukraine), the university dental clinic of Debrecen University (Debrecen, Hungary) in the period 2020-2024.. The study was carried out taking into account the main provisions of the GCP ICH and the Helsinki Declaration on Biomedical Research, the Council of Europe Convention on Human Rights and Biomedicine (2007) and the recommendations of the Committee on Bioethics under the Presidium of the National Academy of Sciences of Ukraine (2002) and the positive opinion of the bioethics commission of the Uzhhorod National University .

Orthopedic treatment of 55 patients aged 29 to 60 years was performed according to the proposed
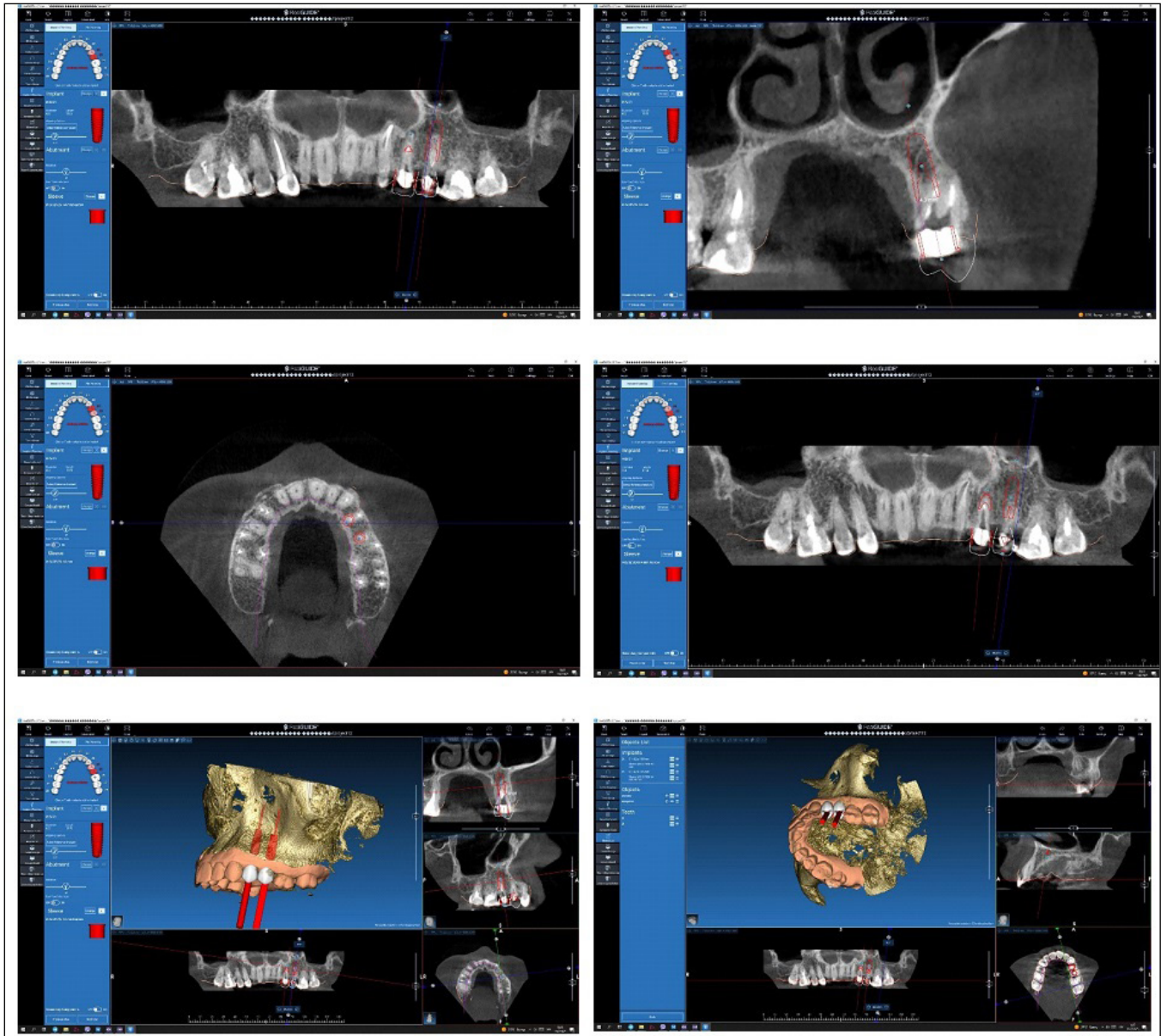
Fig. 2. Cone-beam computed tomography (three-dimensional model of the upper jaw) with virtual simulation of dental implant installation.

treatment protocol. All patients were fitted with dental implants in the defect area of 1-2 teeth on the upper jaw with immediate loading, using intraoperative prosthetics. A total of 65 implants of the ART IMPLANT system were installed (certificate of compliance of products with requirements NoUA.101.MD.3.1024 - 23.00).

Planning and treatment of patients was carried out according to the following algorithm: cone beam computed tomography (CBT) was performed to obtain files in dicom format for further planning of dental implant installation. Later, after intraoral scanning with an optical scanner to obtain a file in STL format, dicom and STL format files were combined in the 3Diagnosis computer program to display the complete clinical picture, which includes all anatomical formations of

the selected area, virtual positioning of the implant and its superstructure was carried out . A virtual model of the surgical template was created using the received data on the state of the implant in the PlastyCAD software. The template was printed using a 3D printer. Milled provisional splinting structures were made beforehand, followed by their rebasing in the oral cavity on temporary titanium abutments fixed to the Multi-unit abutment. Under the condition of using Basis implants of the Art Implant system, temporary splinting structures were made in advance according to the Malevez Chantal method. A feature of the ART IMPLANT system implant thread is a trapezoidal compression shape, which turns into a cutting shape - this, in turn, contributes to the condensation of the cancellous bone and ensures reliable primary
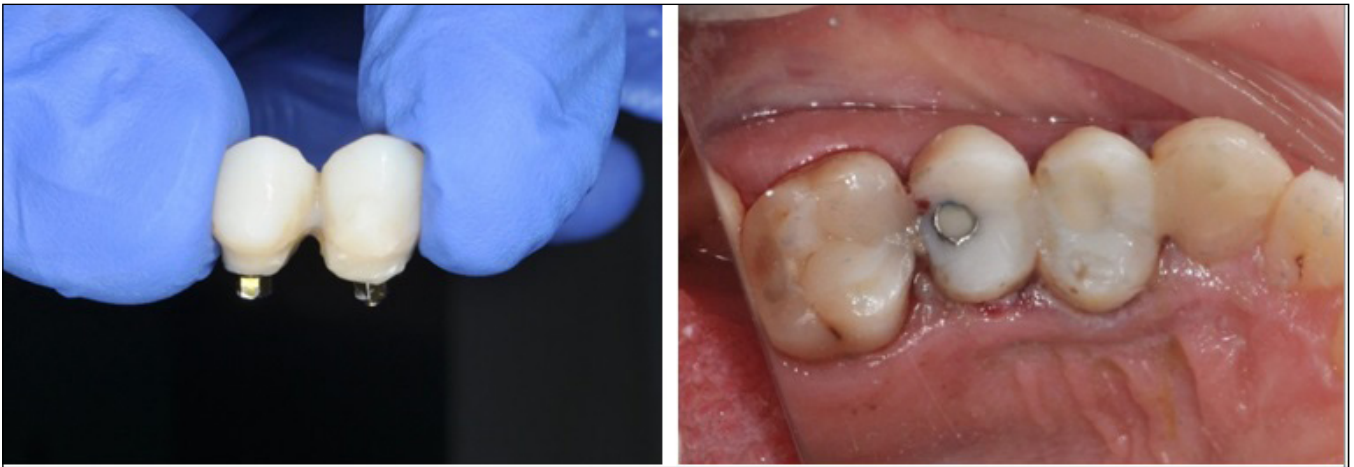
Fig. 3. Clinical situation after one-stage surgical implantation protocol after 14 days.

Fig. 4. Clinical situation after one-stage surgical implantation protocol after 3 months.
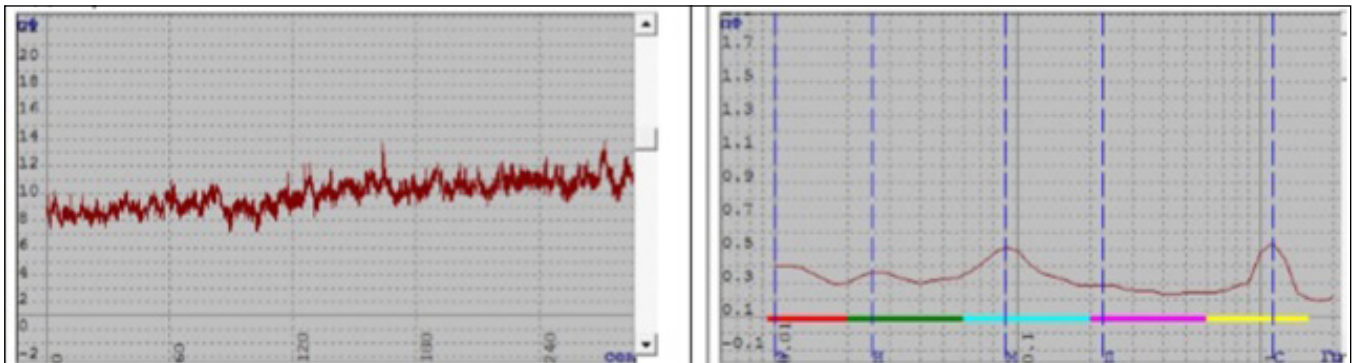
Fig. 5. Indicators of microcirculation according to LDF data in patient A. before dental implantation surgery.

stability, while not losing the self-tapping properties of the implant, which greatly facilitates its installation.

The stability of the implant was assessed by the value of the stability coefficient using the Osstell ISQ device (Integration Diagnostics, Sweden). Also, the patients underwent a study of microcirculation in the peri-implant zone by laser Doppler flowmetry (LDF) using the moor VMS LDF2 system ((785±10) nm, maximum power 2.5 mW) (Moor Instruments Ltd. Millwey Axminster Devon EX13 5HU, Great Britain) and

processed using specialized software (moor VMS-PC, V 3.1 for Vascular Monitor System).

The results of laboratory and clinical studies were processed by methods of variational statistics with determination of the mean value, its errors, Student's t-test for multiple comparisons, using Excel (MS Office 2018, Microsoft, USA) and STATISTICA 6.0 (StatSoft, USA). Differences in indicators at the level of significance $p < 0.05$ were considered statistically significant.
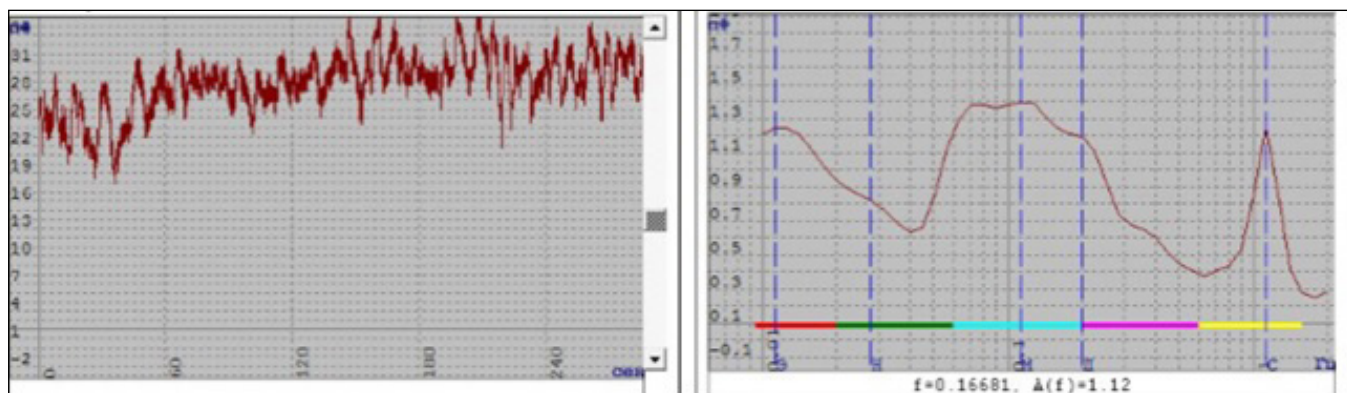
Fig. 6. Indicators of microcirculation according to LDF data in patient A. 3 days after dental implant surgery and immediate intraoperative prosthetics.
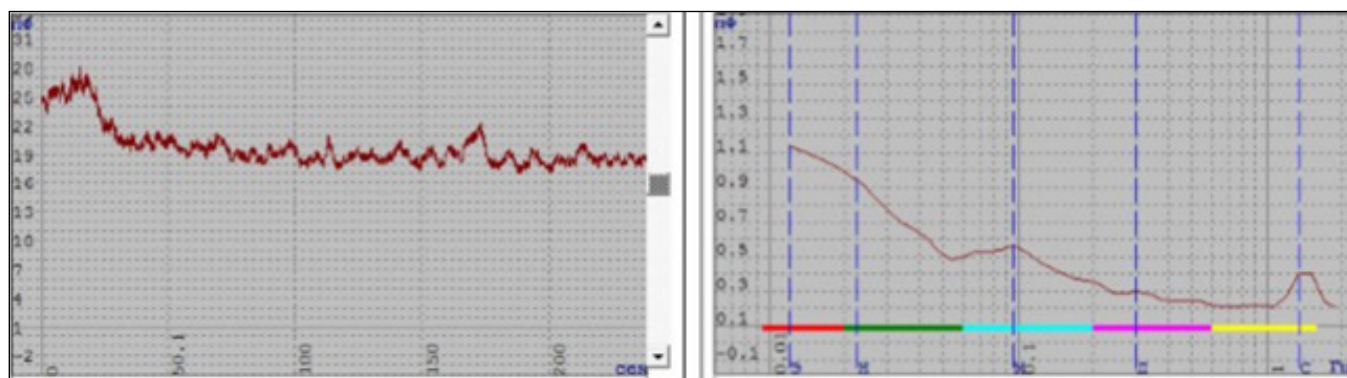
Fig. 7. Indicators of microcirculation according to LDF data in patient A. 10 days after dental implant surgery and immediate intraoperative prosthetics.
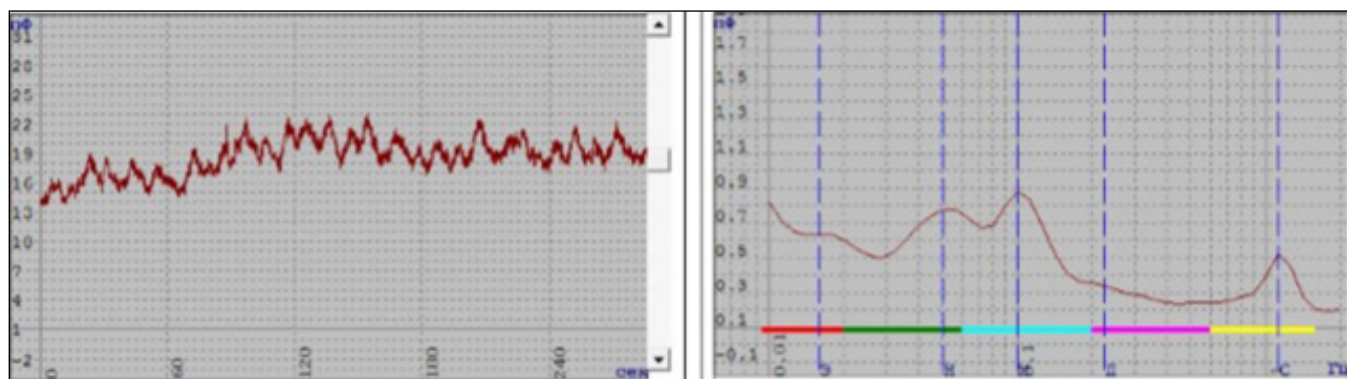
Fig. 8. Indicators of microcirculation according to LDF data in patient A. 1 month after dental implant surgery and immediate intraoperative prosthetics.
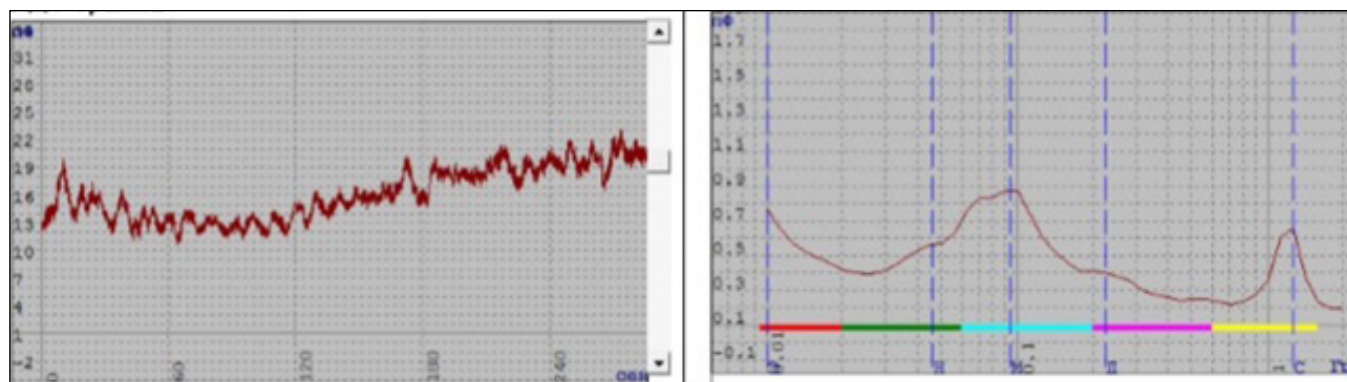
Fig. 9. Microcirculation indicators according to LDF data in patient A. 3 months after dental implant surgery and immediate intraoperative prosthetics.

RESULTS

When examining the dental status, attention was paid to patients' complaints, which were reduced to aesthetic and masticatory insufficiency caused by the presence of defects in the dentition, difficulties in chewing food on the side of the defect. The examination includes an assessment of the state of the periodontal teeth, detection of dentition deformations and determination of the level of oral hygiene. Analysis of patient history data showed that the cause of tooth loss in all patients was a complication of caries.

On the diagnostic models of the jaws, the relationship of the tooth rows during various occlusal movements was studied, some anthropometric parameters were determined - the length of the tooth row defect, the width and height of the edentulous alveolar ridge. In the articulator, with the help of diagnostic models, the structural features of the future dental prostheses were specified, and the relationship of the implant head with the opposing teeth was also determined. Kennedy's classification was used to systematize dentition defects and determine the indications for choosing the type of implantation and the design of a dental prosthesis: 61% of patients were missing one tooth, 39% were missing two teeth. Depending on the type of dentition defect, all patients underwent orthopedic treatment with the manufacture of fixed structures supported by dental implants.

CLINICAL CASE

Patient A., born in 1962, diagnosed with chronic irreversible periodontitis of teeth 2.4, 2.5 (Fig. 1).

A clinical and radiological examination was carried out using cone-beam tomography and computer virtual simulation of the stages of surgical and orthopedic treatment. We also determined the condition and dimensions of the implant, measured the distance to the maxillary sinus, the volume of bone tissue of the alveolar process of the upper jaw in the projection of teeth 2.4, 2.5 (Fig. 2).

After the examination, the patient's treatment plan was drawn up: a one-stage surgical protocol of implantation with Basis dental implants of the ART IMPLANT system with bone plastic and one-moment intraoperative provisional prosthetics with splinting component was carried out (Fig. 3).

Three months after the operation, a permanent metal-ceramic bridge-like prosthesis was made (Fig. 4).

After 1 year, the patient has no complaints, a satisfactory state of oral hygiene is noted, the mucous membrane is pale pink in color, moderately moisturized, without signs of gingivitis. The installed crown is stable, without clinical and radiological signs of peri-implantitis, slight recession of the gingival margin is noted.

The study of the microcirculation of the mucous membrane in the peri-implant zone by the method of laser Doppler flowmetry (LDF) was carried out before the operation, as well as 3 days, 10 days, 1 month and 3 months after implantation (Fig. 5, Fig. 6, Fig. 7, Fig. 8, Fig. 9).

The results of LDF-gram wavelet analysis, shown in Table 1, revealed an increase in the neurogenic tone (NT) of precapillary vessels in the microcirculatory channel of the mucous membrane of the jaws 3 days after dental implantation with immediate loading by 14.77% ($p<0.05$), and after 10 days - a decrease by 28.11% ($p<0.001$) compared to the initial values.

After 1 month (Table 2) after the operation, NT tended to increase, but its values were lower than the initial level by 17.78% ($p<0.05$). After 3 months, NT exceeded the initial level by 30.05% ($p<0.05$). The index of myogenic tone (MT) after dental implantation with immediate loading after 3 days tended to decrease, and after 10 days after the operation, its value increased by 96.86% ($p<0.001$).

1 month after dental implantation with immediate loading, MP values dropped and differed from the preoperative level by 11.07%, and by 21.98% after 3 months ($p<0.05$).

The value of the shunt index (SI) 3 days after dental implantation had a tendency to decrease, and after 10 days its values increased by 3.11 times, which may indicate the reaction of the microcirculation in the mucous membrane to the traumatic injury of the jawbone during dental implantation and immediate intraoperative prosthetics ($p<0.001$). 1 month after the operation, the SI decreased and was higher than the initial level by 38.02% ($p<0.05$), and after 3 months, its values slightly differed from the preoperative level.

When assessing the stability of dental implants using the «Osstell ISQ» device (Sweden), a clinical and functional examination of the state of supporting tissues in the area of the implant on the upper jaw was carried out in 55 patients with a diagnosis of partial absence of teeth (ICD-10 - 08.1), the size of the defect - one - two teeth.

The installed implants reached an average level of stability, in particular for implants of the ART IMPLANT system - the stability coefficient was from 65 to 75 (percentage point), which on average is (71 ± 0.5) percentile. These data indicate that the stability of the implant allows planning the beginning of prosthetics with permanent orthopedic structures (Table 3).

Table 1. State of the basic parameters of the microcirculation of the peri-implant zone of the upper jaw during dental implantation with immediate intraoperative prosthetics according to LDF data for the periods before the operation, after 3 and 10 days

Indexes	Before the operation			3 days after surgery			10 days after surgery		
	MI	σ	Kv	MI	σ	Kv	MI	σ	Kv
n	25			23			23		
M ±m	8,50±0,43	0,90±0,04	10,59±0,48	24,70±1,24	2,80±0,13	11,34±0,52	19,20±0,96	2,01±0,09	10,47±0,48
p <				0,001	0,001		0,001	0,001	

• MI - microcirculation index
• p is the reliability of the differences between indicators before the operation and in different periods after the operation.

Table 2. The state of basic indicators of microcirculation of the peri-implant zone of the upper jaw during dental implantation with immediate intraoperative prosthetics according to LDF data for the periods after surgery in 1 and 3 months

Indexes	1 month after surgery			3 month after surgery		
	MI	σ	Kv	MI	Σ	Kv
n	21			19		
M ±m	16,90±0,85	1,90±0,09	11,24±0,51	11,40±0,57	2,10± 0,09	18,42±0,84
p <	0,001	0,001		0,05	0,001	0,001

• MI - microcirculation index
• p is the reliability of the differences between indicators before the operation and in different periods after the operation.

Table 3. Results of evaluation of the stability of ART IMPLANT implants according to the ISQ index (n=65)

Terms of observation	ISQ index (units)
At the time of installation of the implant	71,00±0,10, p <0,05
14 days after implant placement	65,00±0,08, p <0,05
1 month after implant placement	69,00±0,03, p <0,05
3 months after implant placement	74,00±0,05, p <0,05

Based on the results of frequency resonance analysis, it was established that implants with a high ISQ index (more than 65 u.o.) maintain an appropriate level of stability. However, due to the process of osseointegration, the primary mechanical stability is supplemented by biological stability.

The technology of frequency-resonance analysis provides the doctor with important information about the condition of the "implant-bone" connection area in the dynamics of treatment and control.

Studies have shown that implants with high stability coefficient values at follow-up examinations are successfully integrated, while low stability values may be a sign of implant failure.

DISCUSSION

One of the most important conditions of dental implantology is the creation of conditions for long-term and stable stability of implants. The success of the introduction of non-biological implants into the bone depends on osseointegration, that is, the formation of a direct structural-functional connection between the implant and the bone. Data on the features of osseointegration, bone tissue state processes, bone formation

and resorption around dental implants with different optimized surfaces and its quality attract the attention of specialists in connection with the development of various installation and loading protocols (Zaid MB, O'Donnell RJ, Potter BK, Forsberg JA., 2019).

Fundamental studies of the condition of the bone tissue around the implanted material are important for evaluating the optimal timing of early functional loading of the implant, as early load function promotes osseointegration, however, in the complex context of bone remodeling, their disintegration is possible [11]. There is a study in which 29 articles corresponding to the inclusion criteria were selected out of 889, which is based on the evaluation of different loading protocols (immediate, immediate non-occlusal, early and conventional loading) of dental implants on the processes of osseointegration and marginal bone loss (Sommer M, Zimmermann J,. Grize L, Stübinger S., 2020).

In the experiment, we performed a comparative analysis of the dynamics of remodeling of the bone tissue of the lower jaw of pigs under the conditions of using implants without splinting and with splinting components according to the indicators of the study of bone tissue formation at the interface "implant - bone" (bone implant contact, BIC) and distance (1000 μm) (peri-implantation

area, BIV (bi i vi) to determine osseointegration and predict the long-term stability of implants. We observed that after 3 months after implantation, the BIC was 75%, statistically significantly higher compared to the use of implants without splinting - 47%, it was established, that in conditions without splinting for a period of 3 months after implantation, secondary remodeling occurs with increased osteoclastic resorption, which is a forecast of implant instability. To objectify the data of the morphological study, a morphometric comparative assessment of the integration of the implant with the bone in dynamics was carried out. It was proved that primary osteogenesis does not proceed according to the contact type, that is, starting from the surface of the implant, but according to the remote type from the side of the mother bone [11]. The obtained data are consistent with the research results of Adamantia Byraki, Anca Viochiţa Costea, George Cristian Curcă, Sorin Hostiuc (2019).

In a study by Barbier and colleagues (2011), in which immediate loading of implants installed immediately in sockets of extracted teeth and in sockets after healing was carried out, it was established that the time of implantation does not affect the level of reduction of peri-implant bone tissue (p>0.3).

The average decrease in the height of the bone crest relative to the reference reference point ranged from 0.25 mm to 0.48 mm after 1 year after loading the infrastructure, which corresponds to the scale of success criteria proposed by Albrektsson T. and Zarb G (1989). Considering the obtained indicators and the recorded 100% implant survival rate, the authors concluded that the proposed and described approach of immediate implantation with subsequent immediate loading can be considered successful, based on a 1-year monitoring period [12].

The data obtained by us allow us to conclude that when carrying out immediate loading during dental implantation according to our proposed method, the stability of the implant always remains sufficient for the functioning of the implant.

Similar results were also described in the publication Chranovic B.R., Albrektsson T. and Wennerberg A. (2014), in which the authors noted that the cumulative rate of loss of intraosseous supports installed during the implementation of the immediate implantation protocol reached 4.0%, and exceeded the similar rate of implants, installed in the sockets of the teeth after their healing (3.09%) [12]. Thus, the researchers were able to establish that the relative risk of loss of dental implants, with immediate implantation, is 1.58 with a 95% confidence interval of 1.27-1.95 (p=0.0001). However, the difference between the survival of implants installed according to the immediate and delayed protocol was statistically justified only in cases of analysis of single orthopedic structures.

CONCLUSIONS

The use of a splinting component during immediate intraoperative functional masticatory load accelerates the dynamics of bone tissue remodeling processes around the implant.

The use of laser Doppler flowmetry (LDF) is important for the analysis of capillary hemodynamics of the peri-implant zone, it allows to determine the dynamics of microcirculation indicators, which is the optimal forecast of the rates of osseointegration in various protocols of dental implantation and functional load, which is consistent with high indicators of the coefficient of implant stability.

Thus, it should be stated that the clinical use of a one-stage surgical protocol of implantation with dental implants of the «ART IMPLANT» system in the rehabilitation of patients demonstrates a reliable degree of stability of installed dental implants and allows shortening the waiting period for permanent prosthetics, as well as reducing the duration of the entire treatment, which ultimately leads to increasing optimization of treatment efficiency and patient satisfaction.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Anatoliy Potapchuk
Uzhhorod National University
16a University Street, 88000 Uzhhorod, Ukraine
e-mail: anatoliy.potapchuk@uzhnu.edu.ua

ORCID AND CONTRIBUTIONSHIP
Anatoliy Potapchuk: 0000-0001-9857-1407 Ⓐ Ⓑ Ⓔ Ⓕ
Vasyl Almashi: 0000-0002-2943-4844 Ⓐ Ⓑ Ⓒ Ⓔ
Ievgen Onipko: 0000-0002-3086-4657 Ⓑ Ⓓ
Csaba Hegedűs: 0000-0003-4143-2507 Ⓓ Ⓔ

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

Assessment of the potential effects of l-carnitine and cinnamon supplementation on weight loss and body composition

Qayssar Joudah Fadheel

DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY, COLLEGE OF PHARMACY, UNIVERSITY OF BABYLON, BABYLON, IRAQ

ABSTRACT

Aim: To assess efficacy of L-carnitine and cinnamon alone and in combination on body composition parameters in addition to compare between them.

Materials and Methods: Sample of 28 obese and overweight adults in Babylon city, sample collection includes patients in places, or by internet, where interview take place according to specialize questionnaire height, weight, and body mass index were measured.

Results: A significant differences P<0.05 among gender distribution between male and female. A significant difference between (150-160 cm, 160-170 cm) as compared with (170-180 cm, 180-190 cm). A significant difference between 170-180 cm as compared with 180-190 cm but non-significant differences between 150-160 cm as compared with 160-170 cm. A significant difference between 26-35 as compared with 36-45, 46-55, but non-significant differences between 36-45 as compared with 46-55. A significant difference between body weight, body fat, water content, skeletal muscle, and body mass index after treatment, but non-significant differences between protein, and inorganic salt after treatment and at baseline. A significant difference between body weight, water content, skeletal muscle, and body mass index in group treated with cinnamon as compared with negative control group, but non-significant differences between body fat, protein, and inorganic salt as compared with negative control group.

Conclusions: The prevalence of overweight and obesity within accepted range of that reported in Iraq, important relationship was reported between several life style risk factor, as soon as diagnose increase in weight and education health program for behavior of life style were high recommended.

KEY WORDS: cinnamon, *L-carnitine*, obesity, body mass index, overweight

INTRODUCTION

There has been a significant global increase in obesity rate during the last 50 years. Obesity is defined as when a person has a body mass index (BMI (kg/m^2), dividing a person's weight by the square of their height) greater than or equal to 30, overweight is defined as a BMI of 25.0-29.9. Being overweight or obesity is linked with more deaths than being underweight and is a more common global occurrence than being underweight [1]. Obesity increases the likelihood of various diseases and conditions which are linked to increased mortality. These include Type 2 diabetes mellitus, cardiovascular diseases, metabolic syndrome, chronic kidney disease, hyperlipidemia, hypertension, nonalcoholic fatty liver disease, and certain types of cancer, obstructive sleep apnea, osteoarthritis, and depression (Table 1) [2].

The Body Mass Index is a numerical value that is used as an indicator of fat mass, rather than a direct measurement of fat mass or its associated mechanical and metabolic disruptions. The statement under consideration is both biologically unsound and lacks a suitable phenotype that warrants further investiga-

tion [3]. Indeed, comprehensive examinations have uncovered significant variations among individuals in the correlations between subcutaneous adipose tissue (SAT) and body mass index, skeletal muscle mass, visceral adipose tissue (VAT), biomarkers of inflammation and insulin resistance, as well as secretory activity of adipocyte. There is evident that BMI is insufficient in accurately determining both the presence of excessive fat accumulation and the associated functional impairments. As a result, a proposition has been put forth to redefine obesity by considering the individual's health status. The latter is distinguished by the utilization of

Table 1. Classification of overweight and obesity according to body mass index

Classification	Body Mass Index
Underweight	Less than 18.5
Normal weight	18.5–24.9
Overweight	25.0–29.9
Obesity type I	30.0–34.9
Obesity type II	35.0–39.9
Obesity type III	Equal or more than 40

Table 2. World Health Organization (WHO) cut-off points and metabolic complications risk [7]

Indicator	Cut-off points	Risk of metabolic complications
Waist circumference	More than 94 cm (Men); >80 cm (Women)	Increased
Waist circumference	More than 102 cm (Men); >88 cm (Women)	Substantially increased
Ratio of waist–hip	Equal or more than 0.90 cm (Men); ≥0.85 cm (Women)	Substantially increased

clinical assessment, laboratory and endocrine testing, and comprehensive body composition analysis [4]. The need for further evidence exists to establish a connection between identified health deficits and excess fat, in order to justify the implementation of targeted obesity treatment strategies, even when a comprehensive assessment has been conducted. A comprehensive assessment for composition of the body was conducted using magnetic resonance imaging (MRI) for whole body on a sample of 764 individuals of Caucasian descent who were in good health. The sample consisted of 53% females, with an average age of 40s and a range spanning from 18 to 82s (Table 2) [5, 6].

L-carnitine has the potential to assist in weight loss and address metabolic abnormalities that are commonly associated with it. The role and significance of the carnitine system pertain not solely to the fatty acids metabolic processes, but also to the development of insulin resistance within the body and overall regulation of fat distribution. The role of the carnitine system in the regulation of insulin-mediated fat and glucose metabolism in skeletal muscle has been demonstrated. This system plays a crucial role in body composition determining and relevant risk factors for obesity, cardiovascular disease, hypertension, and type II diabetes [8]. Numerous studies have failed to establish a substantial correlation between the consumption of L-carnitine supplements and the reduction of body weight. A study conducted on individuals with a slight overweight condition assessed the influences of a 10-day ingestion of 3g L-carnitine. The results of this study indicated that there was no significant alteration in composition of body when compared to a placebo treatment [9]. In a controlled experiment, a cohort of 36 rats was allocated into two groups: a treatment group receiving L-carnitine supplementation at a dosage of 5 g/kg, and a control group receiving no supplementation. Both groups were subjected to a calorie-restricted diet. The findings of the study revealed that decrease in weight was observed in both groups, but the disparity in weight loss between the two groups did not reach statistical significance [10]. In contrast, a separate study conducted on feline subjects with obesity demonstrated that the administration of 250 mg of L-carnitine resulted in a notably accelerated weight reduction, in comparison to cats who were adminis-

tered a placebo, while being subjected to a calorie-restricted diet [11]. In a study that followed a randomized, double-blind, placebo-controlled design, a group of 36 moderately obese women were administered either a daily dose of 2 g L-carnitine or a placebo. Additionally, the participants were instructed to engage in a 30-minute walking routine for four days every week. Throughout the duration of the study, no statistically significant alterations were observed in the average total body mass (TBM), fat mass (FM), or resting lipid utilization. Furthermore, no significant differences were found between the two groups in relation to any of the variables examined [12]. However, it is clear that there is a lack of adequately designed clinical studies examining the effects of L-carnitine on weight loss. Several studies have indicated a potential weight loss advantage when L-carnitine is combined with other weight loss supplements, such as choline and caffeine [13, 14]. The current body of evidence regarding the relationship between L-carnitine and weight loss remains inconclusive. Supplementation of L-carnitine has been found to mitigate certain adverse effects associated with excess body weight. The condition of obesity leads to an elevation in the levels of free fatty acids in the bloodstream, subsequently causing a decline in endothelial function. The presence of excess weight can result in endothelial dysfunction, a significant outcome that is associated with heightened cardiovascular mortality rates. The study demonstrated that the administration of L-carnitine had a mitigating effect on endothelial dysfunction induced by free fatty acids and obesity in individuals with obesity [15]. Obesity is additionally correlated with elevated plasma cholesterol levels and an atypical blood lipid profile. Multiple studies have demonstrated that individuals who incorporated L-carnitine supplements into their dietary regimen experienced a notable reduction in their overall cholesterol and triglyceride levels, accompanied by an elevation in high-density lipoprotein (HDL) levels [16, 17]. Previous studies have demonstrated that the administration of L-carnitine supplements can effectively decrease lipoprotein levels in individuals diagnosed with type II diabetes mellitus [18]. The FNB has not yet established dietary reference intakes for carnitine [19]. The majority of studies that indicate the advantages of carnitine utilize dosages ranging from 1 to 3 grams per day. The taxonomic
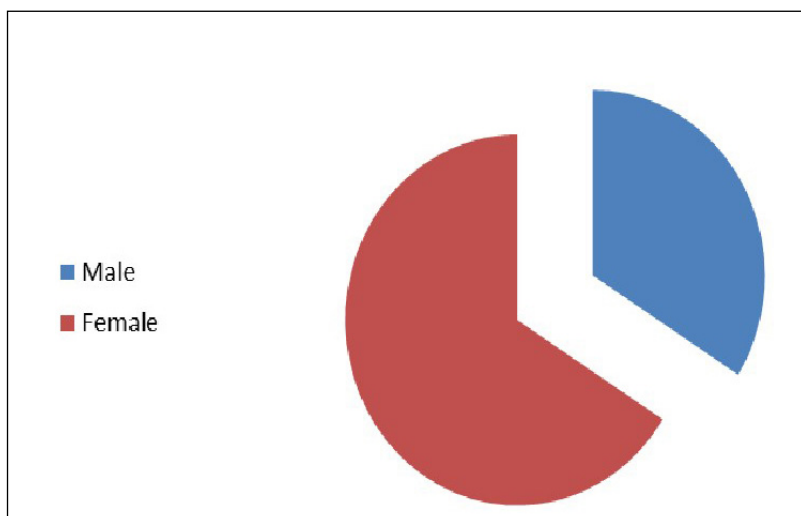
Fig. 1. Gender distribution among study population.
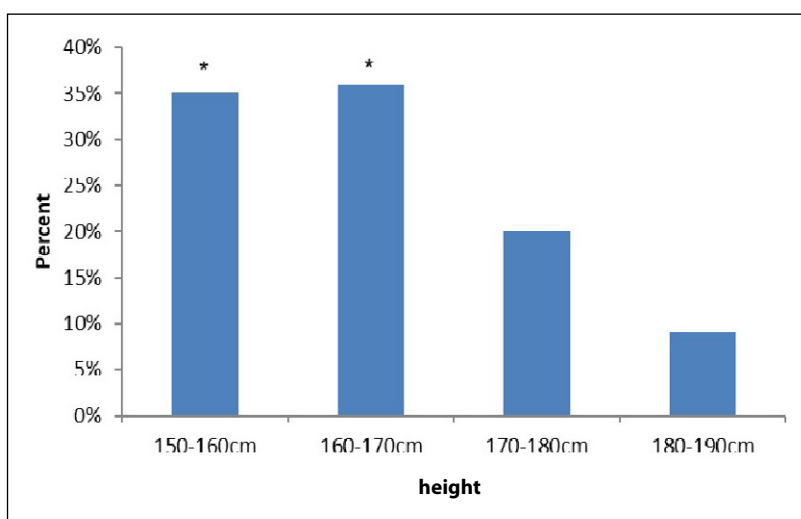
Fig. 2. Height distribution among study population.
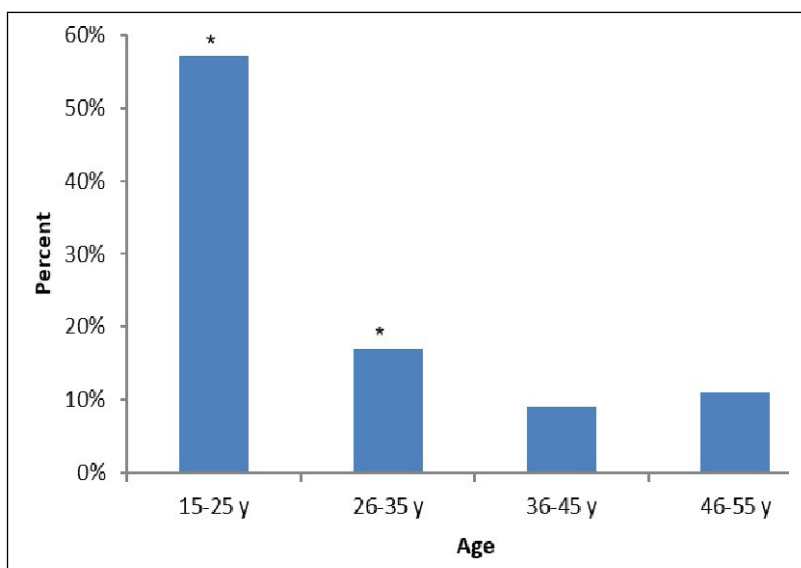
Fig. 3. Age distribution among population of study.

classification of cinnamon is as follows: it belongs to the order *Laurales*, the family *Lauraceae*, the genus *Cinnamomum*, and the species *Cinnamomum zeylanicum*, which is also known as *Cinnamomum verum*. The approval of cinnamon by the European Commission E monographs is based on a variety of factors, which include its extensive historical usage within established systems of traditional medicine. The dried bark of Cinnamomum verum is utilized for the treatment of various gastrointestinal ailments, including loss of appetite,

bloating, dyspepsia, flatulence, and spastic conditions. Cinnamon sensitivity and allergy can potentially lead to mucosal irritation and dermatitis, making it inadvisable for use during pregnancy. Cinnamon is administered internally in various forms, such as bark, essential oil, or cut/ground bark for teas and other medicinal preparations. The recommended dosage ranges from 2-4 g of bark or 0.05-0.2 g of essential oil. Its internal use is attributed to its antibacterial and fungistatic properties, as well as its ability to promote motility [20]. The inclusion of cinnamon flowers in the list of herbs not approved is attributed to the potential for sensitization and the frequent occurrence of allergic reactions in the skin and mucosa resulting from their use. According to the British Herbal Pharmacopoeia, cinnamon essential oil has antifungal, strong antibacterial, larvicidal and antiviral properties. Additionally, its constituents, namely eugenol acetate, eugenol, and methyl eugenol, have been observed to augment activity of trypsin in vitro. Cinnamon bark has demonstrated a significant lipolytic effect, as evidenced by previous research [20]. Cinnamon is utilized as a carminative and stomachic in both the United States and Germany. Additionally, it serves as a constituent in formulations for treating cough, cold, and fever that involve multiple herbs. Cinnamon bark of pharmacopeial grade is required to possess a minimum volatile oil content of 1.2%. In traditional Chinese medicine, cinnamon is utilized to treat the kidney, heart, liver, and spleen meridians. It is regarded as a warming substance and is employed to address various conditions associated with cold, including poor digestion, weak back, and cold or pain sensations in the extremities [21]. Several studies conducted in in vivo and in vitro using animal models have provided evidence of the lipid-lowering properties of cinnamon [22-24]. These studies have identified mechanisms by which cinnamon increases fatty acid oxidation and activates peroxisome proliferator-activated receptors (PPARs). Activation of these receptors has been shown to lead to a reduction in triglyceride (TG) levels and total cholesterol [25, 26]. Previous research has indicated that the consumption of cinnamon (C. spp.) as part of a herb and spice blend, at doses of 0.61 g and 1.11 g, has been associated with a reduction in triglyceride levels in overweight individuals who are otherwise healthy. These effects were observed when the blend was consumed alongside a meal, as reported in studies conducted on human subjects [27, 28]. The findings of a single study indicated that a decrease in postprandial triglyceride levels was observed only when a period of rest followed the consumption of a healthy meal. The authors observed that the presence of stressor tasks during the postprandial period did not result in a reduction of postprandial triglyceride (TG) levels. This finding led the authors to suggest that psychological stress might diminish the potential protective effect of the blend on the risk of cardiovascular disease. The study additionally proposed that the combination of herbs and spices has the ability to impede the digestion of lipids within a living organism. This inhibition was observed in vitro, where the blend demonstrated a dose-dependent inhibition of pancreatic lipase and phospholipase A2, both of which are enzymes involved in the lipid digestion process [29].

AIM
The aim of this study is to evaluate the effectiveness of L-carnitine and cinnamon alone and in combination on body composition parameters, and to compare their effects with each other.

MATERIALS AND METHODS
This clinical study was conducted in one of the medical complexes in Babylon and the study was conducted between December 2022 and January 2023.

INCLUSION CRITERIA
The present study includes an adult women or men with obesity and overweight without possessing any diseases.

EXCLUSION CRITERIA
Patients suffering from hormonal changes can lead to obesity, the presence of other diseases causes obesity, patients cannot make a phone call during observation, patients do not need to continue treatment for personal reasons, patients have chronic diseases such as hypertension, diabetes and others diseases.

SAMPLING AND STUDY GROUPS
The sample of study include 28 obese and overweight adults (men and women) with BMI greater than 25 and 7 adults with BMI between 18 and 25. *Negative control:* include 7 normal patients (BMI between 18 & 25) without take any medication. *Positive control:* include 7 obese patients without take any medication. *Cinnamon group:* include 7 obese patients take cinnamon 500 mg twice daily for 14 days. *L-carnitine group:* include 7 obese patients take L-carnitine 1000 mg once daily for 14 days. *Cinnamon & L-carnitine group:* include 7 obese patients take cinnamon (500 mg) and L-carnitine (1000 mg) once daily for 14 days.
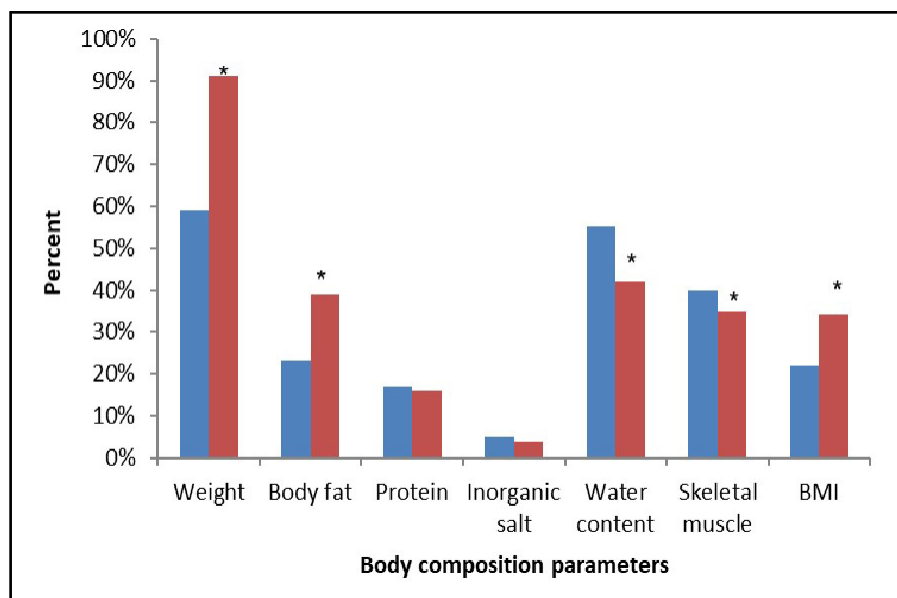
Fig. 4. A comparison between positive and negative control groups among population of study.

Fig. 5. A comparison between parameters of body composition in cinnamon treated patients at baseline and after treatment.

APPARATUS, EQUIPMENT AND MATERIALS USED IN STUDY

Apparatus: body composition analysis. Equipment: not used. Materials: L-carnitine capsule 1000 mg and Cinnamon capsule 500 mg.

ETHICAL ASPECTS AND APPROVAL

The study involved obtaining approvals from various colleges within the University of Babylon, as well as several government schools, in order to secure consent for data collection. The procedure and objectives of the study were clearly communicated to all participants, and specific recommendations were provided regarding preventative measures against obesity. Additionally, the study aimed to investigate the impact of cinnamon and L-carnitine supplements on body composition and weight loss.

COLLECTION OF DATA

The data collection method employed in this study relied on direct personal interviews conducted in educational institutions, specifically within the premises of various colleges in Babylon. The data were gathered using a meticulously designed questionnaire and a structured interview technique. The patients' data were collected from each individual patient in an organized manner, with interviews lasting approximately 15 minutes. Data collection took place on multiple days of the week, and at any time during the day if collected through social media. However, if data were collected from schools and colleges, interviews were conducted in the morning between 8 am and 12 pm.

STATISTICAL ANALYSIS

The statistical analysis involved the utilization of a one-way analysis of variance (ANOVA) test, followed by a
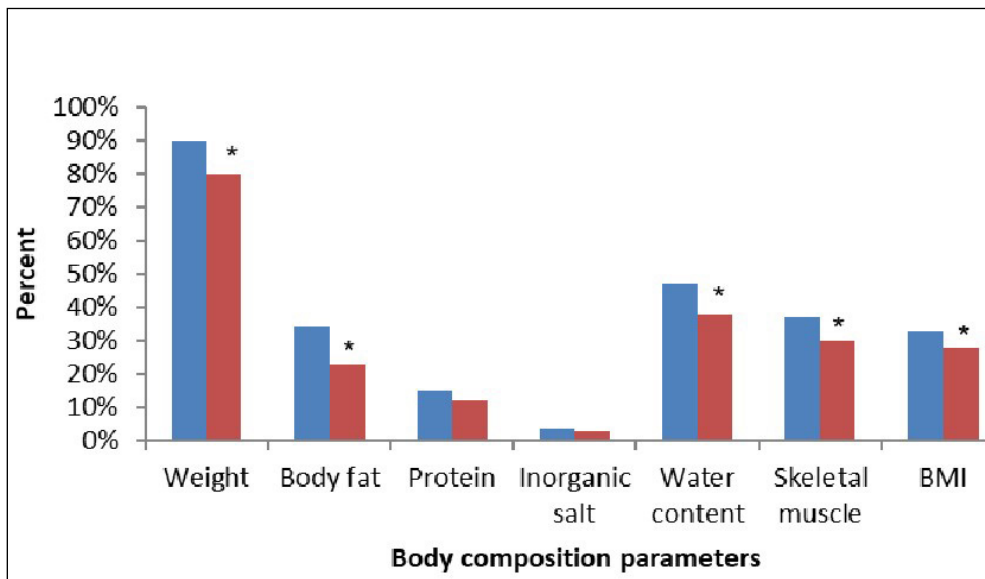
Fig. 6. A comparison between parameters of body composition in L-carnitine treated patients at baseline and after treatment.

Fig. 7. A comparison between parameters of body composition in patients treated with L-carnitine plus cinnamon at baseline and after treatment.

post hoc test to determine significance (P<0.05). This analysis aimed to compare the control group with the treated groups, and subsequently conduct multiple comparisons among the treated groups. The statistical analysis was conducted using the computer program SPSS versions. Microsoft Excel is also the preferred software application.

RESULTS

DEMOGRAPHIC DISTRIBUTION BETWEEN HEALTHY AND OBESE INDIVIDUALS

In present study there was a significant differences P<0.05 among gender distribution between male and female (Fig. 1).

EFFECT OF HEIGHT DISTRIBUTION AMONG STUDY POPULATION

The current study shows a significant difference between 150-160 cm and 160-170 cm as compared with 170-180 cm and 180-190 cm. Also, significant differences between 170-180 cm as compared with 180-190 cm but non-significant differences between 150-160 cm as compared with 160-170 cm (Fig. 2).

EFFECT OF AGE DISTRIBUTION AMONG STUDY POPULATION

The finding of present study reveals a significant difference between 15-25 years as compared with 26-35, 36-45 and 46-55 years. Also, significant differences between 26-35 as compared with 36-45 and 46-55 but non-significant differences between 36-45 as compared with 46-55 (Fig. 3).

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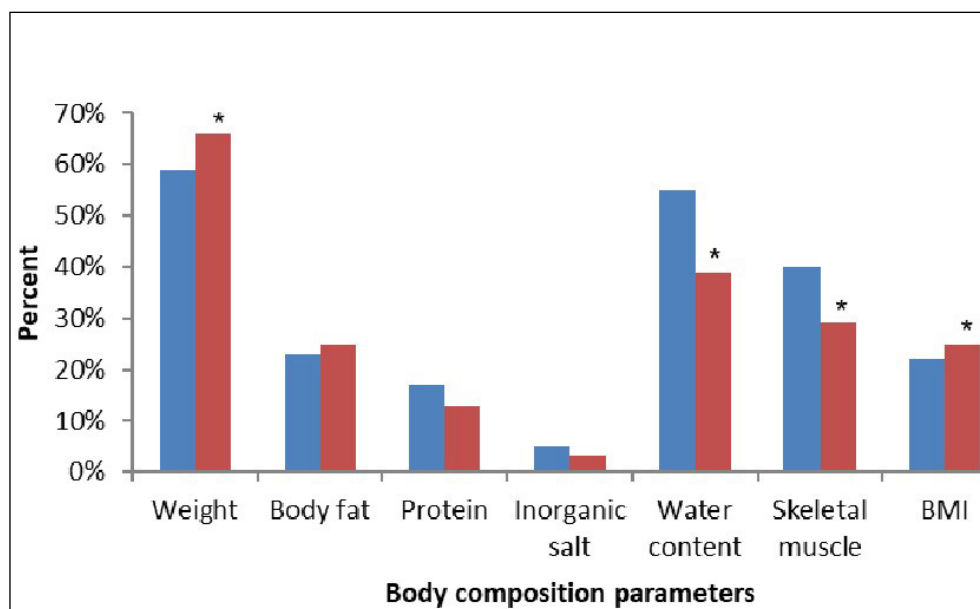
Fig. 8. A comparison between parameters of body composition in patients treated with cinnamon and negative control individuals.
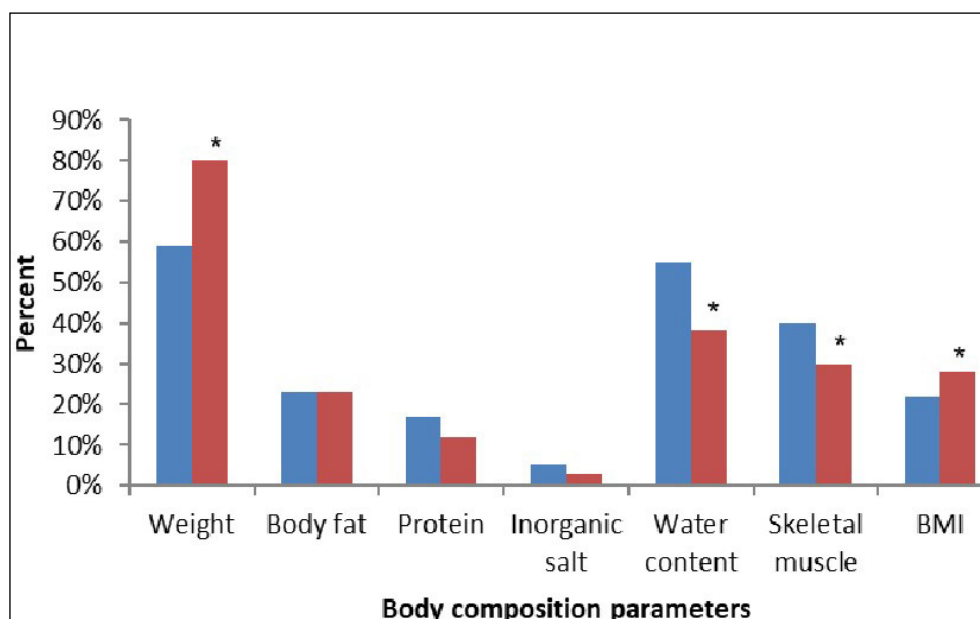
Fig. 9. A comparison between parameters of body composition in patients treated with L-carnitine and negative control individuals.

A COMPARATIVE EFFECT BETWEEN HEALTHY INDIVIDUALS AND THOSE WITH OBESITY

There were significant differences between healthy and obese individuals in body weight, body fat, water content, skeletal muscle, and body mass index, but non-significant differences between protein and inorganic salt in the negative and positive control groups (Fig. 4).

A COMPARATIVE EFFECT BETWEEN BODY COMPOSITION PARAMETERS AT BASELINE AND AFTER TREATMENT WITH CINNAMON

The present study reports a significant difference between weight, body mass index at baseline and after treatment with cinnamon but non-significant differences between body fat, protein, water content, skeletal muscle, and inorganic salt at baseline and after treatment with cinnamon (Fig. 5).

A COMPARATIVE EFFECT BETWEEN BODY COMPOSITION PARAMETERS AT BASELINE AND AFTER TREATMENT WITH L-CARNITINE

The present study shows a significant difference between body weight, body fat, water content, skeletal muscle, and body mass index after treatment and at baseline with L-carnitine, but non-significant differences between protein, and inorganic salt after treatment and at baseline with L-carnitine (Fig. 6).

A COMPARATIVE EFFECT BETWEEN BODY COMPOSITION PARAMETERS AFTER TREATMENT AND AT BASELINE WITH COMBINATION L-CARNITINE PLUS CINNAMON

The current study shows a significant difference between body weight, body fat, water content, skel-
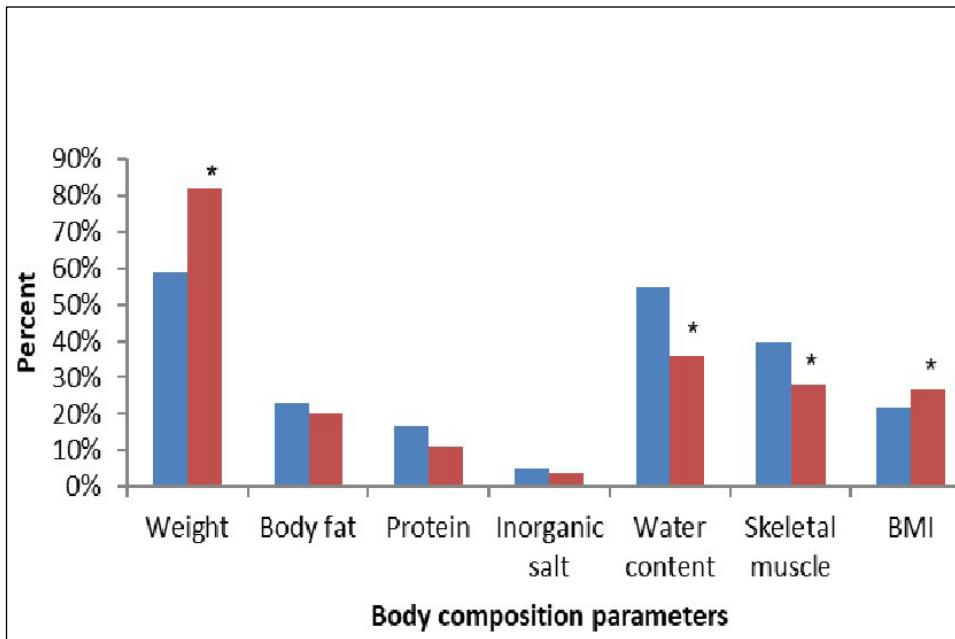
Fig 10. A comparison between parameters of body composition in patients treated with cinnamon plus L-carnitine and negative control individuals.
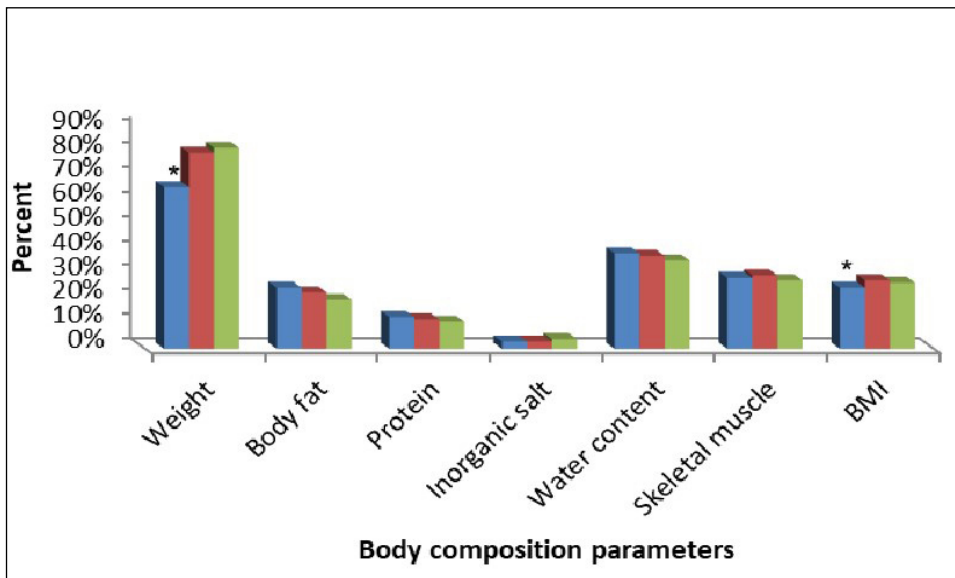
Fig. 11. A comparison between body composition parameters among treated group.

etal muscle, and body mass index after treatment and at baseline with L-carnitine, but non-significant differences between protein, and inorganic salt after treatment and at baseline with L-Carnitine (Fig. 7).

A COMPARATIVE EFFECT BETWEEN BODY COMPOSITION PARAMETERS IN NEGATIVE CONTROL GROUP VERSUS GROUP TREATED WITH CINNAMON

There was a significant difference between body weight, water content, skeletal muscle, and body mass index in group treated with cinnamon as compared with negative control group, but non-significant differences between body fat, protein, and

inorganic salt as compared with negative control group (Fig. 8).

A COMPARATIVE EFFECT BETWEEN BODY COMPOSITION PARAMETERS IN NEGATIVE CONTROL GROUP VERSUS GROUP TREATED WITH L-CARNITINE

The present study reported significant differences between body weight, water content, skeletal muscle, and body mass index in group treated with L-carnitine as compared with negative control group, but non-significant differences between body fat, protein, and inorganic salt as compared with negative control group (Fig. 9).

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A COMPARATIVE EFFECT BETWEEN BODY COMPOSITION PARAMETERS IN NEGATIVE CONTROL GROUP VERSUS GROUP TREATED WITH CINNAMON PLUS L-CARNITINE

The current study reported significant differences between body weight, water content, skeletal muscle, and body mass index in group treated with Cinnamon plus L-Carnitine as compared with negative control group, but non-significant differences between body fat, protein, and inorganic salt as compared with negative control group (Fig. 10).

A COMPARATIVE EFFECT BETWEEN BODY COMPOSITION PARAMETERS IN ALL TREATMENT GROUPS

There was a significant difference between body weight, body mass index in group treated with cinnamon as compared with L-carnitine, cinnamon plus L-carnitine group but non-significant differences between water content, inorganic salt, protein, body fat, skeletal muscle in all treatment groups (Fig. 11).
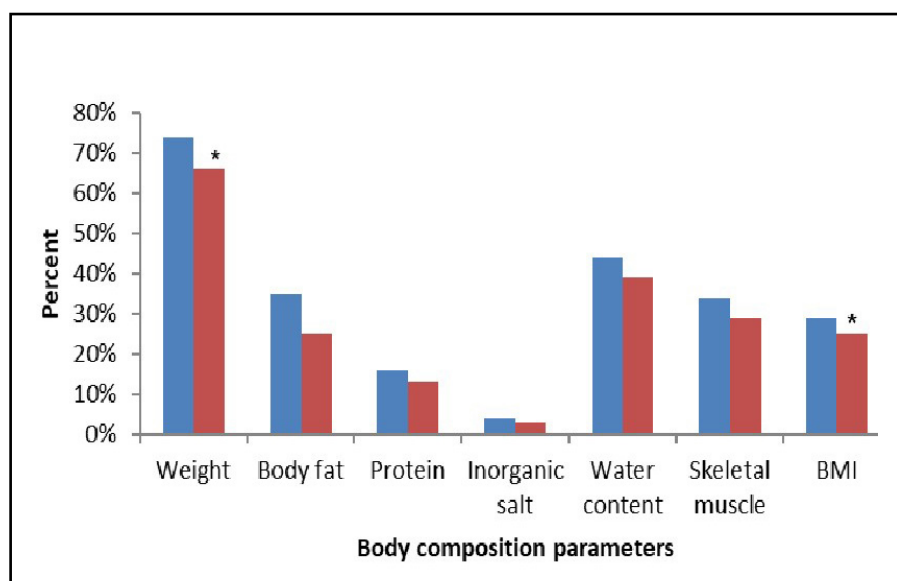
DISCUSSION

A COMPARISON BETWEEN PARAMETERS OF BODY COMPOSITION IN CINNAMON TREATED PATIENTS AT BASELINE AND AFTER TREATMENT

A notable disparity in body weight and body mass index is observed between patients who received cinnamon treatment at the beginning of the study and those who underwent treatment. In terms of body weight and BMI parameters, the cinnamon group, following treatment, exhibits lower values compared to the baseline (Fig. 5). This finding is consistent with a previous study of Bhuyan SS et al. [30] that reported a decrease in BMI and body weight when patients were administered cinnamon supplements. This study demonstrates a notable positive impact of cinnamon supplementation on individuals of Asian Indian descent who have metabolic syndrome. This is evidenced by a significant reduction in hyperglycemia, body weight, overall adiposity, abdominal adiposity, and serum lipid levels when using a daily dose of 3 g of cinnamon, compared to a placebo, over a 16-week period. One notable discovery in the current study is the observation of a statistically significant rise in serum high-density lipoprotein cholesterol HDL-C levels following the administration of cinnamon.

A COMPARISON BETWEEN PARAMETERS OF BODY COMPOSITION IN L-CARNITINE TREATED PATIENTS AFTER TREATMENT AND AT BASELINE

A notable disparity exists in the body weight, body fat, water content, skeletal muscle, and BMI among patients who have undergone treatment with L-carnitine, both at the beginning of the treatment and after its completion. In terms of body weight, body fat, water content, skeletal muscle, and BMI parameters, the L-carnitine group exhibited lower values compared to the baseline following treatment (Fig. 6). This finding is consistent with a previous study of Seburg EM et al. [31] that reported a decrease in body fat, water content, and skeletal muscle when L-carnitine is administered. This study presents findings on the impact of L-carnitine supplementation on the body composition of athletes. Upon analysis and comparison of the data, it was observed that the consumption of L-carnitine prior to training aided athletes in maintaining an optimal body weight and enhancing fat oxidation during exercise. This effect was achieved through the activation of fat metabolism, resulting in a positive impact on the reduction of fat mass. Furthermore, a separate investigation of Danielsson P et al. [32] reported a decrease in body weight and BMI as well. The study examined the impact of L-Carnitine on anthropometric indices. The findings revealed that individuals who received a 1000 mg L-Carnitine supplement over a three-month period experienced weight loss and a decrease in BMI. These outcomes support the hypothesis that L-Carnitine enhances beta-oxidation of fatty acids and increases basal metabolic rate. However, it is important to note that these changes were not statistically significant when compared to the control group. This lack of significance may be attributed to the fact that both study groups followed a low-calorie diet.
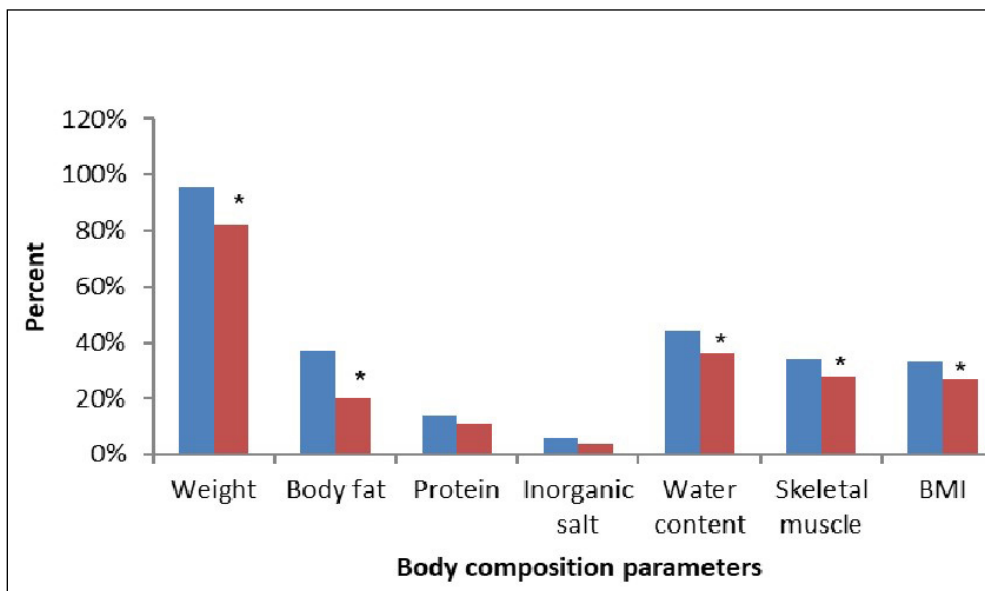
A COMPARISON BETWEEN BODY COMPOSITION PARAMETERS IN PATIENTS TREATED WITH L-CARNITINE PLUS CINNAMON AT BASELINE AND AFTER TREATMENT

There is a significant difference in body weight, body fat, water content, skeletal muscle and body mass index in cinnamon plus L-carnitine treated patients after treatment and at baseline. in body weight, body fat, water content, skeletal muscle, and body mass index parameters the L-carnitine group (after treated) is less than the baseline. This means when used together it will double the effect on body composition (Fig.7).

This result is in consistency with a study of Bhuyan SS et al. [30], which reported that cinnamon have a positive effect on weight loss and body composition and studies [31-33] which stated that L- carnitine have a positive effect on weight loss and body composition parameters.

A COMPARISON BETWEEN PARAMETERS OF BODY COMPOSITION IN CINNAMON TREATED PATIENTS AND NEGATIVE CONTROL INDIVIDUALS

There was a significant difference in water content, body weight, skeletal muscle and body mass index in patients treated with cinnamon when compared with negative control. in body weight and BMI parameters the cinnamon group is higher than the negative control. in water content and skeletal muscle parameters the cinnamon group is lower than the negative control (Fig. 8). This result is in accordance with a study of Benz C et al. [34], which stated that there is a significant difference in body weight between cinnamon group and negative control group. The sedentary and overweight or obese male subjects exhibited good tolerance to the daily consumption of digerlat over a period of six weeks. While the administration of digerlat treatment did not demonstrate any impact on blood pressure, heart rate, or biomarkers related to metabolic function, an interaction between the duration of the treatment period and the treatment itself was observed in terms of changes in body weight. This finding suggests that green tea catechins may have a protective effect against weight gain in situations where there is an excess of energy intake. The influence of COMT genotype on the accumulation of urinary catechins was also demonstrated. Further research is necessary to validate these findings and to delve deeper into the potential influence of genotype.

A COMPARISON BETWEEN PARAMETERS OF BODY COMPOSITION IN L-CARNITINE TREATED PATIENTS AND NEGATIVE CONTROL INDIVIDUALS

There is a significant difference in body weight, water content, skeletal muscle and BMI parameters in patients treated with L-carnitine when compared with negative control. in body weight and BMI parameters the L-carnitine group is higher than the negative control. in water content and skeletal muscle parameters the L-carnitine group is less than the negative control (Fig.9). This result is in agreement with a study of Cole TJ et al. [33], which reported that body weight and BMI in L-carnitine group are higher than the negative control group.

A COMPARISON BETWEEN PARAMETERS OF BODY COMPOSITION IN CINNAMON PLUS L-CARNITINE TREATED PATIENTS AND NEGATIVE CONTROL INDIVIDUALS

There is a significant difference in body weight, water content, skeletal muscle and BMI parameters in patients treated with cinnamon plus L-carnitine when compared with negative control. in body weight and BMI parameters the combination group (cinnamon plus L-carnitine) is higher than the negative control. in water content and skeletal muscle parameters the cinnamon plus L-carnitine group is lower than the negative control (Fig. 10). This result is in agreement with studies of Cole TJ et al. and Benz C et al. [33, 34] which recorded that there a significant difference in body weight and body mass index between the treated group and the negative groups.

A COMPARISON BETWEEN BODY COMPOSITION PARAMETERS AMONG TREATED GROUPS

There was a significant difference in body weight and BMI between the treatment groups (Fig. 11.), but in the cinnamon group was more effective for body weight and BMI, but in the cinnamon plus L-carnitine group was more effective for body fat, and this result was in consistent with all the research that shows that the effects of these supplements on body composition vary, different patients and different environments have different responses to these agents.

CONCLUSIONS

There is a significant difference in body weight and BMI in patients treated with cinnamon at baseline and after treatment. Body mass index and body weight parameters cinnamon group (after treated) is lower than the baseline. Also, there is a significant difference in body fat, body weight, water content, skeletal muscle and BMI in L-carnitine treated patients after treatment and at baseline. in body weight, body fat, water content skeletal muscle and BMI parameters the L-carnitine group (after treated) is lower than the baseline. In addition to that a significant difference in body weight, body fat, water content, skeletal muscle and BMI in patients treated with cinnamon plus L-carnitine at baseline and after treatment. in body weight, body fat, water content, skeletal muscle and BMI parameters L-carnitine group (after treated) is lower than the baseline. That means when using together it will double the effect on body composition.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Qayssar Joudah Fadheel

Department of Pharmacology and Toxicology,
College of Pharmacy, University of Babylon,
Al Najaf's St., Al Hillah, Babylon, Iraq
e-mail: pharm.qayssar.joudah@uobabylon.edu.iq

ORCID AND CONTRIBUTIONSHIP

Qayssar Joudah Fadheel: 0000-0003-3301-8357 (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

ORIGINAL ARTICLE

Analysis and interpretation of Coronavirus infection children's incidence, contributing factors, risks of complications and their relationship

Olesya M. Horlenko, Khrystyna Hechko, Lyubomyra B. Prylypko, Mykhaylo Hechko, Fedir V. Horlenko, Adrian I. Tomey, Agneta V. Lenchenko
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT
Aim: To study and investigate the incidence of Coronavirus infection in children, the course of the disease, the risks of complications and their interrelationships

Materials and Methods: Study included the analysis and observation of children (n=55, aged 14.36±3.62 years) with confirmed Coronerovirus infection, who were observed in the CNE «CMCH» in Uzhgorod in outpatient conditions. A study of clinical presentations, a clinical and laboratory examination followed by a mathematical analysis of the symptoms data in children with an identified Coronavirus infection and in the dynamics up to week 30 (with survey intervals in 3 weeks) from the diagnosis verification was carried out

Results: A dynamic analysis of the clinical manifestation of symptoms in children with an identified Corona virus infection and within 30 weeks (with survey intervals of 3 weeks) from the beginning of the diagnosis verification was carried out. Complaints from the respiratory system were prevailed. The most long-lasting complaint was observed «changes in the sense of taste and smell» (from 35(63.6%) to 6(10.9%) up to 18 weeks inclusive. Other complaints «Cough, Rhinitis, Shortness of breath, Pain in the chest» was observed for 6 weeks. Sore throat, muscular and joint pain were persisted for 3 weeks. Fever was not identified at week 3. Illness with other viral respiratory diseases started at week 9 and was observed until week 30 (from 10(18.2%) to 19(34.5%)) with varying levels. A decrease in cases of IgM identification was observed within 6 weeks (from 55, 100% to 20, 36, 4%). On the 9th week, the presence of IgM was not established. There is also an increase in the number of cases of detection of IgG in patients with a level maximum of 6 weeks.

Conclusions: There is a positive effect of the CRP level on the occurrence of symptoms of cough, rhinitis, shortness of breath, chest pain, change in taste and smell, muscle and joint pain (r=0.33-0.55), with the most significant data for the symptom of pain in chest (p=0.00001). Ferritin level interactions mostly had a negative direction (r=-0.35-0.48, p=0.02-0.00001) on the development of symptoms, with the exception of rhinorrhea (r=0.48, p=0.00002) and chest pains (r=0.39, 0.003). According to multiple logistic regression analysis the chance of the symptom of a change in taste and smell increases due to an increasing in the level of Procalcitonin in 1.48 times. The chance of the symptom of shortness of breath increased due to an increasing in the Ferritin level in 1.025 times

KEY WORDS: Coronavirus infection (SARSCoV-2), symptoms, complication, correlational and multiple logistic regression analysis, children

INTRODUCTION

The study of the prevalence of SARSCoV-2 among children in Ukraine in the first year of the pandemic showed that the part of registered cases was 0.7%, and the morbidity per 100,000 children was determined in 688.64 cases. The most vulnerable to SARS-CoV-2 age group infection among children in Ukraine was teenagers (63.8%). There is a similarity with the results of studies in the USA [1], where children over the age of 12 were predominated [2]. SARS-CoV-2 is characterized by an extraordinary speed of spread due to factors unique to the virus, characteristics of the child, and interaction with the environment [3]. More than 90% of children with COVID-19 have a mild course of the disease and do not require hospitalization. This contrasts with other respiratory viruses, where the disease in children is often more severe. Among the most common clinical signs of SARSCoV-2 in children are classic flu-like symptoms such as fever, sore throat, nasal congestion, and cough. In addition to damage in the upper respiratory tract, other organs can be affected, for example, the gastrointestinal and central nervous systems. Most children were asymptomatic, and only a few cases were severe, in contrast to adult patients [4]. At the same time, it was established that asymptomatic infection is registered in 15-42% of children [5,6]. It is important to note that this is likely an underestimate of the true occurrence, as asymptomatic children are applied much less often for testing than symptomatic children [7].

Table 1. Prospective characteristics of the main symptoms in children with coronavirus infection

Parameters (n=55)	Oneset	3 weeks	6 weeks	9 weeks	12 weeks	15 weeks	18 weeks	21 weeks	24 weeks	27 weeks	30 weeks
IgM	55	55	20(36,4%)	-	-	-	-	-	-	-	-
IgG	-	22(44,0%)	55	55	55	55	55	55	55	55	55
Fever	55	-	-	-	-	-	-	-	-	-	-
Cough	29 (52,7%)	14(25,5%)	2(3,64%)	-	-	-	-	-	-	-	-
Sore throat	8(14,5%)	2(3,64%)	-	-	-	-	-	-	-	–	-
Rhinitis	33(60,0%)	8(14,5%)	4(7,25%)	-	-	-	-	-	-	-	-
Dispnea	21(38,2%)	3(5,45%)	3(5,45%)	-	-	-	-	-	-	-	-
Chest pain	39(70,9%)	23(41,8%)	2(3,64%)	-	-	-	-	-	-	-	-
Desorders in the taste and smell sensation	35(63,6%)	9(16,4)	6(10,9%)	6(10,9%)	6(10,9%)	6(10,9%)	6(10,9%)	-	-	-	-
Muscular and joint pain	48(87,3%)	19(34,5%)	-	-	-	-	-	-	-	-	-
Other viral respiratory diseases	-	-	-	10(18,2%)	14(25,5%)	2(3,64%)	4(7,25%)	1(1,82%)	2(3,64%)	6(10,9%)	19(34,5%)
Hospitalization	3(5,45%)	2(3,64%)	-	-	-	-	-	-	-	-	-

Table 2. Associated pathology in children with coronavirus infection

Associated pathology in children with Covid-19(n=55)	Number (%)
Reccuurnce respiratory diseases in anamneses	8 (14,5%)
Over weight	2(3,64%)
Anemia	5(9,1%)
Chronic respiratory pathology	0
Asthma	2(3,64%)
Cardiio- vascular diseases захворювання	1(1,82%)
Urinary tract pathology патологія	0
Digestivet ract pathology	0
Connective tissue diseases	0

AIM

To study and investigate the incidence of Coronavirus infection in children, the course of the disease, the risks of complications and their interrelationships

MATERIALS AND METHODS

Materials and methods of the study included the analysis and observation of children (n=55, aged 14.36±3.62 years) with confirmed Coronerovirus infection, who were observed in the CNE «CMCH» in Uzhgorod in outpatient conditions. A study of clinical presentations, a clinical and laboratory examination followed by a mathematical analysis of the symptoms data in children with an identified Coronavirus infection and in the dynamics up to week 30 (with survey intervals in 3 weeks) from the diagnosis identification was carried out.

RESULTS

A dynamic analysis of the clinical manifestations of symptoms in children with an identified Coronaviral infection and within 30 weeks (with survey in intervals of 3 weeks) from the beginning of the verification of the diagnosis was carried out (Table 1).

Consideriation of the obtained data presented that complaints from the respiratory system are prevail. The most long-lasting complaint was observed changes in taste and smell sensations (from 35(63.6%) to 6(10.9%) up to and including 18 weeks (Fig. 1).

Other complaints, as cough, rhinitis, dispnea, chest pain has been observed for 6 weeks. The dynamic of chest pain is shown on Fig. 2.

Sore throat and muscle and joint pain lasted 3 weeks. Fever was not identified on the 3rd week. The rate of hospitalization and the presence of complications was observed during the first 6 weeks. Pneumonia was
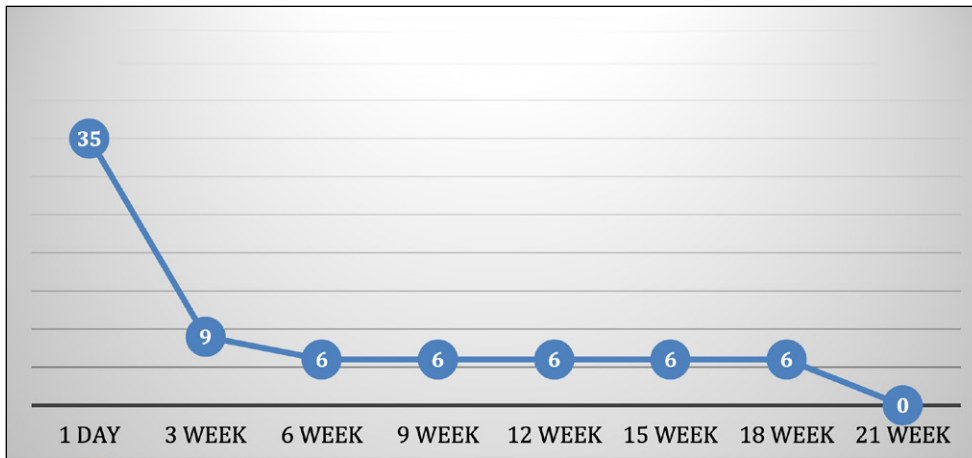
Fig.1. Dinamic of disorders in the taste and smell sensation.
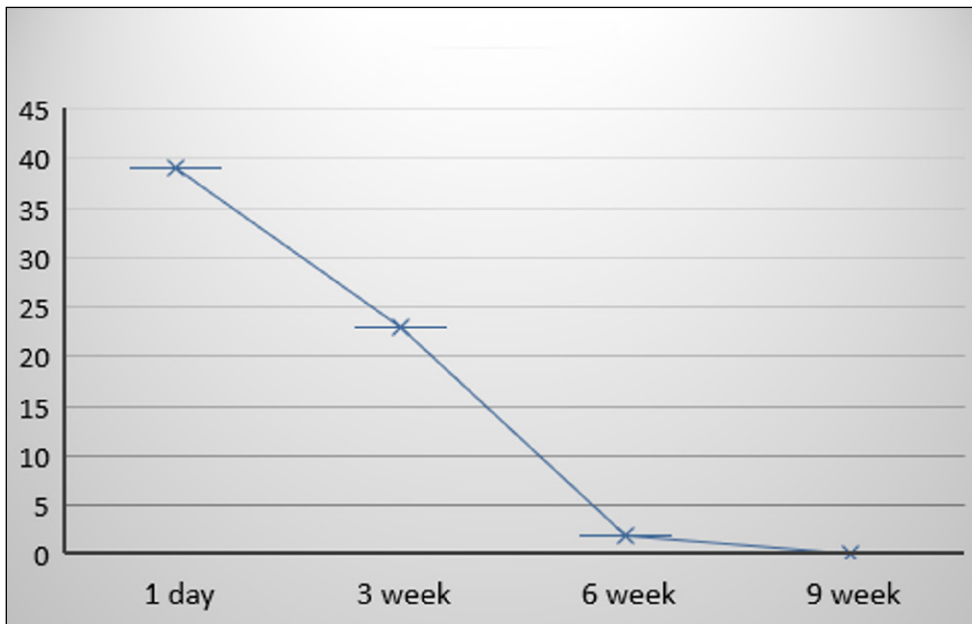
Fig. 2. Dynamics of chest pain.
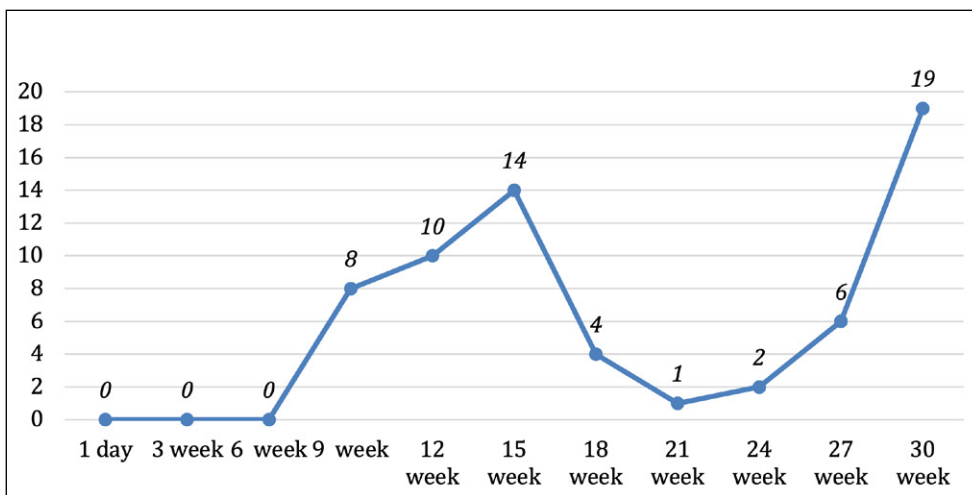
Fig. 3. Dinamics of other respiratory diseases in the postcovid perid.

diagnosed (3.5/45%) in the first 2 weeks and 2 cases (3.64%) in the 3rd week range.

Other viral respiratory diseases began from the 9th week and was observed until the 30th week (from 10 (18.2%) to 19 (34.5%)) with different levels, which are graphically illustrated on Fig. 3.

The accompanying pathology of the studied contingent of children was considered and peasented in Table 2.

According to table II, the most common concomitant

Table 3. Correlation relationships of clinical and laboratory parameters

Parameters		Correlation coefficient (r)	Statistical significance (p)
Cough	CRP	0,44	0,001
	Ferritin	-0,47	0,0004
Sore throat	Total protein	-0,29	0,03
	Na	-0,38	0,004
	Urea	0,34	0,01
	Creatinin	0,36	0,008
	Ferritin	-0,48	0,0002
	antiTPO	-0,51	0,0002
Rhinitis	CRP	0,54	0,00002
	Ferritin	0,48	0,0002
Dispnea	K	0,29	0,03
	CRP	0,33	0,02
	Ferritin	-0,27	0,04
	antiTPO	-0,51	0,00008
Chest pain	CRP	0,55	0,00001
	Ferritin	0,39	0,003
Desorders in the taste and smell sensation	K	0,28	0,04
	CRP	0,44	0,001
	Ferritin	-0,35	0,009
Muscular and joint pain	CRP	0,52	0,00004

diseases were recurrent respiratory diseases in the anamnesis (8, 14.5%) and anemia (5.9.1%). The dynamics of IgM in patients is presented (Fig. 4).

There is a decrease in cases of IgM identification within 6 weeks (from 55/ 100% to 20/36,4%). On the 9th week, the presence of IgM was not established.

There is also an increasing in the number of cases of IgG detection in patients with a maximum of 6 weeks (Fig. 5).

Correlation analysis of the obtained data was carried out and the relationship between clinical symptoms and laboratory indicators was identified (Table 3).

According to Table 3, each considered symptom was correlated with inflammatory markers, in particular CRP and Ferritin, except for Sore Throat and Muscle and Joint pain, separately. There is a positive effect of the CRP level on the occurrence of symptoms of Cough, Rhinitis, Dispnea, Chest pain, change in taste and smell, muscle and joint pain (r=0.33-0.55), with the most significant data for the symptom of Chest pain (p=0.00001). Ferritin level interactions mostly had a negative direction (r=-0.35-0.48, p=0.02-0.00001) on the development of symptoms, with the exception of Rhinitis (r=0.48, p=0.00002) and Chest pain (r=0.39, 0.003). Also interesting is the fact that the level of mineral K has a weak effect on the severity of the symptom of Dispnea and Disorder in taste and smell sinsation. The symptom of Sore Throat is characterized by the most numerous multidirectional relationships, in particular, the negative direction with Total Protein (r =-0.29, p=0.03), Na (r =-0.38, p=0.004), Ferritin (r =-0.48, p=0.000003) antiTPO (r =- 0.51, p=0.0002) and positive direction with Urea (r =0.34, p=0.01), Creatinine (r =0.36, p=0.008). We also performed a multiple logistic regression analysis for interpretation of the development and formation of the symptom of taste and smell sensations change from the studied inflammatory markers [8]. It was proved that the coefficient of Procalcitonin level is statistically significant (p=0.0455). The influence of the Procalcitonin level was evaluated by the value of the Odds ratio (unit ch) (ORu) and the Odds ratio (range) (ORr) for it. The chance of the taste and smell sensation disorder in increases due to an increase in the level of Procalcitonin in 1.48 times (ORu = 1.008-2.184). The range of chances (ORr) for Procalcitonin was 73.846 (1.093-4989.745). It was also found that The coefficient of Ferritin level (p=0.028) in the risk of developing Dispnea in patients with Coronaryvirus disease was statistically significant. The chance of the symptom of Dispnea increases due to an increase in the Ferritin level in 1.025 times (ORu = 1.003-1.047). The chance range (ORr) for Ferritin was 25.12547 (1.437-439.399). Other values of inflammatory markers were not reliable in our sample.
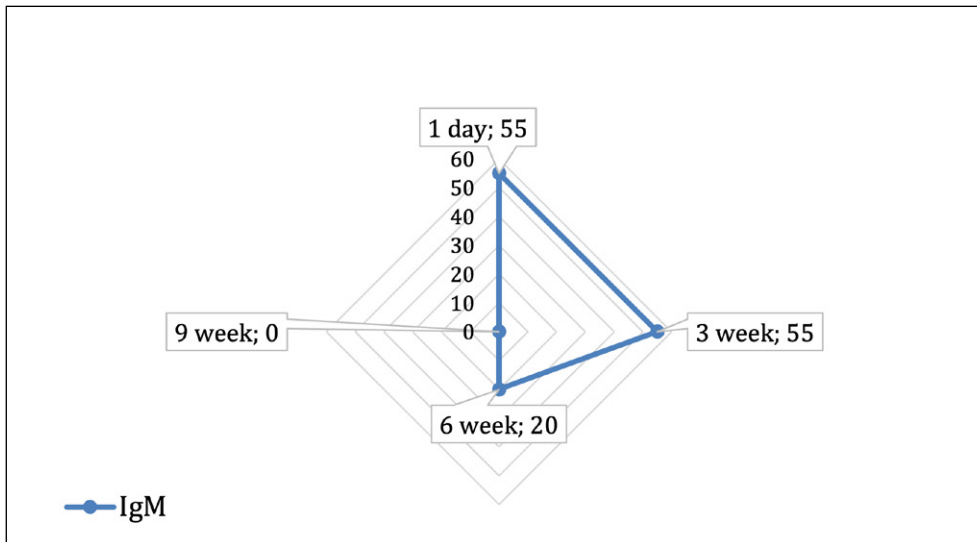
Fig. 4. The dynamics of IgM in patients.
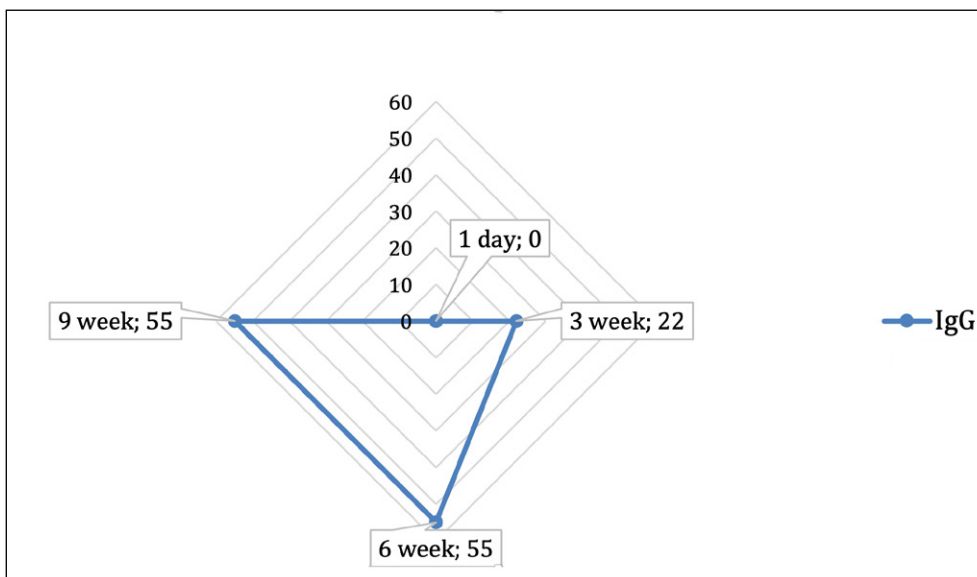
Fig. 5. Dynamics of IgG detection in the examined contingent.

DISCUSSION

SARS-CoV-2 is characterized by a predominance of complaints from the respiratory tract. When the virus binds to the ACE2 receptor, which is present in the lungs as well as in many other organs, the inflammatory response can occur and leading to multiple organ damage and long-term disease symptoms. The reaction of each organism to a viral factor is unique and can be considered as a set of contributing factors for the development of those or other clinical presentations. The persistence of the virus or viral fragments determines the triggering of the immune response, the duration of the disease and the possibility of developing complications [9]. Studies of inflammatory markers of the disease, as levels of Ferritin, CRP, Procalcitonin and proteins of the Acute phase response proved correlations with the severity of Coronaryvirus infection. Research of the functions and influence of each marker on the course of the disease, the intensity of manifestations

and the possibility of complications is ongoing [10]. There are several hypotheses for the relatively low morbidity and mortality of SARS-CoV-2 among children, which are largely related to differences in immune responses compared to adults, as well as differences in the distribution of Angiotensin-Converting enzyme 2 (ACE2), which potentially limits viral entry. and subsequent inflammation, hypoxia and tissue damage [11]. The authors suggest that mucosal immunity in children can prevent SARS-CoV-2 infection [12]. This finding is now supported by further studies showing an increased innate antiviral response in the upper respiratory tract in children compared to adults [13]. Higher levels of IFN-α2, IFN-γ, IP-10, IL-8 and IL-1β, proteins in nasal fluid are also observed in children compared to adults [14]. These results are direct evidence of a more intense early immune response of the Respiratory tract mucous membranes in children compared to adults, and suggest that this contributes to easier There are

several hypotheses for the relatively low morbidity and mortality of SARS-CoV-2 among children, which are largely related to differences in immune responses compared to adults, as well as differences in the distribution of angiotensin-converting enzyme 2 (ACE2), which potentially limits viral entry. and subsequent inflammation, hypoxia and tissue damage [11]. The authors suggest that mucosal immunity in children can prevent SARS-CoV-2 infection [12]. This finding is now supported by further studies showing an increased innate antiviral response in the upper respiratory tract in children compared to adults [13]. Higher levels of IFN-α2, IFN-γ, IP-10, IL-8 and IL-1β proteins in nasal fluid are also observed in children compared to adults [14]. These results provide direct evidence of a more intense early immune response of the respiratory tract mucosa in children compared to adults, and suggest that this contributes to easier clinical outcomes.

CONCLUSIONS

1. The complaints by the Respiratory system prevail according our data. The most long-lasting complaint was observed as taste and smell sensation disorder (from 35/63.6%) to 6/10.9%) up to 18 weeks including. Other complaints, as Cough , Rhinitis Dispnea, Chest Pain were observed for 6 weeks. Sore throat and muscular and joint pain persisted for 3 weeks. Fever was not identified at week 3. Ilness with other viral respiratory diseases have started at week 9 and was observed until week 30 (from 10(18.2%) to 19(34.5%)) with varying levels.

2. A decrease in cases of IgM identification was observed within 6 weeks (from 55/ 100% to 20/36, 4%). The presence of IgM was not revealed on the 9th week,. There is also an increase in the number of cases of detection of IgG in patients with a maximum on 6th week.

3. Each considered symptom correlated with inflammatory markers, in particular with (CRP and Ferritin), with the exception of Sore Throat and Muscle and joint pain and most significant was the symptom of Chest pain (r=0.00001). Positive effects of CRP level on the occurrence of all symptoms (r=-0.33-0.0,55 p=0.02-0.00001), with exception of Sore throat.   Ferritin level interactions mostly had a negative direction (r=-0.35-0.48, p=0.02-0.00001) on the development of symptoms, with the exception of Rhinitis (r=0.48, p=0.00002) and Chest pain (r=0.39, 0.003).

4. According to the data of the conducted Multiple Logistic Regression analysis for the interpretation of the development and formation of the next symptom as Taste and Smell sensations disorder under the influence of the studied inflammatory markers. It was proved that the coefficient of Procalcitonin level is statistically significant (p=0.0455). The influence of the Procalcitonin level was evaluated by the value of the Odds ratio (unit ch) (ORu) and the Odds ratio (range) (ORr) for it. The chance of the taste and smell sensation disorder in increases due to an increase in the level of Procalcitonin in 1.48 times (ORu = 1.008-2.184). The range of chances (ORr) for Procalcitonin was 73.846 (1.093-4989.745).

5. The coefficient of Ferritin level (p=0.028) in the risk of developing Dispnea in patients with Coronaryvirus disease was statistically significant. The chance of the symptom of Dispnea increases due to an increase in the Ferritin level in 1.025 times (ORu = 1.003-1.047). The chance range (ORr) for Ferritin was 25.12547 (1.437-439.399).

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Olesya M. Horlenko
Uzhhorod National University
1 Narodna squ., 88000 Uzhhorod, Ukraine
e-mail: ohorlenko@gmail.com

ORCID AND CONTRIBUTIONSHIP
Olesya M. Horlenko: 0000-0002-2210-5503 Ⓐ Ⓓ Ⓕ
Khrystyna Hechko: 0000-0003-4989-7659 Ⓐ Ⓑ Ⓓ
Lyubomyra B. Prylypko: 0000-0002-4131-5450 Ⓒ Ⓓ
Mykhaylo Hechko: 0000-0003-2793-5044 Ⓑ Ⓒ
Fedir V. Horlenko: 0000-0002-0496-2069 Ⓔ Ⓕ
Adrian I. Tomey: 0000-0002-60958937-2069 Ⓑ Ⓔ
Agneta V. Lenchenko: 0000-0001-7954-7540 Ⓑ Ⓔ

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

ORIGINAL ARTICLE

A case of successful treatment of a rare retinal disease presented by interferon-induced retinopathy

Bohdan M. Patskan, Valerii D. Beliayev, Kateryna V. Bora, Nataliia V. Bondarenko
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

Aim: To showcase a rare retinal lesion and the results of contemporary diagnostic and treatment of interferon-induced retinopathy.

Materials and Methods: We describe a case of a 36-year-old patient with interferon-induced retinopathy, with hepatitis C, that received prolonged interferon treatment. Clinical signs, examination and combined laser and pharmacologic treatment were showcased in the study.

Results: As a result of pharmacologic and laser treatment, the patient's visual acuity increased from 0.1 to 1.0 through the duration of 3 months after treatment. The patients` condition remained stable under dynamic observation.

Conclusions: Because interferon-induced retinopathy is a rare occurrence in routine ophthalmologic practice, combined laser therapy can be used for treatment of preretinal hemorrhage, which leads to improvement of visual functions and stabilization of the retinal processes. This case is an addition to the few described cases of interferon-induced retinopathy.

KEY WORDS: interferon, retinopathy, laser hyaloidotomy, retinal coagulation

INTRODUCTION

Interferon-induced retinopathy is a rare type of retinopathy, which is connected to prolonged systemic usage of in interferon – proteins with mutual properties, which are produced by the body in response to a viral infection. They are used for systemic treatment of some diseases, such as hepatitis B and C, multiple sclerosis, viral infection, malignancies, sarcoma Kaposi, in patients with AIDS, some types of leukemia, myeloma, herpes, mycoplasmas, some types of pneumonia, sepsis, airway and urogenital diseases [1-4]. Historically, interferon was used for treatment of keratitis (Europe), subretinal neovascularization (USA), age-related macular degeneration of the retina (USA) and part of a complex treatment of glaucoma (Australia) [5]. But with the introduction of new and more effective methods and safer medication, treatment of ophthalmologic illness with interferon became a thing of the past [6,7]. Recent data shows that there are 180 million patients with hepatitis C worldwide and 20000 new cases are registered annually [8]. In 2015 a total of 1.75 million new viral infections were observed in patients, that underwent medical procedures and used injected drugs [9]. In the United States of America 1-2% of the population are infected with hepatitis C and it is the leading cause of chronic liver disease. For comparison, in Egypt, the spread of this virus is at 10-20%. Based

on this, the number of complications associated with interferon treatment is increasing.

Interferons were accidentally discovered in 1957 by Alick Isaacs and Jean Lindenmann at the National Institute for Medical Research in London [5]. The types of interferons (depending on the cell that produced them) are as follows: α, β and γ. Interferons alpha-2b (for treatment of severe viral hepatitis) and beta-1a (for the treatment of multiple sclerosis) are particularly important. The mechanism of action of interferons is modulation of the functions of the immune system. Interferons have an antiviral effect, antitumor action and inhibit the proliferation of cells. Despite the fact, that interferons are used for treatment of a multitude of illnesses, the treatment lasts several months and has severe complications. The side effects of interferon treatment for the most part are changes of the central nervous system (depression), cardio-vascular system, gastro-intestinal tract, ocular system (ischemic retinopathy, extraocular muscle paresis, vision loss, retinal hemorrhage etc.), the skin (pruritus, urticaria, dryness, herpes and furunculosis) and the hematopoietic organs [10, 11].

One complication of prolonged interferon treatment is retinopathy – a pathologic state of the retina with hemorrhages, exudative retinal changes and functional disruption of the retina [5, 11-16]. The risk of developing

retinopathy during interferon treatment in patients with systemic conditions (diabetes mellitus, etc.) is 27,7-65,32% [8]. It`s much higher than compared to instances of retinopathy occurring (11.7%) in patients without these risk factors [17]. Other researchers suggest that interferon-induced retinopathy occurs in 19-69% of adult patient with interferon treatment (Narkewicz et al., 2010, Schulman et al., 2001). Usually, the onset of retinopathy starts in 3 to 5 months after the beginning of systemic treatment, however signs of retinopathy may occur in 2 to 6 weeks after start of treatment (Kadayifcilar et al., 1999, Okuse et al., 2006). Interferon-induced retinopathy usually has a mild course and resolves on its own. Differential diagnosis with hypertensive retinopathy, retinopathy associated with leukemia/lymphoma and AIDS, ocular ischemia, Purtscher retinopathy [18].

AIM

To showcase a rare retinal lesion and the results of contemporary diagnostic and treatment of interferon-induced retinopathy.

MATERIALS AND METHODS

A 36 years old patient with interferon-induced retinopathy, received systemic interferons for 2 years as treatment of hepatitis C. Patient`s condition was monitored by an infectious disease doctor. Patient underwent a standard ophthalmologic examination (visual acuity, intraocular pressure, field of view, slit lamp examination and fundoscopy), in addition modern diagnostic methods were conducted at "Transcarpathian center of eye microsurgery" medical center: refractometry (TOPCON KR-800), fundography (TOPCON 3D OCT-2000, Japan), ultrasound (TOMEY UD-800, Japan), retinal tomography (TOPCON 3D OCT-2000, Japan). Patient`s visual acuity was at 0.1, without the means for correction. A massive preretinal hemorrhage in the macular area with areas of retinal ischemia with neovascularization along the vascular arcades was observed during examination. Optimal treatment plan was developed based on these topographic changes in the retina: treatment of the preretinal hemorrhage using dorsal laser hyaloidotomy, treatment of ischemic areas of the retina with elements of neovascularization using laser coagulation of the retina. YAG-laser hyaloidotomy was conducted at "Transcarpathian center of eye microsurgery" medical center using OPTOTEK, OPTOYAG devices (Slovenia) and laser coagulation of the retina was conducted using Alcon, Purepoint (USA). Patient received medication (angio-protectors, retinal protectors, antioxidants, hypotensive treatment – as needed). Laser-surgery procedures on an outpatient basis. The success of treatment was evaluated by the following criteria: stabilization of the process and reduction of the hemorrhages during fundal and visual function examination.

RESULTS

No complications were observed during surgery and post-operative period. During early recovery, day 1 after laser hyaloidotomy a partial evacuation of the preretinal hemorrhage into the vitreous was observed and an increase of visual acuity from 0.1 to 0.5. After 1-month, visual acuity increased to 0.8 with correction, the vitreous hemorrhage partially dissolved, and in 3 months the visual acuity was 1.0. In the latent period (6 months), general condition was stable, visual acuity was 1.0, the fundus was visible, along the vascular arcades ischemic areas of the retina were limited to coagulated parts with pigmentation after laser coagulation of the retina.

On the Fig.1 we see an ophthalmoscopic image of the back part of the eye with a large hemorrhage situated in front of the retina (preretinal) in the macular area and the nasal side near the optic disk. The retina is not visible under the hemorrhage.

Here (Fig.2) we see an ophthalmoscopic image of the back part of the eye with a preretinal hemorrhage after laser hyaloidotomy. Partial evacuation of the preretinal blood into the vitreous is observed, along with horizontal level of the blood. Retina is partially visible near the lower vascular arcade

This ophthalmoscopic image (Fig.3) shows the state of the preretinal hemorrhage 30 minutes after laser hyaloidotomy. It is noted that most of the preretinal hemorrhage has transferred to the vitreous. The macular part of the retina is visible, which led to substantial elevation of central visual acuity. In the vitreous we see a floating blur (partial hemophthalmia).

On the Fig.4 we see the ophthalmoscopic image 2 hours after laser hyaloidotomy for preretinal hemorrhage. The absence of blood in front of the retina is noted, macular and nasal parts of the retina are cloudy during examination because of the blood evacuation into the vitreous (partial hemophthalmia).

DISCUSSION

Interferon-induced retinopathy is one of the rare complications in ophthalmology that can lead to significant decrease in ocular functions. This illness occurs in patients that receive systemic interferon therapy. Retinopathy is a pathologic state of the retina with clinical signs
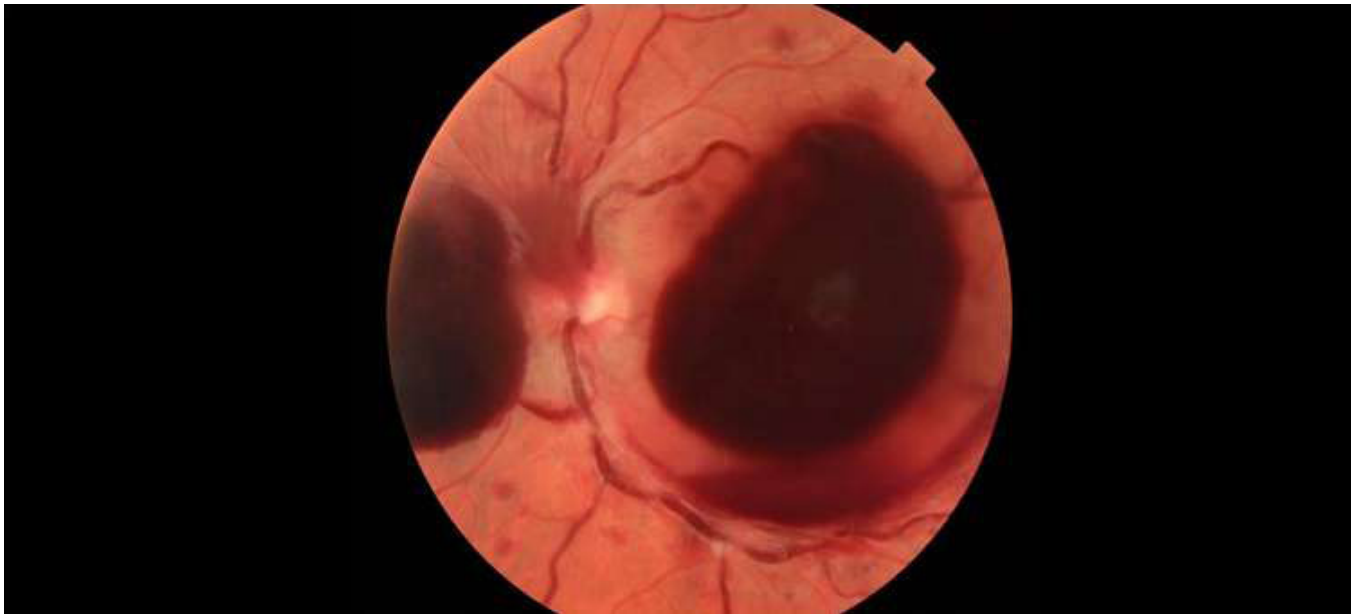
Fig.1. Preretinal hemorrhage of the patient at admission. Visual acuity at 0.07, no correction. Intraocular pressure 15 mmHg.
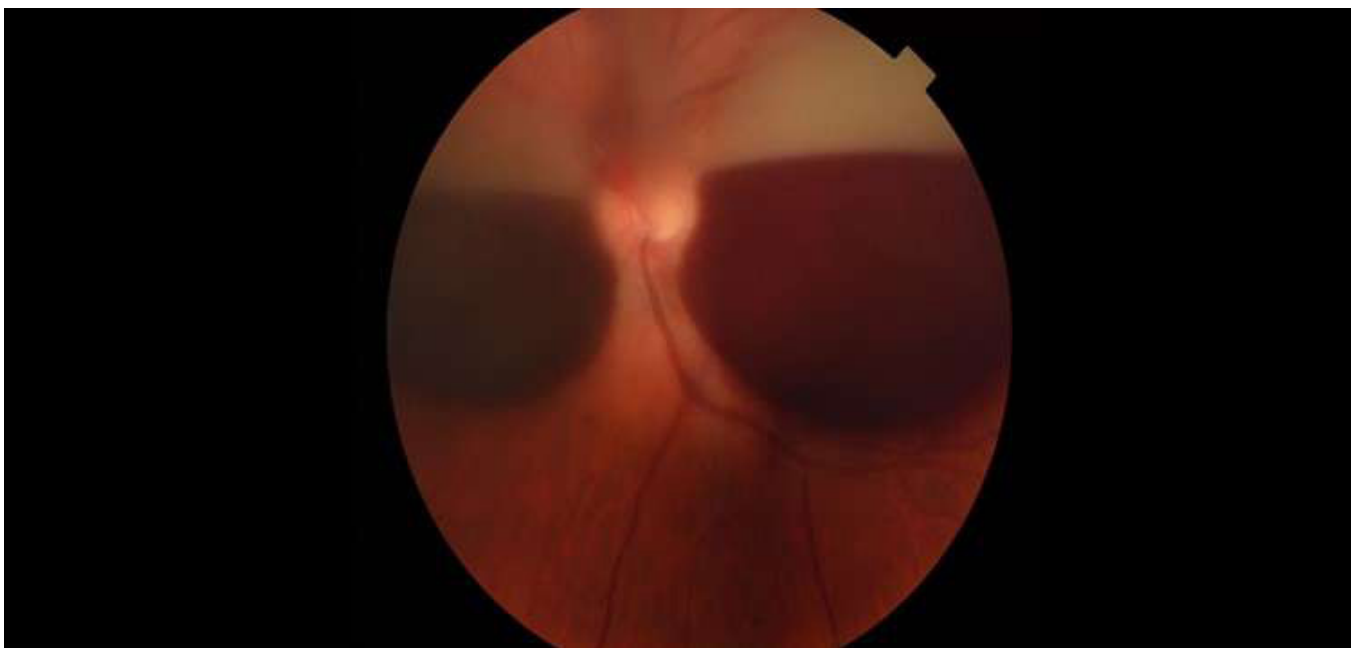
Fig.2. Patient's condition right after laser hyaloidotomy. Visual acuity at 0.1, no correction. Intraocular pressure at 17 mmHg.

of changes in the retinal vessels, exudative changes, intraretinal, subretinal and preretinal hemorrhages. In areas with lesions of the retina, dystrophic, apoptotic and atrophic changes may be seen that can lead to irreversible decrease of ocular functions. Recovery of ocular functions depends on how quickly retinal lesions lower of decrease. Preretinal hemorrhage, as one of the most severe complications of interferon-induced retinopathy, is a massive hemorrhage located between posterior hyaloid membrane of the sclera and the retina. If treatment is not effective or not timely, preretinal hemorrhage can lead to preretinal fibrosis and can lead to retinal traction, tear and detachment with irreversible changes loss of vision. The location of the preretinal hemorrhage in the macular region plays a specific role in central vision. Therefor, timely and adequate treatment of preretinal hemorrhages is important for anatomical and functional recovery of the eyeball [6].

One of the most advanced methods of treatment of preretinal hemorrhages is YAG-laser hyaloidotomy that allows to evacuate blood located in front of the retina, into the vitreum using laser dissection of the posterior hyaloid membrane of the vitreum. The success of the procedure depends on the timeframe of the hemor-
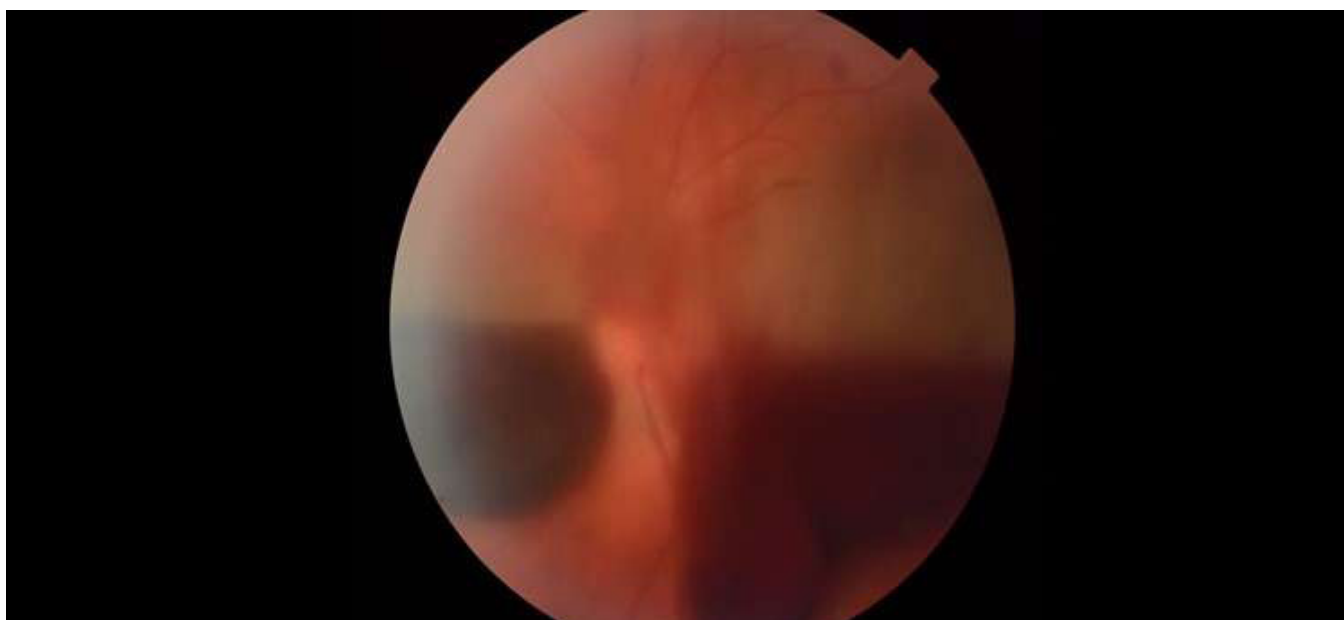
Fig.3. Patient's condition 30 minutes after laser hyaloidotomy. Partial evacuation of blood into the vitreous. Visual acuity at 0.5, no correction. Intraocular pressure at 15 mmHg.
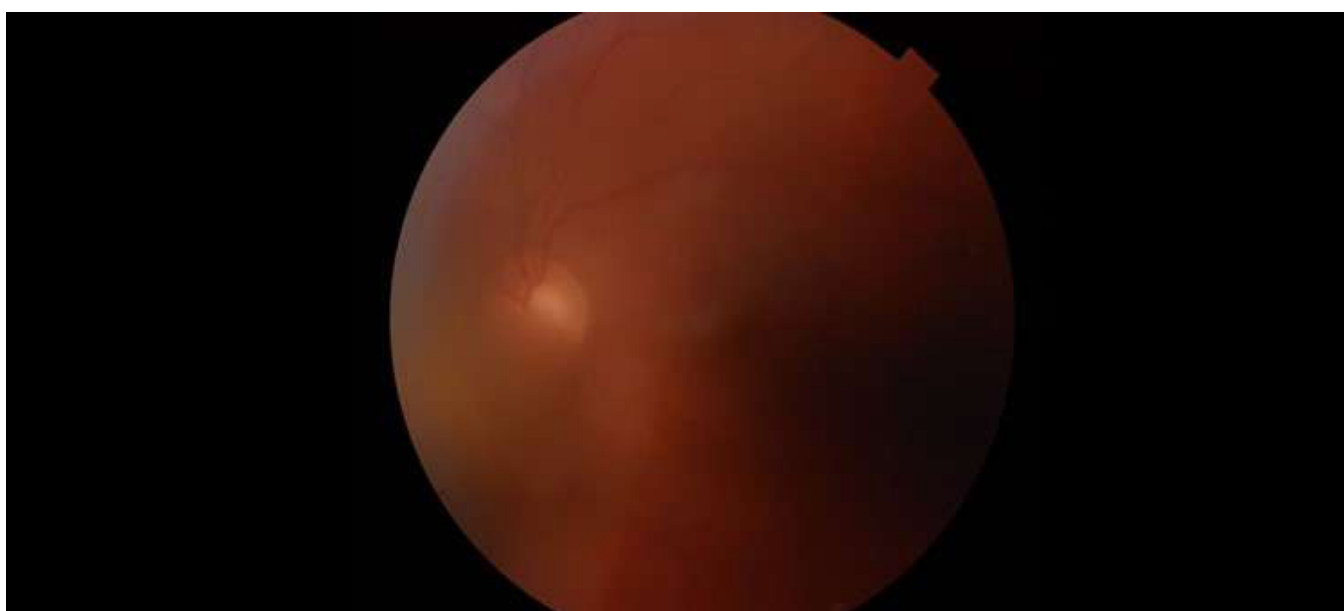
Fig.4. Patient's condition 30 minutes after laser hyaloidotomy. Partial hemophthalmias. Visual acuity at 0.7 with correction -0.5 D=0.8. Intraocular pressure at 20 mmHg.

rhage – laser hyaloidotomy is beneficial before hemorrhages progress to fibrosis. The blood, if evacuated to the vitreum, doesn't produce preretinal fibrosis and dissolves overtime with drug correction. Taking into account that intraretinal hemorrhages produce local ischemia of the retina, it is beneficial to perform laser coagulation of ischemic areas of the retina to prevent the progression of retinopathy and development of neovascularization. In this clinical case the patient underwent laser hyaloidotomy with evacuation of the blood from the preretinal space into the vitreum and, after the hemophthalmia was dissolved, laser coagu-

lation of the performed which led to ocular function recovery and stabilization of the pathologic process [8].

Since interferon-induced retinopathy can have complications, such as preretinal and intraretinal hemorrhages, that may lead to functional disruption of the retina and worsening of the visual functions up to blindness, it is essential to start ophthalmologic treatment on early stages of these complications. This is due to the fact that posterior laser hyaloidotomy has maximum effect at the early stages of preretinal hemorrhage, and laser coagulation of the retina stabilizes the process in the retina with minimization of lesions. Thus,

our conclusion is that an ophthalmologic examination should be recommended to patients with systemic interferon treatment of hepatitis C.

CONCLUSIONS

Modern diagnostic and treatment methods retinal lesions allow us to conduct tomography to diagnose the changes and develop optimal treatment plans of interferon-induced retinopathy. Laser hyaloidotomy was observed to be an effective method for increasing central visual acuity when preretinal hemorrhage is present in patient with interferon-induced retinopathy. Laser coagulation of the retina is an effective method for stabilizing changes in the retina. This case is an addition to the few described cases of interferon-induced retinopathy.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Valerii D. Beliayev
Uzhhorod National University
71 Mynaiska st, 88000 Uzhhorod, Ukraine
e-mail: valeriy.belyayev@uzhnu.edu.ua

ORCID AND CONTRIBUTIONSHIP
Bohdan M. Patskan: 0000-0002-4077-0726 Ⓐ Ⓑ Ⓓ Ⓕ
Valeriy D. Belyayev: 0009-0003-1641-9076 Ⓐ Ⓑ Ⓒ Ⓓ
Kateryna V. Bora: 0009-0009-6294-2523 Ⓐ Ⓑ Ⓓ Ⓔ
Nataliia V. Bondarenko: 0000-0001-6749-4349 Ⓐ Ⓑ Ⓒ Ⓓ Ⓔ

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

ORIGINAL ARTICLE

Effects of CDDO-EA in sepsis-induced acute lung injury: mouse model of endotoxaemia

Mohammed Hamzah Ibadi[1], Sahar Majeed[2], Fadhaa Abdulameer Ghafil[2], Najah R. Hadi[2]
[1]BABYLON HEALTH DIRECTORATE, BABYLON, IRAQ
[2]DEPARTMENT OF PHARMACOLOGY AND THERAPEUTIC, FACULTY OF MEDICINE, UNIVERSITY OF KUFA, KUFA, IRAQ

ABSTRACT
Aim: The aim of this research is to clarify the potential effect of CDDO-EA against experimentally sepsis induced lung injury in mice.
Materials and Methods: Mice have divided into four groups: Sham group CLP group, Vehicle-treatment group, CDDO-EA-treated group: mice in this group received CDDO-EA 2mg/kg intraperitoneally, 1hr before CLP, then the animals were sacrificed 24hr after CLP. After exsAngpuinations, tissue samples of lung were collected, followed by markers measurement including, TNF-α, IL-1β, VEGF, MPO, caspase11, Angp-1and Angp-2 by ELISA, gene expression of TIE2 and VE-cadherin by qRT-PCR, in addition to histopathological study.
Results: A significant elevation (p<0.05) in TNF-α, IL-1β, MPO, ANGP-2, VEGF, CASPASE 11 in CLP and vehicle groups when compared with sham group. CDDO-EA group showed significantly lower levels p<0.05, level of ANGP-1 was significantly lower p<0.05 in the CLP and vehicle groups as compared with the sham group. Quantitative real-time PCR demonstrated a significant decrement in mRNA expression of TIE2&ve-cadherin genes p<0.05 in sepsis & vehicle.
Conclusions: CDDO-EA has lung protective effects due to its anti-inflammatory and antiAngpiogenic activity, additionally, CDDO-EA showes a lung protective effect as they affect tissue mRNA expression of TIE2 and cadherin gene. Furthermore, CDDO-EA attenuate the histopathological changes that occur during polymicrobial sepsis thereby lung protection effect.

KEY WORDS: CLP, sepsis, CDDO-EA, VEGF, cadherin, ANGP/TIE axis, endotoxaemia

INTRODUCTION

Sepsis is defined as a life-threatening condition which occur due to uncontrolled response of body to an infectious agent, which is marked by concurrent unbalanced immune suppression and hyper inflammation [1]. This is a consequence of an infection with a mortality rate exceeding 25%. The lethality of hospital-acquired infection is more than community-acquired sepsis. The most common site of infection is the lung followed by abdominal infection, blood steam infection due to catheter and UTI [2]. Sepsis induce severe inflammatory responses and also increase permeability of vessels, causing acute pulmonary edema leading to ARDS. Recently, many researches focus on the effect of sepsis on ARDS and suggests a direction for future therapeutic intervention [3]. Although a large numbers of organisms can cause sepsis, vascular leak caused by endothelial dysfunction considered a popular route of injury that may lead to lethality in sepsis. Any disturbance to the ANGP /Tie2 pathway cause blood vessels lining cells activation and lead to sepsis. Therefore this axis is included in sepsis and describe its therapeutic and prognostic utility in life-threatening infections [4]. Specific- receptors with their ligands regulate the function and permeability of

vessels including the ANGPT-Tie receptors & vegf with its receptors. Tie receptors of type one &two represent the major receptors of the lining of blood vessels [5]. The binding of ANGPs to Tie2 in a stable vasculature promotes the formation of a Tie1/Tie2 heterodimer, causing Tie2 to be transported to cell-cell junctions. When there are an infections or inflammations, endothelial cells shed the Tie1 ectodomain and antagonism of ANGP2 to Tie2 [6]. During sepsis, the lung are the first deteriorated organ during ALI, that clinically represented as ARDS and is the major cause of lethality during sepsis [7]. CDDO-EA has anti-inflammatory, antioxidant effects, regulates the Nrf2/ARE pathway in animals, and has apoptotic inhibitory activity [8]. It is highly active and has good pharmacodynamic activity in mouse lung [9]. It supposed to decrease endothelial vascular leakage, increase Angp1 and decrease Angp2 [10]. Observed anti-inflammatory activity includes decreased levels of pro-inflammatory chemokines, cytokines, and decreased neutrophilia in the airways. CDDO potentially decrease the oxidative stress of lung, destruction of alveoli, apoptosis of alveolar cell and pulmonary hypertension and also it has protective effect against hyperoxia induced acute lung injury [10].

AIM

This study was undertaken to clarify the protective potential effects of CDDO-EA against experimentally sepsis induced lung injury in mice.

MATERIALS AND METHODS

The study was conducted in the Department of Pharmacology and Therapeutics at the College of Medicine/University of Kufa. The study was authorized by the Bioethics Committee of the University in Kufa, as well as its representation in the College of Medicine. Throughout the proceedings, the Committee's recommendations were followed.

DESIGN OF THE STUDY

Twenty four albino male Swiss mice, 8-12 weeks and weighing 25-30 g obtained from the College of Science, University of Kufa, housed 12:12 light:dark cycles with 25°C room temperature, 60-65% humidity, and free access for water & libitum. Mice were left in the animal's house for 14 days for adaptation and after animals have been allocated into 4 groups (n=6): Sham group: laparotomy without cecal ligation & puncture (CLP); CLP group: laparotomy with CLP; Vehicle group and CDDO-EA treated group: 2mg/kg IP, 1hr before CLP, then the animals were sacrificed 24 hr after [11].

EXPERIMENTAL PROCEDURE

Polymicrobial sepsis has been done in selected group of mice based on previous studies [12]. A double-puncture technique using 20-guage needles was used to induce polymicrobial sepsis, ketamine/xylazine solution is used to anesthetize mice [13]. A 1.5-cm midline incision (laparotomy) was performed and the cecum was exposed just under the ileocecal valve, it was ligated and punctured, a small quantity of stool was squeezed to ensure the patency of the puncture sites, then repositioned anatomically. A 5.0 surgical suture is used to stitch the abdomen. Mice were injected with 20 mL/kg N/S for resuscitation.

PREPARATION OF CDDO-EA

Powder was obtained from Macklin company, and prepared in diluted 10%DMSO and then was given in a dose of 2mg/kg intraperitoneally, 1hr before CLP [11].

COLLECTION OF A TISSUE SAMPLES

Twenty four hours after CLP, animal were sacrificed with anaesthesia by xylazine and ketamine, and then a specific operation was done to get lung tissue. Lung was divided into three parts: first part was kept at deep freeze for homogenization & ELISA study; second part was kept in RNA later solution at deep freeze until qRT-PCR analysis was done; while the other part was fixed with formalin until histopathological analysis [14].

TISSUE HOMOGENIZATION FOR TNF-A, IL-1B, MPO, ANGP-1, ANGP-2, VEGF, CASPASE 11 MEASUREMENT

Briefly, lung tissue samples was cut by using a razor blade, then we weight 0.1gm from each sample, we put samples in ice to prevent warming, then the tissue will be chopped by specific razor, in an icy environments. Then we will add 3ml of phosphate buffer (pH=7.2), then we will homogenize the ice by using a pestle until no more chunks were obvious. After that, we will transfer the resulted samples into Eppendorf tube and did 10000 rpm centrifugation for ten minutes, and then we collect the supernatant accurately. ELISA technique was used to quantify the level of of these markers in lung tissue according to the manufacturer's instructions [14, 15].

TISSUE PREPARATION FOR HISTOPATHOLOGY

The lung tissue samples were cut and fixed in 10% formalin for one week and then embedded in paraffin according to the standard procedure. Paraffin-embedded section (5μm) were stained with hematoxylin and eosin (H&E) for architecture evaluation under a light microscope (Olympus Japan). The degree of lung injury is graded according to scoring system (Mikawa method). Briefly, scoring started from zero to four in 4 categories: congestion of alveoli; hemorrhagic areas; neutrophilic infiltration and thickening of the alveolar wall. The calculation of total lung injury score was done by adding the individual scores for each category [16].

EXPRESSION OF TIE-2 AND VE-CADHERIN LUNG TISSUE BY QRT-PCR

mRNA expression of these two genes are measured by a quantitative PCR (qPCR) or quantitative real-time PCR also called real-time PCR, according to the manufacturer's instructions. It depend on amplification of a target sequence of DNA (TIE2 and VE-cadherin) with quantification of the amount of that DNA species in the reaction. It needs couple of primers which are complementary to the targeted sequence. DNA polymerase enzyme act to extend these complementary sequences (primers) [17, 18].

Table 1. Primers sequences in gene expression quantification of Tie2, VE-cadherin genes and housekeeping gene (HKG)

GENE	F (forward primer)	R (reverse primer)
Tie2	5'-GCCGCGGACTGACTACGAGC-3'	5'-GGAGGAGGGAGTCCGATAGACGC-3'
HKG	TGGCCTTCCGTGTTCCTAC	GAGTTGCTGTTGAAGTCGCA
VE-cad	GCAATGGCAGGCCCTA ACTTTC	CAGCAAACTCTCCTTGGA GCAC
HKG	TGGCCTTCCGTGTTCCTAC	GAGTTGCTGTTGAAGTCGCA

REAL-TIME QUANTITATIVE (RT-Q)PCR

Gene expression of Tie2 and VE-cadherin was measured by Rotor- Gene, Qiagen. The reaction mixture is prepared according to manufacture instructions. we used quantitative real-time PCR of three-step[19]:

45c	10min
94c	30 sec
94c	10sec
60c	20 sec 40-45 cycle
72c	30 sec

PRIMERS PREPARATION

The primers sequences that used in gene expression quantification of the Tie2 and VE-cadherin genes, as well as the housekeeping gene (HKG) are listed in table 1.

STATISTICAL ANALYSIS

SPSS system ver.26 was applicated for statical analysis. Student t-test & ANOVA with LSD post hoc test was used to investigate differences between groups. Statistical significance of the present data was defined as $p \leq 0.05$ [20].

RESULTS

EFFECT OF REGORAFENIB ON TISSUE LEVEL OF TNF-A, IL-1B, MPO, ANGP-2 , VEGF , CASPASE-11 ANGP-2

Our data showed a significant elevation ($p<0.05$) in TNF-α, IL-1β, MPO, ANGP-2, VEGF, Caspase-11 in CLP and Vehicle groups when compared with Sham group.
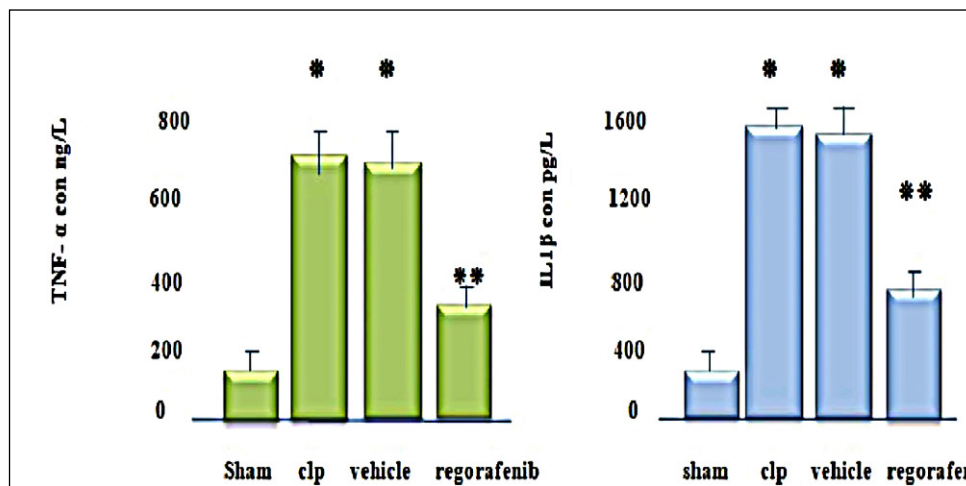
Fig. 1. Mean levels of TNF-α(ng/L) & IL1 β(pg/L) in the tissues of the experimental groups.
*p: significant difference p<0.05 compared with the shame group;
**p: significant difference p<0.05 compared with the CLP group.
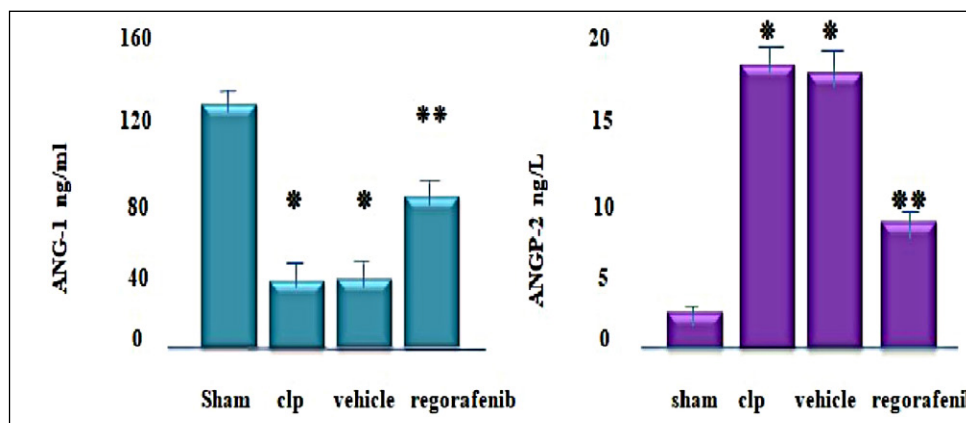
Fig. 2. Mean levels of Angp-1(ng/mL) & Angp-2 (ng/mL) in the tissues of the experimental groups.
*p: significant difference p<0.05 compared with the shame group;
**p: significant difference p<0.05 compared with the CLP group.
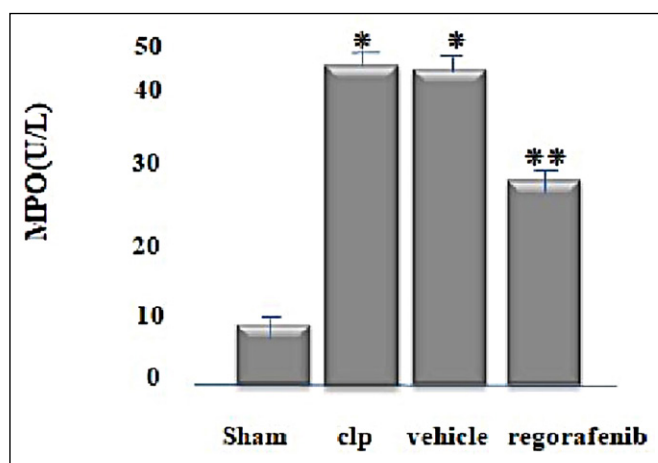
Fig. 4. Mean levels of MPO (U/L) in the tissues of the experimental groups. *p*: significant difference p<0.05 compared with the shame group; **p*: significant difference p<0.05 compared with the CLP group.

Additionally, CDDO-EA group showed significantly lower levels (p<0.05) of these markers if compared with the sepsis - CLP and vehicle groups. More interesting, that the level of ANGP-1 was significantly lower (p<0.05) in the CLP and Vehicle groups as compared with the Sham group. Additionally, CDDO-EA group showed significantly higher levels (p< 0.05) of ANGP-1 when compared with CLP and Vehicle groups (Fig. 1., Fig.2., Fig. 3., Fig. 4.).

EFFECT OF CDDO-EA ON MRNA EXPRESSION OF TIE2 AND VE-CADHERIN

Quantitative real-time PCR demonstrated a significant decrement in mRNA expression of TIE2 & VE-cadherin genes (p<0.05) in Sepsis & Vehicle (CLP) groups when compared with the Sham group. Furthermore, CDDO-EA group showed significantly higher levels of both genes (p<0.05) when compared with the CLP and Vehicle groups (Fig.5.).

EFFECT OF CDDO-EA ON LUNG

HISTOPATHOLOGY

Sham group showed normal lung tissue. Compared with the Sham group, CLP and Vehicle lung tissue had significant damage (P<0.05), with the presence of alveolar congestion, hemorrhage, neutrophils infiltration and thickening of the alveolar wall. These groups had highly severe histological grading (4/4) of normal lung tissue. CDDO-EA group had a significantly less (P<0.05) lung injury (Fig.7). The degree of change rAngped from no change to mild change, and CDDO-EA group had grade (1/4) (Fig.6).

DISCUSSION

Polymicrobial sepsis is a life-threatening condition which may lead to multiorgan dysfunction, that may resulted from abnormal response of the immune system to the invaded microorganisms. The most common characteristics of acute lung injury during sepsis is accumulation of a huge number of neutrophils in the lung and large amounts of inflammatory cytokines are produced [21]. Phagocytosis activation and infiltration of tissue lead to uncontrolled release IL-1β and TNF-α. This uncontrolled secretion cause secondary cytokines, lipid mediators [22]. Consequently, acute injury of lung can be attenuated by pharmacological agents that target one of these mentioned pathways and efforts are still actively being performed for choosing an effective drug candidates [23]. The tissue level of TNF-α and IL1B was significantly higher in the CLP and Vehicle groups as compared with the Sham group. This study agrees with a previous study by Alkharfy KM et al. [24]. The significant elevation of TNF-α & IL-1β level in CLP polymicrobial sepsis is attributed to innate immunity stimulation by PAMPs and DAMPs results in a severe and persistent inflammatory response recognized by over production of inflammatory cytokines such as IL-1b, TNF, additionally, this significant increase in IL-1β and may be explained by the activation of inflammatory pathway followed by TNF-α activation, which acts to
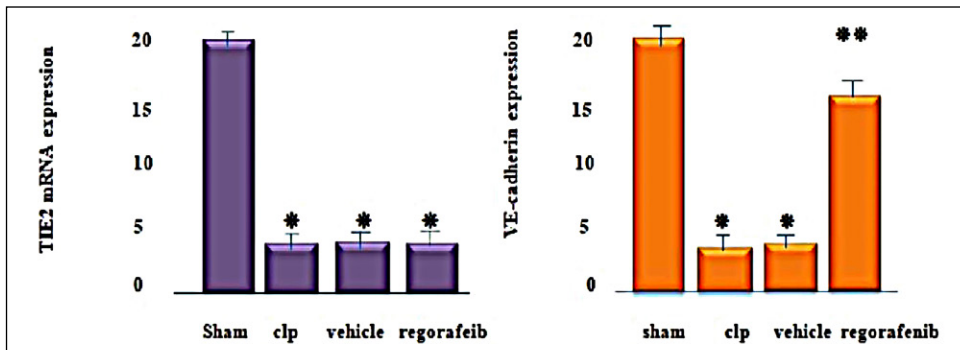
Fig. 5. Mean levels of TIE2 & VE-cadherin mRNA expression in the tissues of the experimental groups. *p*: significant difference p<0.05 compared with the shame group; **p*: significant difference p<0.05 compared with the CLP group.
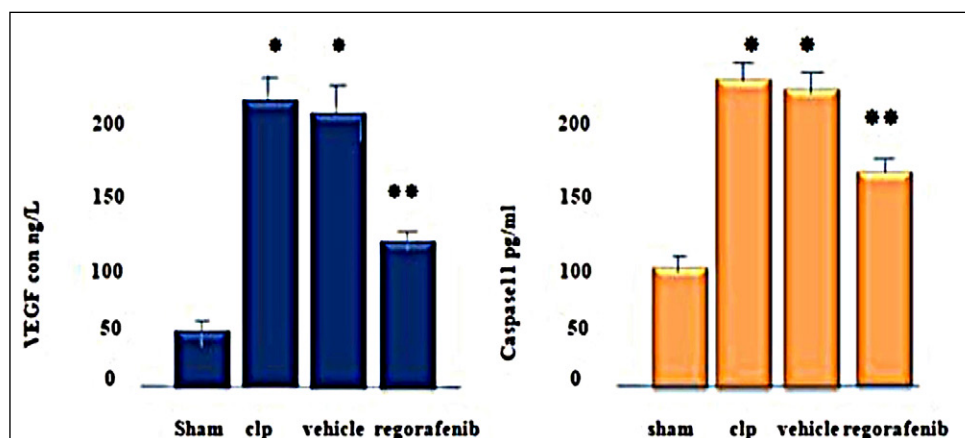
stimulate inflammatory cytokines production as IL-1β [25]. CDDO-EA pretreatment significantly reduced TNF-α and IL1B as compared to the Sepsis and Vehicle groups, and these findings agree with a previous study of Ling H et al. and LiAngp N et al. [26, 27]. This outcome of the lower tissue level of TNF-α &IL-1β in the CDDO-EA group may be attributed to inhibition of the NF-κB and activation of Nrf2 signaling proteins. As these two signaling pathways play an important role in regulating the net inflammatory response in some airway diseases,in addition to its antioxidant capacity in airway epethelial tissue [28]. Furthermore, The current study demonstrated that the tissue level of ANGP-1 was significantly lower in the CLP and Vehicle groups as compared with the Sham group. This study agrees with a previous study of Zhang et al. [29]. Moreover, our results are consistent with *Stensil et al*: they stated that CLP mice have significantly lower levels of Angp-1 compared to the Sham group. This finding may be related to the fact that polymicrobial sepsis leads to production of inflammatory cytokines and VEGF which cause gaps between endothelial cell junctions, loss of endothelial integrity and increase permeability and consequently results in the releasing ANGPT-2, the binding of Angp-2 to Tie2 disrupts protective Angp-1 signaling and promote endothelial inflammation. In sepsis, VEGF levels are associated with increased permeability of endothelium, severe illness and mortality, while Angp-1 act to stabilize the endothelialium and opposes the VEGF effect [30].

More interestingly, our study indicates that the tissue level of Angp-1 significantly raised in the CDDO- EA group, as compared with the CLP group. This finding is consistent with that mentioned by Crowley et al.: they found that CDDO- EA significantly increase Angp-1 level and decrease endothelial activation induced by malaria in mice model [31]. This increament in Angp-1 level due to Nrf-2 activation. Additionally, our study showed that the tissue level of Angp-2 was significantly higher in the CLP and Vehicle groups as compared with the Sham group. This study agrees with a previous study which demonstrated a significant higher tissue level of Angp-

2, twenty four hr after CLP as compared with control group in mice model. Angp-2 is responsible for vascular destabilization, lung leakage, and inflammation which lead to acute lung injury [32]. This increament in Angp-2 level may be related to release the stored form of Angp-2 which occur due to the intraction or binding of toll like receptors to the bacterial cell wall components or due to another mechanisms such as manipulation of NFκ-B and MAPK/activator protein-1 signaling pathway [6]. Furthermore, CDDO-EA act to increase Angp-1, decrease Angp-2 and decrease the vascular leakage in mice and it considered an activator of Nrf2. Nrf2 signaling is essential for preserving an equilibrated endothelial Angpiogenic response, because any disorders in Nrf2 signaling lead to a remarkable imperfect Angpiogenic processes. This finding may be related to CDDO-EA role in inhibiting inflammatory mediators release, VEGF and antioxidant effect which may decrease the pulmonary vascular leakage and attenuating acute lung injury due to sepsis. Notably, the current study demonstrated that tissue level of VEGF was significantly higher in the CLP & Vehicle groups as compared with the Sham group. This study agrees with a previous study which demonstrated a significant high level of VEGF in the CLP mice 24 hr after CLP, and they indicated that sepsis` mortality and VEGF were linked together. This significant increase in VEGF levels in polymicrobial CLP sepsis may be due to inflammatory mediators, and the cytokine storm that occurs during sepsis may increase VEGF levels. More interestingly, our study showed that the tissue level of VEGF significantly lowered in the CDDO- EA group, as compared with the CLP group.

To the best of our knowledge, this finding was the first that demonstrated the effect of CDDO-EA on pulmonary level of VEGF in CLP mice model of sepsis. This decreament may be related to CDDO-EA ability to inhibit NF-kB in early alveolar endothelial cells of lung, which may dysregulated Angpiogenic process. Furthermore, Petronelli et al. indicated that CDDO-EA halts NF-kB enter to nucleus and thereby the VEGF secretion. Our study showed that the lung tissue level of caspase-11 was significantly higher in the CLP & Vehicle groups as
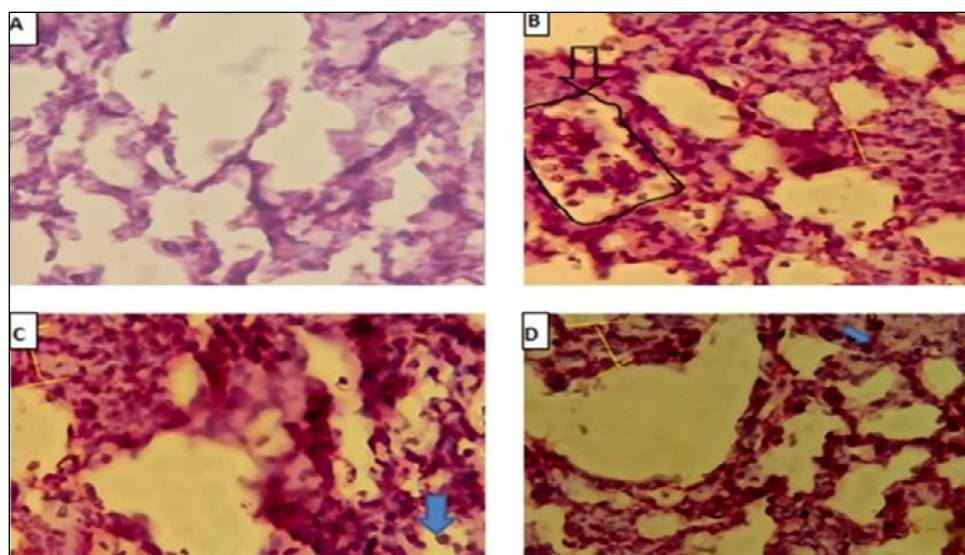
Fig. 6. Histopathological examination of lung tissue: the section stained with hematoxylin and eosin (x400). A: lung tissue section of mice in Sham group: showed normal histology with clear cells borders and structures, grade 0; B-C: lung tissue section of mice in CLP and Vehicle group: showed neutrophilic infiltration (blue arrow), alveolar wall thickening (yellow arrow) and focal areas of congestion (black arrow), grade 4; D: lung tissue section of mice in the Regorafenib group: showed neutrophilic infiltration (blue arrow) with focal areas of alveolar wall thickening (yellow arrow).
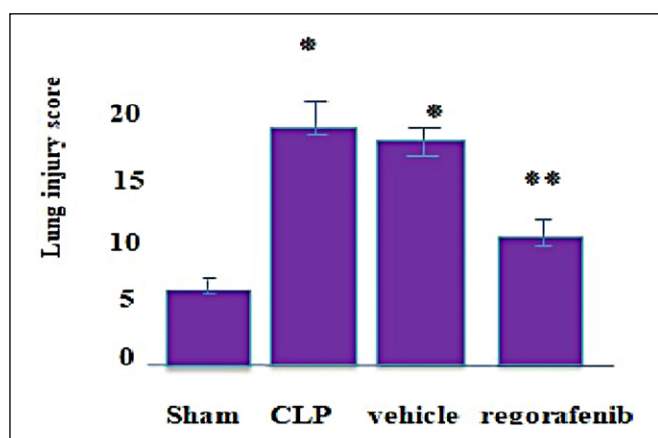
Fig. 7. Difference in mean lung injury rank scores. *p: significant difference p<0.05 compared with the Sham group; **p: significant difference p<0.05 compared with the CLP group.

compared with the Sham group. This study agrees with a previous study of Zhang et al., where they demonstrated that activated form of caspase-1 makes a pores in cell by cleaving gasdermin D leading to pyroptosis process, which is a type of cell death that trigger proinflammatory cytokine release as IL-1β which has detrimental effect on lung tissue and affect endothelial permeability which is a feature of acute lung injury. The significant elevation of caspase-11 level in clp polymicrobial sepsis because of bacterial microvesicles triggers caspase-11 leading to endothelial cell pyroptosis and disturbing its barrier causing pulmonary oedema, proinflammatory cytokines release and leukocyte recruittment. Additionally, during sepsis, complement system is activated and it considered an important contributor to the inflammatory response that happened, as a result of this, complement act to implify caspase-11 associated cell death. Furthermore, within the current study, regarding the CDDO-EA effect on the level of caspase-11, the level of lung caspase-11

significantly decreased in the CDDO-EA group as compared with CLP group. To the best of our knowledge, this study was the first, which demonstrated the effect of this agent on caspase-11 in CLP model of sepsis in mice. This finding may be related to CDDO-EA ability to increase NRF2. As mentioned previously by that overexpression of NRF2 in cellular type of Parkinson` disease model, can repress pyroptosis process. Notably, our study showed that the lung homogenate level of MPO was significantly higher in the CLP & Vehicle groups as compared with the Sham group. This study agrees with a previous study of Yu et al., where they found that MPO trigger proinflammatory responses and upregulate some cytokines and chemokines in clp model of sepsis in rat. This finding may be related to the circulating LPS may induce monocytes and neutrophils to produce MPO which has potent inflammatory and oxidative effect, also neutrophils are the first responder in sepsis, and when stimulated, it will release their components especially MPO. Importantly, regarding the CDDO-EA effect on the level of MPO, the level of lung MPO significantly decreased in the CDDO-EA group as compared with CLP group. To the best of our knowledge, this study was the first, which demonstrated the effect of this agent on MPO in CLP model of sepsis in mice this may be due to its VEGF inhibitory effect and Angp-1 agonist activity.

EFFECT OF ON MRNA EXPRESSION OF TIE2 AND VE-CADHERIN

The current study demonstrated that the mRNA expression of TIE2 & VE-cadherin was significantly lower in the CLP and Vehicle groups as compared with the Sham group. This study agrees with previous studies which demonstrated a significant lower level of TIE2 & VE-cadherin mRNA expression in the CLP mice as compared

with control group at 24 hr post CLP. Such findings may be related to several mechanisms. First, the effect of TNFa, which is a pivotal inflammatory mediator of sepsis, it acts to induce MMP14 to cleave TIE2. Second, decreased microvascular flow due to sepsis can downregulate GATA3 leading to inhibition of TIE2 transcription [20]. In regard to the effect of sepsis on VE-cadherin expression such finding may be related to several mechanisms. First, the effect of IL1B which is a pivotal inflammatory mediator of sepsis, it acts indirectly to decrease VE-cadherin transcription. Second, Angp-2 that triggered during sepsis act induce ve-cadherin phosphorylation, leading to decrease its expression. Within the current study, regarding the CDDO-EA effect on the level of TIE2 and VE-cadherin expression, their lung levels significantly increased in the CDDO-EA group as compared with CLP group. To the best of our knowledge, this study was the first, which demonstrated the effect of this agent on TIE2 and cadherin expression in CLP model of sepsis in mice. This result may be due to its antiinflammatory effect and Angp-2 inhibitory action. Additionally, Lee et al. stated that Angp-1 act to dephosphorylate VE-cadherin. As mentioned previously, we found that CDDO-EA by its positive effect on Angp-1 level it may protect the lung during sepsis and decreasing endothelial permeability by enhancing VE-cadherin level.

EFFECTS ON LUNG HISTOPATHOLOGY

CLP & Vehicle groups showed a significant histopathological change when compared with the Sham group, and CLP group demonstrated the development of contraction bands, inflammatory cell infiltration, necrosis, and oedema. These observations are consistent with that obtained by Aziz et al. [14]. Notably, in the current study, by comparison to the CLP, the CDDO-EA group significantly reduced lung tissue injury. The histopathological damage scores for the CDDO-EA group were usually nearly normal in which there was a mild degree of inflammation, neutrophil infiltration, necrosis and oedema. To the best of our knowledge, this study was the first, that demonstrating CDDO-EA protective effect on lung tissue of mice model of sepsis. This result may be attributed to its antiinflammatory effects.

CONCLUSIONS

CDDO-EA has lung protective effects possibly through their anti-inflammatory and antiAngpiogenic effects. Additionally, CDDO-EA show a lung protective effect as it affects tissue mRNA expression of TIE2 and cadherin gene. Furthermore, it attenuates the histopathological changes that occur during polymicrobial sepsis thereby lung protection effect.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Najah Rayish Hadi
Department of Pharmacology and Therapeutic,
Faculty of Medicine, University of Kufa,
299G+HPX, Kufa, Iraq
e-mail: drnajahhadi@yahoo.com

ORCID AND CONTRIBUTIONSHIP
Mohammed Hamzah Ibadi: 0000-0002-8492-0191 Ⓑ
Sahar Majeed: 0000-0002-7296-4998 Ⓒ
Fadhaa Abdulameer Ghafil: 0000-0002-1998-9459 Ⓓ Ⓔ
Najah Rayish Hadi: 0000-0002-8415-5311 Ⓐ Ⓔ Ⓕ

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

ORIGINAL ARTICLE   CONTENTS ↗

Results of the study of the attitude of women of reproductive age to the integrated gynecological care provision by family physicians

Nina G. Goida, Zhanna S. Oktysiuk
SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE

ABSTRACT

Aim: To study and analyze the attitude of women of reproductive age to the integrated gynecological care provision by family physicians, their readiness to receive some gynecological services from family physicians, as well as to analyze the level of women's support and readiness for the integrated provision of gynecological care depending on age and level of education.

Materials and Methods: For the survey, anonymous questionnaires containing questions on the attitude of women of reproductive age to the integrated provision of certain types of gynecological care by family physicians were developed. 181 women from the Kyiv region took part in the survey.

Results: Support of more than 80% of respondents regarding the integrated gynecological care provision by family physicians received the following questions: counseling on the prevention of sexually transmitted infections and human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) (92,3%); counseling on family planning and prevention of unwanted pregnancy (83,4%); counseling on the use of various methods of contraception (82,3%); examination and palpation of mammary glands (80,1%); referral of women to a higher level of obstetric and gynecological care (if necessary (86,2%).

Conclusions: The majority of respondents (67,4%) are ready or better ready than not ready to receive certain types of gynecological care services from family physicians. Almost the same percentage ratio (more than 60%) of women of each age group and all levels of education are ready or better ready than not ready to receive gynecological care services, which they supported, from family physicians.

KEY WORDS: female, reproduction, integrated health care systems, family practice

INTRODUCTION

Integrated care, as defined by the World Health Organization (WHO) (2002), is a concept of providing medical care that ensures the coordination of the actions of individual providers of medical services in order to achieve economic efficiency, improve quality, and increase the level of satisfaction of users and providers of medical care [1,2].

The new health policy of the WHO Regional Office for Europe «Health 2020» calls for a system-wide and society-wide approach to meet the challenges of the 21st century and create people-centered health care systems [2].

In the health care of many countries of the European Union and the United States of America, there is a search for organizational and economic mechanisms for integrating the work of individual medical services for ensuring continuity of treatment and achieving, on this basis, intra-system savings and higher clinical results of the industry as a whole [1,3-5].

More than a buzzword for the 21st century,

coordinated/integrated health care is a necessity [6,7]. Due to demographic changes and the rise of chronic diseases to the constant threat of infectious diseases, combined with modern technology, increasing patient expectations and the constant context of financial constraints, for new and innovative approaches to healthcare provision, should be given the highest priority, that ensure high quality and efficient services and provide them according to human needs [8-11].

The center of attention of integrated systems is the patient and the satisfaction of his needs in medical care [12-14]. Doctors in the system should be effectively integrated at all its levels and play a leadership role in the development, implementation and operation of comprehensive services from all aspects of treatment [5, 15-22]. Complexity of services means providing the patient with the entire continuum of necessary medical interventions (from primary to tertiary medical care), as well as cooperation between medical institutions and social care organizations [1, 23-25].
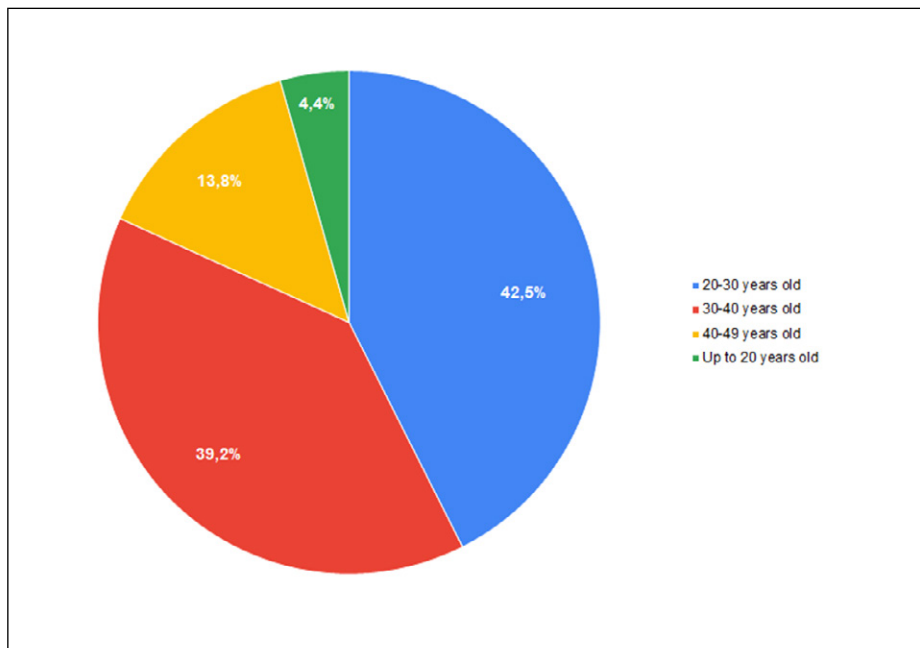
Fig. 1. Age of women of reproductive age, who took part in the survey.
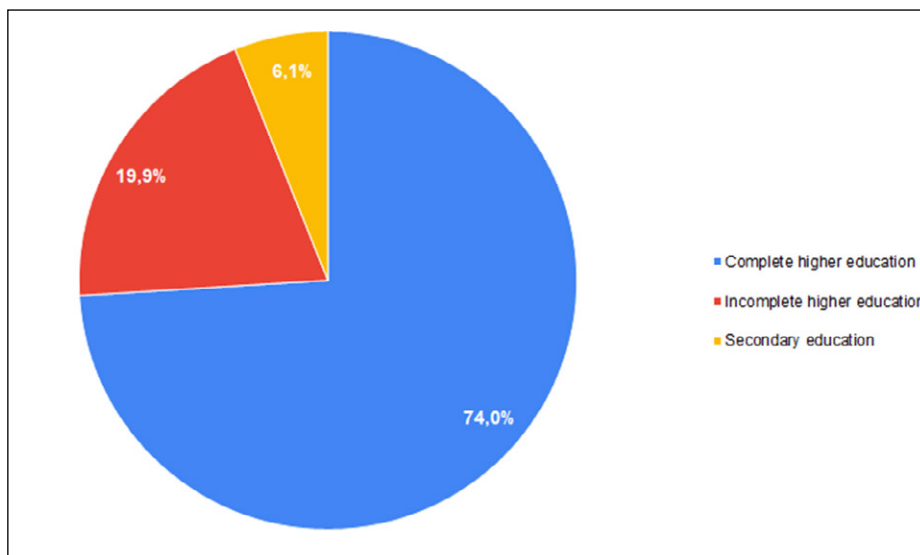
Fig. 2. Education level of women of reproductive age, who took part in the survey.

AIM

To study and analyze the attitude of women of reproductive age to the integrated gynecological care provision by family physicians, their readiness to receive some gynecological services from family physicians, as well as to analyze the level of women's support and readiness for the integrated provision of gynecological care depending on age and level of education.

MATERIALS AND METHODS

In 2023, a sociological survey of women of reproductive age was conducted to study the issues of integrated gynecological care provision by family physicians. For the survey, anonymous questionnaires, containing questions on the attitude of women of reproductive age to the integrated provision of certain types of gynecological care services by family physicians were developed. The conduct of the study was approved by Protocol № 1 dated 10.01.2022 of the commission on ethics of the Shupyk National Healthcare University of Ukraine.

Questionnaires with an anonymous survey were distributed to women living in Kyiv region, using a Google form. Local Internet groups of residents were used to distribute the questionnaires.

The obtained results were analyzed using Google Docs and Microsoft Excel 2010 software. Descriptive statistics methods were used for data analysis. For qualitative indicators, an analysis of their frequency characteristics was performed with the determination of the absolute number of observations and distribution in
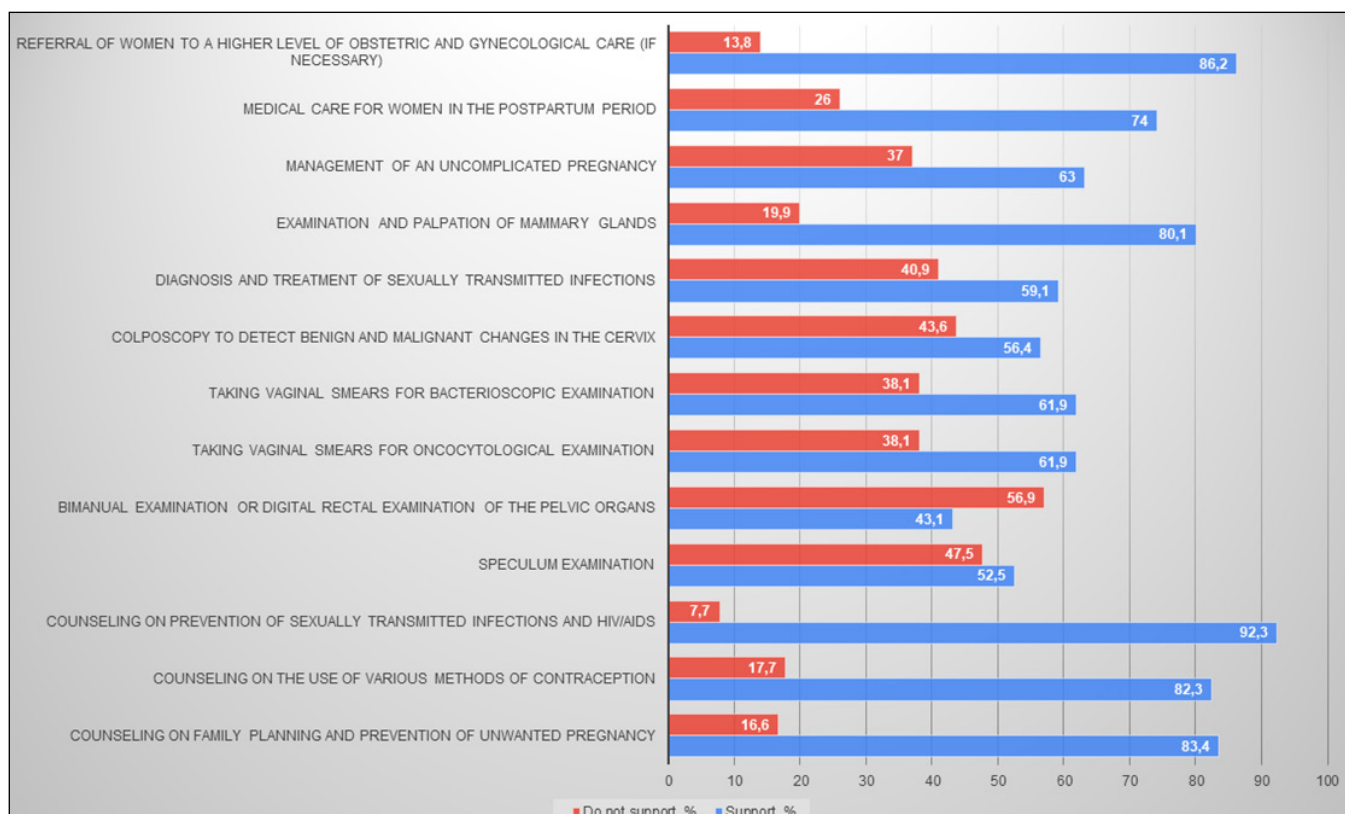
Fig. 3. Graphic representation of the general results of the survey of women of reproductive age, n=181.
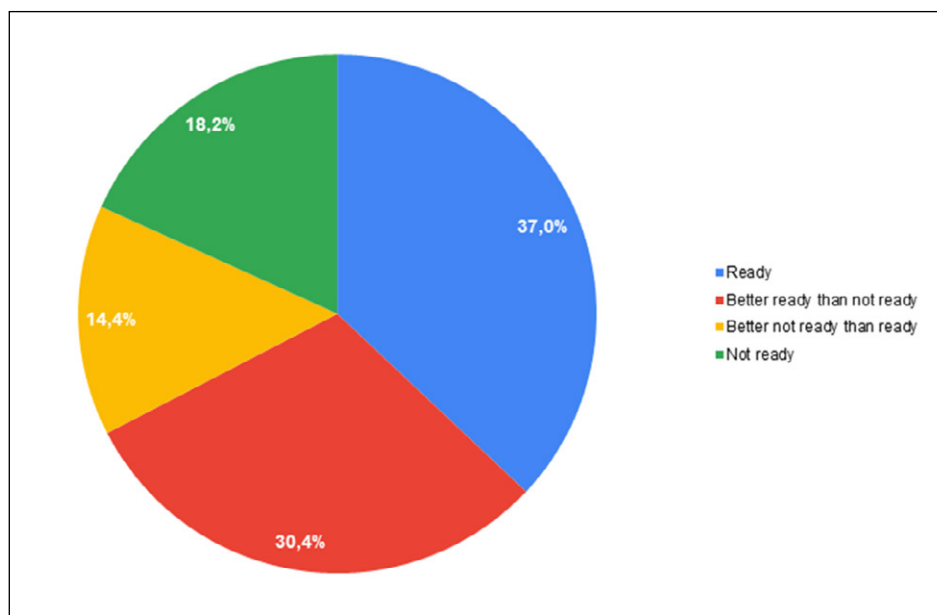
Fig. 4. General results of readiness of women of reproductive age to receive gynecological services, provided by family physicians, n=181.

percentage (%). Comparative analysis of the statistical significance of the difference between the studied groups when comparing frequency characteristics was carried out using the Pearson's χ2 test. In some cases, Fisher's exact test was used to compare parameters that were rarely found in the studied groups (a small number of observations in subgroups - 5 or less) to assess the significance of the intergroup difference according to frequency distributions.

For all calculated statistical estimates, their statistical significance was checked at a significance level of at least 95,0% (p<0.05).

181 women took part in the survey. 77 (42,5%) of them were 20-30 years old, 71 (39,2%) were 30-40 years old, 25 (13,8%) were 40-49 years old, and 8 (4,4%) were under 20 years old (Fig. 1); 134 (74%) of women have a complete higher education, 36 (19,9%) have an incomplete higher education, 11 (6,1%) have secondary education (Fig. 2).

Table 1. General results of the survey of women of reproductive age, n=181

Gynecological services	Support, abs (%)	Do not support, abs (%)	p
Counseling on family planning and prevention of unwanted pregnancy	151 (83,4)	30 (16,6)	<0,05*
Counseling on the use of various methods of contraception	149 (82,3)	32 (17,7)	<0,05*
Counseling on prevention of sexually transmitted infections and HIV/AIDS	167 (92,3)	14 (7,7)	<0,05*
Examination of the cervix in mirrors	95 (52,5)	86 (47,5)	0,501
Bimanual examination or digital rectal examination of the pelvic organs	78 (43,1)	103 (56,9)	0,066
Taking vaginal smears for oncocytological examination	112 (61,9)	69 (38,1)	0,0018*
Taking vaginal smears for bacterioscopic examination	112 (61,9)	69 (38,1)	0,0018*
Colposcopy to detect benign and malignant changes in the cervix	102 (56,4)	79 (43,6)	0,0876
Diagnosis and treatment of sexually transmitted infections	107 (59,1)	74 (40,9)	0,0160*
Examination and palpation of mammary glands	145 (80,1)	36 (19,9)	<0,05*
Management of an uncomplicated pregnancy	114 (63,0)	67 (37,0)	<0,05*
Medical care for women in the postpartum period	134 (74,0)	47 (26,0)	<0,05*
Referral of women to a higher level of obstetric and gynecological care (if necessary)	156 (86,2)	25 (13,8)	<0,05*

* - statistical significance is confirmed by the χ^2 test.

Table 2. Level of support of women of reproductive age depending on the age, n=181

Questions	Up to 20 years old, abs (%) n=8	20-30 years old, abs (%) n=77	30-40 years old, abs (%) n=71	40-49 years old, abs (%) n=25
Counseling on family planning and prevention of unwanted pregnancy	7 (87,5)	69 (89,6)	57 (80,3)	18 (72,0)
Counseling on the use of various methods of contraception	7 (87,5)	68 (88,3)	56 (78,9)	18 (72,0)
Counseling on prevention of sexually transmitted infections and HIV/AIDS	8 (100,0)	75 (97,4)	62 (87,3)	22 (88.0)
Speculum examination	7 (87,5)	49 (63,6)	25 (35,2)	14 (56,0)
Bimanual examination or digital rectal examination of the pelvic organs	6 (75,0)	40 (51,9)	19 (26,8)	13 (52,0)
Taking vaginal smears for oncocytological examination	7 (87,5)	56 (72,7)	36 (50,7)	13 (52,0)
Taking vaginal smears for bacterioscopic examination	7 (87,5)	56 (72,7)	36 (50,7)	13 (52,0)
Colposcopy to detect benign and malignant changes in the cervix	7 (87,5)	53 (68,8)	29 (40,8)	13 (52,0)
Diagnosis and treatment of sexually transmitted infections	7 (87,5)	55 (71,4)	31 (43,7)	14 (56,0)
Examination and palpation of mammary glands	7 (87,5)	69 (89,6)	52 (73,2)	17 (68,0)
Management of an uncomplicated pregnancy	7 (87,5)	56 (72,7)	35 (49,3)	16 (64,0)
Medical supervision of women in the postpartum period	7 (87,5)	61 (79,2)	49 (69,0)	17 (68,0)
Referral of women to a higher level of obstetric and gynecological care (if necessary)	8 (100,0)	71 (92,2)	56 (78,9)	21 (84,0)

The attitude of women to the integrated gynecological care provision by family physicians was studied on the following issues: family planning counseling and prevention of unwanted pregnancy; counseling on the use of various methods of contraception; counseling on prevention of sexually transmitted infections and human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS); examination of the cervix in mirrors; bimanual examination or digital rectal examination of the pelvic organs; taking vaginal smears for oncocytological examination; taking vaginal smears for bacterioscopic examination; colposcopy to detect benign and malignant changes in the cervix; diagnosis and treatment of sexually transmitted infections (STIs); examination and palpation of mammary glands; managing an uncomplicated pregnancy; medical care of women in the postpartum period; referral of women to a higher level of obstetric and gynecological care (if necessary).

Table 3. Level of support of women of reproductive age depending on the education level, n=181

| Questions	Complete higher education, abs (%) n= 134	Incomplete higher education, abs (%) n=36	Secondary education, abs (%) n=11
Counseling on family planning and prevention of unwanted pregnancy	106 (79,1)	34 (94,4)	11 (100,0)
Counseling on the use of various methods of contraception	105 (78,4)	33 (91,7)	11 (100,0)
Counseling on prevention of sexually transmitted infections and HIV/AIDS	120 (89,6)	36 (100,0)	11 (100,0)
Speculum examination	59 (44,0)	25 (69,4)	11 (100,0)
Bimanual examination or digital rectal examination of the pelvic organs	45 (33,6)	24 (66,7)	9 (81,8)
Taking vaginal smears for oncocytological examination	71 (52,9)	30 (83,3)	11 (100,0)
Taking vaginal smears for bacterioscopic examination	71 (52,9)	30 (83,3)	11 (100,0)
Colposcopy to detect benign and malignant changes in the cervix	63 (47,0)	28 (77,7)	11 (100,0)
Diagnosis and treatment of sexually transmitted infections	68 (50,7)	28 (77,7)	11 (100,0)
Examination and palpation of mammary glands	101 (75,4)	33 (91,7)	11 (100,0)
Management of an uncomplicated pregnancy	75 (55,9)	28 (77,7)	11 (100,0)
Medical supervision of women in the postpartum period	92 (68,7)	31 (86,1)	11 (100,0)
Referral of women to a higher level of obstetric and gynecological care (if necessary)	111 (82,8)	34 (94,4)	11 (100,0)

Table 4. Level of readiness of women of reproductive age to receive gynecological services, provided by family physicians depending on the age, n=181

Readiness	Up to 20 years old, abs (%) n=8	20-30 years old, abs (%) n=77	30-40 years old, abs (%) n=71	40-49 years old, abs (%) n=25	p
Ready	4 (50,0)	26 (33,8)	26 (36,6)	11 (44,0)	p=0,693
Better ready than not ready	1 (12,5)	27 (35,1)	19 (26,8)	8 (32,0)	p=0,480
Better not ready than ready	2 (25,0)	11 (14,3)	10 (14,1)	3 (12,0)	p=0,837
Not ready	1 (12,5)	13 (16,9)	16 (22,5)	3 (12,0)	p=0,615

RESULTS

The question for women was as follows: «Estimate your attitude towards the services that can be provided by family physicians.»

The survey results are presented in Table 1 and Fig. 3.

Most of the presented gynecological services received a high percentage of support from respondents. 12 out of 13 proposed questions received support of more than 52,5%.

More than 80% of 181 women received support from the following gynecological services: counseling on prevention of sexually transmitted infections and HIV/AIDS - 167 (92,3%) women; counseling on family planning and prevention of unwanted pregnancy - 151 (83,4%); counseling on the use of various methods of contraception - 149 (82,3%); examination and palpation of mammary glands - 145 (80,1%); referral of women to a higher level of obstetric and gynecological care (if necessary) - 156 (86,2%) women.

The lowest percentage of support received bimanual examination or digital rectal examination of the pelvic organs – 78 (43,1%) women.

Statistical significance is confirmed by the χ2 test for all questions (p<0,05), with the exception of the following: examination of the cervix in mirrors (p=0,501), bimanual examination or digital rectal examination of the pelvic organs (p=0,066), colposcopy to detect benign and malignant changes in the cervix (0,0876).

We analyzed the level of support for the above questions depending on the age and education level of responders. The results of the analysis are presented in Tables 2 and 3.

12 of the 13 above-mentioned questions received the support of 87,5% and more among women under 20 years old; 10 of the 13 above-mentioned questions - of 71,4 % and more among women of 20-30 years old. 9 of the 13 above-mentioned questions got the support of 50,7% and more of women 30-40 years old; all 13 questions - of 52,0% and more of women 40-49 years old.

Therefore, more than 87,5% of respondents under 20 years old and more than 71,4% of respondents 20-30 years old, support the integrated provision of most of the offered gynecological care services by family physicians.

Table 5. Level of readiness of women of reproductive age to receive gynecological services, provided by family physicians depending on the educational level, n=181

Readiness	Complete higher education, abs (%) n= 134	Incomplete higher education, abs (%) n=36	Secondary education, abs (%) n=11	p
Ready	44 (32,8)*	15 (41,7)	8 (72,7)	0,026*
Better ready than not ready	41 (30,6)	11 (30,6)	3 (27,3)	0,974
Better not ready than ready	21 (15,7)	5 (13,9)	0 (0,0)	0,361
Not ready	28 (20,9)	5 (13,9)	0 (0,0)	0,170

* - statistical significance is confirmed by the χ2 test with with Yeats' correction.

12 of the 13 above-mentioned questions got the support of 100% of women with a secondary education; 11 of the 13 questions - of 77,7 % and more among women with incomplete higher education; 10 of the 13 questions - of 50,7% and more among women with complete higher education.

To the question: "Are you ready to receive the above-mentioned types of services, which you have supported, from family physicians?" 122 (67,4%) of the interviewed women answered positively, 67 (37,0%) of them are ready, 55 (30,4%) are better ready than not ready (Fig. 4).

Results of the analysis of level of readiness depending on the age and education level of women are presented in Tables 4 and 5.

More than 60% of women of every age group are ready or better ready than not ready to receive the above types of services that they supported (62,5% of women under 20 years, 68,9% - from 20 to 30 years old, 63,4% - from 30 to 40 years old, 76,0% - from 40 to 49 years old).

Comparative analysis of the statistical significance of the difference between the studied groups, depending on the age, was carried out using the χ2 test with Yeats' correction and Fisher's exact test. Statistical significance for groups, depending on the age, was not confirmed. Therefore, readiness to receive certain gynecological services which they have supported, does not depend on the age of the respondents.

100% of women with secondary education, 72,3% of women with incomplete higher education and 63,4% of them with higher education are ready or better ready than not ready to receive the above mentioned types of services that they supported.

Analyzing the above-mentioned table, statistical significance is confirmed only for answer "ready", depending on level of education (p=0,026). Therefore, readiness to receive gynecological services, which they have supported, does not depend on the educational level of the respondents, except for the value "ready".

DISCUSSION

According to results of the study, most of the presented gynecological services received a high percentage of support. More than 80% of 181 respondents received support from the following gynecological services: counseling on prevention of sexually transmitted infections and HIV/AIDS; counseling on family planning and prevention of unwanted pregnancy; counseling on the use of various methods of contraception; examination and palpation of mammary glands; referral of women to a higher level of obstetric and gynecological care (if necessary).

Almost the same percentage ratio of responders of each age group and all levels of education are ready to receive gynecological care services, which they supported, from family physicians.

The data of this study confirm and complement the data of other scientists regarding the need for the implementation of integrated provision of medical services, where the central figure is the patient and the satisfaction of his needs in the necessary medical care [6, 8-10].

This necessitates the addition of curricula and training programs for family doctors in terms of acquiring practical skills in providing gynecological care, as well as conducting certain targeted trainings, for the further possible widespread implementation of integrated provision of some gynecological care services for women of reproductive age.

CONCLUSIONS

The research data on the attitude of women of reproductive age to the integrated gynecological care provision by family physicians proved:

1. The majority of respondents (67,4%) are ready or better ready than not ready to receive certain types of gynecological care services from family physicians.
2. Women under the age of 30 showed the highest support for the integrated provision of individual gynecological care services by family physicians.
3. Almost the same percentage ratio (more than 60%) of women of each age group and all levels of education are ready or better ready than not ready to receive gynecological care services, which they supported, from family physicians.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Zhanna S. Oktysiuk
Shupyk National Healthcare University of Ukraine
9 Dorohozhytska St., 04112 Kyiv, Ukraine
e-mail: Zhannaoktysiuk@gmail.com

ORCID AND CONTRIBUTIONSHIP
Nina H. Goida: 0000-0001-6625-0078 Ⓐ Ⓔ Ⓕ
Zhanna S. Oktysiuk: 0000-0003-3604-9183 Ⓐ Ⓑ Ⓒ Ⓓ

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

ORIGINAL ARTICLE   CONTENTS ↗

Molecular docking, synthesis, characteristics and preliminary cytotoxic study of new coumarin-sulfonamide derivatives as histone deacetylase inhibitors

Ammar Abdul Aziz Alibeg, Mohammed Hassan Mohammed

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, COLLEGE OF PHARMACY, UNIVERSITY OF KUFA, KUFA, IRAQ

ABSTRACT

Aim: To evaluate the cytotoxic activity of newly synthesized a series of novel HDAC inhibitors comprising sulfonamide as zinc binding group and Coumarin as cap groups.

Materials and Methods: The utilization of sulfonamide as zinc binding group and Coumarin as cap groups known to possess antitumor activity in the designed of new histone deacetylase inhibitors and using the docking and MTT assay to evaluate the compounds.

Results: Four compounds have been synthesized and characterized successfully by ART-FTIR, NMR and ESI-Ms. The synthesized compound assessed for their cytotoxic activity against hepatoblastoma HepG2 (IC_{50}, I=0.094, II=0.040, III=0.032, IV=0.046, SAHA=0.141) and human colon adenocarcinoma MCF-7 (IC_{50}, I=0.135, II=0.050, III= 0.065, IV=0.059, SAHA=0.107). The binding mode to the active site of [HDAC6] were determined by docking study which give results that they might be good inhibitors for [HDAC6].

Conclusions: The synthesized compounds (I, II, III and IV) showed a comparable cytotoxic result with FDA approved drug (SAHA) toward HepG2 and MCF-7 cancer cell lines and their docking analysis provided a preliminary indication that they are viable [HDAC6] candidates.

KEY WORDS: HDAC, HDAC inhibitor, cytotoxic sulfonamide derivatives, coumarin derivatives

INTRODUCTION

Cancer and microbial resistance constitute the highest public health risks around the world. Their incidence rate was hugely increased worldwide since 1990 [1-3]. The development of drug resistance is a crucial threat to general public health and represents a challenge to a broad area of field, comprises control of infection, mechanism of resistance, gene virulence and designing of drugs [4]. The antimicrobial and antitumor pathogens considered the most common frequent cases of drug resistance [5]. Additionally, drug resistance is also one of the biggest challenges in cancer diagnosis research that results in the failure of tumor treatment, a recurrence of the illness, or even the patient's death. Drug resistance may exist at the time of diagnosis or may appear after the tumor has been treated. However, the development of new drugs continues to offer promise for preventing the tumor cell lines' resistance capacities [6]. The creation of novel materials with intriguing biological properties has recently been a top priority in the fight against drug resistance. Due to their distinctive pharmacological and biological properties,

sulfonamide molecules are among the most sought-after substances and are known as one type of "privileged medicinal scaffolds" [7]. The recent advancements of sulfonyl or sulfonamide-based compounds in a wide range of therapeutic applications, such as antiviral, antidiabetic, antimicrobial, antileishmanial, anti-inflammatory, anticonvulsant, antitubercular, antimalarial, carbonic anhydrase, and other medicinal agents, show tremendous medicinal behaviors in derivatives of these molecules, such as antitumor behavior [8]. Sulfa medicines, which are compounds with sulfonamide moieties, have also been recognized for their extraordinary uses in biology and medicine [9]. Through covalent chemical alterations of DNA and covalent post-translational modifications (PTMs) of histones, the epigenetic control plays a significant role in the regulation of gene expression [10]. Histone acetylation is one type of PTM that may be reversed, and it is controlled by enzymes called histone acetyltransferases (HATs) and histone deacetylases (HDACs). The lysine residues in the core histone tail are the target of both HATs and HDACs. Histone, which is positively charged and is identified
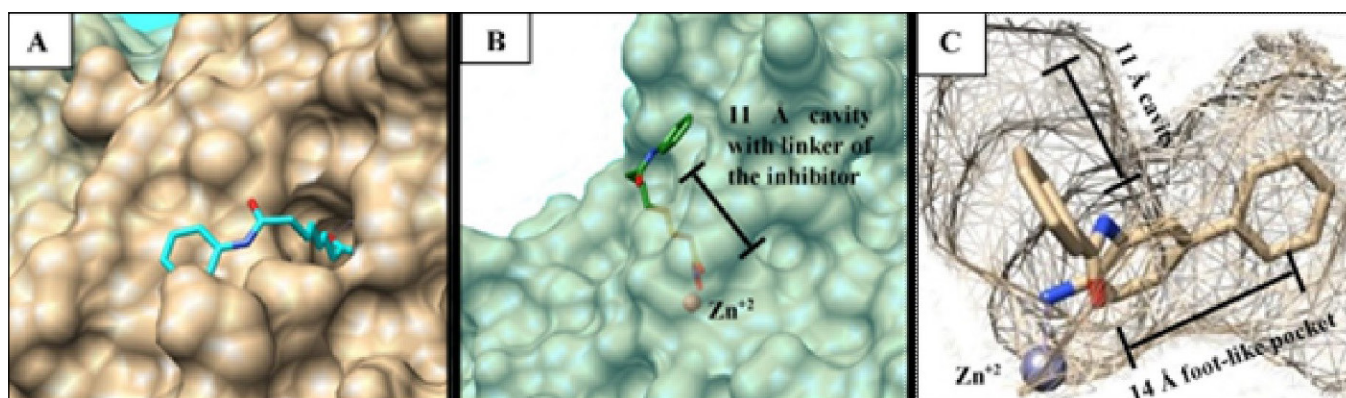
Fig.1. The binding site of HDAC2. (A): The linker of the inhibitor (cyan) is lying in the tunnel cavity's aperture, which is depicted. (B): In this side view of the binding site, 30% of the surface is transparent, making it possible to observe the inhibitor's linker (in forest green) and the whole length of the tunnel (11 Å). At the deepest end of the tunnel, zinc ions may be seen. (C): The inhibitor is visible on the binding site's mesh surface inside a 14 Å foot-long hole [15].

by the lysine residue's -amino group, binds strongly to negatively charged DNA. The lysine residues' charge at the N-position is neutralized by acetylation of histone, which transforms the compacted heterochromatin into the relaxed euchromatin [11]. Active DNA transcription results from the binding of RNA polymerase and gene regulatory proteins to this unfolded euchromatin, thus, through controlling histone acetylation, HDACs and HATs exert control over the epigenetic process. HDACs regulate the acetylation of a few non-histone proteins, including tubulin, p21, and p53, in addition to histones, it implies that these enzymes take part in other biological processes. According to studies, HDACs are overexpressed in a wide range of illnesses, including inflammatory, neurodegenerative, and different cancer types [12]. Thus, HDAC inhibitors can be used therapeutically. Based on their subcellular location, sequence homology, and catalytic activity, Mammalian HDACs may be divided into four groups. Class I, II, and IV HDACs, also referred to as conventional zinc-dependent HDACs, have the cofactor Zn2+ at their catalytic site. Comparatively, class III HDACs are NAD+-dependent and contain sirtuins 1–7 [13]. As a method for treating cancer, the zinc-dependent classes are thoroughly researched and put through inhibitory investigations [14]. HDAC2 is a well-known and traditional example due to its well-resolved crystallographic structure [10]. According to earlier research on the binding site, it is formed like an 11 Å internal tubular (tunnel) hollow. A zinc ion may be found at the bottom of this cavity, followed by a pocket that is 14 Å in size [15-20]. Figure 1 shows that histone lysine residues can be deacetylated as they fit inside the tunnel (Fig. 1) [14].

As a result, a zinc binding group should be included in the development of zinc-dependent HDAC inhibitors. ZBG chelates the zinc ion at the base of the catalytic domain by including hydroxamate, benzamide, sulfon-

amide, and thiol motifs., a hydrophobic cap to identify and interact with the outside surface, and a linker of 4-6 carbon atoms length to fit into the cavity [12]. The classical inhibitor is now the greatest example of them. Different forms of cancer have been treated with a number of HDAC inhibitors that have received FDA approval. These substances include FK22841 [21], Panobinostat, Belinostat [22], and Suberoylanilide Hydroxamic Acid [SAHA] [23] (Fig. 2).

Using of Coumarin molecule as capping groups which possess activity against a variety of cancer cell lines and possess cytotoxic activity beside to its activity as antiviral, antimalarial and antimicrobial activity [25].

In this research four novel substituted sulfonamide derivatives (compounds I, II, III and IV), were synthesized as inhibitors for zinc-dependent histone deacetylase enzymes (fig. 3). They have sulfonamide group as ZBG, 5-carbon atoms linker and coumarin as cap groups. The MCF7 human colon adenocarcinoma and the HepG2 hepatoblastoma were used in an in vitro cytotoxicity experiment to show whether or not these chemicals would have cytotoxic activity. In addition, docking research against [HDAC6] was performed on the produced molecules.

AIM

The aim of study to synthesis and evaluate the cytotoxic activity of new series of sulfonamide derivatives as HDAC inhibitors to treat cancer disease.

MATERIALS AND METHODS

CHEMICALS AND DRUGS
4-Amino benzene sulfonamide, monoethyl pimelate, 7-amino coumarin, coumarin-3-carboxylic acid and
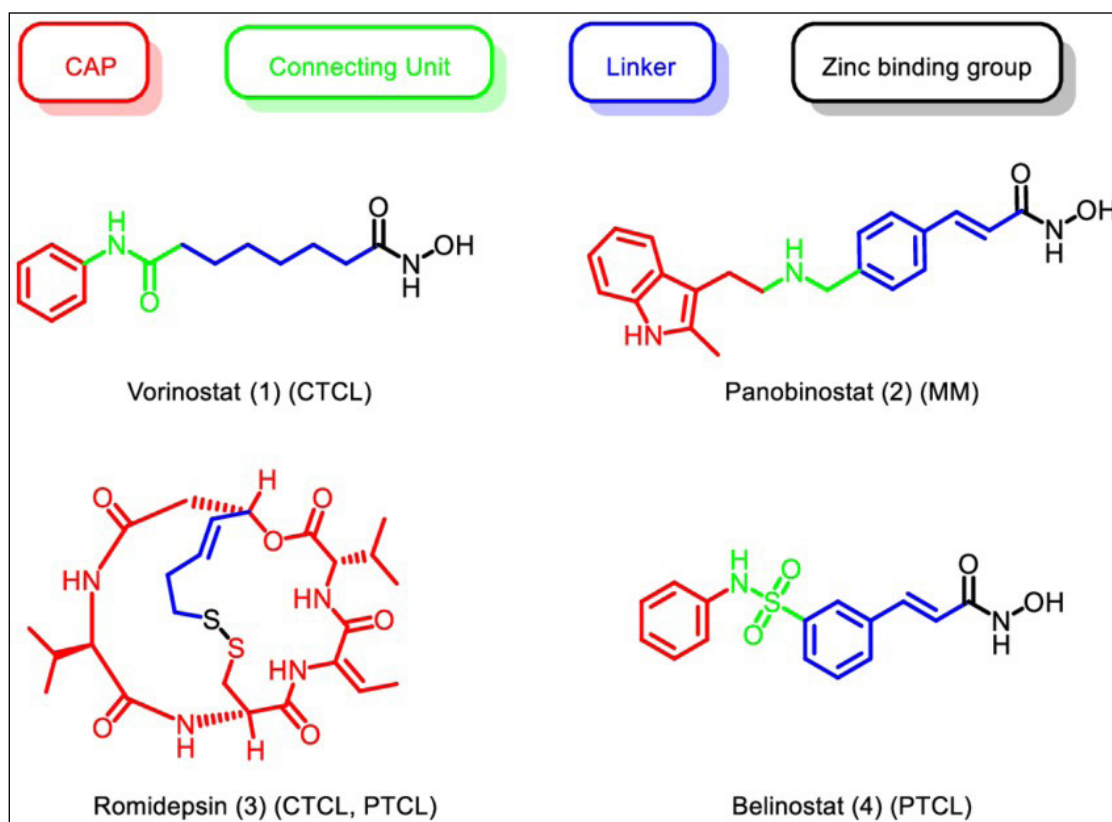
Fig. 2. Chemical compositions of HDAC inhibitors authorized by the FDA [24].
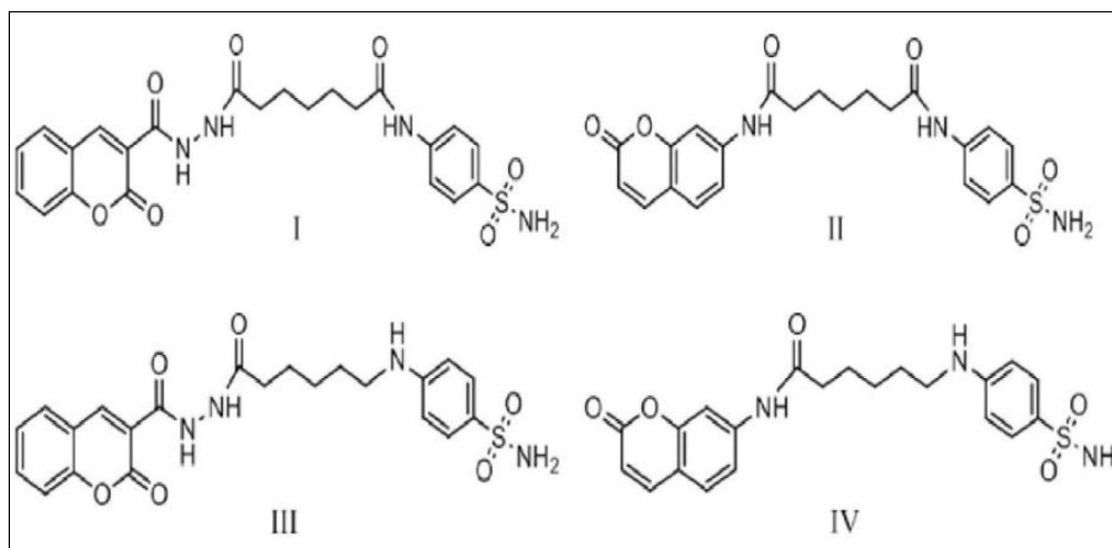
Fig.3. The designed compounds have a zinc binding group with sulfonamide functionality and coumarin as cap groups.

ethyl 6-bromohexanoate was purchased from BLD pharm, China, the source of the hydrazine hydrate 80% was Alpha Chemika in India. The solvent used was absolute and without further purification. By Stuart SMP30 Electronic Melting Point Apparatus melting points (m.p.) were measured. The progression and ending of reactions were monitoring and evaluated using UV light and thin-layer chromatography (TLC) on Merck silica gel 60F254, the solvent system that used as mobile phase was Ethyl acetate: Hexane (3:7)

[26] determination of functional groups in compounds was done by the FT-IR spectra which were recorded on FTIR spectrophotometer/ Shimadzu, Japan, supplied by Specac® Quest ATR (diamond)- UK (College of Pharmacy, University of Baghdad), 1H-NMR (1HNMR spectroscopy (400 MHz) (Bruker Avance II) , C^{13} NMR [^{13}C-NMR spectroscopy (100 MHZ) (Bruker Avance II)], Mass Spectroscopy [(Electrospray Ionization) compact™ ESI QTOF Mass Spectrometer Bruker Daltonics, Germany] In the BU-Ali Research center, Mashhad University

of Medical Science, Mashhad, Iran in Silico Molecular Docking Study was done by MOE software program. Cytotoxicity assay was performed at the BU-Ali Research center, Mashhad University of Medical Science, Mashhad, Iran. We bought HepG2 (human liver cancer) and MCF7 (human breast cancer) cell lines from the Iranian National Cell Bank (Pasteur Institute). Gibco's RPMI-1640 and DMEM: F12 media, each containing 10% FBS and antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin), were used to culture the cells. Using trypsin/EDTA (Gibco) and phosphate-buffered saline (PBS) solution, cells were passaged. and kept at 37 °C in humidified air containing 5% CO_2.

SYNTHESIS PROCEDURES OF COMPOUND IA [27]

The synthetic procedure was done by preparing of two-mixture solution. The first one prepared by dissolving carboxylic acid (monoethyl pimelate) (0.18 mL, 0.001 moles) in 20 ml of DMF then cooling the mixture to 0^0C, after that by adding EDCI (0.2 gm, 0.0012 moles), and then HoBt (0.2 gm, 0.0012 moles) was added after 15 min. The reaction combination was stirred for 1hr at 0^0C. Then a second solution was prepared by dissolving 4-aminobenzenesulfonamide (0.002 mol, 0.34 g) in 5 ml of DMF and then gradually (at 0°C) the second mixture was added to the first one. Then, the final mixture was stirred continuously at room temperature for 48 hrs. The termination of the reaction was checked by TLC using a solvent system (hexane: ethyl acetate (3:2 v/v), Rf value=0.75). The DMF was evaporated under reduced pressure on a rotary evaporator using co-evaporator solvent (toluene), the obtained residues was washed by 5% HCl, 5% sodium bicarbonate, and then recrystallization from methanol, giving pale off white solid. Yield: 72%; m.p. 189-191 C^0; FTIR (ú, cm^{-1}): 3244 (N-H, amine), 3142 (N-H, amine), 3107 (C-H, aromatic ring), 1722 (C=O, ester), 1688 (C=O, amide), 1595 (C=C, aromatic ring), 1168 (C-N, amide).

SYNTHESIS OF 2-OXO-2H-CHROMENE-3-CARBOHYDRAZIDE (IB) [28]

At room temperature, 1.9 g (10 mmol) of coumarin-3-carboxylic acid was dissolved in 60 ml of dichloromethane and stirred. Following that, 1.6 g (11.84 mmol) of HOBt (hydroxybenzotriazole) was added followed by the addition of 2.3 g (12 mmol) of EDCI (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide). The mixture was stirred for a whole night at room temperature before being progressively added to a solution of hydrazine (60 mmol ,0.64g) and cyclohexene (1 mL) in DCM 5mL while

the temperature was kept between 0-10°C. The reaction was completed at the end of the addition, and this was approved by TLC (hexane: ethyl acetate (3:2 v/v), Rf value=0.68). The organic layer was washed with 15 ml of 5% sodium carbonate solution to get rid of the extra acid and HOBt. The hydrazide was produced when the solvents were removed at decreased pressure and then recrystallized from methanol, giving green yellow solid. Yield (74%); m.p. 204-206 C^0; FTIR (ú, cm-1): 3321, 3278 (N-H, str, of hydrazide, NH_2), 3022(C-H, of aromatic), 1710 (C=O, of coumarin, 1681 (C=O, of amide).

SYNTHESIS OF ETHYL 6-((4-SULFAMOYLPHENYL) AMINO) HEXANOATE (IC) [29]

Sulfanilamide (0.01mole) was dissolved in 50ml of absolute ethanol containing (0.01mole) of pyridine. Ethyl 6-bromohexanoate 0.01mole was added slowly with stirring, and then the reaction mixture was reflux for 24 hours. The solution was concentrated to half and crushed ice was added. After 24 hours, the crude material was filtered off, washed with cold water and recrystallized from water, giving white crystals. Yield: 57%; m.p. 193-195 C^0; FTIR (ú, cm^{-1}): 3394,3361 (N-H, amine), 3269 (N-H, of 2nd amine), 3093 (C-H, aromatic ring), 2978 (C-H str. Vibration of CH_2), 1730 (C=O Str. vibration band of ester).

SYNTHESIS OF COMPOUNDS I-IV [27]

The final compounds were synthesized by dissolving 1mmol of compound Ia or Ic in 30 ml of solvent mixture (Ethanol: DMF), then adding 1mmol of compound Ib or 7-amino coumarin, then the reaction mixture was refluxed for 12 hours. The final solution was evaporated to a halfand crushed ice was added. After 24 hours, the resulting precipitate was removed using filtration, rinsed with cold water, and recrystallized from water, giving pale gray, off white, gray, gray white powder. Yield: (56%, 49%, 39%, 35%); m.p. 219-221, 235-237, 215-218, 226-228 C^0 respectively.

7-oxo-7-(2-(2-oxo-2H-chromene-3-carbonyl) hydrazinyl)-N-(4-sulfamoylphenyl) heptan- amide, Compound I

FT-IR (ATR; u, cm^{-1}): 3240, 3219 (NH_2 Str. Vib. of amine), 3154 (NH Str. Vib. of amide), 2974, 2941 (C-H Asymmetric, Symmetric str. Vib. Of CH_3), 2877 (C-H str. Vibration of CH_2), 1736 (C=O Str. Of coumarin), 1685 (C=O Str. vibration band of amide); ^{1}HNMR (400 MHz, DMSO-d6), ppm: 1.44 (m, 2H, CH_2), 1.62 (m, 4H, CH_2), 2.24 (tt, 4H, CH_2CO), 7.06 (s, 2H, SO_2NH_2), 7.34-7.81 (mm, 8H, H-5,6, and H-8 of coumarin and 4H of Ar sulfanilamide), 8.99

(s, 1H, H-4 of coumarin), 9.86 (m, 2H, of 2NHCO), 11.33 (d, 1H, NH-NHCO); ^{13}CNMR (100 MHz, DMSO-d6), ppm: 24.37, 24.39, 25.36, 27.36, 35.33, 37.39, 116.98, 118.38, 118.43, 118.99, 124.17, 124.75, 127.76, 129.11, 130.91, 130.93, 136.90, 145.55, 152.84, 160.22, 165.00, 172,13, 174.72; and the ESI-MS for C$_{23}$H$_{24}$N$_4$O$_7$S calculated 500.1; found 501.1[M+1]$^+$.

N1-(2-oxo-2H-chromen-7-yl)-N7-(4-sulfamoylphe-nyl) heptanediamide, Compound II

FT-IR (ATR; υ, cm^{-1}): 3408, 3226 (NH2 Str. Vib. of amine), 3112 (NH Str. Vib. of amide), 2927, (C-H Asymmetric, Symmetric str. Vib. Of CH3), 2868 (C-H str. Vibration of CH2), 1728 (C=O Str. Of coumarin), 1708,1678 (C=O Str. vibration band of amide); ^{1}HNMR (400 MHz, DMSO- d6), ppm: 1.49 (m, 2H, CH2), 1.70 (m, 4H, CH2), 2.33 (td, 4H, CH2CO), 6.32 (d, 1H, H-3 of coumarin), 7.05 (s, 2H, SO2NH2), 7.33 (d, 1H, H-6 of coumarin), 7.42-7.81 (m, 6H, H-4, H-5 of coumarin and 4H of Ar. Sulfanilamide), 7.99 (s, 1H, H-8 of coumarin), 9.93 (ss, 2H, of 2NHCO); ^{13}CNMR (100 MHz, DMSO-d6), ppm: 24.38, 24.41, 27.38, 27.91, 27.94, 104.36, 106.44, 113.43, 115.51, 118.95, 118.98, 127.73, 127.82, 129.79, 136.93, 140.61, 141.85, 144.75, 155.27, 161.33, 172.03, 172.12; and the ESI-MS for C$_{22}$H$_{23}$N$_3$O$_6$S calculated 457.5; found 458.1[M+1]$^+$.

4-((6-oxo-6-(2-(2-oxo-2H-chromene-3-carbonyl) hydrazinyl) hexyl) amino) benzenesulfon- amide, Compound III

FT-IR (ATR; υ, cm^{-1}): 3376, 3296 (NH2 Str. Vib. of amine), 3219 (NH Str. Vib. of amide), 2974, 2941 (C-H Asymmetric, Symmetric str. Vib. Of CH3), 2877 (C-H str. Vibration of CH2), 1721 (C=O Str. Of coumarin), 1685,1637 (C=O Str. vibration band of amide); ^{1}HNMR (400 MHz, DMSO-d6), ppm: 1.44 (m, 2H, CH2), 1.69 (m, 4H, CH2), 2.28 (t, 2H, CH2CO), 3.14 (m, 2H, CH2NH), 6.31 (t, 1H, NH), 6.41 (s, 2H, SO2NH2), 6.68 (dd, 2H of Ar. Sulfanilamide), 7.08 (m, 3H, H-5,6 and 7 of coumarin), 7.11 (d, 1H, H-8 of coumarin), 7.44 (dd, 2H of Ar. Sulfanilamide), 8.01 (s, 1H, H-4 of coumarin), 9.87 (d, 1H, of NHCO), 10.32 (d,1H, of NHNHCO); ^{13}CNMR (100 MHz, DMSO-d6), ppm : 24.27, 25.94, 28.69, 33.29, 43.25, 111.85, 111.86, 117.14, 118.28, 124.78, 124.79, 127.97, 129.15, 130.96, 135.50, 152.74, 153.12, 160.22, 165.00, 174.71; and the ESI-MS for C$_{22}$H$_{24}$N$_4$O$_6$S calculated 472.1; found 473.1[M+1]$^+$.

N-(2-oxo-2H-chromen-7-yl)-6-((4-sulfamoylphe-nyl) amino) hexanamide, Compound IV

FT-IR (ATR; υ, cm^{-1}): 3387, 3311 (NH2 Str. Vib. of amine), 3241 (NH Str. Vib. of amide), 2922 (C-H Asymmetric, Symmetric str. Vib. Of CH3), 2850 (C-H str. Vibration of CH2), 1720 (C=O Str. of coumarin), 1688 (C=O Str. vibration band of amide); ^{1}HNMR (400 MHz, DMSO-d6), ppm: 1.48 (m, 2H, CH2), 1.55 (m, 4H, CH2), 2.31 (t, 2H, CH2CO), 3.16 (m, 2H, CH2NH), 6.32 (d, 1H, H-3 of coumarin), 6.43 (t,1H of NH), 6.81 (s, 2H, SO2NH2),

7.11 (dd, 2H of Ar. Sulfanilamide), 7.40 (d, 1H, H-6 0f coumarin), 7.63 (m, 3H, H-5 of coumarin and 2H of Ar. Sulfanilamide), 7.82 (m, 2H, H-4 and H-8 of coumarin), 9.82 (s, 1H, of NHCO); ^{13}CNMR (100 MHz, DMSO-d6), ppm: 24.24, 25.89, 28.69, 37.87, 43.29, 106.49, 111.46, 111.47, 113.61, 115.44, 117.12, 120.58, 127.72, 127.73, 135.83, 140.61, 144.71, 153.15, 155.27, 161.33, 170.07; and the ESI-MS for C$_{21}$H$_{23}$N$_3$O$_5$S calculated 429.14; found 430.1[M+1]$^+$.

IN VITRO CYTOTOXICITY STUDY

MAINTENANCE OF CELL CULTURES

The Iranian National Cell Bank (Pasteur Institute) provided MCF7, a human breast cancer cell line, and HepG2, a human liver cancer cell line. Gibco's RPMI-1640 and DMEM: F12 media, each containing 10% FBS and antibiotics (100 U/ml penicillin and 100 μg /ml streptomycin), were used to culture the cells. Cells were passaged in phosphate-buffered saline (PBS) solution and trypsin/ EDTA (Gibco) solution while being maintained at 37 °C in humidified air containing 5% CO2 [30].

CYTOTOXICITY ASSAY

Cell growth and viability were assessed using the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide] (Sigma-Aldrich) assay. Using a monolayer culture method, the cells were collected, adjusted to a density of 1.4*10^4 cells/well, and seeded into 96-well plates with 200 μl of fresh media per well. The assay was conducted for 24 hours. After the cells had formed a monolayer, they were treated for 24 hours at 37 °C in 5% CO2 with 600-7.4 μg/ml of the produced compounds. 24 hours after the treatment ended, the supernatant was removed from the mono layer culture and then adding 200 μl/well of MTT solution [0.5 mg/ml in phosphate-buffered saline (PBS)], then the plate was incubated for 4 hours at (37) °C. MTT solution was created by removing the cell supernatant and adding 100 μl of dimethyl sulfoxide to each well. Crystals were totally dissolved in cells after being shaken at 37°C for an extended period of time. We determined the cell viability using an ELISA reader and a 570 nm absorbance measurement. The concentration of the compounds that induced 50% of cell death (IC50) was derived from the appropriate dose-response curves [31].

FLUORESCENT STAINING

Fluorescent staining study is done by using ethidium bromide (EB)/ acridine orange (AO) (Sigma- Aldrich).
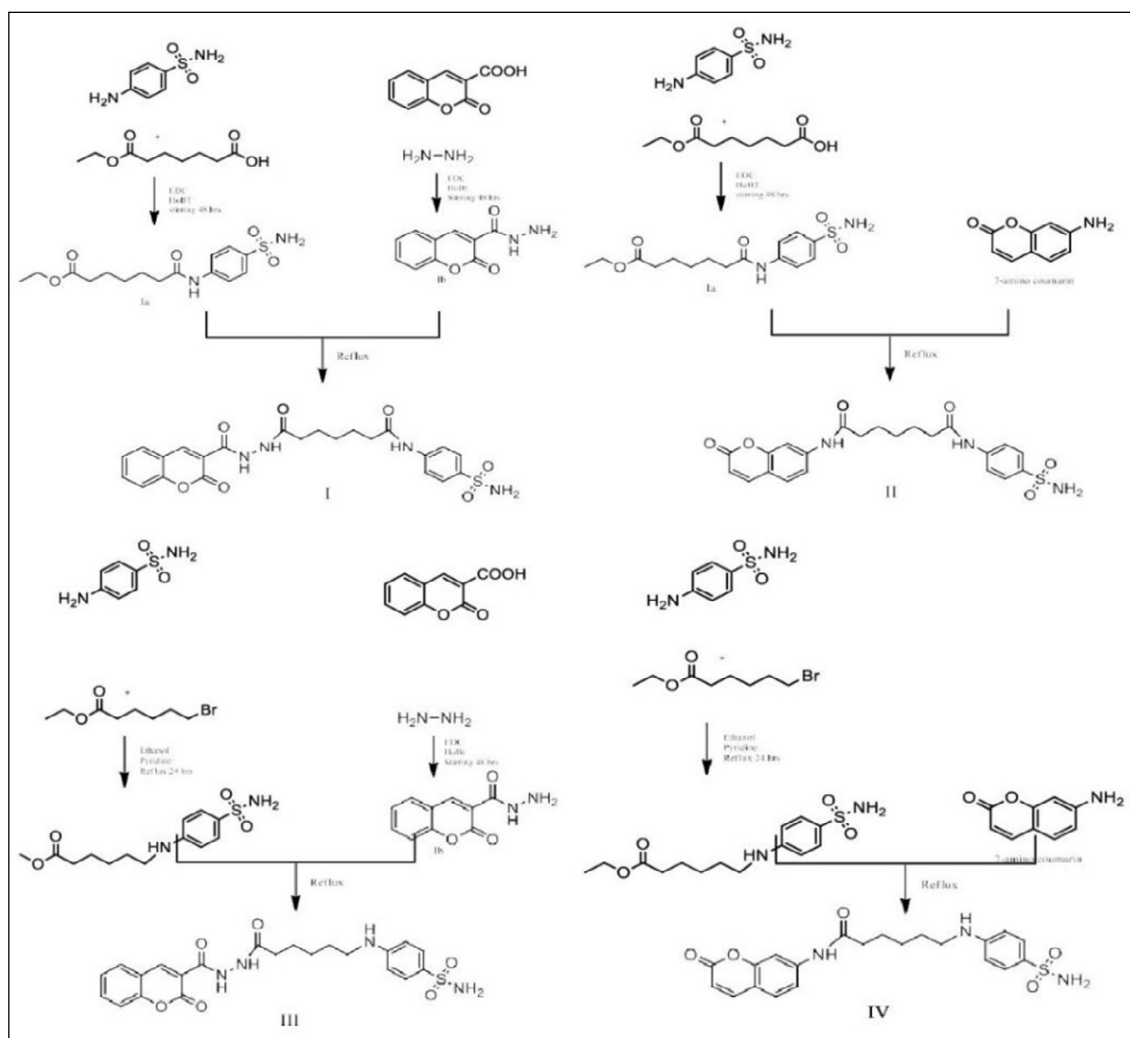
Fig. 4. Illustration of synthesis of target compounds (I -IV) cytotoxicity assay.

The goal of the study is to evaluate the viability (Live/Dead) rates of cells. Initially HepG2 and MCF-7 cells were seeded in a 12 well cell culture plates and treated with 120 µg/ml of the compounds in 24 hours. The stained cells were then instantly seen and captured on camera using a fluorescent microscope (Axioskop 2 plus, Ziess, Germany) after being washed with PBS and a solution containing EB/AO was added. Based on the differential in the permeability of dye into an intact cell membrane, it is possible to distinguish between live, necrotic, and apoptotic cells. The viable cells represented by green mean stained with AO only. Necrotic cells are stained orange by EB whereas early and late apoptotic cells have condensed chromatin and are labeled orange and green by AO and EB [with a little alteration in membrane permeability].

MOLECULAR DOCKING

Studying the mode of binding of the designed ligands that have biological activity explain the affinity of bind-ing and types and nature of interaction with desired protein [HDAC6]. The docking can be reverse docking, by using the ligands that is docked against [HDAC6] to predict the novel target for these ligands and finally study its mode of binding [23]. The docking study was performed by using Molecular Operating Environment [MOE] software version 2015.10. [HDAC6]'s X-ray crystal structures was taken from protein data bank [PDB] under the code [5EDU]. All water molecules were removed and then add hydrogen atoms to the prepared protein. The optimization of the shape of chemical to the binding site was done by using the MOE-Dock algorithm. The initial scoring method selected was the London ΔG and the final scoring method used is the Rigid Receptor. The best poses of each ligand were scored. Finally, by using MOE pose utility Complexes docked were examined for geometry [23]. In agreement with the docking study results the synthesized compounds showed cytotoxic activity by inhibition the histone deacetylase enzyme [HDAC6] when compared with the standard inhibitor suberoylanilide hydroxamic acid [SAHA].

Control MCF 7 cells

Control HepG2 cells

MCF-7 Treated with compound I

HepG2 Treated with compound I

MCF-7 Treated with compound II

HepG2 Treated with compound II

MCF-7 Treated with compound III

HepG2 Treated with compound III

MCF-7 Treated with compound IV
IV

HepG2 Treated with compound

MCF-7 Treated with SAHA

HepG2 treated with SAHA
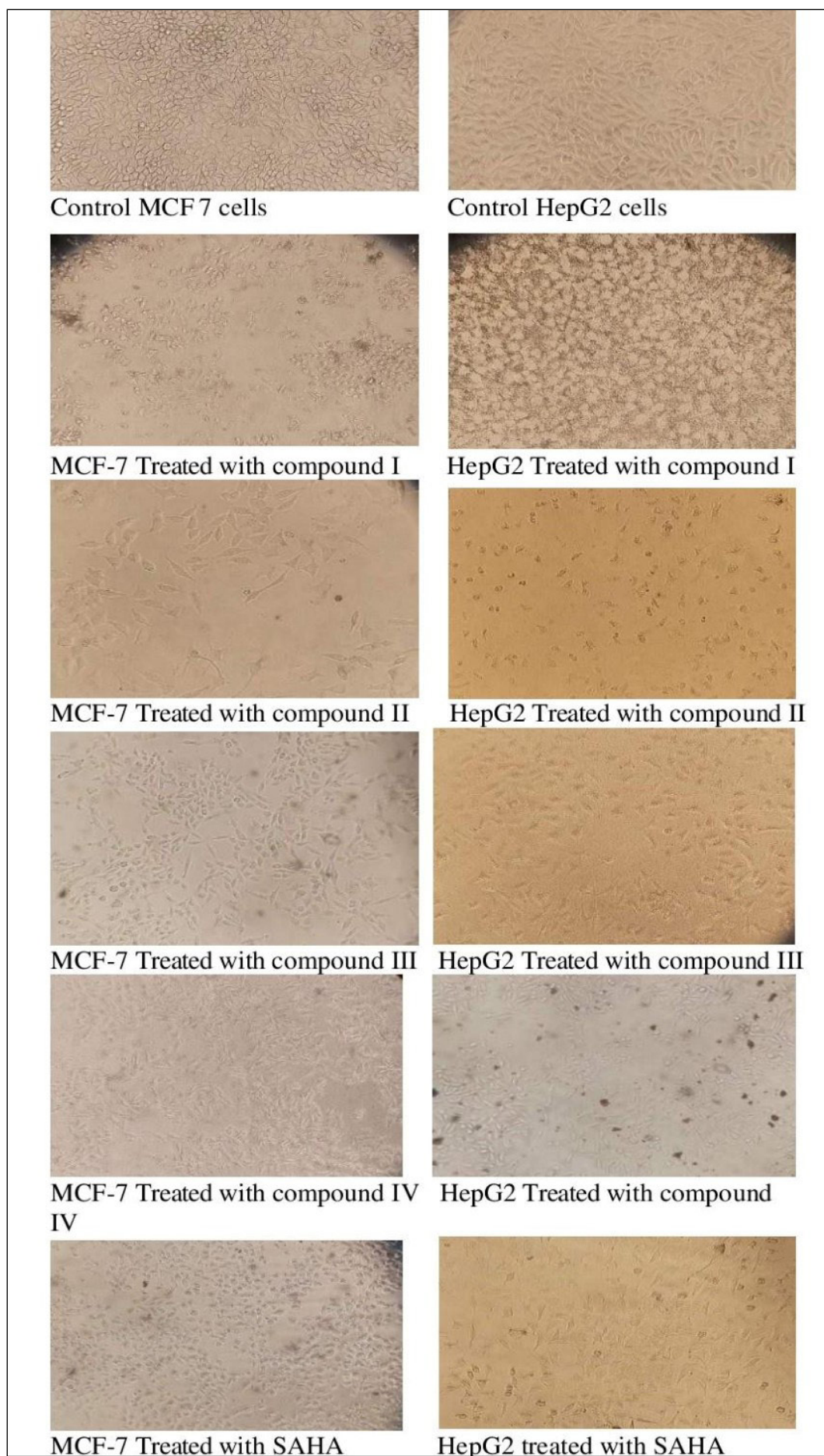
Fig. 5. Cell line morphology after treatment with compounds I, II, III and IV.
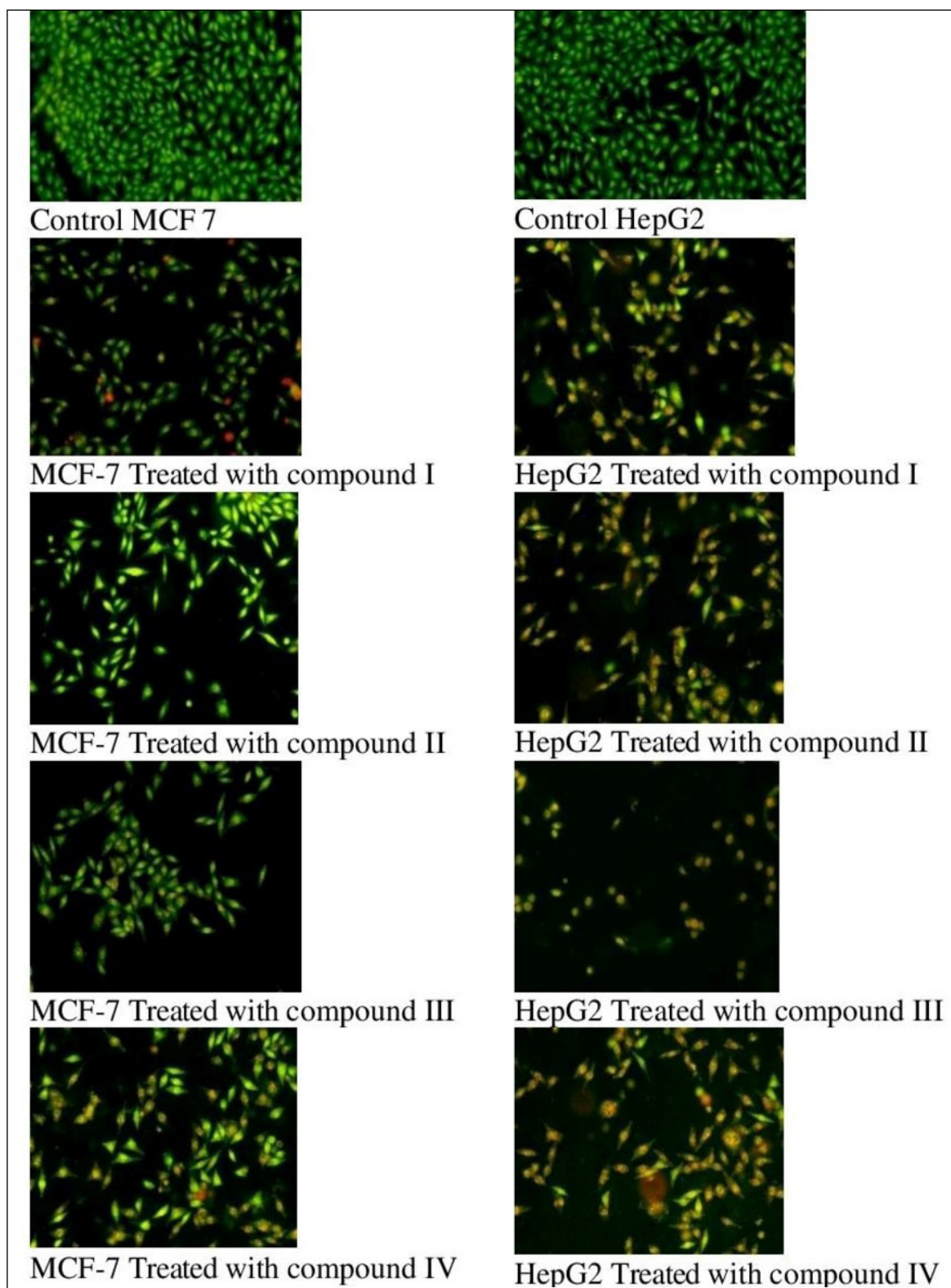
Fig. 6. Fluorescent images of selected cell lines.

RESULTS

The target compounds I–IV are synthesized according to Fig. 4. Compound (Ia) was synthesized by the reaction of 7-ethoxy-7-oxoheptanoic acid with 4-amino benzene sulfonamide using EDCI (1) and HOBt (2) as coupling agents.

CYTOTOXICITY ASSAY RESULTS

In vitro cytotoxicity of synthesized compounds (I, II, III and IV) are assessed at micro concentrations (7.4, 22.22, 66.66, 200, 600 µg/ml) by using MTT assay. HepG2 (human hepatic carcinoma) and MCF-7 (breast cancer cell line) were employed as they overexpress HDAC6. The
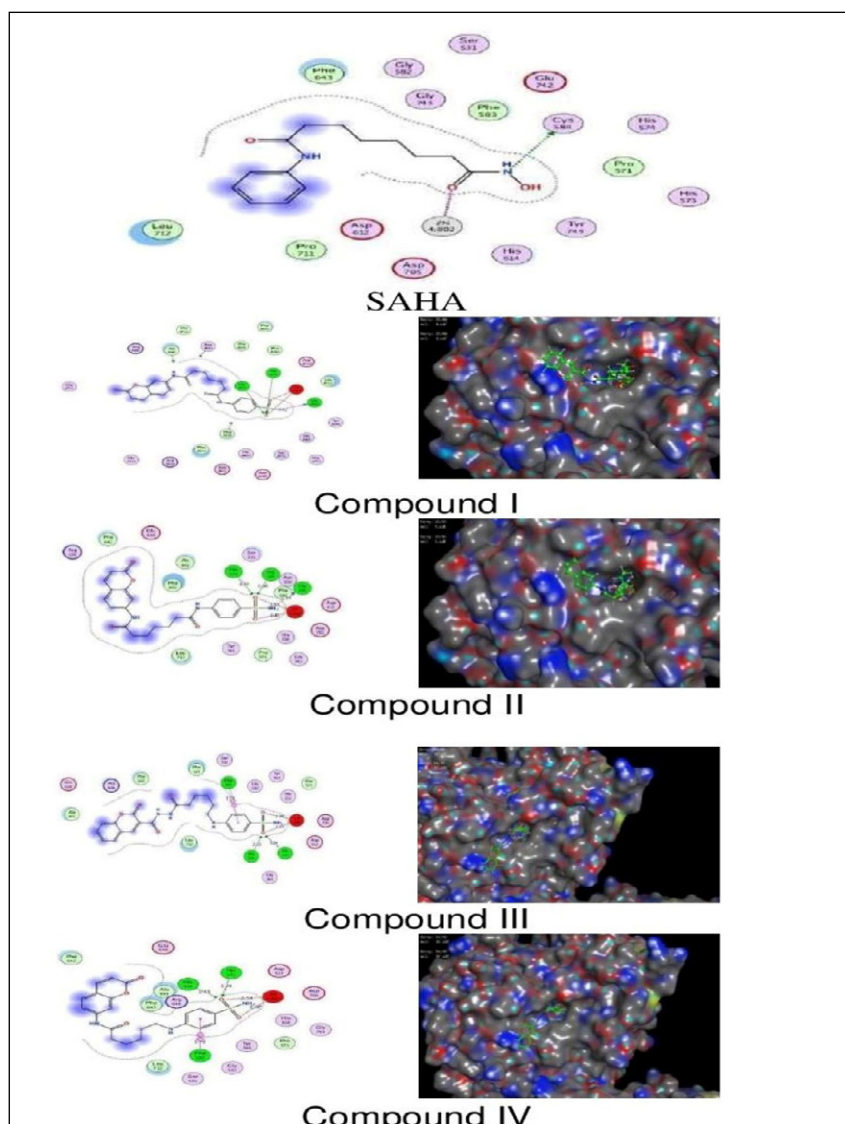
Fig 7. The occupation of the binding of compound I–IV and SAHA to HDAC6.

cell proliferation and cytotoxic effect of the synthesized compounds was measured by MTT assay method. The synthesized compounds activity was studied by testing their inhibition ability against cancer cells proliferation.

The IC50s were calculated to show how these chemicals inhibited the development of the two cell lines (Table 1). Fig. 5 display how well some cell lines respond to chemicals I, II, III, and IV as compared to SAHA.

FLUORESCENT STAINING

Depending on how differently a dye penetrates an undamaged cell membrane, it is possible to distinguish between live, apoptotic, and necrotic cells. Necrotic cells are orange and stained with EB as shown in Fig. 6. Only AO is used to stain green cells, which are alive cells. Early and late apoptotic cells are represented by green and orange cells with condensed chromatin; these cells are stained with both AO and EB (with a little change in membrane permeability).

DOCKING STUDY RESULTS

For comparison purposes, Molecular docking investigations were carried out using the program MOE 2015.10 with Compounds I–IV and SAHA against [HDAC6] (PDB code: 5EDU).

In vitro cytotoxicity results agreed with molecular docking studies, which provided a rationale for the greatest inhibitory activity. Table 2 lists the outcomes of docking with this isoform. Fig. 7 shows pictures of target substances and SAHA occupying HDAC6's binding pocket.

DISCUSSION

The synthesized compound gave the characterized amid band at 1688. The amide derivative of coumarin Compound (Ib) was synthesized by reaction of 2-oxo-2H-chromene-3-carboxylic acid with hydrazine through using EDCI (1) and HOBt (2) as coupling agents. While compound (Ic) was synthesized by reaction of 6-bromo-1-ethoxy-1-oxohexan-3-ylium with 4-amino benzene sulfonamide. Compounds I-IV were obtained from the

Table 1. IC50 values of target compounds I, II III and IV

Compound	μg/mL	
	HepG2 cell line	MCF-7 cell line
I	47.52	67.59
II	18.53	23.33
III	15.44	30.88
IV	19.81	25.63
SAHA	37.50	28.41

Table 2. Docking results of compounds, I, II, III, IV and SAHA against HDAC6

Ligand	Energy of binding ΔG (KJ/mol)	Amino acid
SAHA	-7.07	Cys 584 (3.68 A^0)
I	-7.12	His 574 (2.64A0), His 573 (3.31A0), Gly 584 (3.39A0)
II	-6.89	His 574 (2.66A0), His 573 (3.27A0), Gly 582 (3.35A0)
III	-6.91	His 574 (2.65A0), His 574 (3.24A0)
IV	-6.68	His 574 (2.65A0), His 573 (3.24A0), Gly 582 (2.65A0), Phe 643 (3.24A0)

reaction of compound Ia or Ic with compounds Ib and 7-amino coumarin r through nucleophilic substitution reaction. The IR spectrum shows the disappearance of ester band and appearance of 3240, 3219 (NH2 Str. Vib. of amine of sulfonamide), 3154 (NH Str. Vib. of amide) and 1710 (C=O Str. Of coumarin), 1719,1685 (C=O Str. vibration band of amide). ^{1}HNMR shows the presence of 2.24 (tt, 4H, CH2CO), 7.06 (s, 2H, SO2NH2), 7.34-7.81 (mm, 8H, H-5,6, and H-8 of coumarin and 4H of Ar sulfanilamide), 8.99 (s, 1H, H-4 of coumarin), 9.86 (m, 2H, of 2NHCO), 11.33 (d, 1H, NH- NHCO); 13CNMR shows 160.22 (COO of coumarin), 165.00 (ArCO-NH), 172.13 (CO-NH-NH), 174.72 (ArNH-CO-CH2) and ESI-MS for C23H24N4O7S calculated 500.1; found 501.1[M+1]+. For compound I. while for compound II also the IR spectrum show disappearance of ester band and appearance of 3408, 3226 (NH2 Str. Vib. of amine), 3084 (NH Str. Vib. of amide), 1728 (C=O Str. Of coumarin), 1708,1678 (C=O Str. vibration band of amide); ^{1}HNMR shows the presence of .33 (td, 4H, CH2CO), 6.32 (d, 1H, H-3 of coumarin), 7.05 (s, 2H, SO2NH2), 7.33 (d, 1H, H-6 of coumarin), 7.42-7.81 (m, 6H, H-4,H-5 of coumarin and 4H of Ar. Sulfanilamide), 7.99 (s, 1H, H-8 of coumarin), 9.93 (ss, 2H, of 2NHCO); 13CNMR shows 161.33 (COO of coumarin), 172.03 and 172.12 of 2(ArNH-CO) and ESI-MS for C22H23N3O6S calculated 457.5; found 458.1[M+1]+. For compound III the IR spectrum show disappearance of ester band and appearance of 3376, 3296 (NH2 Str. Vib. of amine), 3219 (NH Str. Vib. of amide), 1721 (C=O Str. Of coumarin), 1685,1637 (C=O Str. vibration band of amide); ^{1}HNMR shows the presence of 2.28 (t, 2H, CH2CO), 3.14 (m, 2H, CH2NH), 6.31 (t, 1H, NH), 6.41 (s, 2H, SO2NH2), 6.68 (dd, 2H of Ar. Sulfanilamide), 7.08 (m, 3H, H-5,6 and 7 of coumarin), 7.11 (d, 1H, H-8 of coumarin), 7.44 (dd, 2H of Ar. Sulfanilamide), 8.01 (s, 1H, H-4 of coumarin), 9.87 (d, 1H, of NHCO), 10.32 (d,1H, of NHNHCO); ^{13}CNMR shows 43.25 (CH2-NH), 160.22 (COO of coumarin), 165.00 (CO-NH), 174.71 (CH2CONH); and the ESI-MS for C22H24N4O6S calculated 472.1; found 473.1[M+1]+. For final compound IV the IR spectrum show disappearance of ester band and appearance of 3387, 3311 (NH2 Str. Vib. of amine), 3241 (NH Str. Vib. of amide), 1720 (C=O Str. Of coumarin), 1688 (C=O Str. vibration band of amide); ^{1}HNMR shows the presence of 2.31 (t, 2H, CH2CO), 3.16 (m, 2H, CH2NH), 6.32 (d, 1H, H-3 of coumarin), 6.43 (t,1H of NH), 6.81 (s, 2H, SO2NH2), 7.11 (dd, 2H of Ar. Sulfanilamide), 7.40 (d, 1H, H-6 0f coumarin), 7.63 (m, 3H, H-5 of coumarin and 2H of Ar. Sulfanilamide), 7.82 (m, 2H, H-4 and H-8 of coumarin), 9.82 (s, 1H, of NHCO); ^{13}CNMR shows 43.29 (CH2-NH), 140.61 (ArNHCO), 153.15 (ArNH), 161.33 COO of coumarin), (CONH); and the ESI-MS for C21H23N3O5S calculated 429.14; found 430.1[M+1]+.

CONCLUSIONS

Four new compounds (I, II, III and IV) was synthesized and achieved successfully starting from 4-aminobenzene sulfonamide, monoethyl pimelate, ethyl-6-bromo hexanote, coumarin-3- carboxylic acid and 7-amino coumarin in acceptable yields and their structures approved by FTIR, 1HNMR, 13 CNMR and ESI Mass spectroscopy They showed cytotoxicity toward MCF-7 and HepG2 cancer cell lines. Their docking analysis and cytotoxicity findings provided a preliminary indication that they are viable [HDAC6] candidates. These substances have coumarin as the cap group and sulfonamide functionality as a ZBG. As a result, we are motivated to broaden the focus of our ongoing research in order to look into the pharmacokinetic profile, conduct in vivo toxicity investigations, and apply the cytotoxicity test to different cancer cell lines.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Ammar Abdul Aziz Alibeg
Department of Pharmaceutical Chemistry,
College of Pharmacy, University of Kufa,
299G+HPX, Najaf 54003, Iraq
e-mail: ammara.alibeck@uokufa.edu.iq

ORCID AND CONTRIBUTIONSHIP

Ammar Abdul Aziz Alibeg: 0009-0009-4975-7462 **B** **C** **D**
Mohammed Hassan Mohammed: 0000-0001-9125-1611 **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

ORIGINAL ARTICLE

Features of exocrine pancreatic insufficiency in patients with non-alcoholic fatty liver disease in combination with type 2 diabetes and COVID-19

Yelyzaveta S. Sirchak, Vasyl V. Kalytych, Erica S. Dobryanska (Opalenyk), Volodimir G. Mihlyas, Ivan I. Kohutych, Valeriya V. Brych, Mykhailo M. Ivachevskyi
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT
Aim: The aim of the research was to study the features of pancreatic exocrine insufficiency (EPI) in patients with nonalcoholic fatty liver disease (NAFLD) and type 2 diabetes mellitus (DM) at COVID-19.
Materials and Methods: 72 patients with NAFLD and COVID-19 were examined. The patients have been divided into two groups: group 1 included 42 patients with NAFLD and insulin resistance (IR); group 2 consisted of 30 patients with NAFLD in the combination with type 2 DM. EPI was detected by ^{13}C-mixed triglyceride breath test (^{13}C-MTBT) in all the patients.
Results: The result of ^{13}C-MTBT indicates EPI in the examined subjects of the 2 group. A significant decrease in the maximum concentration of $^{13}CO_2$ between 150 and 210 min was also diagnosed in group 1 patients. research (up to $8.2 \pm 0.9\%$ – $p < 0.05$), however, the total concentration of $^{13}CO_2$ at the end of 360 min. the study reached only $27.7 \pm 1.1\%$ ($p < 0.05$).
Conclusions: Based on the results of laboratory-instrumental methods of research, patients with NAFLD and type 2 diabetes with COVID-19 were diagnosed with severe EPI. The results of ^{13}C-MTBT in NAFLD and IR with COVID-19 indicate a decrease in the functional reserves of the pancreas and the formation of its EPI.

KEY WORDS: nonalcoholic fatty liver disease, insulin resistance, type 2 diabetes mellitus, COVID-19, exocrine pancreatic insufficiency; ^{13}C-mixed triglyceride breath test

INTRODUCTION

In December 2019, China reported an outbreak of pneumonia of unknown causes in Wuhan, the capital city of Hubei province. Using unbiased next-generation sequencing, an unknown betacoronavirus was discovered from lower respiratory tract samples of these patients. Human airway epithelial cells were used to isolate the virus that was named 2019–novel Coronavirus (2019–nCoV). Phylogenetically, the novel coronavirus was found to be more similar to two bat derived coronavirus strains (~88% similarity) than coronaviruses which infect humans including SARS (~79% similarity) and MERS (~50% similarity) [1]. The World Health Organization (WHO) used the term 2019 novel coronavirus to refer to a coronavirus that affected the lower respiratory tract of patients with pneumonia in Wuhan, China on 29 December 2019/ The WHO announced that the official name of the 2019 novel coronavirus is coronavirus disease (COVID-19) [2].

The most common clinical symptoms of the COVID-19 patients are fever, cough, shortness of breath, and other breathing difficulties in addition to other nonspecific symptoms including headache, dyspnea, fatigue, and muscle pain and digestive symptoms such as diarrhea and vomiting [3]. The gastrointestinal tract can be a potential site for virus replication and feces a source of transmission [4].

The leading hypothesis for the mechanism of transmission of SARS-CoV-2 is the aerosol route, which occurs through droplets released into the air from the respiratory tract. When a person comes into contact with the pathogen, the virus binds to angiotensin-converting enzyme 2 (ACE2) receptors in the lungs. The SARS-CoV-2 adhesion glycoprotein binds to the ACE2 receptor and ensures effective cellular entry, which leads to virus replication and spread throughout the body. The epithelium of the intestine (mainly the absorptive enterocytes of the ileum and colon) and oesophagus also highly express ACE2 receptors. In addition, glandular cells in the stomach and duodenum express ACE2, so SARS-CoV-2 can infect intestinal epithelial cells via ACE2 receptors. ACE2 receptors

in the gastrointestinal tract play a regulatory role in amino acid homeostasis, the gut microbiome and innate immunity. Therefore, binding of SARS-CoV-2 to ACE2 receptors in the gastrointestinal tract may lead to gastrointestinal symptoms such as abdominal pain and diarrhoea [5].

Therefore, the study of the features of the combined pathology of the organs of the digestive system, including damage to the liver and pancreas in patients with COVID-19, is of interest to the medical community.

AIM

To study the features of pancreatic exocrine insufficiency (EPI) in patients with nonalcoholic fatty liver disease (NAFLD) and type 2 diabetes mellitus (DM) at COVID-19.

MATERIALS AND METHODS

The study and treatment of patients was carried out at the clinical base of the Department of Propedeutics of Internal Medicine, School of Medicine, State Higher Educational Institution "Uzhhorod National University". The study included 72 patients with NAFLD at the stage of outpatient follow-up after COVID-19. All patients included in the study had previously been hospitalized in the department for treating COVID-19 infected patients based on communal non-profit enterprise «The Transcarpathian Regional Clinical Hospital Named after A. Novak» of Transcarpathian Regional Council during the period between March 2021 and October 2023 and had a confirmed diagnosis of COVID-19 pneumonia (the positive polymerase chain reaction (PCR test) to SARS-CoV-2 RNA (the RdRP SARS-CoV-2 gene, the E SARS-CoV-2 gene) and did not require patients be connected to the artificial ventilator. The hospitalised patients were treated in accordance with the standards of medical care for patients with COVID-19, including antiviral therapy, glucocorticoids, anticoagulants, vitamin D3, zinc and antibiotic therapy.

Exclusion criteria for the study include patients with NAFLD and carbohydrate metabolism disorders with COVID-19. Criteria for inclusion from the study were acute respiratory infection with COVID-19 at the time of the examination, the presence of alcohol, autoimmune, viral (hepatitis B, C, D viruses) liver damage.

The patients with NAFLD have been divided into two groups depending on the presence of insulin resistance (IR) or type 2 DM, namely:

- group 1 included 42 patients with NAFLD and IR (among them there were 24 men (57.1 %), 18 women (42.9 %); the average age was 48.6 ± 6.3 years)

- group 2 consisted of 30 patients with NAFLD in the combination with type 2 diabetes mellitus (there were 18 men (60.0 %) among them, 12 women (40.0 %; the average age was 47.3 ± 5.8 years). The examined patients of 2 group were diagnosed with type 2 diabetes mellitus of mainly mild and moderate severity, which was characterized by the absence of hypoglycemic reactions, the level of glucose in the fasting blood up to 8.5 mmol/l, after eating - up to 10 mmol/l, HbA1c - did not exceed 7% .

The control group included 30 healthy individuals (there were 17 men (56.7 %), 13 women (43.3 %) with the average age 47.8 ± 5.8 years).

All research studies were performed with the consent of the patients (written consent), and the methodology was in line with the Helsinki Declaration of Human Rights of 1975 and its revision of 1983, the Council of Europe Convention on Human Rights and Biomedicine, and the legislation of Ukraine.

Patients underwent general clinical, anthropometric, instrumental and laboratory tests. To make a diagnosis, complaints and medical history were assessed. Patients underwent an ultrasound examination of the abdominal cavity according to the generally accepted method. Standard general and biochemical tests based on blood serum were performed to determine the functional state of the liver (alanine aminotransferase, aspartate aminotransferase, total bilirubin, alkaline phosphatase, gamma-glutamyltransferase), indicators of lipid metabolism (total cholesterol, triglycerides, high-density lipoproteins, low-density lipoproteins, very-low-density lipoproteins, the atherogenic index was calculated), carbohydrate metabolism (glucose, insulin, glycated hemoglobin (HbA1c,%), calculated the insulin resistance index (HOMA-IR)). Also, the level of α-amylase in the blood serum of the patients was identified.

NAFLD was diagnosed in accordance with the EASL-EASD-EASO guidelines for the diagnosis and treatment of NAFLD [6]. The degree of liver damage was determined using the online calculators NAFLD fibrosis score (NFS), Fibrosis 4 calculator (FIB-4), as well as FibroTest and liver elastography results.

The diagnosis of type 2 diabetes was established in accordance with the recommendations of the IDF (2005) and criteria European Association for the Study of Diabetes (EASD) i *American Diabetes Association (ADA)* [7]. The severity of type 2 diabetes was assessed by the level of HbA1c.

All patients underwent coprological examination.

A ^{13}C -mixed triglyceride breath test was also carried out. The respiratory samples were analyzed with the infrared spectroscopy of IRIS (Izinta, Hungary). The diagnostic value of the ^{13}C -mixed triglyceride breath

Table 1. Distribution of the surveyed depending on BMI

Indicator	Examined		
	Control group (n=30)	Patients with NAFLD and COVID-19	
		I group (n=42)	II group (n=30)
Normal weight (BMI: 18.5 – 24.9)	76.7 %**	11.9 %	3.3 %
Overweight (BMI: 25.0 – 29.9)	23.3 %	54.8 %*++	20.0 %
Obese Class I (BMI: 30.0 – 34.9)	–	16.7 %	40.0 %++
Obese Class II (BMI: 35.0 – 39.9)	–	11.9 %	26.7 %+
Obese Class III (BMI: >40)	–	4.7 %	10.0 %

Note: the difference between the indicators of the control group and the examined patients is statistically significant: * – p<0.01; ** – p<0.001; the difference between indicators in patients of the I and II groups is significant: + - p<0.05; ++ - p<0.01.

test is that with the help of this test we determine the number of lipase, which is in the lumen of the duodenum, set the number of enzymes necessary for a particular patient to eliminate exocrine pancreatic insufficiency (EPI), and also allows to distinguish the pancreatic steatore from the intestinal. During the test, 13 respiratory samples received: the initial, before the test and after the test breakfast (100 g of white bread and butter (from 0.5 d / kg of body weight), with added a mixture of triglycerides (fatty acids labeled with non-radioactive carbon isotope - 13 ° C 42 mg / kg body weight) and another 12 samples for 10 hours (every 30 min each) [8].

Triglycerides containing various fatty acids are the main components of natural fats. The active pharmacological substance is 1,3-distearol-2- (1-13C) octanoil glycerol, labeled with a steady carbon isotope. It is metabolized in two stages. The first stage involves the cleavage of 1-13C caprylic acid at positions 1,3, which occurs mainly under the action of pancreatic lipase entering the lumen of the duodenum. he second stage involves the absorption of cleaveaprylic acid molecules and 2-(1-13 C) mono-octanoyl glycerol, which may be preceded by its cleavage to caprylic acid.. Upon entering the small intestine, caprylic acid is rapidly absorbed, binds to blood albumin, and is transported to the liver via the portal blood flow system or the lymphatic system in the general circulation as part of lipoproteins. The main channel of caprylic acid metabolism is mitochondrial beta-oxidation, which leads to the formation of bicarbonate ion containing carbon-13 (^{13}C) and replenishes the bicarbonate pool of the blood. This leads to an increase in the proportion of ^{13}C in the carbon dioxide (CO_2) of exhaled air. The percentage of 13C released depends on the activity of pancreatic lipase. In case of exocrine insufficiency, the amount of

lipase produced decreases or is absent, which means that triglycerides are broken down less intensively and less $^{13}CO_2$ is released. Depending on the concentration of $^{13}CO_2$ in different samples, a curve is built, the nature of which reflects the presence and degree of pancreatic exocrine insufficiency. Pancreatic exocrine insufficiency accompanied by lipase deficiency was detected by analyzing the curve reflecting the concentration of $^{13}CO_2$ in breath samples (maximum concentration between 150 and 210 minutes of the study and total concentration after 360 minutes of the study). Normally, the maximum concentration of $^{13}CO_2$ between 150 and 210 minutes of the study is more than 8%, and the total concentration at 360 minutes of the study is 30-35% of 13CO2. In case of exocrine insufficiency of the pancreas, there is a decrease in the activity of intraduodenal pancreatic lipase with a maximum concentration between 150 and 210 minutes of the study of less than 8% $^{13}CO_2$ and a total concentration of $^{13}CO_2$ at the end of 360 minutes - less than 23% [8].

The data were analysed and processed using STATISTICA 10.0 (StatSoft Inc, USA) using parametric and non-parametric methods of evaluating the results.

RESULTS

In all examined patients with NAFLD and COVID-19 were determined BMI changes, especially increasing in weight (table 1).

As the data obtained, among the patients of group 1, the majority were overweight persons (54.8% 0 and the obesity class I (16.7%) - p <0.01, while in the group2 , there were more persons with obesity class I (40.0% - p <0.01) and obesity class II (26.7% - p <0.05).

According to the results, all patients with NAFLD and COVID-19 had complaints of digestive system (table 2)

Table 2. Clinical symptoms from the digestive organs in examined patients with NAFLD and COVID-19

Symptoms	Examined patients with NAFLD and COVID-19	
	I group (n=42)	II group (n=30)
Pain	42.9 %	60.0 % *
Periodicity		
Constant	28.6 %	80.0 %**
Periodic	71.4 %**	20.0 %
Localization		
- in the upper parts of the abdomen (left hypochondrium, epigastric area)	28.6 %	56.7 %**
- in the right hypochondrium	57.1 %**	16.7 %
- without clear localization, diffuse character	14.3 %	26.6 %*
Flatulence	52.4 %	80.0 %**
Defecation disorders:	61.9 %	73.3 %*
- diarrhea	30.8 %	72.7 %**
- constipation, which was later replaced by diarrhea	69.2 %**	27.3 %
- polyfaecalia	33.3 %	63.3 %**
Nausea	61.9 %**	26.7 %
Vomiting	19.0 %	23.3 %
Bitterness in the mouth	57.1 %**	20.0 %

Note: statistically significant difference between indicators in patients of the I and II groups: * – p<0.05; ** – p<0.01.

Table 3. The results of laboratory research methods in the examined

Indicator	Control group (n=30)	Examined patients with NAFLD and COVID-19	
		group 1 (n=42)	group 2 (n=30)
Coprological research			
Steatorrhea (+)	-	71.4 %	90.0 %++
Amylorrhea (+)	-	52.4 %	60.0 %
Creatorrhoea, due to muscle fibers that preserved transverse striations (+)	-	42.9 %	53.3 %+
Soap	3.3 %	54.8 %	66.7 %**
Amylase in blood serum (normal range: 0 - 115 (U/L)	58.7 ± 3.2	98.7 ± 3.1*	112.4 ± 2.4**+

Note: statistically significant difference between the indicators of the control group and the examined patients: * – p < 0.05; ** – p < 0.01; statistically significant difference between indicators in patients of the I and II groups: + - p < 0.05; ++ - p < 0.01.

There is a difference between the recurrence of the pain symptom in the examined patients, namely: in patients of the group 2 (NAFLD in combination with type 2 diabetes and COVID-19), the pain was constant, less severe (in 80.0% of cases – p<0.01)), while in the patients of the 1st group, there was a repeated and recurrent character (in 71.4% of cases – p<0.01). In patients of the group 2 , the pain was more often localized in the left hypochondrium and epigastric area (in 56.7% of observations – p<0.01), while in patients of the group 1, it was more often in the right hypochondrium (in 57.1% of observations – p<0 ,01).

Flatulence was more often diagnosed in patients of the group 2 (in 80.0% of cases – p<0.01). Defecation disorders were detected in patients of the group 2 more often, which was manifested mainly by diarrhea (in 72.7% of patients) and polyfecality (63.3% of the examined) – p<0.01. In patients of the 1st group, constipation alternating with diarrhea was more often established (in 69.25 cases – p<0.01). – p<0.01

It should be noted that signs of biliary dyspepsia (nausea, bitterness in the mouth) were more often diagnosed in patients of the group 1 (in 61.9% and in 57.1% of the examined, respectively – p<0.01). The peculiarity of biliary dyspepsia in patients of the group 2 was that nausea in almost all patients was accompanied by vomiting, which did not bring relief to the patients.

All patients who were under our observation had a coprological examination and determination of the

Table 4. Results of 13C -mixed triglyceride breath test in the examined patients and the control group

Indicator	Control group (n=30)	Patients with NAFLD and COVID-19	
		I group (n=42)	II group (n=30)
The maximum concentration of $^{13}CO_2$ between 150 and 210 min. research	16.9 ± 0.8 %	8.2 ± 0.9 %*	6.1 ± 0.4 %**
Total concentration of $^{13}CO_2$ at the end of the study (360 min.)	34.1 ± 1.2 %	27.7 ± 1.1 %*	17.8 ± 0.9 %**+

Note: $+ -$ a statistically significant difference was found between the indicators of the control group and the examined patients: $* - p < 0.05$; $** - p < 0.01$; statistically significant difference between indicators in patients of the I and II groups: $+ - p < 0.05$.

level of amylase in blood serum. The level of amylase in the blood serum of the examined patients of both groups was statistically significantly different from the indicators of the control group, but at the same time it did not go beyond the reference values – Table 3.

According to the results of the coprological study, signs of exocrine insufficiency of the pancreas were found in patients with NAFLD and impaired carbohydrate metabolism (IR or type 2 diabetes) with COVID-19. More pronounced changes during the coprologic examination of the EPI were found in patients of the group 2, namely - in 90.0% of patients, steatorrhea due to fatty acids was detected, in 60.0% - amylorrhea (the presence of starch grains) and in 53.3% - creatorea due to muscle fibers , which preserved the transverse banding.

Changes in the secretory pancreatic function were also found in patients of the group 1, but less severe than in the group 2 of subjects. In patients of the group 2, a more severe increase in soap was also found during coprological examination, which indicates the dysfunction of the biliary system in patients with type 2 diabetes and indicates the biliary genesis of the lesion of the pancreas in these patients.

For a more accurate study of the EPI in patients with NAFLD and impaired carbohydrate metabolism in COVID-19, ^{13}C -mixed triglyceride breath test was performed - Table 4.

The analysis of the ^{13}C -mixed triglyceride breath test data indicates a severe EPI in the examined patients of the group 2. Patients of the group 2 showed a significant decrease in the maximum concentration of $^{13}CO_2$ between 150 and 210 min. research, as well as the total concentration of $^{13}CO_2$ at the end of 360 min. research - p<0.01. A significant decrease in the maximum concentration of $^{13}CO_2$ between 150 and 210 min was also diagnosed in group I patients. research (up to 8.2 $\pm$ 0.9% – p < 0.05), however, the total concentration of $^{13}CO_2$ at the end of 360 min. study decreased to only 27.7 $\pm$ 1.1% - p < 0.05. Therefore, according to the results of highly informative ^{13}C -mixed triglyceride breath test, EPI was detected in patients with NAFLD and type 2 diabetes, and also a violation of exocrine insufficiency of pancreas was established in patients with NAFLD and IR in case of COVID-19.

DISCUSSION

The proportion of diabetics among COVID-19-positive patients varies according to the regions in the world. For example, in Italy as many as 36% of those seriously ill, having a positive result from a COVID-19 test, were burdened with diabetes, and in the United States, this same phenomenon was noted in as many as 58% of patients. The vast majority of infected patients present a mild form of COVID-19, but some develop a severe form of infection that can be fatal [9]. Type 2 diabetes is associated with low-grade chronic inflammation induced by the excessive visceral adipose tissue. This inflammatory condition affects the homeostatic glucose regulation and peripheral insulin sensitivity. Chronic hyperglycemia and inflammation can determine an abnormal and ineffective immune response [10]. Diabetic patients with COVID-19 are at higher risk of being in an excessively hypercoagulable state and uncontrolled inflammation responses, which may contribute to a poorer outcome [11].

Damage to the pancreas can lead to loss of blood glucose control. The β-cells of the pancreas in type 2 diabetes are depleted over time as a result of compensatory insulin secretion in insulin resistance. The negative impact of hyperglycaemia on the secretory function of the islets of Langerhans is increasingly discussed [12].

During the SARS-CoV-2 pandemic, patients with reduced pancreatic function are at high risk of contracting the virus. High blood glucose levels in patients with and without diabetes mellitus have been shown to increase the risk of mortality in COVID-19, and hyperglycaemia impairs the immune response and negatively affects the excessive cytokine response, and thus has a strong pro-inflammatory effect [12].

ACE2 receptors, which are also present in the pancreas, are targeted by SARS-CoV-2 in the body, which can lead to acute failure of both the islets of Langerhans and exocrine cells. This can lead to an uncontrolled hyperglycaemic state, especially in patients with dia-

betes mellitus. Consequently, hyperglycaemia leads to a more severe course of COVID-19 and viral infection complicated by secondary infections. In patients with diabetes, the coexistence of other risk factors (atherosclerosis, hypertension, obesity) should also be taken into account, which leads to a worse prognosis and course of COVID-19 [13, 14].

The impact of coronavirus on exocrine pancreatic function is not fully understood, and the available literature cannot clearly answer whether tissue damage leading to acute pancreatitis is a consequence of direct SARS-CoV-2 infection or a syndrome of systemic multiorgan dysfunction with elevated amylase and lipase levels. A study by Liu et al. involving 121 patients with COVID-19 with an average age of 57 years and variable course of infection found that amylase and lipase levels were higher than normal in 1-2% of patients with moderate COVID-19 and 17% of patients with severe COVID-19. This confirms the hypothesis that the SARS-CoV-2 virus has a destructive effect on both the endocrine and exocrine parts of the pancreas [13, 15, 16].

As indicated by the data obtained by us, patients with NAFLD, regardless of the form of impaired carbohydrate metabolism (either type 2 diabetes or IR) with COVID-19, were diagnosed with clinical and laboratory-instrumental signs of EPI. At the same time, exocrine pancreatic insufficiency in these patients has a number of features, namely, in patients with type 2 diabetes, it is clinically manifested by diarrhea and polyfecaly, while in patients with NAFLD and IR, signs of biliary dyspepsia (nausea, bitterness in the mouth) are more often diagnosed. The data of the coprological study indicate a severe EPI, and the results of 13C -mixed triglyceride breath test confirm the depletion of the functional reserves of the pancreas in patients with NAFLD and type 2 DM with COVID-19.

At the same time, it should be noted that patients with NAFLD and IR with COVID-19 have signs of the formation of exocrine insufficiency of the pancreas, and the results of 13C -mixed triglyceride breath test with high accuracy confirm the compromise of not only the endocrine part, but also the exocrine part of the pancreas in these patients, which requires timely correction in order to prevent its EPI

CONCLUSIONS

1. Patients with NAFLD and impaired carbohydrate metabolism with COVID-19 have signs of intestinal and biliary dyspepsia. At the same time, the patients of the group 1 have clinically more pronounced signs of biliary dyspepsia, while the patients of the group 2 have clinical manifestations of EPI.
2. Based on the results of laboratory-instrumental methods of research, patients with NAFLD and type 2 diabetes with COVID-19 were diagnosed with severe EPI
3. The results of ^{13}C -mixed triglyceride breath test in NAFLD and IR with COVID-19 indicate a decrease in the functional reserves of the pancreas and the formation of its EPI.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Yelyzaveta S. Sirchak
Uzhhorod national university
1 Narodna sqr., 88000 Uzhhorod, Ukraine
e-mail: sirchakliza777@gmail.com

ORCID AND CONTRIBUTIONSHIP
Yelyzaveta S. Sirchak: 0000-0001-6738-0843 Ⓐ Ⓕ
Vasyl V. Kalytych: 0009-0005-5482-3293 Ⓑ Ⓒ Ⓓ
Erica S. Dobryanska (Opalenyk): 0000-0001-6014-1110 Ⓓ
Ivan I. Kohutych: 0000-0002-3064-8292 Ⓑ
Volodimir G. Mihlyas: 0000-0001-6133-8257 Ⓓ Ⓔ
Valeriya V. Brych: 0000-0003-3741-6002 Ⓑ Ⓒ
Mykhailo M. Ivachevskyi: 0000-0002-9254-7163 Ⓑ

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

ORIGINAL ARTICLE

Endoscopic and morphological features of chronic gastritis in patients with lumbar spine osteochondrosis

Stepan S. Filip, Vitaliy M. Kochmar, Vasyl V. Stoika, Ivan I. Pushkash
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

Aim: of the study is to determine the endoscopic and morphological features of chronic gastritis (CG) in patients with lumbar spinal OC.

Materials and Methods: 102 patients with lumbar spine OC and CG were examined. The patients were diagnosed with Helicobacter pylori (HP) infection, according to which the patients were divided into two groups: the first group included 92 HP-positive patients, the second group consisted of 10 HP-negative patients.

Results: Among HP infected patients with lumbar spine OC, erosive gastropathy was most often diagnosed (in 40 (43.5%) of the examined), as well as erosive-papular and erosive-hemorrhagic gastropathy (in 14 (15.2%) and in 16 (17, 4 %) of patients, respectively), while erythematous gastropathy was more often diagnosed among HP-negative patients (in 7 (70.0 %) cases, respectively).

Conclusions: 1. 90.2% of patients with lumbar spine OC and CG have been diagnosed with HP infection. 2. Endoscopically, the lesion of the stomach MM in patients with lumbar spine OC corresponds mainly to erosive and erosive-hemorrhagic forms of gastropathy. 3. During histological examination of stomach MM, mainly 2nd and 3rd degrees of inflammation were established, especially in patients with erosive, erosive-papular and erosive-hemorrhagic forms of gastropathy.

KEY WORDS: lumbar spine osteochondrosis; chronic gastritis; Helicobacter pylori; diagnostics

INTRODUCTION

Degenerative and dystrophic diseases of the spine in the structure of disability account for 20.4%. Disability occurs with a relatively short duration of the disease, and, as a rule, in people of working age [1]. The most common cause of vertebral pain syndrome is osteochondrosis (OC) of the spine, which is a manifestation of a degenerative lesion of the intervertebral disc with the involvement of vertebral bodies, intervertebral joints, and later - the bodies of adjacent vertebrae, connective and muscular apparatus. Osteochondrosis of the spine is a systemic multifactorial process, in the development of which traumatic, age-related, dyshemic, hormonal-endocrine, immunological, hereditary and some other factors play a role [2].

Degradation and loss of articular cartilage is one of several signs of OC, which leads to severe pain, loss of joint function, and deterioration of quality of life [3, 4]. As a rule, pain prevails in the clinical symptoms, regardless of the nature of the spinal damage. An important component of the treatment of DD and inflammatory lesions of the spine should be prevention of the degenerative process in the articular cartilage, reduction of pain syndrome and inflammation, as well as prevention

of disability and improvement of the patient's quality of life [5-7].

In patients with chronic back pain, pharmacological treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) should be considered as first-line therapy [8, 9, 10]. NSAIDs are widely distributed in the world, but numerous studies have revealed their negative effects on various body systems, including the gastrointestinal tract, biliary and hematological systems, as well as NSAIDs inhibit osteogenesis and can lead to delayed or non-union of bones [11, 12].

Therefore, the study of the features of endoscopic changes in the mucous membrane (MM) of the stomach and its histological evaluation in patients with OC who have been taking drugs from the group of NSAIDs for a long time is an urgent and unresolved issue that requires a consolidated approach of various specialists in the management of patient data.

AIM

The aim of the study is to determine the endoscopic and morphological features of chronic gastritis in patients with lumbar spine OC.

MATERIALS AND METHODS

102 patients with lumbar spine OC were examined on the clinical bases of the Department of General Surgery of the Medical Faculty of the State Medical University "UzhNU" in 2021-2023. All examined patients were diagnosed with chronic gastritis (CG) (according to clinical and endoscopic examination). There were 62 (60.8%) men and 40 (39.2%) women among the examined patients with lumbar spine OC. The average age was 44.7±6.2 years. The control group included 20 practically healthy people (12 (60.0%) men, 8 (40.0%) women). The average age was 42.9±4.7 years.

All studies were performed with the consent of the patients (written consent was obtained from all patients regarding the appropriate diagnostic and therapeutic measures), and the methodology of their conduct was in accordance with the Helsinki Declaration of Human Rights of 1975 and its revision of 1983, the Council of Europe Convention on Human Rights and biomedicine and legislation of Ukraine.

Examination of the spine included inspection, palpation, and objective assessment of pain. The diagnosis of lumbar spine OC was made on the basis of physical, general clinical examination methods, as well as given imaging methods (x-ray examination, computer tomography, magnetic resonance imaging) of the relevant section of the spine. The motor activity of the spine was determined by means of a functional assessment of the mobility of the spine and muscle strength of the back and abdominal press (Schober test, spine streching, "fingers-to-the-floor" test, functional tests to determine the strength endurance of the back extensor muscles and abdominal press), as well as according to the Leken indices and the Harris test in accordance with the recommendations of the American Academy of Orthopedic Surgeons (AAOS, 2018), the International Society for the Study of Osteoarthrosis (OARSI, 2019) [6].

All patients with lumbar spine OC underwent fibroesophagogastroduodenoscopy (FEGDS) using endoscopy equipment with a video processor "Pentax" EPM-3300 using flexible fibroendoscopes of the company "Pentax" E-2430, GIF-K20. A test for rapid diagnosis of Helicobacter pylori (HP) infection based on the urease activity of a biopsy obtained during an endoscopic examination of the gastric mucosa (MM). After obtaining a biopsy from the stomach, it was placed on the surface of the test in the center. The result was evaluated by changing the color of the indicator from yellow to blue after 3 minutes.

Also, the degree of microbial insemination of HP was determined according to the scale of L.Y. Aruin from biopsies obtained during an endoscopic examination of MM of the stomach, which were fixed in formalin and then embedded in paraffin. Sections were coloured according to Romanovsky – Giemse. The degree of insemination was determined by the bacterioscopic method according to the scale of L.Y. Aruin: 0 – there are no bacteria in preparation, 1 – a low degree of insemination (up to 20 microbial bodies in the field of view), 2 – an average degree of insemination (from 20 to 50 microbial bodies in the field of view), 3 – a high degree of insemination (more than 50 microbial bodies in the field of view) [13].

Helicobacter pylori infection was also diagnosed using immunochromatographic analysis with visual recording of the result for the qualitative detection of HP antigens in feces samples (Pharmasco, Ukraine). The patients underwent C^{13}- urease breath test (C^{13}-UBT) (IZINTA, Hungary) to determine HP.

Histological sections were prepared from the taken biopsy material of the MM of the stomach, which were stained with hematoxylin-eosin and diagnosed according to the Sydney system. To make a diagnosis of chronic gastritis, morphological changes in the MM of the stomach, namely dystrophic changes in the epithelium, inflammatory infiltration of the MM and remodelling of the glands [14] were taken into account.

With the help of a visual-analogue scale to assess the severity of HP colonization, the activity of chronic inflammation, atrophy and metaplasia of the MM of the stomach: HP colonization - if HP is found in at least one of the biopsies, the diagnosis of "HP-associated chronic gastritis" is established; infiltration by neutrophils is the main indicator of activity; chronic inflammation - presence of at least 1-2 plasma cells in the field of vision indicates chronic inflammation; atrophy - in the field of view of high magnification, at most 2 transversely cut glands (normally there are more of them); intestinal metaplasia (anatomical assessment - complete (small intestine) and incomplete (large intestine); quantitative assessment (damage to 5% of CO of the stomach - mild, up to 20% - moderate, more than 20% - pronounced metaplasia) [15].

The criteria for the inclusion of patients in the study were lumbar spine OC; clinical, endoscopic, morphological signs of chronic gastritis.

Exclusion criteria from the study: stomach ulcer; stomach cancer; patients who underwent surgery on the stomach, duodenum, and any other surgery within the last month; spine fractures; tuberculosis of lungs, bones; acute infectious diseases, including a history of respiratory infection COVID-19 (up to 6 months).

Analysis and processing of the results of the examinations was carried out by the computer program Statistics for Windows v.10.0 (StatSoft Inc, USA) using

Table 1. Distribution of patients with OC of the spine depending on the severity of damage to the MM of the stomach during FEGDS

Type of gastropathy detected in FEGDS	Patients with OC and CG were examined	
	1st group HP-positive patients n=92	2nd group HP-negative patients n=10
Erythematous gastropathy	8 (8.7%)	7 (70.0%)
Erosive gastropathy	40 (43.5%)	2 (20.0%)
Erosive-papular gastropathy	14 (15.2%)	–
Erosive and hemorrhagic gastropathy	16 (17.4%)	–
Reflux gastritis	14 (15.2%)	1 (10.0%)

Note: due to the small number of patients in the 2nd group, it was not possible to calculate the reliability between the signs by group.

Table 2. Results of a histological examination of the gastric mucosa in accordance with the endoscopic diagnosis of gastric lesions in patients with lumbar spine OC

Endoscopic diagnosis		Inflammation				Process activity	Atrophy	Intestinal metaplasia
		St. 0	St. 1	St. 2	St. 3			
Erythematous gastropathy	1st gr. n = 8	1 (12.5%)	1 (12.5%)	4 (50.0%)	2 (25.0%)	2 (25%)	0 (0%)	0 (0%)
	2nd gr. n=7	2 (28.6%)	4 (57.1%)	1 (14.3%)	0 (0%)	2 (28.6%)	0 (0%)	0 (0%)
Erosive gastropathy	1st gr. n=40	2 (5.0%)	4 (10.0%)	26 (65.0%)	8 (20.0%)	28 (70.0%)	0 (0%)	0 (0%)
	2nd gr. n=2	1 (50.0%)	1 (50.0%)	0 (0%)	0 (0%)	1 (50.0%)	0 (0%)	0 (0%)
Erosive-papular gastropathy	1st gr. n=14	0 (0%)	0 (0%)	5 (35.7%)	9 (64.3%)	12 (85.7%)	0 (0%)	0 (0%)
	2nd gr. –	–	–	–	–	–	–	–
Erosive and hemorrhagic gastropathy	1st gr. n=16	2 (12.5%)	0 (0%)	5 (31.3%)	9 (56.2%)	4 (25.0%)	0 (0%)	0 (0%)
	2nd gr. –	–	–	–	–	–	–	–
Reflux gastritis	1st gr. n=14	2 (14.3%)	3 (21.4%)	4 (28.6%)	5 (35.7%)	10 (71.4%)	0 (0%)	0 (0%)
	2nd gr. n=1	0 (0%)	1 (100.0%)	0 (0%)	0 (0%)	1 (100.0%)	0 (0%)	0 (0%)

Note: due to the small number of patients in the 2nd group, it was not possible to calculate the reliability between the signs by group.

parametric and non-parametric methods of evaluation of the results.

RESULTS

As indicated by the results of the obtained data (summarized data of the conducted tests for the detection of HP), HP infection was detected in 90.2% of patients with lumbar spine OC and chronic gastritis.

All patients underwent FEGDS with biopsy, the results of which are presented in Table 1. At the same time, the severity of gastric MM changes in patients with lumbar spine OC was assessed depending on the presence or absence of HP infection, according to which the patients were divided into two groups: group 1 included 92 patients with OC and CG infected with HP, and group 2 there were 10 HP-negative patients.

Evaluating the obtained results presented in Table 1 when comparing the signs of gastritis with FEGDS in pa-

Table 3. The degree of MM insemination of the stomach with HP in patients with OC and CG according to L.Y. Aruin

Degree of MM insemination of the stomach with HP	1st group HP-positive patients with CG and spine OC n=92
High	74 (80.4%)
Average	14 (15.2%) *
Low	4 (4.4%)
There are no bacteria in preparation	0

Note: the difference between indicators in patients with high and medium degrees of insemination is significant: * - p <0.01.

tients with lumbar spine OC depending on the infection of HP, it was established that erythematous gastropathy was detected mainly in HP-negative patients (in 7 (70.0%) cases), and only in 8 (8.7%) patients with OC infected with HP, respectively. Erosive gastropathy was most often diagnosed among HP-infected patients with lumbar spine OC (in 40 (43.5%) examined and only in 2 (20.0%) cases in HP-negative patients). Erosive-papular and erosive-hemorrhagic gastropathy was not diagnosed in HP-negative patients with FEGDS, while in HP-infected patients it was detected in 14 (15.2%) and 16 (17.4%), respectively. Signs of reflux gastritis were found in only one HP-negative patient with lumbar spine OC, and in 14 (15.2%) patients with a positive HP.

Histological evaluation was performed from the biopsy of the MM of the stomach in the examined patients with OC according to the Sydney evaluation system, shown in Table 2.

Analysis of the obtained data indicates a difference between the indicators of the degree of inflammatory process and the activity of the stomach MM process at various endoscopic diagnoses and depending on the presence or absence of HP infection in patients with lumbar spine OC. Thus, the highest degree of activity was found in 85.7% of patients with erosive-papular gastropathy, in 70.0% of patients with erosive gastropathy and in 71.4% of those examined with 1st group reflux gastritis. In HP-negative patients, only minor activity was found in erythematous gastropathy and reflux gastritis. Therefore, there is a tendency towards more pronounced activity of the process in patients from the 1st group.

According to the results regarding the activity of the stomach MM process, it was established that a higher activity of CG was more often observed in patients from the 1st group compared to the 2nd group, regardless of the type of gastropathy. Comparing the degrees of the inflammatory process in HP-infected patients of the 1st group, it was found that the inflammatory process of the 2nd and 3rd degree was more often established in patients with erosive, erosive-papular and erosive-hemorrhagic gastropathy.

During the examination regarding the infection of HP patients, 92 patients who were HP-infected underwent determination of the degree of MM insemination of the HP stomach according to the method of L.Y. Aruin et al. - Table 3.

It was established that in patients with lumbar spine OC and CG, a high degree of insemination was detected significantly more often in comparison - 80.4%, respectively. The average and low degree of insemination was found only in 15.2% and 4.4% of the examined, respectively (p<0.05).

Therefore, in patients with OC and CG, a high frequency of HP infection was found, which, accordingly, will lead to a more pronounced lesion of the MM of the stomach in these patients.

DISCUSSION

HP is one of the most common bacterial infections, with about half of humanity infected with it. The results of recent studies have shown that in several European regions the level of HP infection remains high; the lowest level of prevalence of HP was in Northern Europe, and the highest was observed in Eastern and Southern Europe [16-18].

H. pylori colonizes the gastric mucosa, causing chronic active gastritis, which in a small percentage of cases eventually progresses to gastric cancer [19]. There is a direct relationship between factors such as alcohol consumption, smoking, use of medications such as NSAIDs and HP infection. It was found that alcohol and tobacco have a direct necrotic effect, contributing to oxidative stress in the cells of the gastric mucosa [20, 21]. These changes are especially pronounced against the background of periodic or constant use of NSAIDs.

The results of our research indicate a high frequency of infection in patients with OC who have been taking NSAIDs for a long time. At the same time, as indicated by the obtained results, in patients with lumbar spine OC and CG endoscopically, more pronounced changes in the MM of the stomach were established precisely when infected with HP. The results of our research in-

dicate a more pronounced degree of inflammation of the MM of the stomach during histological examination in HP-infected patients. In patients with HP-positive CG, erosive-papular and erosive-hemorrhagic forms of gastropathy are associated with 2 and 3 degrees of inflammation, especially in patients with a high degree of MM insemination of the stomach.

CONCLUSIONS

1. 90.2% of patients with lumbar spine OC and CG have been diagnosed with HP infection, this is a high de-
gree of insemination of HP in stomach MM (in 80.4% of the examined - p < 0.01).
2. Endoscopically, the lesion of the stomach MM in patients with lumbar spine OC corresponds mainly to erosive and erosive-hemorrhagic forms of gastropathy - in 43.5% and 17.4% of the examined, respectively.
3. During histological examination of stomach MM in HP-positive patients with lumbar spine OC, mainly 2nd and 3rd degrees of inflammation were established, especially in patients with erosive, erosive-papular and erosive-hemorrhagic forms of gastropathy.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Vitaliy M. Kochmar
Uzhhorod national university
1 Narodna sqr., 88000 Uzhhorod,. Ukraine
e-mail: stepan.filip@uzhnu.edu.ua

ORCID AND CONTRIBUTIONSHIP
Stepan S. Filip: 0000-0002-6549-3892 Ⓐ Ⓕ
Vitaliy M. Kochmar: 0000-0002-0902-1743 Ⓑ Ⓒ Ⓓ Ⓔ
Vasyl V. Stoika: 0000-0003-2219-2544 Ⓑ Ⓔ
Ivan I. Pushkash: 0000-0002-5728-2980 Ⓓ

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

ORIGINAL ARTICLE

Evaluation of functional interaction masseter and sternocleidomastoid muscles affected by TMJ dysfunction

Victoria V. Vovk, Vadym S. Skibitskyi

O. O. BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

ABSTRACT

Aim: To evaluate the functional connection and the bioelectrical state of the m.masseter and m. sternocleidomastoid using functional tests before and after treatment.

Materials and Methods: The sample consisted of 21 individuals with temporomandibular joint dysfunction. Examinations were carried out before and after treatment using repositioning splint therapy and in seated/standing positions.

Results: M. masseter - $p=0.072$ before treatment and $p=0.821$ after treatment. Symmetry is also maintained after treatment. After treatment, a significant difference is noted at the level of significance $p<0.001$ for the right chewing muscle. In seated and standing positions before treatment did not reveal a statistically significant difference ($p=0.07$, $p=0.143$) and after ($p=0.272$, $p=0.623$).M. sternocleidomastoid- $p<0.001$ when comparing right and left sides. After treatment, there was no difference between the right and left sides ($p=0.169$). No statistical difference was found when assessing indicators separately for the right and left muscles in seated and standing positions ($p=0.304$, $p=0.611$, $p=0.089$, $p=0.869$). When comparing the bioelectric potentials of the right muscle before, after treatment, a statistically significant difference was found $p=0.001$.

Conclusions: Biostatistical analysis of the indicators of bioelectrical activity of m. masseter and sternocleidomastoid indicates no changes in muscle microvolt indicators with changes in body position in patients. However, repositioning splint therapy is associated with reduced muscle tone in initially more spasmodic muscles. It is worth noting that the symmetry of interaction between muscles improves.

KEY WORDS: EMG, muscles, splint, repositioning, computed tomography, TMJ

INTRODUCTION

Parafunctions of the chewing muscles are one of the most common pathologies, represented separately and together by myofascial pain syndrome or symptoms [1]. Modern examination methods allow for a more precise consideration of these pathologies and dysfunctions, which include the disease.

Muscle research using electromyography is one of the most common methods [2]. Thus, it has been investigated that patients with dysfunction of the right temporomandibular joint predominantly exhibit left-sided chewing and vice versa [3,4]. During maximal opening tests, a larger mean number of intersections between interincisal O-C paths during mastication and smaller asymmetry between working and balancing sides have been observed [5]. Some authors have investigated, for example, myofascial trigger points within the upper trapezius in patients with TMD. They noted that changes in this muscle are related to changes in electromyographic patterns of masticatory muscles [6,7,8,9]. However, other studies have shown a lack of correlational interactions [10]. Further research focusing on the adjacent spinal section and functional connection and interaction with the stomatognathic apparatus will improve the provision of qualified dental care.

AIM

The aim of the research is to evaluate the functional connection and the bioelectrical state of m. masseter and m. sternocleidomastoid using functional tests before and after treatment.

MATERIALS AND METHODS

The sample consisted of 21 individuals. Inclusion criteria were age 18-35 years, dysfunction of the temporomandibular joint, specifically, persistent displacement of the lower jaw, and informed patient consent. Exclusion criteria included connective tissue diseases, injuries to the maxillofacial and cervical spine regions, neurological disorders, intake of muscle relaxants, visits to physical rehabilitation doctors, and any influences from adjacent specialists. Biostatistical analysis was performed using the EZR v. 1.54 software pack-

age (a graphical interface to R statistical software v. 4.0.3, R Foundation for Statistical Computing, Vienna, Austria). Joint head position analysis was conducted using cone-beam tomography and Planmeca Romexis Viewer software. The bioelectrical activity of chewing muscles and muscles of the cervical spine, namely m. masseter and m. sternocleidomastoideus was evaluated. The study was conducted with functional tests in sitting and standing positions. Mandatory recommendations included refraining from lifting heavy objects, visiting the gym, wearing new shoes, and performing any interventions in the head or neck area one week before the examination. The investigation used a portable computerized 8-channel electromyograph, "Bio-EMG III" (BioRESEARCH Associates. Inc., USA). An advantage is that the characteristics of this device allow for the simultaneous synchronization of all muscles under investigation during tests in standing and sitting positions. The study was conducted twice: at the beginning of treatment and afterward. Treatment was carried out using anterior-lateral, repositioning, and rigid splints. Indicators of each muscle were evaluated separately and compared with body position after treatment. It is worth noting that special attention was paid to muscle symmetry. In doing so, the difference in bioelectrical potentials between symmetric muscles in sitting and standing positions was calculated before and after treatment.

RESULTS

In the sitting position, the following indicators of individual muscle groups were recorded:

1. M. masseter - Student's t-test corresponds to $p=0.072$ before and $p=0.821$ after treatment. This indicates no statistically significant difference in symmetry between the right and left muscles before treatment. Symmetry is also preserved after treatment. However, after treatment, a significant difference is noted at the level of significance $p<0.001$ for the right chewing muscle, where initially the highest indicators were observed. The average indicators are presented in Table 1.

When comparing the indicators of bioelectrical activity of the right and left chewing muscles in the sitting and standing positions before treatment, no statistically significant difference was found, with $p=0.07$ and $p=0.143$, respectively. After treatment, there were no significant differences in sitting (right, $p=0.272$) and standing (left, $p=0.623$) positions on either side. This indicates that neither before nor after treatment does body position statistically affect the tone distribution in the area of the chewing muscles. Comparing the symmetry difference between sitting and standing positions before and after treatment yielded a significance level of $p<0.001$. This indicates an improvement in the symmetry of the functioning of the chewing muscles.

2. M. sternocleidomastoid - a difference was found at a significance level of $p<0.001$ when comparing the bioelectrical potentials of the right and left muscles. This indicates unilateral biomechanical overload of the myofascial chain element. However, after treatment, there was no difference between the right and left sides, with $p=0.169$. This indicates a balance in symmetry. The data are presented in Table 2.

It should be noted that when comparing the bioelectrical potentials of the right muscle before and after treatment, a statistically significant difference was found at a significance level of $p=0.001$. Initially, the highest µV indicators were explicitly recorded on the right side. When assessing the indicators separately before and after treatment, sitting and standing, for both the right and left muscles, no statistical difference was found, with $p=0.304$, $p=0.611$, $p=0.089$, and $p=0.869$, respectively. Therefore, in patients, there are no changes in the µV indicators of muscles with changes in body position. The differences in the symmetry of the investigated muscle in these body positions also remain unchanged, with $p=0.053$ before and $p=0.284$ after treatment, respectively.

DISCUSSION

Parafunctions of the chewing muscles are one of the most prevalent manifestations, both as persistent displacement of the lower jaw and as a separate nosological form. The biofunctional connection between adjacent areas is described in the literature as postural myofascial chains [11-13]. It should be noted that the correlation interactions between the chewing muscles and the muscles of the cervical spine are also based on common innervation [14,15]. This explains clinical manifestations such as referred pain. Based on these facts, we assessed the functional connection of some of the strongest muscles in the stomatognathic apparatus - m. masseter and the equally important muscle of the cervical spine - m. sternocleidomastoid. To do this, we used cone-beam tomography and electromyography. Comparison was made with functional tests in sitting and standing positions, and before and after treatment. It is important to emphasize the indicators of bioelectrical potentials of each muscle separately and the difference in symmetry of interactions between them, as described in this article.

The initial augmentation in muscular tone was evident bilaterally within the jaw muscles, with a notable predominance on the right side within the neck muscle. This asymmetrical manifestation indicates an initial discordance in the orchestration of movements, potentially impinging upon functional dynamics, notably influencing head rotational patterns.

Repositioning splint therapy served as the cornerstone of our treatment approach, offering a powerful intervention avenue. Ensuring the integrity and robustness of our experiment, we meticulously adhered to stringent exclusion criteria.

Table 1. Average bioelectrical potentials of m. masseter µV before and after treatment

Before Treatment	Median	I Quartile	III Quartile	Median Absolute Deviation	Left (95%CI)	Right (95%CI)
R	4.4	3.6	4.7	0.611	3.6	4.7
L	2.1	1.9	4.4	0.589	1.9	4.4
After Treatment						
R	2.4	2	2.9	0.274	2	2.9
L	2.6	1.5	3.5	0.279	1.5	3.5

Table 2. Average bioelectrical potentials of m. sternocleidomastoid µV before and after treatment

Before Treatment	Median	I Quartile	III Quartile	Median Absolute Deviation	Left (95%CI)	Right (95%CI)
R	3.9	2.9	5.4	0.787	2.9	5.4
L	2.8	2.2	3.2	0.801	2.2	3.2
After Treatment						
R	1.9	1.4	2.7	0.391	1.4	2.7
L	1.7	1.2	4.3	0.556	1.2	4.3

Specifically, patients were prohibited from using muscle relaxants, and visits to various specialists, spanning from physical rehabilitation doctors to chiropractors, and others were not allowed. This rigorous adherence to protocol underscored our commitment to methodological rigor and the unequivocal pursuit of scientific accuracy.

Ensuring the accuracy of setting the precise movement dynamics across three planes - axial, coronal, and sagittal - yielded positive impact. Additionally, certain redistribution of myofascial tone among muscles in adjacent anatomical regions was observed. Notably, areas exhibiting heightened tone demonstrated a subsequent reduction post-treatment. Furthermore, noteworthy finding emerged regarding the appearance of symmetry in synergistic muscles following treatment, notably evident in m.scm (p=0.169). Importantly, the preservation of symmetry in the jaw muscles persisted even after treatment.

Henceforth, the findings underscore the efficacy of split therapy in modulating neck muscle function, highlighting its potential as a transformative treatment modality. Furthermore, the consistent electromyographic readings throughout the study, regardless of body position, underscore the robust nature of muscle tone regulation. Even post-treatment, when subjects underwent varied body positioning, no fixed changes were noted. Hence, it can be assumed that the tone of m.masseter and m.sternocleidomastoid is not dependent on the body position and does not participate in a vertical stabilization of the postural complex. These observations challenge conventional paradigms of postural stability and beckon for a deeper investigative approach. Moving forward, expanding the sample size and integrating additional functional assessments will be instrumental in elucidating the intricate correlational dynamics inherent in these findings.

CONCLUSIONS

A characteristic feature was increased bioelectrical potential indicators observed in both investigated muscles - m. masseter and scm - in patients with persistent displacement of the lower jaw. However, asymmetry was predominantly observed in m. scm. The indicators changed significantly after introducing functional dynamics of lower jaw movements using the method of repositioning splint therapy. The most significant changes occurred predominantly at the right chewing muscle level. Meanwhile, at the level of m. scm, symmetry indicators were balanced. An interesting fact is that when measuring the bioelectrical potentials of an individual muscle synergist on the right and left sides in sitting and standing positions, no difference was observed. Discovering new biomechanical interactions between muscles of different anatomical regions requires further in-depth study.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Victoria V. Vovk
O.O. Bogomolets National Medical University
1 Zoolohichna st, 03057 Kyiv, Ukraine
e-mail: viktorija.viktorovna.vovk@gmail.com

ORCID AND CONTRIBUTIONSHIP
Victoria V. Vovk: 0000-0001-5658-1287 **B** **C**
Vadym S. Skibitskyi: 0009-0009-1425-2261 **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

ORIGINAL ARTICLE

Dynamic indicators of acute respiratory diseases treatment in children after correction

Olesya M. Horlenko[1], Iryna Yu. Pikina[1], Lyubomyra B. Prylypko[1], Gabriella B. Kossey[1], Ihor H. Roshko[2], Valeriya V. Brych[1], Antonina V. Kedyk[1]

[1]UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE
[2]CNE "UZHHOROD CITY CHILDREN'S CLINICAL HOSPITAL», UZHHOROD, UKRAINE

ABSTRACT

Aim: To study the Respiratory pathology of the upper respiratory tract, markers of the inflammatory response of the organism, Oxidative stress, Metabolic adaptation and possibilities of correction.

Materials and Methods: The study group (n=111) included school-aged children (10-14 years old). The general group of inflammatory diseases of the respiratory tract (J000-J06) was considered, with a diagnosis of acute respiratory infection (ARI) of viral and bacterial origin and included local inflammation of the upper respiratory tract with presentation of acute pharyngitis (68.0%), acute bronchitis (22,0%), acute tonsillitis (10,0%).

Results: Dynamic observation of groups of children who received optimized (group 1, n=60) and basic (group 2, n=51) treatment was carried out. The level of the erythrocyte pool correlated with IL-1 (r=-0,29, p=0,03), IL-4 (r=0,32, p=0,01), TNF-α (r=-0,35 , p=0,006). Creatinine value correlated with IL-10 (r=0,3, p=0,005), γ-IFN (r=0,42, p=0,001), TNF-α (r=0,25, p=0,05). Correlations of ferritin presented positive correlation values with the level of total protein (r=0,26, p=0,04) and TNF-α (r=0,41, p=0,001).

Conclusions: After the optimized treatment, there was a significant decrease in the reliable levels of CRP and γ-IFN by 7 and 4,4 times (by groups) and 5,8 and 3,2 times (by groups), respectively. Correlation relationships of urea levels with IL-2,4 were detected. The level of the erythrocyte pool correlated with IL-1,4, TNF-α, Ferritin presented positive correlation values with the level of total protein,TNF-α .

KEY WORDS: acute respiratory diseases of the upper respiratory tract (acute pharyngitis, acute bronchitis, acute tonsillitis), markers of inflammation, indicators of antioxidant protection, metabolic adaptation, correction, children

INTRODUCTION

Infection of a child is a complex multicomponent dynamic process of interaction of infectious pathogenic agents with the organism, characterized by the development of a complex of typical pathological reactions and systemic functional disorders. Research shows that the complex interaction between the respiratory microbiome and the child's immune response affects the pathogenesis and severity of the course of infectious diseases [1].

The action of infectious pathogenic factors leads to the development of direct and cytokine-mediated systemic, functional and metabolic disorders that form the basis of the prodromal period of the main manifestations of an infectious disease [2]. Characteristic nonspecific metabolic signs that unfold in the prodromal period and during the period of severe clinical manifestations are changes in protein homeostasis: increased synthesis of acute-phase proteins by hepatocytes and macrophages (fibrinogen, CRP, cerusoplasmin, antihemorrhagic

globulin, VI and XI coagulation factors, anticoagulants, alpha-2 -macroglobulin, etc.). Together with the increase in the synthesis of these positive markers of the acute phase of inflammation, there is a decrease in the synthesis of albumins and transferrin - negative markers of the systemic inflammatory response syndrome [2].

The respiratory tract is a complex system of organs, that is responsible for the exchange of oxygen and carbon dioxide, and the respiratory tract microbiota is believed to act as a gatekeeper that provides resistance to colonization by respiratory pathogens. Respiratory microbiota can also participate in maintaining the homeostasis of respiratory physiology and immunity [3].

Oxidative processes and the formation of free radicals are an integral part of human metabolism. Oxidative-reduction biology encompasses events involving a shift in the balance between reactive oxygen species (ROS) and their removal [4]. Infection with respiratory viruses is usually associated with cytokine production,

Table 1. Dynamics of biochemical indicators under the influence of various treatment methods

Indicator	Group 1 (optimized treatment) (n=60)		Group 2 (basic treatment) (n=51)		Control group (n=25)
	Before treatment	After treatment	Before treatment	After treatment	
1	2	3	4	5	6
Total protein, g/l	66,49 ± 7,04	74,42 ± 3,98 (p_1<0,01; p_2=0,20)	67,72 ± 7,30	71,09 ± 3,18 (p_3=0,003; p_4=0,06; p_5<0,01)	73,03 ± 5,68
Total bilirubin, μmol/l	15,72 ± 9,34	12,68 ± 2,91 (p_1=0,02; p_2=0,19)	15,98 ± 9,01	14,75 ± 3,95 (p_3=0,37; p_4=0,31; p_5=0,002)	13,74 ± 4,26
Creatinine, μmol/l	79,66 ± 21,63	72,97 ± 8,57 (p_1=0,03; p_2=0,05)	78,77 ± 17,93	71,51 ± 6,38 (p_3=0,008; p_4=0,15; p_5=0,32)	69,14 ± 7,03
Urea, mmol/l	4,72 ± 1,26	3,87 ± 0,82 (p_1<0,01; p_2=0,77)	4,92 ± 1,37	4,27 ± 1,05 (p_3=0,008; p_4=0,07; p_5=0,03)	3,81 ± 0,99
Glucose, mmol/l	5,59 ± 0,91	4,79 ± 0,67 (p_1<0,01; p_2=0,18)	5,39 ± 0,83	4,96 ± 0,56 (p_3=0,003; p_4=0,005; p_5=0,15)	4,58 ± 0,53
ALT, μmol /л	34,67 ± 24,08	23,15 ± 7,59 (p_1<0,01; p_2=0,003)	34,43 ± 17,47	27,82 ± 5,84 (p_3=0,01; p_4<0,01; p_5<0,01)	18,05 ± 5,31
Alkaline phosphatase, Un/l	349,14 ± 149,12	165,73 ± 58,71 (p_1<0,01; p_2=0,08)	325, 36 ± 138,07	177,95 ± 53,97 (p_3<0,01; p_4=0,004; p_5=0,26)	143,46 ± 31,22

Notes: p_1 – reliability of differences in group 1 between the values of indicators before and after treatment; p_2 – reliability of differences between the values of indicators of the control group and group 1 after treatment; p_3 – reliability of differences in group 2 between the values of indicators before and after treatment; p_4 – reliability of differences between the values of indicators of the control group and group 2 after treatment; p_5 – reliability of differences between the values of indicators of groups 1 and 2 after treatment.

inflammation, cell death, and other pathological processes[5]. Increased levels of oxidation products were detected not only during the active course, but also 3 months after recovery [6].

The respiratory tract occupies the second place after the gastrointestinal tract in terms of the area of the mucous membrane. Therefore, understanding the unique nature of the immune system of the respiratory mucosa is extremely important [7]. According to observations, the frequency of anemia in children who are often sick varied from 9,3 to 20,8%, depending on the presence of chronic tonsillitis in them [8].

Respiratory virus infection, which is associated with cytokine production, inflammation, cell death, and other pathological processes, can be triggered by oxidative stress, changes in the content of certain vitamins and trace elements, which creates conditions for the development of a number of pathological conditions, including acute and recurrent respiratory diseases. A summation of mutual influences is often found between individual indicators of cellular immunity, which is probably of significant importance for the functioning of the protective systems of the organism [9]. Thus, the mucous membranes of the nasopharynx and oropharynx are the entrance gates of respiratory infections. Aggressive environmental conditions, the presence of chronic foci of infection, disruption of the microbiocenosis of saprophytic microflora disrupt the colonization resistance of MALT of the respiratory tract of children. Therefore, the possibility of activating their own protective forces is a way not only to treat, but also to prevent infections of the upper respiratory tract (rhinitis, sinusitis, nasopharyngitis, tonsillitis, pharyngitis, tonsillopharyngitis).

Table 2. Dynamic indicators of mineral metabolism in children

Indicator	group 1 (n=60)		group 2 (n=51)		Control group (n=25)
	Before treatment	After treatment	Before treatment	After treatment	
1	2	3	4	5	6
K, mmol/l	$4,17 \pm 0,52$	$4,39 \pm 0,41$ (p_1=0,01; p_2=0,47)	$4,21 \pm 0,51$	$4,27 \pm 0,38$ (p_3=0,48; p_4=0,71; p_5=0,10)	$4,31 \pm 0,56$
Na, mmol/l	$141,77 \pm 6,33$	$141,66 \pm 5,17$ (p_1=0,92; p_2=0,29)	$141,69 \pm 6,79$	$142,72 \pm 6,22$ (p_3=0,43; p_4=0,08; p_5=0,33)	$140,47 \pm 3,35$
Ca, mmol/l	$2,03 \pm 0,08$	$2,18 \pm 0,18$ (p_1<0,01; p_2<0,01)	$2,02 \pm 0,09$	$2,07 \pm 0,09$ (p_3=0,008; p_4<0,01; p_5<0,01)	$2,39 \pm 0,08$
Fe, µmol/l	$51,55 \pm 23,28$	$79,07 \pm 19,72$ (p_1<0,01; p_2<0,01)	$51,93 \pm 19,95$	$72,14 \pm 14,03$ (p_3<0,01; p_4<0,01; p_5=0,04)	$95,17 \pm 13,52$
Zn	$7,78 \pm 2,23$	$13,58 \pm 1,87$ (p_1<0,01; p_2=0,008)	$7,87 \pm 2,09$	$10,09 \pm 1,27$ (p_3<0,01; p_4<0,01; p_5<0,01)	$15,19 \pm 3,62$
Cu	$69,82 \pm 29,13$	$115,43 \pm 10,98$ (p_1<0,01; p_2=0,30)	$70,60 \pm 28,93$	$92,43 \pm 13,59$ (p_3<0,01; p_4<0,01; p_5<0,01)	$118,57 \pm 16,47$

Notes: p_1 – reliability of differences in group 1 between the values of indicators before and after treatment; p_2 – reliability of differences between the values of indicators of the control group and group 1 after treatment; p_3 – reliability of differences in group 2 between the values of indicators before and after treatment; p_4 – reliability of differences between the values of indicators of the control group and group 2 after treatment; p_5 – reliability differences between the values of indicators of groups1 and 2 after treatment.

AIM

To investigate the respiratory pathology of the upper respiratory tract, markers of the inflammatory response of the child's orgnaism, oxidative stress, metabolic adaptation in them, the possibilities of correction.

MATERIALS AND METHODS

The research group included school-age children (10-14 years old). The general group of inflammatory diseases of the respiratory tract (J000-J06) was considered, with a diagnosis of acute respiratory infection (ARI) of viral and bacterial origin and included local inflammatory lesions of the upper respiratory tract with presentation of acute pharyngitis (68,0%), acute bronchitis (22,0%), acute tonsillitis (10,0%), and a control group (n=25), identical in age and gender, using the Unified clinical protocol of primary and medical care for adults and children «Severe respiratory infections», protocol No. 85, dated February 11, 2016.

Researches were conducted at the clinical base of the Uzhhorod City Council KNP «Uzhhorod City Children's Clinical Hospital». General clinical and immunological studies, analysis of markers of the inflammatory response of the child's body in dynamics under the influence of the addition of mineral-vitamin complex and lysozyme preparations were carried out. Statistical analysis of patient examination data was performed using the Statistics for Windows v.10.0 computer program (StatSoft Inc, USA). The evaluation of the obtained results was carried out using parametric and non-parametric methods.

RESULTS

Dynamic monitoring of groups of children who received optimized (group 1, n=60) and basic (group 2, n=51) treatment was carried out, according to the protocols of the Ministry of Health and guidelines.

Dynamic indicators of biochemical research in children is presented in Table 1.

According to Table 1, dynamic changes in indicators under the influence of various treatment methods are

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Table 3. Indicators of the inflammatory response in children after treatment

Indicator	Group 1 (optimized treatment) (n=60)		Group 2 (basic treatment) (n=51)		Control group (n=25)
	Before treatment	After treatment	Before treatment	After treatment	
1	2	3	4	5	6
IL-1 (0-11, pg/ml)	25,87 ± 9,94	2,11 ± 1,86 (p_1<0,01; p_2=0,94)	26,13 ± 9,53	3,98 ± 1,78 (p_3<0,01; p_4<0,01; p_5<0,01)	2,08 ± 0,49
IL-2 (0-10, pg/ml)	9,12 ± 5,81	1,64 ± 0,65 (p_1<0,01; p_2<0,01)	9,48 ± 5,99	5,50 ± 3,15 (p_3<0,01; p_4<0,01; p_5<0,01)	0,41 ± 0,05
IL-4 (< 0,5 ng/ml)	6,06 ± 3,23	0,99 ± 0,69 (p_1<0,01; p_2<0,01)	5,93 ± 3,21	2,43 ± 1,16 (p_3<0,01; p_4<0,01; p_5<0,01)	0,37 ± 0,14
IL-6 (0-10, pg/ml)	16,46 ± 7,09	3,60 ± 1,29 (p_1<0,01; p_2=0,26)	16,77 ± 7,28	5,42 ± 1,34 (p_3<0,01; p_4<0,01; p_5<0,01)	3,29 ± 0,67
IL-10 (0-20, pg/ml)	15,57 ± 11,60	3,63 ± 1,70 (p_1<0,01; p_2<0,01)	15,46 ± 12,42	5,97 ± 4,03 (p_3<0,01; p_4<0,01; p_5<0,01)	2,13 ± 0,31
γ-IFN (< 15, pg/ml)	52,28 ± 49,62	9,29 ± 7,79 (p_1<0,01; p_2=0,03)	51,58 ± 46,24	16,83 ± 12,68 (p_3<0,01; p_4<0,01; p_5<0,01)	5,65 ± 0,85
TNF-α (< 6, pg/ml)	15,63 ± 21,49	6,04 ± 2,03 (p_1<0,01; p_2<0,01)	15,72 ± 2,03	6,74 ± 1,42 (p_3<0,01; p_4<0,01; p_5=0,04)	3,43 ± 0,47
Neopterin (< 10, nmol/l)	88,66 ± 55,89	30,81 ± 18,53 (p_1<0,01; p_2<0,01)	91,32 ± 54,56	37,66 ± 15,03 (p_3<0,01; p_4<0,01; p_5=0,04)	6,34 ± 1,14
Cortisole (110-692, nmol/l)	531,52 ± 133,23	338,91 ± 58,41 (p_1<0,01; p_2<0,01)	548,11 ± 131,68	518,43 ± 60,89 (p_3=0,15; p_4<0,01; p_5<0,01)	251,46 ± 118,82
Ferritine (10-291, ng/ml)	60,97 ± 27,17	75,29 ± 23,80 (p_1=0,003; p_2=0,46)	60,18 ± 28,24	64,23 ± 14,01 (p_3=0,36; p_4=0,02; p_5=0,004)	71,59 ± 10,19
CRP (<5, mg/l)	23,84 ± 2,78	3,84 ± 1,33 (p_1<0,01; p_2=0,02)	23,62 ± 2,87	5,07 ± 1,13 (p_3<0,01; p_4<0,01; p_5<0,01)	3,09 ± 1,16

Notes: p_1 – reliability of differences in group 1 between the values of indicators before and after treatment; p_2 – reliability of differences between the values of indicators of the control group and group 1 after treatment; p_3 – reliability of differences in group 2 between the values of indicators before and after treatment; p_4 – reliability of differences between the values of indicators of the control group and group 2 after treatment; p_5 – reliability of differences between the values of indicators of groups 1 and 2 after treatment.

observed. It is especially worth noting that there is a predominance of positive probable differences in the group of children with an optimized therapy scheme in the levels of total bilirubin ($p5$=0,002), total protein ($p5$<0,01), ALT ($p5$<0,01), urea ($p5$=0,03).

Changes in the levels of indicators of mineral

Table 4. Correlation relationships of indicators of children with respiratory pathology after treatment using optimized therapy

Laboratory parameters		Correlation coefficient (r)	Statistical significance (p)
IL-1	Erythrocytes	-0,29	0,03
IL-2	ESR	0,29	0,02
	Urea	0,30	0,02
IL-4	Erythrocytes	0,32	0,01
	Urea	0,26	0,05
	IL-6	0,31	0,02
	TNF-α	-0,30	0,02
IL-6	IL-4	0,31	0,02
IL-10	ESR	-0,27	0,04
	Glucose	-0,27	0,04
	Creatinine	0,36	0,005
	Neopterine	0,26	0,04
γ-IFN	Creatinine	0,42	0,001
TNF-α	ESR	-0,30	0,02
	Erythrocytes	-0,35	0,006
	Creatinine	0,26	0,05
	Ferritin	0,41	0,001
	IL-4	-0,30	0,02
Neopterin	IL-10	0,26	0,04
CRP	K	-0,39	0,002
Ferritin	Total protein	0,26	0,04
	TNF-α	0,41	0,001

metabolism of blood serum in the studied groups of children are considered in Table 2.

According to the data in Table 2, there are also positive effects of optimized therapy on the levels of microelements copper (p5<0,01), zinc (p5<0,01), iron (p5=0,04) and macroelement calcium (p5<0,01). An increase in the level of zinc is observed, which is part of approximately 300 different proteins and plays an extremely important role in the functioning of the organism, promoting the reaction of antioxidant protection, stabilizing biological membranes and functioning of the glands of internal secretion. The trace element copper is involved in the immune reactions of the children's organism, tissue respiration, mechanisms of enzymatic catalysis, supports the processes of cell proliferation, which determines it's importance in the regeneration of the mucous membrane. It's biological significance is due to the fact that Cu+ and Cu2+ ions are components of numerous enzymes and proteins, about 20 are currently known, in particular Cytochrome C oxidase is a terminal protein complex that plays a decisive role in regulating the functioning of the entire respiratory chain.

Also, Cu/Zn-dependent superoxide dismutase is the starting chain of the antioxidant defense system of the child's organism. Interdependence with iron exchange is reflected in the presence of copper in the composition of ceruloplasmin. The existence of physiological antagonism between copper, on the one hand, and zinc, molybdenum, and magnesium, on the other, has also been scientifically proven. Iron participates in the synthesis of hemoglobin and myoglobin, catalase and peroxidase, in direct and indirect oxidative processes (includes 72 enzymes), ensuring normal functioning of the immune system [10]. Therefore, according to the obtained data, it can be stated that the imbalance of mineral metabolism indicators in children diminshes after treatment, positive effects on immune processes and strengthening of antioxidant protection are revealed.

The analysis of inflammatory markers is an important research chain in respiratory pathology (Table 3).

According to Table 3, there are significant changes in all parameters after treatment, in particular, IL-1 (p5<0,01) in group 1 (from 25,87 ± 9,94 to 2,11 ± 1,86 pg/ml, p1< 0,01; p2=0,94 to the data of group 2 (from 26,13 ± 9,53 to 3,98 ± 1,78 pg/ml, p3<0,01; p4<0,01) and in comparison with the data of the control group (2,08 ± 0,49 pg/ml) to the limit of reference values; IL-2(p5<0,01) with variations within physiological limits; IL-4(p5<0,01) in group 1 (from 6,06 ± 3,23 to 0,99
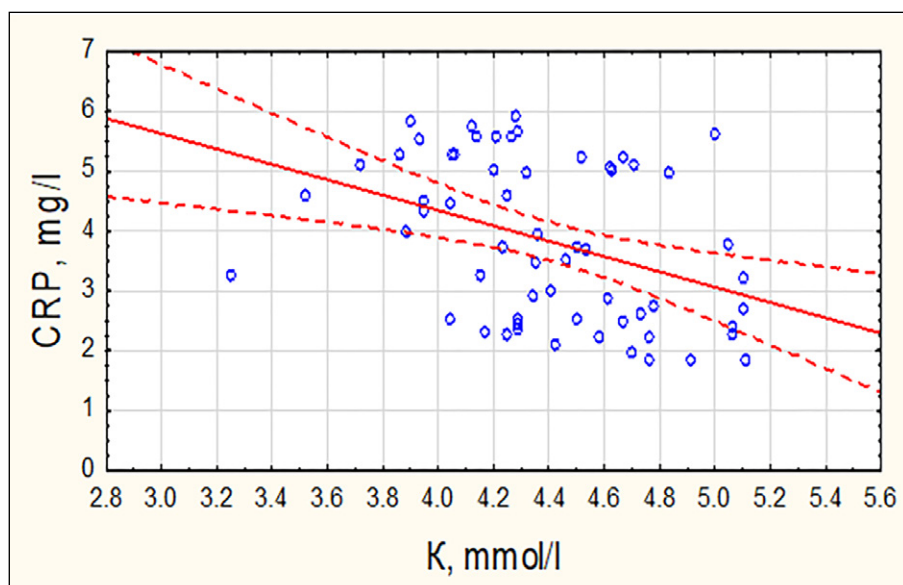
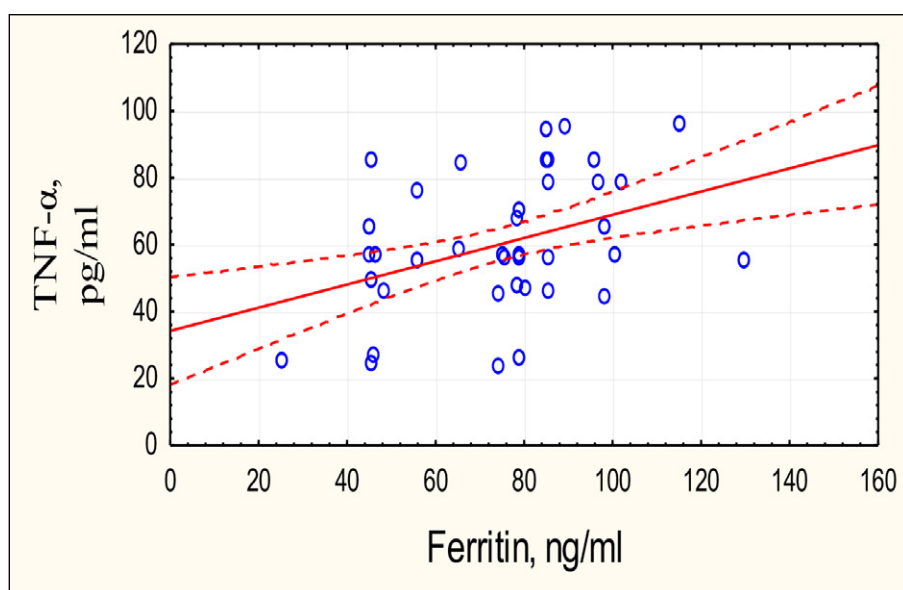**Fig. 1.** Correlation between the levels of CRP and K in the blood (r= -0,39; p=0,002).

Fig. 2. Correlation between the levels of TNF-α and ferritin in the blood (r= 0,41; p=0,001).

± 0,69 ng/ml, p1<0,01; p2<0,01) and group 2 (from 5,93 ± 3,21 to 2,43 ± 1, 16 ng/ml, p3<0,01, p4<0,01) compared to the data of the control group (0,37 ± 0,14 ng/ml) did not reach the values of the upper limit of reference values after treatment; IL-6 (p5 <0,01) and IL-10 (p5<0,01), the data varied within the reference range with a tendency to decrease. The lowest levels of reliability were observed for the dynamic indicators of TNF-α (p5<0,04) and Neopterin (p5<0,04), and did not reach the upper reference limit, especially the value of eopterin. The level of cortisol (p5<0,01) and ferritin (p5<0,004) varied within the reference range. There was a decrease in the reliable levels of CRP and γ-IFN after treatment by 7 and 4,4 times (by groups) and 5,8 and 3,2 times (by groups, respectively).

Correlation analysis of indicators of the group with optimized therapy was carried out (Table 4).

Positive correlations are observed between the levels of urea, IL-2 (r=0,30, p=0,02) and IL-4 (r=0,26, p=0,05). The level of the erythrocyte pool correlates with IL-1 (r=-0,29, p=0,03), IL-4 (r=0,32, p=0,01), TNF-α (r=-0,35, p=0,006). Creatinine value correlated with IL-10 (r=0,3, p=0,005), γ-IFN (r=0,42, p=0,001), TNF-α (r=0,25, p=0,05). Correlations of ferritin presented positive correlation values: with the level of total protein (r=0,26, p=0,04) and TNF-α (r=0,41, p=0,001).

Indicative correlograms of the obtained interrelationships between markers of inflammation were studied (Fig. 1., Fig. 2.)

The inflammatory cascade of reactions is determined by the protective mechanisms of the child's organism, initiated by infectious triggers of the development of the pathological process.

DISCUSSION

Respiratory diseases initiated by infectious factors create an environment of struggle for spheres of influence in the organism. The condition of the antioxidant system is decisive in this process. The relationship between iron and glucose metabolism has been proven. The expanding role of iron in infection is based on its role in a variety of metabolic processes, including electron transport, DNA synthesis, and protection against oxidative stress [11]. To set up a metabolic adaptation to fight infection, the patient must activate the glycolytic program to enhance immune function and promote antagonistic defense during infection. According to our correlational data, when the level of anti-inflammatory IL-10 increases, the level of glucose decreases (r=-0,27, p=0,04).

Differentiated immune responses of the child's organism must be combined with specific metabolic processes to support the energy needs of these inflammatory reactions, as well as adaptation to certain forms of cellular stress, which are also caused by existing triggers [11,12].

Metabolic interactions during infection between the patient's antioxidant system and the pathogen are natural defenses against the pathological consequences of infection. Currently, there are numerous researches on metabolic adaptation during infection, but this process should be considered as a whole, including both the characteristics of the pathogen and the capabilities of the patient. The term «immunometabolism» and the study of its components are very promising in the analysis of the organism's protective processes and the possibility of preventing pathological effects and correcting harmful consequences.

CONCLUSIONS

1. Changes in indicators under the influence of various treatment methods presented a predominance of positive probable differences in the group of children with an optimized therapy scheme in the levels of total bilirubin (p5=0,002), total protein (p5<0,01), ALT (p5<0,01) and urea (p5=0,03).
2. The prevailing positive effects of optimized therapy on the condition of the levels of trace elements copper (p5<0,01), zinc (p5<0,01), iron (p5=0,04) and macroelement calcium (p5<0,01) are noted, which indicates elimination of imbalance of mineral metabolism indicators in children after treatment and formation of positive effects on immune processes and strengthening of antioxidant protection in the organism.
3. According to the dynamics of inflammatory markers, significant changes are observed in all indicators after treatment, in particular, IL-1 (p5<0,01) in group 1 (from 25,87 ± 9,94 to 2,11 ± 1,86, p1< 0,01; p2=0,94 pg/ml) to the data of group 2 (from 26,13 ± 9,53 to 3,98 ± 1,78 pg/ml, p3<0,01; p4<0,01) and in comparison with the data of the control group (2,08 ± 0,49 pg/ml) to the limit of reference values; IL-2 (p5<0,01) with variation within physiological limits; IL-4 (p5<0,01) in group 1 (from 6,06 ± 3,23 to 0,99 ± 0,69 ng/ml, p1<0,01; p2<0,01) and group 2 (from 5,93 ± 3,21 to 2.43 ± 1,16 ng/ml, p3<0,01, p4<0,01) compared to the data of the control group (0,37 ± 0,14 ng/ml), but did not reach the values of the upper limit of reference values after treatment; IL-6 (p5<0,01) and IL-10 (p5<0,01) data varied within the reference range with a tendency to decrease. Dynamic indicators of TNF-α (p5<0,04) and Neopterin (p5<0,04) were observed with the lowest levels of confidence, and did not reach the upper reference limit, especially the value of Neopterin. The level of cortisol (p5<0,01) and ferritin (p5<0,004) varied within the reference range. There was a 7- and 4.4-fold (by groups) and 5,8- and 3,2-fold (by groups) reduction in reliable levels of CRP and γ-IFN after treatment, respectively.
4. Positive correlations of urea levels with IL-2 (r=0,30, p=0,02) and IL-4 (r=0,26, p=0,05) are presented. The level of the erythrocyte pool correlated with IL-1 (r=-0,29, p=0,03), IL-4 (r=0,32, p=0,01), TNF-α (r=-0,35, p=0,006). The value of creatinine correlated with IL-10 (r=0,3, p=0,005), γ-IFN (r=0,42, p=0,001), TNF-α (r=0,25, p=0,05). Correlations of ferritin presented positive correlation values: with the level of total protein (r=0,26, p=0,04) and TNF-α (r=0,41, p=0,001). The inflammatory cascade of reactions is determined by the protective mechanisms of the child's organism, initiated by infectious triggers of the development of the pathological process.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Olesya M. Horlenko
Uzhhorod National University
1 Narodna squ., 88000 Uzhhorod, Ukraine
e-mail: ohorlenko@gmail.com

ORCID AND CONTRIBUTIONSHIP
Olesya M. Horlenko: 0000-0002-2210-5503 Ⓐ Ⓓ Ⓕ
Iryna Yu. Pikina: 0000-0003-1565-8174 Ⓑ Ⓓ
Lyubomyra B. Prylypko: 0000-0002-4131-5450 Ⓒ Ⓓ
Gabriella B. Kossey: 0000-0003-0811-4929 Ⓓ Ⓕ
Ihor H. Roshko: 0009-0005-0622-5328 Ⓑ Ⓔ
Valeriya V. Brych: 0000-0003-3741-6002 Ⓒ Ⓓ
Antonina V. Kedyk: 0000-0002-7021-9644 Ⓑ Ⓔ

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

ORIGINAL ARTICLE

Obesity and heart failure with preserved ejection fraction

Tetyana M. Ternushchak, Marianna I. Tovt-Korshynska, Oksana M. Moskal, Vasyl V. Kaliy, Taras I. Griadil, Snizhana V. Feysa

UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

Aim: To perform an overall assessment of heart failure with preserved ejection fraction (HFpEF) adults with central obesity.

Materials and Methods: We enrolled HFpEF patients with central obesity (n = 73, mean age 52.4 ± 6.3 years) and without obesity (n = 70, mean age 51.9 ± 7.1 years) and compared with an age-matched healthy subjects who had not suffered from HF (n = 69, mean age 52.3 ± 7.5 years). Physical examination, routine laboratory tests such as fasting blood glucose, fasting insulin, insulin resistance (HOMA) index, serum lipids, haemoglobin, creatinine, ALT, AST, uric acide, hs CRP, TSH, N-terminal proB-type natriuretic peptide (NT-proBNP) and standard transthoracic echocardiogram (2D and Doppler) examinations were performed and assessed.

Results: The average values of diastolic blood pressure (DBP), glucose and lipid profiles, uric acide, hs CRP were found to be significantly higher among obese patients with HFpEF than non-obese**.** Despite more severe symptoms and signs of HF, obese patients with HFpEF had lower NT-proBNP values than non-obese patients with HFpEF (129±36.8 pg/ml, 134±32.5 pg/ml vs 131±30.4 pg/ml, 139±33.8 pg/ml respectively; p < 0.05). However, it was found that patients with high central (visceral) adiposity have more pronounced obesity-related LV diastolic dysfunction, lower E/e' ratio, lower mitral annular lateral e' velocity, an increased LV diastolic dimension and LV mass index. Compared with non-obese HFpEF and control subjects, obese patients displayed greater right ventricular dilatation (base, 35±3.13 mm, 36±4.7 mm vs 33±2.8 mm, 34±3.2 mm and 29±5.3 mm, 30±3.9 mm; length, 74±5 mm, 76±8 mm vs 67±4 mm, 69±6 mm and 60±3 mm, 61±5 mm respectively; p < 0.05), more right ventricular dysfunction (TAPSE 16±2 mm, 15±3 mm vs 17±2 mm, 17±1 mm and 19±2 mm, 20±3 mm respectively; p < 0.05).

Conclusions: Obese patients with HFpEF have higher diastolic BP, atherogenic dyslipidemia, insulin resistance index values and greater systemic inflammatory biomarkers, despite lower NT-proBNP values, which increase the risk of cardiovascular events in future. Echocardiography examination revealed not only significant LV diastolic dysfunction, but also displayed greater RV dilatation and dysfunction.

KEY WORDS: heart failure with preserved ejection fraction, obesity, N-terminal proB-type natriuretic peptide

INTRODUCTION

Heart failure with preserved ejection fraction (HFpEF) is considered to be one of the major issue in healthcare system, due to its increasing prevalence among aged adults, high mortality and morbidity [1].

As a multifactorial and multisystemic disorder HFpEF represents more than 50% of all heart failure cases, but its pathophysiology remains very complex and there are very limited therapeutic options [2].

It's s associated with multiple cardiovascular and non-cardiovascular comorbidities, such as metabolic syndrome, atrial fibrillation, obesity, diabetes, sleep apnea, chronic obstructive pulmonary disease, chronic kidney disease and anaemia [3].

Obesity has been considered a major triggering factor for the development of HFpEF, but the mechanisms remain unclear [4].

The experimental and clinical studies have revealed a significant role of abdominal obesity in promoting low-grade systemic inflammation, related oxidative stress, insulin resistance and dyslipidemia leading to myocardial remodeling [5,6].

Also, recent study have been shown varies systemic triggers for HFpEF, such as proinflammatory conditions, arterial and microvascular dysfunction, cardiac hypertrophy, impaired systolic and diastolic function, and interstitial cardiac fibrosis [7].

Moreover, obesity may effect the progression of HFpEF, including increased myocardial loading, worsening of arterial hypertension, damage of the left ventricular structure and the diastolic and/or systolic function [8].

It has been noted that centrally obese patients have often increased epicardial adipose tissue, that promotes chronic myocardial inflammation and fibrosis via local secretion of pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumour necrosis factor (TNF)-α, which may directly cause remodelling and
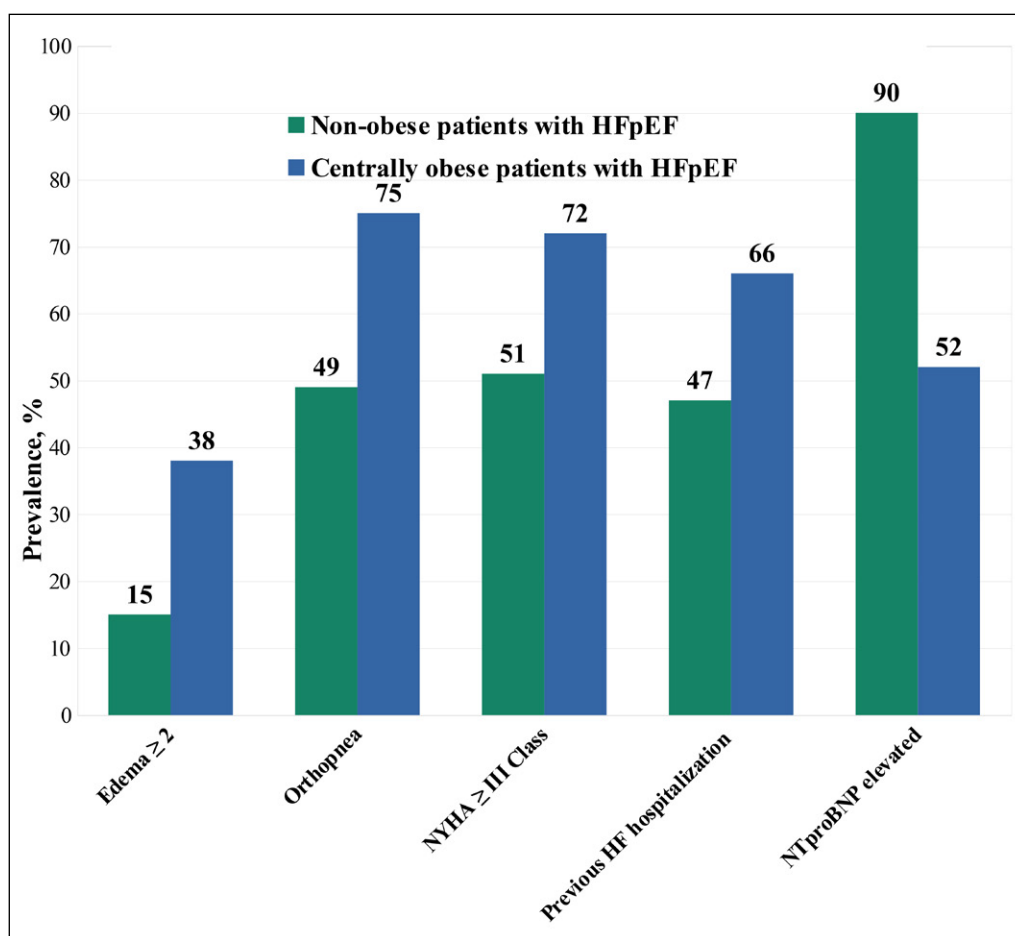
Fig.1. Proportion of patients suffering the symptoms and signs of heart failure.

dysfunction in both cardiac and skeletal muscle through mitochondrial dysfunction, capillary rarefaction and was associated with adverse prognosis in patients with HFpEF [9,10].

Obese patients with HFpEF as compare with non-obese in addition to increased wedge pressure have higher right-to-left-sided filling pressures. This constrictive pattern positively correlates with increased epicardial adipose tissue [11].

Phenotyping patients into pathophysiologically homogeneous groups may enable better targeting of treatment.

AIM

We aimed to perform an overall assessment of HFpEF adults with central obesity.

MATERIALS AND METHODS

We enrolled 143 patients with HFpEF, which were recruited to the study from September to December 2023. The eligible subjects at screening required an age over 50 years, signs and symptoms of heart failure, central obesity (n =73, mean age 52.4 ± 6.3 years), non-obese (n =70, mean age 51.9 ± 7.1 years) compared with

an age-matched healthy subjects who had not suffered from HF (n = 69, mean age 52.3 ± 7.5 years).

There was no statistically significant difference between the study groups and subject controls regarding age, gender or smoking status.

HFpEF was diagnosed according to current HF guidelines: symptoms ± signs of HF, elevated levels of B-type natriuretic peptide and relevant structural heart disease including LV hypertrophy and/or left atrial (LA) enlargement, and/or evidence of diastolic dysfunction on echocardiography.

Body mass index was calculated as weight in kilograms divided by height in meters squared (kg/m^2). General obesity was defined as a BMI ≥ 30 kg/m^2. Central obesity was defined as WC ≥ 88 cm for women and ≥102 cm for men.

Physical examination was performed and routine laboratory tests such as fasting blood glucose, fasting insulin, serum lipids, haemoglobin, creatinine, ALT, AST, uric acid, hs CRP, TSH, NT-proBNP were analyzed at the local hospital laboratory.

Insulin resistance was assessed from the fasting glucose and insulin concentrations by using the HOmeostasis Model Assessment (HOMA) formula: fasting insulin (μU/ml) x fasting glucose (mmol/l)/22.5.

Table 1. Baseline characteristics

	Centrally obese patients with HFpEF (n = 73)		Non-obese patients HFpEF (n = 70)		Control Subjects (n = 69)	
	Female (n = 36)	**Male (n = 37)**	**Female (n = 34)**	**Male (n = 36)**	**Female (n = 34)**	**Male (n = 35)**
Systolic BP (mmHg)	134.2±20.4	136.1±28.5	133.7±29.2	135.1±30.4	113.8±22.5	115.7±20.6
Diastolic BP (mmHg)	79.8±12.5*	80.1±9.5*	74.5±11.3*	77.5±10.2*	61.3±9.8*	69.6±8.5*
Heart rate (beats/min)	69 ± 17	71 ± 19	68 ± 15	73 ± 14	63 ± 12	71 ± 13
Haemoglobin (g/dl)	12.8 ± 2.2	13.1 ± 2.5	12.9 ± 2.3	13.9 ± 2.7	12.8 ± 2.6	13.4 ± 2.3
Fasting glucose (mmol/l)	6.5 ±1.2*	6.9 ±1.1*	5.9 ± 1.7*	6.1 ±1.4*	3.8 ± 1.5*	4.1± 1.3*
Creatinine (mg/dl)	1.02±0.5	1.1±0.4	1.09 ± 0.7	1.1 ± 0.8	0.9 ±0.5	1.0±0.6
NT-proBNP (pg/ml)	129±36.8*	134±32.5*	131±30.4*	139±33.8*	95±23.7*	101±20.2*
LDL-C (mmol/l)	3.52±1.69*	3.82±1.93*	3.47±1.54*	3.75±1.83*	2.15±1.20*	2.33±1.65*
HDL-C (mmol/l)	1.02±1.73	0.97±1.28	0.89±1.07	0.99±1.51	1.12±1.19	1.17±1.43
Triglyceride (mmol/l)	1.97±0.8*	2.2±0.5*	1.45±0.9*	1.57±0.7*	1.14±0.6*	1.23±0.8*
Uric acid (µmol/L)	395±22.1*	481±19.3*	373±20.5*	460±18.2*	324±21.4*	409±17.3*
TSH (mIU/mL)	3.6 ± 1.52*	3.1±1.29*	3.0±1.14*	2.9±1.76*	2.4±1.52*	2.7±1.91*
AST (U/L)	20.2 ± 4.76	24.5±5.08	22.3 ± 4.12	25.1± 5.29	21.3±6.19	26.2±4.57
ALT (U/L)	33.4±7.92	35.9±8.50	29.5±6.33	31.2±7.41	30.5±5.08	31.5±9.12
hs CRP (mg/L)	3.3 ±1.5*	3.4±1.3*	3.0±1.7*	3.2 ±1.9*	1.3±1.2*	1.5±1.1*
HOMA-IR	3.82±1.1*	3.35±1.2*	2.67±1.3*	2.95±1.2*	1.50±1.1*	1.73±1.2*
LV diastolic dimension (mm)	51.3 ± 6.5*	52.7 ±5.3*	50.4 ±7.2*	51.9 ±4.8*	47.8 ±6.1*	48.3 ±5.2*
LV mass (g)	195± 47	202± 41	163±37	175±49	150± 33	151± 38
LV mass index (g/m2)	55 ± 14*	57 ± 12*	43±11*	45±13*	36±8*	37±10*
Mitral annular e' (cm/s)	7±2*	7±1*	8±2*	8±1*	10±2*	9±1*
E/e ' ratio	12±1*	11±2*	13±2*	14±2*	6±2*	7±1*
LA volume index (ml/m2)	23.5±7.3*	25.1±6.4*	27.8±6.1*	28.5±7.3*	20.3±3.8*	21.3±4.5*
EF, %	60.1±4.1	59.4±3.2	60.7±4.5	61.5±3.9	62.1±5.3	62.9±4.7
TAPSE, mm	16±2*	15±3*	17±2*	17±1*	19±2*	20±3*
RV basal dimension, mm	35±3.1*	36±4.7*	33±2.8*	34±3.2*	29±5.3*	30±3.9*
RV longitudinal dimension, mm	74±5*	76±8*	67±4*	69±6*	60±3*	61±5*
RV fractional area change, %	45±8*	46±9*	47±8*	48±7*	52±6*	55±4*

*: p < 0.05 compared to the control group

Standard transthoracic echocardiogram (2D and Doppler) examinations were performed using commercially available equipment (GE Healthcare, Chicago, IL, USA) according to the current guidelines. Cardiac morphology was assessed in standard four- and two-chamber views. The biplane Simpson method was used to determine the left ventricular ejection fraction. The degree of diastolic dysfunction was stratified to one out of four grades [Grade I (impaired relaxation), Grade II (pseudonormal), Grade III (reversible restricted), and Grade IV (fixed restricted)]. Assessment of the RV included RV basal and longitudinal dimensions, fractional area change (FAC), tricuspid annular plane systolic excursion (TAPSE).

Statistical analyses were carried out in SPSS 22.0 Statistical Package Program for Windows (SPSS Inc., Chicago, Illinois).

Continuous variables were presented as the mean ± standard deviation (SD) and were compared using an independent samples t test. The differences between groups were checked by Chi-square test for categorical variables and by independent t-test for continuous variables.

RESULTS

Obese patients with HFpEF had greater peripheral edema (38% vs 15%; $p < 0.05$), more orthopnea (75% vs 49%; $p < 0.05$), worse New York Heart Association class (Class ≥ III).

(72% vs 51%; $p < 0.05$), more previous HF hospitalization (66% vs 47%; $p < 0.05$), but less elevated NT-proBNP values (52% vs 90%; $p < 0.05$) as compared with non-obese (Fig. 1).

The average values of DBP, glucose and lipid profiles, uric acide, hsCRP were found to be significantly different among obese patients with HFpEF and non-obese (Table 1).

Obese patients with HFpEF had a higher DBP than non-obese controls.

Group analyses showed that this difference was greater in centrally obese patients with HFpEF than without obesity HFpEF (79.8±12.5 mmHg and 80.1±9.5 mmHg vs 74.5 ± 11.3 and 77.5 ± 10.2 respectively; $p < 0.05$).

However, the values of SBP and HR did not significantly differ among obese and non-obese patients with HFpEF ($p > 0.05$).

The haemoglobin, creatinine, AST, ALT values were almost similar in all groups, but TSH values - significantly higher mainly in obese female patients with HFpEF than in non-obese female (3.6 ± 1.52 mIU/mL vs 3.0±1.14 mIU/mL respectively; ($p < 0.05$).

A significant increased of fasting glucose values and HOMA-IR indexes were observed in patients with HFpEF as compared with control subjects (5.9 ± 1.7 mmol/l, 6.1 ±1.4 mmol/l and 2.67±1.3, 2.95±1.2 vs 3.8 ± 1.5 mmol/l, 4.1± 1.3 mmol/l and 1.50±1.1, 1.73±1.2 respectively; $p < 0.05$), especially with central obesity (6.5 ±1.2 mmol/l, 6.9 ±1.1 mmol/l and 3.82±1.1, 3.35±1.2 respectively; $p < 0.05$).

As the prevalence of insulin resistance (HOMA-IR) was higher in female patients with central obesity than in male, then they have an increased risk of developing prediabetes and type 2 diabetes.

With regard to lipid parameters, the significant differences were detected among patients with HFpEF and control subjects ($p < 0.05$).

The LDL-C values were almost similar among study groups, but triglyceride levels were higher in obese males (2.2±0.53 mmol/l vs 1.57±0.7 mmol/l respectively; $p < 0.05$) as compare with non-obese males with HFpEF.

Moreover, the group analyses revealed that serum hsCRP and uric acid levels were also higher in obese patients with HFpEF than in non-obese (3.3 ±1.5 mg/L, 3.4±1.3 mg/L vs 3.0±1.7 mg/L, 3.2±1.9 mg/L and 395±22.1μmol/L, 481±19.3μmol/L vs 373±20.5μmol/L,460±18.2 μmol/L respectively; $p < 0.05$), indicating greater systemic inflammation.

Despite more severe symptoms and signs of HF, obese patients with HFpEF had lower NT-proBNP values than non-obese patients with HFpEF (129±36.8 pg/ml, 134±32.5 pg/ml vs 131±30.4 pg/ml, 139±33.8 pg/ml respectively; $p < 0.05$).

However, it was found that patients with high central (visceral) adiposity have more pronounced obesity-related LV diastolic dysfunction.

Thus, obese patients had lower E/e' ratio (12±1,11±2 vs.13±2, 14±2 respectively; $p < 0.05$), lower mitral annular lateral e' velocity (7±2 cm/s, 7±1 cm/s vs 8±2 cm/s 8±1cm/s respectively; $p < 0.05$) and an increased LV diastolic dimension (51.3 ± 6.5 mm, 52.7 ±5.3 mm vs 50.4 ±7.2 mm, 51.9 ±4.8 mm respectively; $p < 0.05$) and an increased LV mass index (55 ± 14 g/m^2, 57 ± 12 g/m^2 vs 43±11 g/m^2, 45±13 g/m^2 respectively; $p < 0.05$) as compare with non-obese HFpEF.

Left atrial volume index values were lower in obese patients with HFpEF than in non-obese (23.5±7.3 ml/m^2, 25.1±6.4 ml/m^2 vs 27.8±6.1 ml/m^2, 28.5±7.3 ml/m^2 respectively; $p < 0.05$).

There was no clinically significant difference between the EF values among study groups ($p < 0.05$).

Compared with non-obese HFpEF and control subjects, obese patients displayed greater right ventricular dilatation (base, 35±3.13 mm, 36±4.7 mm vs 33±2.8 mm, 34±3.2 mm and 29±5.3 mm, 30±3.9 mm; length, 74±5 mm, 76±8 mm vs 67±4 mm, 69±6 mm and 60±3 mm, 61±5 mm respectively; $p < 0.05$), more right ventricular dysfunction (TAPSE 16±2 mm, 15±3 mm vs 17±2 mm, 17±1 mm and 19±2 mm, 20±3 mm respectively; $p < 0.05$).

DISCUSSION

Obesity is a commonly seen in HFpEF disorder, affects the NT-proBNP values.

Especially, NT-proBNP as a response to myocardial stretching due to volume overload, is negatively correlated with BMI after accounting for potential confounders [12,13].

It has been hypothesized that obesity-associated lowering of NT-proBNP may primarily be due to suppression of NP production/release rather than increased degradation [14]. This is because NT-proBNP, unlike BNP, is not cleared via NP receptor-C or through neprilysin-mediated mechanisms [15].

By contrast, markers of systemic inflammation (hsCRP, procalcitonin), myocardial injury (cardiac troponins), tissue fibrosis (galectin-3) and thrombosis (plasminogen activator inhibitor-1) are known to be elevated in obese individuals [16].

The current data suggests that typical symptoms of HF and elevated level of brain natriuretic peptides might be less sensitive for obese patients with HFpEF [17].

As previous studies had shown the diastolic function was impaired by increased chamber stiffness in patients with HFpEF regardless of adiposity [18].

Obese patients with HFpEF displayed more profound hemodynamic derangements on exercise, greater RV dysfunction, impaired pulmonary vasodilation, plasma volume expansion, more biventricular remodeling and worse exercise capacity [19].

The recent studies had shown a strong positively correlation between an increased epicardial adipose tissue in obese patients with HFpEF and pulmonary artery systolic pressure, right-sided filling pressures; thus it has been suggested that an abundance of epicardial adipose tissue surrounding the heart may lead to right heart overload in particular [20].

Moreover, RV dysfunction in these patients with HFpEF is strongly associated with worse outcome.

CONCLUSIONS

Obese patients with HFpEF have higher diastolic BP, atherogenic dyslipidemia, insulin resistance index values and greater systemic inflammatory biomarkers, despite lower NT-proBNP values, which increase the risk of cardiovascular events in future.

Echocardiography examination in obesity-related HFpEF patients revealed not only significant LV diastolic dysfunction, but also displayed greater RV dilatation and dysfunction.

Nevertheless, further prospective investigations is warranted to investigate the potential mechanisms.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Tetyana M. Ternushchak
Uzhhorod National University
14 University St, 88000 Uzhhorod, Ukraine
e-mail: tatyana.xs38@gmail.com

ORCID AND CONTRIBUTIONSHIP

Tetyana M. Ternushchak: 0000-0001-6308-5716 Ⓐ Ⓑ Ⓒ Ⓓ Ⓔ Ⓕ
Marianna I. Tovt-Korshynska: 0000-0002-8763-334X Ⓐ Ⓑ Ⓔ Ⓕ
Oksana M. Moskal: 0000-0001-8615-9933 Ⓐ Ⓑ Ⓔ Ⓕ
Vasyl V. Kaliy: 0000-0001-5260-32-82 Ⓑ Ⓒ Ⓔ
Taras I.Griadil: 0000-0002-1048-0656 Ⓒ Ⓔ Ⓕ
Snizhana V.Feysa: 0000-0002-5064-8222 Ⓓ Ⓔ Ⓕ

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

ORIGINAL ARTICLE

Professional self-determination of future dentists in the modern realities of Ukraine

Anna Dobrovolska

IVANO-FRANKIVSK NATIONAL MEDICAL UNIVERSITY, IVANO-FRANKIVSK, UKRAINE

ABSTRACT

Aim: To research how the future dentists' professional self-determination (reflects the cognitive-reflexive component of higher medical education applicants' readiness to use digital technologies in their professional activities) develops within the formation of information technology competence in the modern realities of Ukraine.

Materials and Methods: The author's questionnaire consisted of 15 questions. The questionnaire surveys covered 98 future dentists who studied 'Medical Informatics' and 'Information Technology in Dentistry' at the Ivano-Frankivsk National Medical University in the September-December 2017-2018 and September-December 2022-2023 academic years. The research results were assessed according to the algorithm described. The research used such methods as analysis, synthesis, comparison, concretisation, systematisation, and generalisation, as well as methods of mathematical statistics for evaluating data, namely correlation analysis, Kolmogorov-Smirnov test, Cronbach's alpha, Fisher's test (F-test of equality of variances), Student's t-test and ranking.

Results: The research found positive dynamics of the professional self-determination levels (in 2022 compared to 2017, the low level decreased by 20.5%, the satisfactory level – by 19.0%, the average level increased by 20.6%, the high level – by 18.9%) and their quality, which within the research increased by 39.5%.

Conclusions: By forming information technology competence, future dentists changing the priorities of professional self-determination in the modern realities of Ukraine and acquiring readiness (within the cognitive-reflexive component) to use digital technologies in professional activities.

KEY WORDS: digital technologies, cognitive-reflexive component of readiness, information technology competence, professional self-determination

INTRODUCTION

On the territories of Ukraine, the large-scale and brutal war brings destruction, physical and mental pain, and suffering to citizens of different age groups and social statuses. Therefore, the viability and livelihoods of the Ukrainian society as a whole and the health care system of our country, in particular in the realities that quite often are beyond the limit of survival, are impossible without specialists capable of responsible, independent decisions in any situation within of professional activity, taking into account its purpose.

In today's extreme conditions caused by the war, the future dentists' preparation for professional activities in institutions of higher medical education acquires a special meaning. Given the total digitalization of life in society at its various stages and the significant impact of digital technologies in the course of socioeconomic processes in Ukraine, both in peacetime and wartime, such preparation is a series of complex actions that encourage the higher medical education applicants to professional self-determination [1, 2].

Domestic and foreign researchers [3-15] studied various aspects of the professional self-determination problem of an individual. Considering the complexity and multifaceted phenomenon of 'professional self-determination', its generally accepted definition still does not exist. Therefore, the concept of 'professional self-determination' is not interpreted unambiguously in modern scientific literature [16].

By researching, we believed that the future dentists professional self-determination within the learning of '*Medical Informatics*' ('*MI*') and '*Information Technology in Dentistry*' ('*ITD*') during higher medical education was the complex and multifaceted process of personality development of each future specialist [1]. This process is being implemented:

– By the independent, conscious acquisition by higher medical education applicants of readiness for the use of digital technologies in professional activities through the information technology competence (*IT* competence) formation;

– Due to the internal resources of the educational process subjects, which provide both the development of their professional qualities that don't contradict the capabilities of the speciality, they are pursuing

and the requirements for it and of planning for the prospects for a professional future [1].

In our opinion, the future dentists' professional self-determination during higher medical education is influenced by socioeconomic conditions and situational factors, etc., and directed at the self-realization of each subject of the educational process in future professional activity [2, 16].

At the moment, during the complicated realities of wartime, transformations are taking place in Ukrainian society that we didn't expect before. Therefore, transformational changes in the personality of every future specialist in the health care system who is getting higher medical education in Ukraine under the conditions caused by the war are predictable. Our earlier research relating to the future doctors' professional self-determination who have formed *IT* competence when studying the '*MI*' [17] took place at a time when there was not even an idea of a possible large-scale war on the territory of Ukraine. By observing the future dentists who studied the '*MI*' and '*ITD*' and also by conversing with them outside the training sessions, we understood that their participation in the educational process of the Ivano-Frankivsk National Medical University and life in society during the difficult trials of the war are interconnected. We realized that by acquiring a profession and preparing for the application of digital technologies in professional activities in such realities, future specialists must show extraordinary perseverance, as well as professionally self-determine, acquiring knowledge, skills, and abilities during the *IT* competence formation when studying the '*MI*' and '*ITD*' in the difficult conditions of wartime. Considering this, the study of future dentists' professional self-determination as the cognitive-reflexive component of their readiness to use digital technologies in professional activities [16] was and remains relevant for us during the higher education of future specialists in the health care system.

AIM

The purpose of scientific research was to study how future dentists' professional self-determination (reflects the cognitive-reflexive component of the readiness of higher medical education applicants to use digital technologies in their professional activities) develops if they acquire IT competence in the modern realities of the Ukrainian state.

MATERIALS AND METHODS

The research took place based on the Department of Medical Informatics, Medical, and Biological Physics of the Ivano-Frankivsk National Medical University within the teaching and studying the '*MI*' and '*ITD*' in the amount of two modules (in the 2017-2018 academic year – 105 hours, the 2022-2023 academic year – 90 hours) during one semester. The research involved 98 future dentists, among which 68 respondents (The *control group*, namely the *CG.*) and 30 respondents (The *experimental group*, namely the *EG.*) studied in the sp eciality 221 'Dentistry' in the 2nd year of the Faculty of Dentistry of the Ivano-Frankivsk National Medical University, respectively in September-December of the 2017-2018 (the research ascertaining stage), and 2022-2023 (the formative stage) academic years and answered the questionnaire voluntarily after studying these disciplines. Thus, the samplings (the *CG* and the *EG)* were formed by random selection.

The author's questionnaire for interviewing research participants contained 15 questions [16, 18]. In the questions of the questionnaire, the author focused on the fact that by studying the '*MI*' and '*ITD*' and also professionally self-determining, future specialists outline the range of professional interests, identify their professional inclinations, assess their professional suitability, and look for the meaning of future professional activities, taking into account the needs of society for dentists who can carry out professional activities according to the standard within of the formed *IT* competence.

Let us note that the content of the questions for the questionnaire survey reflects the normative, real, and psychological aspects, as well as the content-structural, activity and productive-resultant components of future dentists' professional self-determination [16]. The selection of such questions also was determined by the experience of the author's pedagogical activity. The answers were evaluated on a point system ranging from zero to two (0, 1, or 2).

The levels (*high*, *medium*, *satisfactory*, *low*) of future dentists' professional self-determination have been characterized in advance and evaluated by such an indicator as *the sum of points* (*SP*) within the predefined algorithm [16, 18].

The questionnaire survey was implemented in the 2017-2018 and 2022-2023 academic years using Microsoft Forms (Microsoft Office 365).

The questionnaire survey data have been processed using Microsoft Excel (Microsoft Office 365) according to the described algorithm [16, 18].

The research used such methods as analysis, synthesis, comparison, concretization, systematization, and generalization, as well as methods of mathematical statistics for the evaluation of data, namely correlation analysis, Kolmogorov-Smirnov test, Cronbach's alpha,

Table 1. The correlation analysis results (by the number of respondents' answers to the questionnaire questions)

The criteria and conclusions		CG, n = 68 (2017)	EG, n = 30 (2022)
Pearson's r	r	− 0.58	− 0.94
	r*	0.51	
The conclusion		│ r │ ≥ r*, there is statistically significant correlation	
Student's t-test	t	− 2.60	− 10.14
	t*	2.16	
The conclusion		│ t │ > t*, there is linear correlation	

Table 2. The questionnaire survey results (number of questionnaire questions' answers (%) that were evaluated by the respondents from zero to two points (0, 1, or 2), respectively) and the questions' ranks by the number of positive answers to them

The questionnaire question	CG, n = 68 (2017)			EG, n = 30 (2022)			The ranks	
	The points used to evaluate the respondents' answers						*2017*	*2022*
	2	**0**	**1**	**2**	**0**	**1**		
The question 1: Do you understand the purpose of using *IT* competence in the future professional activity of dentists?	**45.6**	22.1	32.3	**66.7**	6.6	26.7	*4*	*11*
The question 2: Do you know when studying the '*MI*' and '*ITD*' about the basic requirements regarding the formation of *IT* competence of future dentists?	**32.4**	32.4	35.2	**63.3**	20.0	16.7	*14*	*12*
The question 3: Do you know what knowledge, skills and abilities a future dentist must have to carry out the professional activity within the confines of the formed *IT* competence?	**39.7**	23.5	36.8	**60.0**	16.7	23.3	*7*	*13*
The question 4: Are you familiar with the primary responsibilities that future dentists must perform within the confines of *IT* competence formed while studying the '*MI*' and '*ITD*'?	**47.1**	22.0	30.9	**76.7**	3.3	20.0	*3*	*3*
The question 5: Do you agree that the formed *IT* competence is significant during the future professional activity of a dentist?	**42.7**	22.0	35.3	**70.0**	3.3	26.7	*6*	*7*
The question 6: Do you think it's necessary to independently form *IT* competence for professional development while studying the '*MI*' and '*ITD*'?	**38.2**	30.9	30.9	**73.4**	13.3	13.3	*12*	*6*
The question 7: Do you think it's necessary to form professionally oriented *IT* competence outside of studying the '*MI*' and '*ITD*'?	**48.5**	20.6	30.9	**70.0**	0.0	30.0	*2*	*7*
The question 8: Are you interested in forming professionally oriented *IT* competence while studying the '*MI*' and '*ITD*'?	**36.8**	20.6	42.6	**70.0**	0.0	30.0	*13*	*7*
The question 9: Are you trying to find new ways of performing professionally oriented tasks by forming *IT* competence while studying the '*MI*' and '*ITD*'?	**39.7**	14.7	45.6	**56.7**	10.0	33.3	*7*	*14*
The question 10: Are you interested in non-standard ways of forming *IT* competence under the condition of its use during future professional activity?	**39.7**	25.0	35.3	**70.0**	3.3	26.7	*7*	*7*
The question 11: Do you like to learn new facts about future professional activity by forming *IT* competence while studying the '*MI*' and '*ITD*'?	**54.4**	14.7	30.9	**83.3**	0.0	16.7	*1*	*1*
The question 12: Do you rely on the experience gained during the formation of *IT* competence by performing professionally oriented tasks?	**30.9**	26.5	42.6	**76.7**	0.0	23.3	*15*	*3*
The question 13: Do you think that the knowledge, skills, and abilities acquired within the confines of the formation of *IT* competence while studying the '*MI*' and '*ITD*' will allow you to become a highly qualified specialist?	**45.6**	13.2	41.2	**83.3**	0.0	16.7	*4*	*1*
The question 14: Do you ask for help if problems arise at runtime of professionally oriented tasks if you form *IT* competence by studying the '*MI*' and '*ITD*'?	**39.7**	22.1	38.2	***0.0***	60.0	40.0	*7*	*15*
The question 15: Do you learn new terminology by forming *IT* competence and studying the '*MI*' and '*ITD*' that will use in future professional activity?	**39.7**	14.7	45.6	**76.7**	6.6	16.7	*7*	*3*

Table 3. Assessing the internal consistency of the questionnaire survey results (analysis of the respondents' answers to the questionnaire questions)

The criterion and conclusion		CG, n = 68 (2017)	EG, n = 30 (2022)
Cronbach's alpha	α	0.83	0.82
The conclusion		0.8 < α < 0.9, the internal consistency is good	

Table 4. The Kolmogorov–Smirnov test results (according to the questionnaire survey results SP)

The criterion and conclusion		CG, n = 68 (2017)	EG, n = 30 (2022)
Kolmogorov–Smirnov test	d	0.08	0.17
	d*	0.20	0.30
The conclusion		d < d*, there is a normal distribution	

Table 5. The results of statistical testing of the hypotheses (according to the questionnaire survey results SP)

The criteria and conclusions		CG (n = 68) and EG (n = 30)
F-test of equality of variances	f	1.90
	f*	1.64
The conclusion		f > f*, the variances are not the same
Student's t-test	t	4.92
	t*	1.99
The conclusion		$\lvert t \rvert$ > t*, the distribution centres of the two populations are not equal

Table 6. The future dentists' professional self-determination levels (%) and the dynamics of their quality (The quality of levels characterise the medium and high levels in total.) [1]

The levels of the professional self-determination	CG, n = 68 (2017)	EG, n = 30 (2022)	The dynamics
low	33.8	13.3	− 20.5
satisfactory	32.4	13.3	− 19.0
medium	29.4	50.0	+ 20.6
high	4.4	23.4	+ 18.9
The quality of the levels	33.8	73.4	+ 39.5

Fisher's test (F-test of equality of variances), Student's t-test and ranking [18, 19].

This research was carried out under the Ethical Guidelines for Educational Research of the British Educational Research Association (fourth edition, 2018) and of the Code of Ethics of the American Educational Research Association (approved by the AERA Council, 2011).

RESULTS

We found out that:
– The research participants answered the formulated questions carefully and motivated as there is a statistically significant negative linear correlation ($\lvert r \rvert \geq r^*$, r < 0, $\lvert t \rvert$ > t* in Table 1) between the number of their positive and negative answers (Note that the positive and negative answers were evaluated by two and zero points, respectively, Table 2);
– The internal consistency of the questionnaire survey results, conducted among the respondents of the *CG* and the *EG*, was good (Table 3);
– The results *SP* of the questionnaire survey are subject to the normal distribution with the probability p ≥ 0.99 (p = 1 – α, the type I error probability α = 0.01, Table 4).

We wanted to find out whether the *IT* competence formation when studying the '*MI*' and '*ITD*' affects the development of future dentists' professional self-determination. Therefore, we checked whether the hypothesis about the equality of the normally distributed populations' distribution centres is true, namely sample populations of the *CG* and the *EG*, at the ascertaining and formative stages of the research.
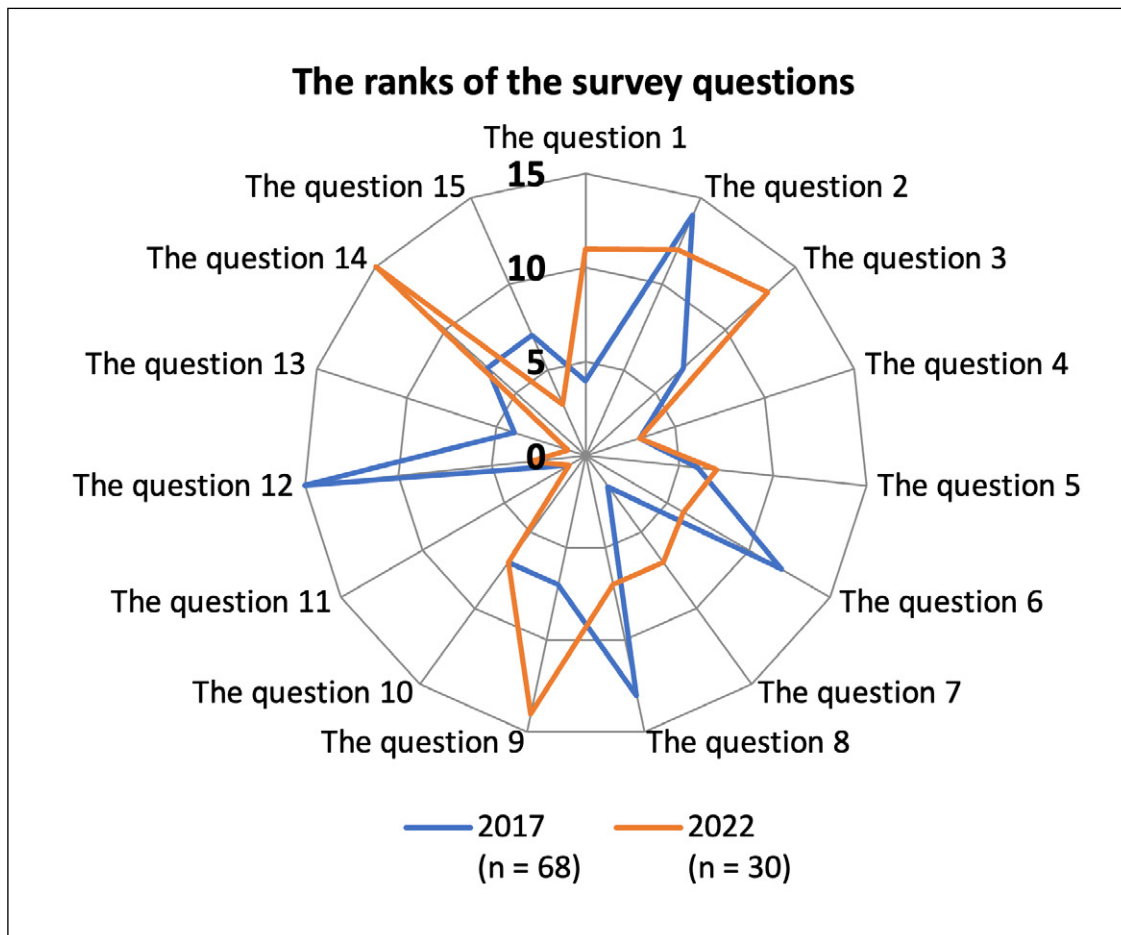
Fig. 1. The ranks of the survey questions by the number of positive answers (%) to them.

Note that we have chosen a criterion for testing the hypothesis, given that the variances of the populations are biased (Table 5).

We affirm (the probability p ≥ 0.95, p = 1 – α, the type I error probability α = 0.05) that in 2022, compared to 2017, the educational process in general, as well as the learning of the '*MI*' and '*ITD*' in particular, which were implemented by the scientific and pedagogical staff of the Department of Medical Informatics, Medical, and Biological Physics of the Ivano-Frankivsk National Medical University in the conditions of the war in Ukraine, thanks to several of organizational and methodical measures, differed in the ability of future dentists to define themselves professionally subject to the development of *IT* competence during higher medical education ($|t| > t^*$ – the distribution centres of the two populations are not equal, Table 5) and most fully contributed to the development of the cognitive-reflexive component of the future specialists' readiness to use digital technologies in the professional activities, despite the modern challenges of Ukrainian society.

Given the *IT* competence acquisition when learning the '*MI*' and '*ITD*' in 2022 at the formative stage of the research, there was a positive dynamic of the levels of

research participants' professional self-determination and their quality in comparison with the ascertaining stage of the research in 2017. We established that the professional self-determination low level decreased by 20.5%, the satisfactory level – by 19.0%, the average level increased by 20.6%, the high level – by 18.9%, and the quality of the levels increased by 39.5% (Table 6) [1].

Such positive dynamics should be explained by the fact that the course of the educational process as a whole was accompanied by organizational measures implemented by employees of the administration and departments of the Ivano-Frankivsk National Medical University, considering the challenges of Ukrainian society in the conditions of a brutal war.

We emphasize that the learning of the '*MI*' and '*ITD*' at the Department of Medical Informatics, Medical, and Biological Physics was provided by [16]:
– The educational and methodical complexes of the '*MI*' and '*ITD*';
– The learning technologies (technologies of modular, multi-level, active, problem-developmental, interactive, person-oriented, resource-oriented learning, individualization of learning);
– The learning methods (information-receptive,

reproductive, heuristic methods, the methods of problem-based presentation, problem-developmental learning, interactive learning, moderation, active learning, expediently selected tasks, demonstration examples, integrated learning, control, and self-control).

We are inclined to the opinion that the constant use of the developed manuals [20, 21] by the research participants in the 2017-2018 and 2022-2023 academic years when learning the '*MI*' and '*ITD*' effectively ensured the *IT* competence formation and contributed to the development of the professional self-determination as the cognitive-reflexive component of future dentists' readiness to use digital technologies in the professional activities [16].

In our opinion, the positive dynamics of the quality of the levels of professional self-determination (+39.5%, Table 6) for the research participants in 2022 are also explained by the fact that with the beginning of the large-scale war on February 24, 2022, they [1]:

– Were responsible for their own higher medical education, valuing every moment favourable for learning;

– Persistently studied the '*MI*' and '*ITD*' outside the classrooms in special conditions at a time when there were no air alarms but the Internet and light were available, the hardware of the information centre of the Ivano-Frankivsk National University and the gadgets of future specialists worked stably, there was uninterrupted access to educational resources, textbooks, methodical materials, etc.

It's worth mentioning that the teaching of the '*MI*' and '*ITD*' took place with the sensitive attitude of scientific and pedagogical workers and support staff to each higher medical education applicant in the difficult conditions of our country today.

By analysing the survey results according to the ranks (Table 2 and Fig. 1.) assigned to the questionnaire questions at the formative stage of the research, we concluded that in 2022, during the difficult trials of society associated with a large-scale war in Ukraine, compared to the 2017 (the ascertaining stage of the research) the higher medical education applicants' emphasis changed within the professional self-determination, namely priorities during the development of the cognitive-reflexive component of their readiness to use digital technologies in professional activities.

In 2017, future specialists who studied the '*MI*' and '*ITD*' primarily considered it necessary (Table 2) to get acquainted with new facts about future professional activity, as well as to form professionally oriented *IT* competence outside of studying these disciplines because they knew about the main duties that a future dentist should perform within the formed *IT* competence [2].

In 2022, the range of priorities for the educational process participants, who learned new facts about their future professional activities and professionally self-determined during the *IT* competence formation, expanded and changed. Primarily, the future dentists were convinced that the knowledge, skills, and abilities acquired when studying the '*MI*' and '*ITD*' would allow them to become highly qualified specialists. When performing professionally oriented tasks, they relied on the experience that has been gained within the *IT* competence formation and also on terminology that would be used in future professional activities (Table 2) [2].

By analysing the information in Table II, we concluded that when studying the '*MI*' and '*ITD*' in 2022, in comparison with 2017:

– 1.53 times more of the higher medical education applicants had the intention to learn new facts about future professional activity, by forming *IT* competence (83.3% versus 54.4%);

– 1.83 times more respondents believed that the knowledge, skills, and abilities acquired within the *IT* competence formation would allow becoming highly qualified specialists (83.3% compared to 45.6%);

– 2.48 times more respondents relied on the experience gained during the *IT* competence formation when performing professionally oriented tasks (76.7% versus 30.9%);

– 1.63 times more participants when studying were familiarized with the main duties that a future dentist should perform within the formed *IT* competence (76.7% against 47.1%);

– 1.93 times more respondents, by forming *IT* competence, mastered new terminology used during future professional activity (76.7% compared to 39.7%);

– 1.92 times more respondents considered it necessary to independently acquire *IT* competence for professional development (73.4% against 38.2%);

– 1.44 times more respondents expressed a desire to form professionally oriented *IT* competence outside of studying the '*MI*' and '*ITD*' (70.0% versus 48.5%).

DISCUSSION

The lack of scientific research by other scholars in the context of this study's aim narrows the scope of discussion only to our reflections.

The results obtained in this research allow us to state that in 2022 when faced with problems during the performance of professionally oriented tasks, given

that *IT* competence is formed when studying the '*MI*' and '*ITD*' [2]:

– 60.0% (against 22.1 % in 2017) of higher medical education applicants didn't consider it necessary to ask for help;

– The number of future dentists (40.0% in 2022 compared to 38.2% in 2017) who applied for help irregularly almost didn't change (Table 2).

In our opinion, the implementation of the search approach within the developed adaptive model of learning the '*MI*' and '*ITD*' provided the independent acquisition of new knowledge, skills, and abilities by the educational process participants [22] that in 2022 contributed to the solving of problems during carrying out of professionally oriented tasks.

In 2022, the following results were significant for respondents who studied the '*MI*' and '*ITD*' (Table 2):

– The higher medical education applicants didn't doubt that the knowledge, skills, and abilities acquired within the *IT* competence development would allow them to become highly qualified specialists because in 2017, such doubts were in 13.2% of the respondents;

– All future dentists performing professionally oriented tasks relied on the experience gained during the *IT* competence development, but in 2017 26.5% of respondents had doubts about it;

– There were no education process participants who would not like to learn new facts about their future professional activity by forming *IT* competence, but 14.7% of respondents in 2017 had no such interest;

– All the higher medical education applicants were interested in forming professionally oriented *IT* competence, but in 2017, 20.6% of respondents didn't have such emotional displays;

– All future dentists considered it necessary to form professionally oriented *IT* competence outside the study of the '*MI*' and '*ITD*', although, in 2017, 20.6% of respondents didn't have such a desire.

We think that the change in 2022 of the priorities of future dentists during professional self-determination within the confines of its normative, real, and psychological aspects, as well as content-structural, activity and productive-resultant components [16] due to the motivated understanding of the importance of acquiring *IT* competence when learning the '*MI*' and '*ITD*' for the successful implementation of future professional activity, which involves the use of digital technologies, especially in wartime, that is the time of the extreme tests of Ukrainian society.

We claim that the essence of professional self-determination of future specialists reflects the meaning of their personal growth, which is enhanced by the formation of the image of the professional '*I*' [23, 24] when acquire *IT* competence, based on an independent search for the goals and content of the future professional activity, taking into account the needs of the health care system of the Ukrainian state because they change their attitude towards the professional activities of a dentist in the modern realities of Ukraine by studying the '*MI*' and '*ITD*' in higher medical education institutions.

CONCLUSIONS

1. In times of difficult trials, Ukraine, courageously defending its territorial integrity and the right to development in the world community of democratic states, provides citizens with the opportunity to obtain higher medical education and to professional self-determination during such learning.

2. The active use by future dentists of information technologies in the modern realities of Ukraine actualised the acquisition of IT competence on the way to obtaining higher medical education, which became a benchmark for their professional growth for quality improvement of medical service of future patients.

3. The IT competence acquisition in higher medical education institutions, whose education processes are often limited by the modern realities of Ukraine, productively ensures the development by future dentists of readiness (within the cognitive-reflexive component) to use digital technologies in professional activities by changing the priorities of professional self-determination and by increasing its levels (in 2022 compared to 2017 the low level decreased by 20.5%, the satisfactory level – by 19.0%, the average level increased by 20.6%, the high level – by 18.9%) and their quality, which within the research increased by 39.5%.

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CORRESPONDING AUTHOR
Anna Dobrovolska
Ivano-Frankivsk National Medical University
2 Halytska st, 76018 Ivano-Frankivsk, Ukraine
e-mail: AnnaD68@meta.ua

ORCID AND CONTRIBUTIONSHIP
Anna Dobrovolska: 0000-0003-0841-8076 Ⓐ Ⓑ Ⓒ Ⓓ Ⓔ Ⓕ

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

Constitutional and legal status of the subject during biomedical research

Serhii S. Pyroha[1], Bohdana B. Shandra[2], Ruslana P. Naturkach[2], Maryna G. Kohut[2]
[1]AUGUSTIN VOLOSHIN CARPATHIAN UNIVERSITY, UZHHOROD, UKRAINE
[2]UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT
Aim: To find out the peculiarities of constitutional and legal status of the subject during biomedical research.
Materials and methods: A synergistic approach helps predict possible fluctuations and vectors of development, taking into account various social and technical processes of influence on the status of the subject; comprehensive - involves the analysis of the research subject within the framework of a combination of different scientific schools, concepts and methods and provides opportunities for the development of unified standards, benchmarks, principles and general norms of legal regulation.
Conclusions: The constitutional-legal status of the subject is the position of the subject (patient, object of research) established and established by the norms of constitutional law, which distinguishes him as a special subject of legal relations in the process of conducting biomedical research and consists of a set of rights and obligations and specifics of the legal liability of its participants.

    KEY WORDS: human rights and freedoms, law, biomedical research, human rights protection, subject during biomedical research

INTRODUCTION

The second half of the 20th and the beginning of the 21st century were characterized by serious scientific and technical discoveries in the field of biology and medicine, and the rapid rise of medical technologies to a qualitatively new level. Cloning, genetic engineering, organ and tissue transplantation – this is not a complete list of research that can change the life of mankind and find the possibility of treating many diseases. Scientific research in the field of medicine today represents the most important sphere of human activity, the main goal of which is to preserve the life and health of people. In recent years, medical science has made great strides; it has become capable of penetrating the deep processes occurring in the human body, influencing reproductive health, dying processes, genetic status, etc. The objective emergence of completely new knowledge-intensive technologies, the expansion of the horizons of human activity in the field of medical and biological sciences gives rise to the emergence of new relationships between researcher and subject, which undoubtedly leads to the need for legal regulation of these relations, the emergence of new profound scientific developments that can influence the formation of adequate legislative bases in this area [1].

At the present stage, our society, having gone through a serious path of development, is forced to admit that there are a lot of issues the solution of which is not yet within the control of man. Each new evolutionary stage is accompanied by the emergence of a mass of new diseases and viruses that claim many lives and pose a serious threat. HIV (AIDS), cancer, COVID are just the tip of the iceberg of the most serious diseases, the treatment of which is not yet possible even in our time of rapid development of science [2].

Disappointing statistics inevitably emphasize the clear need to combat these diseases and search for fundamental means of their prevention, diagnosis and treatment. In this regard, this area is one of the priority tasks of modern medicine, the implementation of which, in turn, is unthinkable without conducting experimental studies with human participation [3].

In order to test the theoretical principles put forward by scientists, with a view to testing them in practice and ensuring the safety of the developed treatment methods, the need invariably arises to conduct biomedical experiments on humans. At the same time, to date, legal science has not formed a final position on the issue of protecting human rights in their implementation. Conducting experimental research is

very closely interconnected with interference in the sphere of private interests of a person, with the right to life, personal integrity, respect for private life, which necessitates detailed regulation of the possibility of permissible interference in this area, as well as the development, taking into account humanistic trends, theoretical and legal the basics of the relationship with the subject during the study [4].

The need for a serious and more careful consideration of the legal relations that arise during biomedical research involving humans is thus determined by the dynamic development of science and the corresponding complication of social relations. In this regard, the study of these relations should be based on ensuring human rights, which in itself, to one degree or another, is a manifestation of the multifaceted and multidimensional principle of humanism, which, in essence, implies considering a person as the highest value. It is the extent to which the implementation of human rights and freedoms, their recognition and maximum respect will occur that directly determines whether our state will truly be legal [5].

AIM

The aim is to find out the peculiarities of constitutional and legal status of the subject during biomedical research.

MATERIALS AND METHODS

The research methodology is based on a complex of methodological approaches and methods of scientific knowledge of social phenomena and processes.

A synergistic approach helps predict possible fluctuations and vectors of development, taking into account various social and technical processes of influence on the status of the subject; comprehensive - involves the analysis of the research subject within the framework of a combination of different scientific schools, concepts and methods and provides opportunities for the development of unified standards, benchmarks, principles and general norms of legal regulation; humanistic - normalizes manifestations of coercion in the construction of the constitutional system and human value in the theory of constitutionalism in the formation and functioning of the system for the protection of the rights of the subject; posthumanistic - revealed through the positioning of changes in the status of the subject as an alternative transformation that has already taken root in the constitutional and legal reality. A complex of methods was used, among which: dialectical - when analyzing the phenomenon through its normative-legal

and law enforcement genesis; transcendental - to reflect the dominance of the primacy of human interests in the functioning of the system for the protection of the subject's rights; hermeneutic - when interpreting normative legal acts, proposals for improving the conceptual and categorical apparatus; constitutional comparativistics - when distinguishing a group of states according to the level of technology implementation, which makes it possible to reflect the connection between the status of the subject and the democracy of the government; legal forecasting - to determine the prospects for the further development of constitutional law in the modern conditions of constitutionalism, to identify directions for the development of the status of the subject.

REVIEW AND DISCUSSION

Analyzing the ethical aspects of conducting research on humans in the 1970s, G. Jonas noted that the time will soon come when the scientific community will have to overcome the huge temptation to move to regular, daily experiments on the human body. Becoming an ordinary event, such experiments will bring great danger to humanity [6]. Even half a century ago, the basic norm of the Nuremberg Code of 1947 regarding the possibility of conducting an experiment on a person only in case of extreme necessity was an absolute imperative of the scientific ethnos, the internal moral censorship of world science did not allow it to be reconsidered [7].

The Geneva Declaration of 1948, adopted by the World Medical Assembly, and the 1949 International Code of Medical Ethics assume, at least implicitly, that an experiment on a person is possible only in the absence of another alternative to obtaining knowledge of great importance to society. As we have already noted, these normative acts do not contain a definition of a medical experiment, but the principles laid down in them have a direct impact on the ethics of its conduct. Fundamental knowledge is an indisputable value of society and often serves as a justification for risking the physical and social well-being of mankind. In any scientific research, there are ethical principles that regulate the scientist's attitude to the subject of his activity. For a scientist who works in the field of experimental medicine, the object of research is a person, and the ethical norms laid down in the process of their interaction are a guarantee of the success of future results [7].

In the context of the topic of our study, we note that scholars in the field of constitutional law have sufficiently investigated the issue of the constitutional and legal status of a person and a citizen, but little attention has been paid to the study of the constitutional and legal status of an object in the field of biomedical research.

It should be noted that among legal theoreticians and constitutionalists, there is no unified view regarding the understanding of the concept of "legal status" and its components (sub judĭce). So, in particular, the category "status" translated from the Latin language means the position, condition of something or anyone: "status of a person", "status of an individual", "status of a citizen", "status of an organ" [8]. In modern domestic explanatory dictionaries, you can find such a definition of status as: "legal status of persons or organizations, institutions, etc.; the position of an individual or a group of individuals in relation to other individuals or groups in the social system; the position of its subjects, the totality of their rights and obligations established by the norms of law" [2].

Undoubtedly, one should fully agree with the statement of one of the leading researchers in the field of constitutional law, M. Gromovchuk, that the concept of "status" is one of the basic concepts in legal science, as it allows determining the place of legal subjects in the system of social relations, their rights and obligations relationships with other subjects [9-11]. Status in constitutional law has several expressions depending on the role it plays in determining and fixing the position of participants in constitutional-legal relations and approaches to its characteristics. But in any of its manifestations, the constitutional and legal status has the qualities of stability, relative stability, internal coherence and systemic significance [12]. In the science of constitutional law, status is a theoretical construction that combines normative characteristics, theoretical ideas and the practice of implementing legal institutions [13]. The term "status" is widely used both in legislation and in special legal literature [12-15].

According to the successful statement of Z. Makarova, the actual legal category "constitutional and legal status" allows to establish the place of one or another institution in the system of distribution of power, to show all the multifaceted relationships between this institution and other government institutions [14].

Starting to solve one of the tasks of our research, it is necessary, first of all, to reveal the meaning of more general legal definitions - "legal status", "constitutional status" and "constitutional-legal status". Note that the existence of several approaches to the definition of scientific terminology is a generally accepted phenomenon both in legal science and in constitutional law. Therefore, to reveal the issue of the constitutional and legal status of the subject in a biomedical experiment, it is necessary first of all to use the terminological apparatus, while it is important to outline the key theoretical and methodological approaches to its analysis.

Many domestic and foreign researchers (Y. Bysaga, D.Byelov, M. Savchyn, M. Matuzov, A. Lebedev, Y. Todyka, N. Shuklina), the term "legal status" and "legal provision" are usually used as synonyms and used to characterize the subject's place in legal society. At the same time, A. Syrota notes in this regard that: "Despite the different approaches to this phenomenon, the difference in views on the concept of this legal phenomenon, all authors are united in the fact that the legal status of any subject of legal relations is reflecting and enshrining in law its real, factual position in the system of social relations" [15].

In constitutional law, the status category was most fully explored by N. Bohdanova, who pointed out that status in constitutional law has several incarnations depending on the role it plays in defining and fixing the participants of constitutional-legal relations and approaches to its characterization. In any of its manifestations, the constitutional-legal status has such qualities as: stability, relative constancy, internal consistency and system-forming significance [13].

It should be noted that both legal theorists and constitutionalists do not have a single point of view in the understanding of such a category as "legal status". Thus, in the dictionary of terms from constitutional law, the term "legal status" is defined as the set of rights, duties and responsibilities of its subjects - citizens, authorities, institutions, organizations, etc., established by law. [16]. Here P. Shlyakhtun understands the term "constitutional status" as the set of rights, duties and responsibilities of a subject of constitutional law established by the norms of the constitution [2]

V. Chetvernin defines legal status as "a legally established permanent position of a subject of a certain type in society and the state. Legal status, in his opinion, consists of primary rights and legal obligations. In other words, the rights and obligations that constitute the legal status of an entity are such rights and obligations that are constantly, always present in every entity of a certain type" [17].

N. Onishchenko under the category "legal status" understands the system of legislatively established and state-guaranteed rights, freedoms, legal interests and obligations of the subject of public relations [18]. V. Korelsky considers legal status as a multifaceted category, which, firstly, has a general, universal character, includes the statuses of various subjects of legal relations: the state, society, individuals, etc.; secondly, it reflects the individual characteristics of the subjects and their real position in the system of multifaceted social relations; thirdly, legal status cannot be realized without duties corresponding to rights, without legal responsibility in necessary cases, without legal guarantees; fourth, the

legal status category defines the rights and obligations of subjects in a systematic way, which makes it possible to carry out a comparative analysis of statuses [19].

It should be noted that in the scientific legal literature the legal status of subjects is distinguished: a) natural persons; b) legal entities; c) the state; d) foreigners, stateless persons; e) status of refugees; e) status of Ukrainian citizens residing abroad; g) professional and job status [20].

Thus, legal status can be defined as the legally established position of the subject, therefore legal sources sometimes also speak of legal status. However, in our dissertation research, we will consider the legal status.

Investigating the general theoretical principles of the legal status of a legal entity, I. Okunev singles out such components as: 1) legal personality; 2) the system of rights, obligations and legal interests; 3) system of guarantees of rights and obligations of legal subjects; 4) legal responsibility. At the same time, the researcher believes that this construction of legal status: firstly, can have a universal character, which allows it to be formed on the basis of the construction of the legal status of a legal subject of a specific field of law; secondly, it is capable of acting as a universal basis for legal regulation of the legal status of a legal entity; thirdly, it can be applied to specific types of legal status of legal subjects [21].

A. Kolodiy and A. Oliynyk adhere to the position according to which they consider the most complete structure of legal status, which contains the following elements: 1) statutory legal norms and legal relations; 2) subjective rights, freedoms and legal obligations; 3) citizenship; 4) legal principles and legal guarantees; 5) legitimate interests; 6) legal personality; 7) legal responsibility [22].

At the same time, we share the position of B. Damdinov, who believes that the constitutional status is determined by the normative characteristics enshrined in the Constitution, for subjects of legal relations of the same kind (type), it is general. The constitutional-legal status is broader in content than the constitutional one and involves characteristics contained, in addition to the Constitution, in the norms of other sources of constitutional law. This type of status takes into account the specific characteristics of subjects of legal relations of the same kind (type) and in this sense it is synonymous with a special legal status. The latter is characterized by the greatest breadth of parameters, determined by the sources of various branches of law. Therefore, the most general scope has a legal status, the basis of which is the constitutional-legal status, the core of which is the constitutional status [23].

As we have already seen from the above, for almost all branches of law, the concept of "status of the subject of law" is one of the main ones, it reveals the main branch institu-

tions, since the concepts of "subject of law" and "subject of legal relations" are considered identical . We believe that this approach should be used in the study of the problem of the constitutional and legal status of the probationer.

The constitutional-legal status of the subject can be characterized from the point of view of constitutional-legal, administrative-legal, criminal-legal, family-legal, civil-legal and other branches of law. But at the same time, taking into account the object of our research, the constitutional and legal status of the subject will be analyzed. Taking into account the above, depending on the typological or individual characteristics that individuals have as subjects of law, normative legal acts, which provide for statuses, completeness of legal status, they are divided into certain types. Namely: a) general is a legal status provided by the constitutional law for any person or citizen; b) special – prescribed by separate legal acts for certain categories of people or citizens (judges, prosecutors, teachers); individual – legal status provided by individual legal acts for a specific person [22].

Thus, the general constitutional and legal status of a probationer is determined by the general status of a person and a citizen, because in constitutional legislation, with few exceptions, there are almost no norms that directly mention the rights of a probationer. And the general theory of law does not develop the features of a special subject of law, which is, due to the peculiarity of its legal nature, the subject. In Chapter II of the Constitution of Ukraine "Rights, Freedoms and Duties of Man and Citizen" the term "undertrial" does not appear at all. In the norms, the probationer acts as the subject of legal relations and is included in the terms "everyone", "everyone", "citizen".

CONCLUSIONS

The next type of constitutional and legal status is the special status of a probationer, which is established by the norms of the relevant branches of law (administrative, criminal, civil, medical, etc.) and international legal acts that regulate the variety of spheres of public life in which the probationer participates, as well as conditions that affect its legal status. That is why the special status of the subject, which distinguishes him as a special subject of legal relations, is based on the sign of participation in biomedical research. However, the understanding of the limits of this feature and, accordingly, of legal personality has led to fairly significant variations in different areas of law. That is, different branches of law use several concepts that characterize a person according to this feature, namely: subject, patient, object of research, and in this connection approach the issue of legal personality of the specified categories of persons in different ways.

Thus, the constitutional-legal status of the subject is the position of the subject (patient, object of research) established and established by the norms of constitutional law, which distinguishes him as a special subject of legal relations in the process of conducting biomedical research and consists of a set of rights and obligations and specifics of the legal liability of its participants.

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CORRESPONDING AUTHOR
Serhii S. Pyroha
Voloshin Carpathian University
4 Hoydy st, 88000 Uzhhorod, Ukraine
e-mail: belov_dimon@yahoo.com

ORCID AND CONTRIBUTIONSHIP
Serhii S. Pyroha: 0000-0002-7093-3207 Ⓓ Ⓔ
Bohdana B. Shandra: 0000-0003-4104-2744 Ⓑ Ⓕ
Ruslana P. Naturkach: 0000-0002-3324-2040 Ⓔ Ⓕ
Maryna G. Kohut: 0000-0001-7885-7416 Ⓐ Ⓔ

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

REVIEW ARTICLE

Informed consent of the patient for medical intervention for conducting biomedical research

Vitaliy M. Herzanych, Taras O. Datso, Oksana I. Matviienko, Viktoriia Yu. Svyshcho
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT
Aim: is to find out the peculiarities of informed consent of the patient for medical intervention during biomedical research.
Materials and Methods: The dialectical method was used as a universal and general scientific method, which made it possible to consider the peculiarities of the content of the patient's informed consent. Using the logico-semantic method, the essence and features of biomedical research with the patient's participation were determined. The formal-legal method is used to analyze adaptation processes of biomedical research. System-structural method were applied when comparing the content of the patient's informed consent in separate legislation. The work also used such methods of cognition as comparative-legal, systemic-logical, and logical-legal.
Conclusions: "Informed consent" includes not only the concept of consent itself, i.e. the free decision of a person, but also an explanation of a specific case or research procedure. And it largely depends on the specialist. Will he be able to convey and explain the patient's problem, illness, actions correctly, fully and in an accessible form? Yes, free consent is given by a person, but the doctor also influences this decision to some extent. Patients' freedom in choosing medical care methods is somewhat limited.

KEY WORDS: human rights, medicine, biomedical research, subject during biomedical research, informed consent of the patient

INTRODUCTION

The right to life is the main and basic human right. All civilized, conscious society recognizes human rights as fundamental and tries to preserve them. The mission of doctors is to save, heal, help.

However, in various complex medical cases, a person faces a difficult choice, and only with his consent can doctors begin their actions [1].

In order for a person to fully understand his situation in which he is, first of all, the doctor must give him an informative and accessible explanation about the nature of this disease and about the consequences that may occur when the recommended treatment is carried out or when such treatment is refused [2].

But before starting treatment, it is necessary to carry out all prescribed medical examinations and establish the correct diagnosis.

It is the patient who makes the decision to conduct this or that biomedical research.

It would seem that every patient seeks help voluntarily, in need of medical assistance. But, in practice, we observe that almost every medical intervention requires the patient's written consent. Sometimes this does not create any problems, but in most patients, it causes a

feeling of wariness - there are probably high risks if it is necessary to sign such a piece of paper [3].

The patient's rights are absolute, regardless of whether assistance is provided on the basis of state guarantees of free medical care or on the basis of paid contracts for the provision of medical services. In other words, whether it is a state or commercial medical institution, doctors cannot ignore these rights. Moreover, the patient himself cannot ignore them.

AIM

The aim is to find out the peculiarities of informed consent of the patient for medical intervention during biomedical research.

MATERIALS AND METHODS

The methodological basis is a system of methods and techniques of scientific knowledge. The dialectical method was used as a universal and general scientific method, which made it possible to consider the peculiarities of the content of the patient's informed consent. Using the logico-semantic method, the essence and

features of biomedical research with the patient's participation were determined. The formal-legal method is used to analyze adaptation processes of biomedical research. System-structural and system-functional methods were applied when comparing the content of the patient's informed consent in separate legislation. The work also used such methods of cognition as comparative-legal, systemic-logical, and logical-legal.

REVIEW AND DISCUSSION

The legislation does not contain a clear definition of the term "patient's informed consent to medical intervention during biomedical research". However, based on the content of Article 28 of the Constitution of Ukraine, Article 39, 43 of the Law of Ukraine "Basics of Ukrainian legislation on health care" (hereinafter - Basics), Article 284 of the Civil Code of Ukraine (hereinafter - CCU), it can be concluded that: the patient's informed consent for medical intervention during biomedical research is a free, informed decision of the person who sought medical help or his legal representatives regarding the selection and application of methods of diagnosis, prevention and treatment, which is based on receiving from the doctor in an accessible form information about his state of health, the purpose of the proposed research and treatment measures, the prognosis of the possible development of the disease, including the presence of a risk to life and health [4].

The decision to give the patient's consent to medical intervention must be free. It cannot be the result of external coercion or active persuasion by anyone, including a doctor.

The decision should only be the result of a personal choice based on full health information. The medical worker is obliged to provide the patient in an accessible form with information about his health, the purpose of the proposed research and treatment measures, the prognosis of the possible development of the disease, including the existence of a risk to life and health [5].

If information about a patient's illness can worsen his health or the health of his representatives, harm the process of treatment, medical workers have the right to provide incomplete information about the patient's health, limit their access to individual medical documents [6].

According to the general rule, consent to medical assistance is provided by a person who needs medical assistance - from the age of 14 [7] or legal representatives - if patients under the age of 14 and patients recognized as incompetent need medical assistance.

In the event that a person between the ages of 14 and 18 needs medical intervention, it is advisable to notify

their legal representatives. This is due to the need to comply with the provisions of Art. 31, 32, 37, 39 of the CCU and Article 150 of the Family Code of Ukraine [8].

The Ministry of Health of Ukraine has provided and approved a unified form of such patient consent. Namely, we are talking about the Form of primary accounting documentation No. 003-6/o and the instructions for filling it out, approved by the order of the Ministry of Health No. 110 of 02.14.2012 [9, 10].

The latest changes to form 003-6/o and the instructions were made by the order of the Ministry of Health dated 09.12.2020 No. 2837: "Informed voluntary consent of the patient for diagnosis, treatment and for surgery and anesthesia and for the presence or participation of participants in the educational process".

The medical documentation - form 003-6/o - is filled out by the patient who applied to the health care institution and gives his consent to diagnosis, treatment, if necessary, to surgical intervention and anesthesia, the presence or participation of participants in the educational process in the presence of the attending physician.

The patient himself indicates his last name, first name, patronymic; the attending physician provides him with information regarding the diagnosis and treatment plan, provides in an accessible form information about the probable course of the disease, the consequences of refusing treatment.

The patient's consent to the proposed treatment and diagnosis (form No. 003-6/o) is certified by the signatures of the attending physician and the patient [10].

Consent to medical intervention is not taken if there are signs of a threat to the patient's life and it is not possible for objective reasons to obtain consent to the intervention. In this case, the doctor makes the decision regarding medical intervention.

If it is established that a person is unable to give consent, the authorized person (representative, relatives) must make a decision exclusively in the interests of this person. Such a decision must be as close as possible to the likely decision of the person to be interfered with, if he had the opportunity to express it in any way.

A person has the right to refuse intervention at any stage of the process. She must also be informed about the possible consequences of withdrawing consent. Her decision must be respected. Protection against discrimination should be guaranteed for the decision to intervene [11].

A patient over 18 years of age, whose legal capacity is not limited, or legal representatives of patients under 18 years of age and patients recognized as incompetent or whose legal capacity is limited, has the right to refuse treatment [7].

If the lack of consent can lead to serious consequences for the patient, the doctor is obliged to explain this. If the patient refuses treatment even after that, the doctor has the right to take a written confirmation from him, and if it is impossible to receive it, to certify the refusal with an appropriate act in the presence of witnesses. In the event that the legal representative's refusal of treatment may have serious consequences for the patient, the doctor must inform the guardianship and guardianship authorities [9].

In Ukraine, there is no separate normative document regarding the rights of patients. The Constitution of Ukraine guarantees every citizen the right to health care, medical assistance and medical insurance. At the same time, the state creates conditions for effective and accessible medical care for all citizens. The relationship between the doctor and the patient is regulated by a wide range of legal acts.

In turn, the law of Ukraine "Fundamentals of the legislation of Ukraine on health care of citizens" provides that every citizen has the right to health care, a standard of living, including food, clothing, housing, medical care and social services and provision, which is necessary for maintaining human health; a natural environment that is safe for life and health; sanitary-epidemic well-being of the territory and settlement where he lives; safe and healthy conditions for work, study, living and recreation; qualified health care, including the free choice of a doctor, the choice of treatment methods in accordance with his recommendations and the health care institution [7].

For many conditions and diseases, there are one or more established effective treatments. Doctors and hospitals may use different methods to treat the same condition. However, the relative merits of these treatments are often unknown.

Comparative effectiveness studies, as well as systematic reviews, have received increasing attention over the past few years. A comparative effectiveness study directly compares two or more interventions that are considered the standard of care. This research may help determine which standard of care has better outcomes and more acceptable risks.

The risks of standard care procedures do not necessarily qualify as minimal simply because the treatment has become standard practice. Risks to participants must be minimized and properly balanced against the prospects for potential individual benefit or social value of the research [12-23].

The principle of voluntary informed consent implies that every person has the right to access information about the purpose and nature of such an intervention, as well as about the possible consequences and risks for his life and health.

Such consent contains an important component of the person's awareness of the decision made on the basis of the information provided to him (regarding the content, order and consequences of the intervention).

At the same time, it should be taken into account that consent as an element of the voluntariness of decision-making and its result often depends on many objective and subjective factors (for example, it is given under the influence of pain, fear, etc.), which can significantly affect a person's assessment of the situation in which he finds himself, as well as on reducing control in making one's own decisions [11].

The legal basis for conducting biomedical interventions consists of numerous international legal acts, which are based on the principle of voluntary and informed consent: the Convention on the Protection of Human Rights and Dignity in the Use of Biology and Medicine; The 1997 Convention on Human Rights and Biomedicine (also known as the Oviedo Convention) [15]; Additional Protocol to the Convention on Human Rights and Biomedicine on Transplantation of Human Organs and Tissues of 2002 (hereinafter - Additional Protocol on Transplantation) [16]; Additional Protocol to the Convention on Human Rights and Biomedicine in the Field of Biomedical Research of 2005 (hereinafter - Additional Protocol in the Field of Biomedical Research) [17]; Additional Protocol to the Convention on Human Rights and Biomedicine concerning Genetic Testing for Medical Purposes of 2008 (hereinafter - Additional Protocol on Genetic Testing) [18]; The 1997 Universal Declaration on the Human Genome and Human Rights [19], the 2005 Universal Declaration on Bioethics and Human Rights [20], as well as the 2000 Charter of Fundamental Rights of the European Union [21] and a number of EU directives, recommendations and regulations.

The Oviedo Convention defines a general rule according to which: "Any intervention in the field of health may be carried out only after the voluntary and informed consent of the person concerned". Such a person is provided with appropriate information in advance about the purpose and nature of the intervention, as well as about its consequences and risks" [15], "persons on whom research is conducted must be informed about their rights and guarantees established by law for their protection; the necessary consent provided for in Article 5 must be given clearly, specifically, and must be documented".

The Universal Declaration of Bioethics and Human Rights proclaimed the important principle of consent: "Any medical intervention for preventive, diagnostic or therapeutic purposes must be carried out only with the prior, free and informed consent of the person concerned on the basis of adequate information. Consent in appropriate cases must be clearly expressed" [19].

A person's awareness of decision-making is one of the essential conditions for biomedical intervention. At the same time, in addition to the general provisions for the protection of persons capable of giving consent, special protection is needed for persons who are unable to give their consent. Thus, according to the Oviedo Convention, "... intervention with regard to a person who is incapable of giving consent may be carried out only on the condition that it will have a direct benefit for such person"[15].

Provisions regarding consent to biomedical interventions are contained in the Charter of Fundamental Rights of the European Union in the context of the right to the integrity of the person: "In the fields of medicine and biology, the following shall be respected, in particular: the voluntary and informed consent of the person concerned, in accordance with procedures established by law"[21].

Enshrining the principle of voluntary informed consent in numerous international legal acts is an important component of the right to life in international law, as well as the protection of human rights in judicial practice [24].

Informed consent is a process. The initiation of this process requires the provision of appropriate information to the potential participant, ensuring that the individual has adequately understood the relevant facts and consented or refused to participate without coercion, undue influence, or misrepresentation [25].

Informed consent is based on the principle that individuals who are capable of giving informed consent have the right to voluntarily decide whether to participate in research. Informed consent protects individuals' freedom of choice and respects individual independence.

CONCLUSIONS

Having studied, analyzed and researched the issue of "informed consent of the patient for conducting biomedical research", it is possible to come to the unequivocal position that this issue is very relevant and important today. It is mentioned and constantly improved in both international and national legislation. A person repeatedly needs medical assistance during his life.

The right to life and health is guaranteed to everyone by the Constitution of Ukraine and should be unhindered for a person to exercise this right. The process of diagnosis and treatment depends only on the person himself and his free decision. Only with the patient's consent is this or that study conducted. International legal acts indicate that a person's failure to consent to medical intervention is considered an interference in his personal private life, a violation of his dignity and freedom.

"Informed consent" includes not only the concept of consent itself, i.e. the free decision of a person, but also an explanation of a specific case or research procedure. And it largely depends on the specialist. Will he be able to convey and explain the patient's problem, illness, actions correctly, fully and in an accessible form? Yes, free consent is given by a person, but the doctor also influences this decision to some extent. Patients' freedom in choosing medical care methods is somewhat limited. The patient can choose only among those methods of medical intervention recommended by the attending physician or refuse treatment.

That is why the question of "informed patient consent" will continue to be one of the most debated issues and will repeatedly be an excellent topic for research by young scientists.

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The Authors declare that there is no conflict of interest

CORRESPONDING AUTHOR
Vitaliy M. Herzanych
Uzhhorod National University
26 Kapitulna st., 88000 Uzhhorod, Ukraine
e-mail: belov_dimon@yahoo.com

ORCID AND CONTRIBUTIONSHIP
Vitaliy M. Herzanych: 0000-0002-5344-6095 Ⓐ
Taras O. Datso: 0000-0001-7737-245X Ⓓ Ⓕ
Oksana I. Matviienko: 0000-0001-8106-1355 Ⓑ Ⓕ
Viktoriia Yu. Svyshcho: 0000-0002-6810-8267 Ⓐ Ⓕ

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

REVIEW ARTICLE

Biliary atresia in children (analytical literature review and review of own observation)

Olga Gorbatyuk[1], Halyna Kurylo[2]
[1]SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE
[2]DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE

ABSTRACT

Aim: to review information resources and analysis of the own experience on this problem for the provision of modern knowledge in the pathogenesis of the pathology, the latest diagnostic and treatment technologies, with consideration of the need to adhere to a single strategy in the management of patients with BA.

Materials and Methods: The analysis of the data regarding the results of existing studies evaluating the clinical benefit and safety of diagnostic and treatment methods in Biliary atresia.

Conclusions: BA is the leading cause of neonatal cholestasis development. Early diagnostics of BA, based on the complex evaluation of clinical-laboratory, instrumental and morphological signs of the pathology, has a significant meaning. Surgical correction during the first 2 months of life – the Kasai procedure, as well as dynamic post-surgery follow-up significantly prolong the life of children and allow postponing liver transplantation. The highest patient survival both at the first stage of treatment - conduction of the Kasai procedure and the stage of liver transplantation may be achieved by joined work of surgeons and pediatricians, which allows considering the whole row of possible problems.

KEY WORDS: biliary atresia, infants, diagnostics, treatment

INTRODUCTION

Biliary atresia (BA) is one of the most frequent causes of the development of neonatal cholestasis. It is an intrahepatic and/or extrahepatic inflammatory cholangiopathy of still uncertain pathophysiology leads to biliary duct obliteration during the first weeks of life, progressive liver fibrosis, secondary biliary cirrhosis, and eventually death, if not treated in a timely fashion. [1, 2] Nowadays, BA remains one of the pathologies of infants, which is extremely difficult to treat. Despite the number of studies dedicated to the problem of BA, the number of publications related to clinical recommendations for this pathology treatment is limited. Progressive obliteration of extrahepatic bile ducts with gradual involvement of intrahepatic bile duct injury and formation of biliary liver cirrhosis lies at the basis of the disease [3]. Without surgical intervention, BA causes the death of the child during the first 2 years of life. In recent decades, significant progress in the treatment of children with BA was achieved – timely Kasai's operation, and in case of its inefficiency – liver transplantation increased the survival of patients with BA to 90% [4-6].

AIM

The aim of the article is the review of information resources and analysis of the own experience on this problem for the provision of modern knowledge in the pathogenesis of the pathology, the latest diagnostic and treatment technologies, with consideration of the need to adhere to a single strategy in the management of patients with BA.

MATERIALS AND METHODS

The analysis of the data regarding the results of existing studies evaluating the clinical benefit and safety of diagnostic and treatment methods in Biliary atresia.

REVIEW AND DISCUSSION

EPIDEMIOLOGY

According to the data of epidemiological studies, the frequency of cholestatic jaundice is approximately 1:2500 infants and the incidence of BA in different

countries of the world varies from 1:3500 to 1:20000 live-born children [7]. BA is more frequent in Taiwan and Japan, compared to North America and Europe. Several studies have found that the highest incidence of BA is in African-American children. The evidence concerning gender differences in BA incidence is not convincing. BA amounts to approximately 8% of all defects of internal organs in children. According to some data, BA incidence is higher in premature infants [6-8]. Children with BA and developmental defects in other body systems are not frequent; there is also data about single episodes of association of BA with chromosomal abnormalities [9]. In most episodes, BA is isolated or related to other hepatobiliary pathologies and developmental defects [10].

HISTORICAL ASPECTS

The first notice about BA was published by J. Burns in 1817 [11]. The author wrote that the presence of jaundice during the first months of life may indicate a serious danger to the life of the child, especially if the white colour stool is observed, and the most possible reason for this is biliary system obstruction. Later, in 1852, C. West presented a detailed description of clinical symptoms of this disease in a 13-week-old girl, who was born to healthy parents, and on the 3rd day of life, she developed jaundice, which was gradually increasing, as well as white-color cream-like stool and dark urine. In 1891, J. Thomson defended a thesis, dedicated to the issues of the clinical presentation and pathological anatomy of congenital obliteration of the bile ducts. The work was based on his experience of observation of 50 children with this pathology. The author concluded about the primary lesion of extrahepatic bile ducts with gradual involvement of the intrahepatic bile system with the formation of biliary liver cirrhosis [12]. In 1916, J. B. Holmes dedicated the work to diagnostics and treatment of congenital obliteration of bile ducts, in which the author paid attention to the danger of the pathology which leads to biliary cirrhosis with lethal outcomes [13].

In 1928, W. E. Ladd et al. made the first attempt at surgical correction of BA in Boston. 11 children underwent surgeries, and it was concluded that in the case of conduction of surgical intervention conduction at the age before 4 months of life clinical and laboratory manifestations of the disease decreased [14]. By 1946, 146 with BA underwent surgery in Boston Children's Hospital. During surgery, the doctors managed to staple anastomosis between bile ducts and duodenum only in 27 patients. Herewith, jaundice disappeared only in 12 patients, and the condition of 15 children

deteriorated after surgery and led to lethal outcomes [15]. Hepatoportoenterostomy operation, which was developed by M. Kasai and S. Suzuki in the 50s of the last century, remains the main method for the treatment of children with BA [16]. The surgical intervention, which nowadays is named after Kasai, is based on the idea that the intrahepatic bile ducts persist in early childhood and are located in the cone-shaped fibrous tissue of the porta hepatis. In the 1970s, professor M. Kasai (Japan) studied pathological changes in intrahepatic and extrahepatic bile ducts in children with BA, as the result of which two important conclusions were made: 1) the process of destruction of intrahepatic bile ducts with the development of a pseudo-ductular proliferation of the portal tracts is progressively increasing during the period of 2nd to 12th week of the life; 2) histological signs, indicating on the preservation of the integrity of intrahepatic bile ducts in the area of the porta hepatis, is a favorable indicator of the prognosis. In this situation, renewal of the patency of the extrahepatic bile ducts prevents further obliteration of the intrahepatic bile ducts [17, 18].

In 1974 M. Kasai offered the classification, which described two types of BA: type I – BA, which can be corrected (bile ducts or cystic formations with thick bile in porta hepatis) and type II – BA, which cannot be corrected (is characterized by the absence of bile ducts in porta hepatis [17].

AETIOLOGY AND PATHOGENESIS

The extrahepatic bile system is known to originate from the caudal part of the liver bud approximately at 4 weeks of gestation. Hepatocytes and cells of intrahepatic ducts stem from the cranial part. The bile begins to secrete into the duodenal tube before 12–14 weeks. Development of BA may be the result of abnormal morphogenesis of bile ducts or lesions of normally formed ducts. The process of obliteration of extrahepatic bile ducts was found to take place during the first trimester of pregnancy. Later, on the further terms of intrauterine development, circulation of the bile of the foetus occurs with the participation of the placenta and intestines of the mother. Only a small amount of it comes into non-formed bile ducts, causing the inflammatory reaction of surrounding tissues. Therefore, the most vivid presentation of the disease develops after birth, when the mother's organism is excluded from the process of bile circulation [19, 20]. Nowadays, the etiology and pathogenesis of BA remain not studied completely [10, 21]. Different theories are discussed: theory of developmental abnormality, viral, genetic, etc. According to the theory of developmental abnormality, the absence

of canalization of the primary epithelial lining in the embryonic stage may take place. The possibility of a combination of several defects of development indicates this theory. However, defects of the bile system may be the result of infection, intoxication, and other pathological factors, influencing morphogenesis in the early terms of intrauterine development [22]. Most children with BA have meconium, coloured with bile, which allows assuming normal primary development of bile ducts and practically excludes the theory of development abnormality [23, 24].

Data from experimental studies indicate an interconnection between the persistence of cytomegalovirus (CMV), respiratory syncytial virus, Epstein-Barr virus, human papillomavirus, and reovirus type 3 and the development of BA. A relation between virus hepatitis A, B, C and the development of BA was not found [25-27]. The infectious etiology of the pathological process was first assumed in 1965 by

H. Stern and S.M. Tucker, who distinguished CMV in a patient with BA [28]. The infectious etiology of BA was studied by B.H. Landing, who developed the conception of perinatal obliterating inflammation of the bile system with the involvement of both extrahepatic and intrahepatic ducts. The author assumed that neonatal hepatitis, BA, and cyst of the common bile duct are manifestations of one inflammatory process developing at different stages of intrauterine development [29].

Nowadays, studies on the role of the immune system in the pathogenesis of BA are conducted. Some observations indicate the possibility of genetic predisposition to the development of BA [30, 31]. The main etiological cause of the embryonic type of BA is considered to be gene mutation, which causes pathological morphogenesis of the biliary tree. According to many researchers, perinatal BA is acquired, and not the congenital pathology. In the case of perinatal BA, the biliary system develops regularly, but later it undergoes the influence of provocative factors, leading to obliteration of the biliary tract (viruses, toxic ecological factors, blood supply disorders). The most frequent factor of BA formation is CMV, the DNA of which is found in liver biopsy slides in 80% of episodes. Based on the literature data, it is possible to conclude that according to its etiology, BA is a heterogeneous disease and shall be considered a general condition of various disorders [10].

CLINICAL MANIFESTATIONS OF BA

In most cases, children with BA are born at full-term and have anthropometric indicators corresponding to the normal range. Obtaining a detailed family history (neonatal cholestasis in parents or siblings, fetal loss or early death of a child in the anamnesis, hemolytic diseases, etc.), prenatal and postnatal history (prenatal results of USD, cholestasis of pregnancy, acute fatty liver of pregnancy, maternal infections), data about neonatal screening (general health condition disorders, neonatal infection, stool and urine color, hemorrhage, etc.), having increased risk of probable BA is compulsory. Based on our observations, family history was burdened in 20% of children with BA.

Jaundice develops on the 2nd – 3rd day of life, which is in regular terms for physiological jaundice. Approximately 23 studied patients had a "light period" – reduction of jaundice intensity by the end of 1–2 weeks of life with its gradual increase and development of the greenish shade by the end of the 1st month. Acholic stools are the earliest and constant clinical symptom of the disease. The urine is dark.

The characteristic feature of BA is the absence of hepatomegaly at birth with further enlargement of the dimensions of the liver and changes in its consistency from elastic to dense during the first 2 months of life [4, 33].

Development of haemorrhagic syndrome (hemorrhage from mucous membranes of the gastrointestinal tract, umbilical wound, intracranial hemorrhages), caused by the deficiency of vitamin K-dependent coagulation factors as the result of the violation of the process of vitamin K absorption in the intestine is possible approximately at the 1st month of life, what we observed in 60% of patients. Absorption of fat-soluble vitamins is known to take place with the participation of bile, which does not come into the intestinal tract in the case of BA. The weight deficit, as a rule, is formed at the age of 1–2 months, and the degree of its expression depends on the nutrition of the child. The most expressed deficit is observed in the case of breastfeeding or the use of milk formula, recommended for feeding healthy infants. In the case of nutritional care, weight deficit can be absent or minimally expressed [32].

Without surgical treatment, signs of portal hypertension appear yet before 5–6 months together with skin itching and xanthomas, which progressively grow and indicate the formation of biliary liver cirrhosis [33].

The earliest laboratory indicator of BA is bilirubin increase due to the direct fraction in the blood serum, which amounts to 20% of the level of bilirubin total. An increase in other biochemical markers of cholestasis (gamma-glutamyl transferase GGT, β-lipoproteins, cholesterol, alkaline phosphatase, bile acids, etc.) is typical, and the level of its expression increases in dynamics from the minimal increase in first 2–3 weeks of life to significant increase before 2-3 month of life. GGT ferment is especially indicative, as its level may be exceeded more than 20 times. Cytolysis ferments (ALT,

AST) are moderately increasing, which is delayed as a rule. In most episodes of BA, these parameters remain within the normal range during the first 2–3 weeks after birth. Parameters, reflecting the protein-synthetic function of the liver (albumin, fibrinogen, PTI, etc.) change at the early stages of the disease. The finding of low levels of PTI in these patients reflects disturbances of the processes of vitamin K absorption in the intestinal tract, which are renewed well in the case of vikasol use [34].

Special attention should be given to the fact that patients with BA do not have specific anamnestic or clinical symptoms. A thorough examination has a decisive meaning for the appropriate evaluation of jaundice in a neonate and infant. Diagnostic examination in any child with prolonged jaundice (after 14 days in full-term infants and after 21 days in premature infants with a clear tendency to reduction) shall include evaluation of the family history, pregnancy anamnesis, clinical examination and monitoring of the stool color. While evaluating the color of the stool, it is important to remember that the use of certain formulas may influence the color of the stool. In such a way, according to our observations, Humana low-lactose with medium chain triglycerides, Alfare, Pregestimil, Nutrillon, etc. give the stool different shades of gray – from light to dark gray.

LABORATORY DIAGNOSTICS OF BA

During BA diagnostics, obtaining of detailed family history of neonatal cholestasis in parents or siblings, fetal loss or early death of a child in the anamnesis, haemolytic diseases, etc.), prenatal and postnatal history (prenatal results of USD, cholestasis of pregnancy, acute fatty liver of pregnancy, maternal infections), data about neonatal screening (general health condition disorder, neonatal infection, stool and urine color, hemorrhage, etc.), having increased risk of probable PA is compulsory.

An increased level of direct (conjugated) bilirubin for more than 20 umol/l or for more than 20% of the level of the bilirubin total is observed in the blood of children with BA. Conjugated bilirubin is found in urine. Urine does not contain urobilin, as it is not formed and cannot come into the intestinal tract [35].

Cytolysis ferments (ALT, AST) are moderately increasing with a delay. In most episodes of BA, these parameters remain within the normal range during the first 2–3 weeks after birth, and then they gradually increase with the progression of hepatocyte cytolysis. The increased level of biochemical markers of cholestasis – plasma beta-lipoproteins, cholesterol, alkaline phosphatase, and bile acids. In coagulogram - signs of hepatic coagulopathy: reduction of prothrombin, fibrinogen, prothrombin time, and activated partial thromboplastin time [36].

Determination of the level of GGT (gamma-glutamyl transferase) in blood serum, which is significantly increased in case of this disease (almost 20 times) has significant meaning in establishing the diagnosis of BA [2, 4, 37]. EIA test for the detection of intrauterine infection detects CMV infection in 80% of the cases [38].

INSTRUMENTAL DIAGNOSTICS

ULTRASOUND
A characteristic triad of ultrasound signs of BA: hypoplasia or absence of the gallbladder; triangular cord sign at the fibrous area in the porta hepatis above the portal vein bifurcation associated with periportal fibrosis of the liver parenchyma, increase in the diameter of the hepatic artery.

Ultrasound of the abdominal cavity is appointed to exclude a choledochal cyst and gallstone disease, which cause obstruction of extrahepatic biliary ducts Contraction of the gallbladder after feeding excludes BA. In some cases of BA, extended intrahepatic bile ducts are detected, cysts in porta hepatis are less frequent. It is important to note that the informativeness of ultrasound significantly decreases in children after 3 months of life [33]. Among children with BA, studied by us, 2 patients developed polysplenia.

HEPATOBILIARY SCINTIGRAPHY
In BA, absorption and accumulation of nucleotides by hepatocytes are rapid, however, there is no excretion into the intestinal tract, even on the postponed scans. Analysis of the excretion of isotopes is not sufficiently informative to differentiate BA from other cholestatic liver diseases, which may lead to the delay in diagnosis establishing dues to the necessity of pre-test administration of phenobarbital. However, demonstration of the present bile excretion may be valuable while establishing a diagnosis [34].

MRI. The criteria of BA are considered to be the bile duct, which is not visualized, and the small dimensions of the gallbladder. Certainly, MRI is a prospective method of hepatobiliary system visualization. MRI is the most accurate non-invasive examination method, allowing diagnosis to be established almost without error [33, 35].

Intraoperative liver biopsy – wedge-shaped excision of the particle of parenchyma with the purpose of histological examination – applied in all children with BA [36].

Prenatal diagnostics. Low GGT level in amniotic fluid at the 18th week of gestation may be a sign of BA[35, 37].

Table 1. Causes of neonatal cholestasis

Extrahepatic cholestasis
Atresia of extrahepatic bile ducts (biliary atresia)
Common bile duct cyst
Gallstones and bile duct stones
Compression of the common bile duct

Intrahepatic cholestasis
Progressive familial intrahepatic cholestasis (PFIC)
Type 1 (Byler disease)
Type 2 (Byler syndrome)
Type 3 (MDR3 deficiency)
Benign familial intrahepatic cholestasis
Metabolic disorders
Alpha-1 antitrypsin (AAT) deficiency
Galactosemia
Fructosemia
Tyrosinemia
Bile acid synthesis disorders (BASD) due to enzyme defects
Peroxisome deficiency (Zellweger syndrome),
Neonatal haemochromatosis
Niemann-Pick disease type C
Mitochondrial insufficiency
Infectious diseases (virus, bacterial, caused by protozoa)
Endocrine disorders
Hypopituitarism
Hypothyroidism
Chromosomal disorders
Trisomy 13, 17 or 18 chromosomes
Cholestasis induced by total parenteral nutrition
Drug-Induced Cholestasis
Other
Alagille syndrome
Non-syndromic intrahepatic bile duct hypoplasia.
Perinatal sclerosing cholangitis
Idiopathic neonatal hepatitis

Liver macroscopy in biliary atresia. The color of the external surface of the liver changes from green to dark green in 81% of clinical observations. In 19% of cases, the color of the liver varies from yellow-green to red-brown. The surface of the liver and parenchyma are fine-grained. In 43% of cases, the extension of intrahepatic bile ducts occurs, and cyst cavities closer to the porta hepatis contain thick biliary sludge. The choledoch duct has either a form of fibrous cord (76.2% cases) or is hypoplastic (23.8% of observed cases). The gallbladder has either a form of fibrous cord (66.7% of cases) or hypoplastic (33.3% of cases).

HISTOLOGICAL STUDIES OF THE LIVER IN BILIARY ATRESIA

The most characteristic histological changes of the liver in BA are:
- cholestasis in hepatocytes and bile ducts
- proliferation of bile ducts
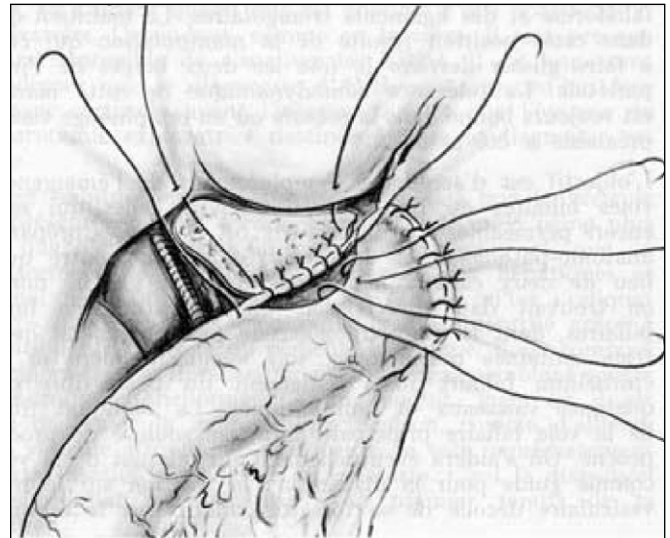- giant transformation of hepatocytes
- portal oedema

Fig. 1. Kasai Procedure (hepatoportoenterostomy).

- liver fibrosis and cirrhosis
- absence of diagnostic signs of other diseases of the liver of an infant.

The degree of morphological changes depends on the age of the child and increases with age [38].

Diagnostic accuracy of the examination methods of BA:
- Liver biopsy - 96.9%
- Clinical-laboratory study - 70.8%
- Ultrasound 69,2%
- Scintigraphy – 58.5% [33, 34, 38].

Examination by medical specialists Purposeful establishment of the diagnosis in neonates and infants with jaundice should also be based on the examination by the medical specialists aimed at differential diagnosis conduction of the pathology: geneticist, ophthalmologist, cardiologist, infectious disease specialist, etc. Radiography of the lungs and heart, diagnosis of vertebral and spinal anomalies, echocardiography, etc. are recommended.

Differential diagnosis of BA is conducted with other liver and bile duct diseases, which manifest as cholestasis syndrome (Table 1).

SURGICAL CORRECTION

Surgical modifications of the Kasai (hepatoportoenterostomy) include removing the structures, located in the area of porta hepatis, and conducting the resection of the fibrous residue of the bile duct at this level. This resection opens the lumen of the preserved bile ducts, which remain passable [14, 15, 32]. For applying biliary-intestinal Y-anastomosis the loop of the small intestine (Fig. 1).

The efficiency of this procedure depends on several factors: age of the child, expression of cirrhosis changes, number of functioning bile ducts in porta hepatis,

presence of developmental defects of other organs, surgeon's experience, post-surgery follow-up of the patient, etc.

One of the main factors influencing the efficiency of the Kasai procedure is the age of the child at the moment of surgical intervention. The most optimal age for the procedure is the first two months of life. According to R. Ohi et al., jaundice clearance after the surgery is observed in 80% of children at the age under 2 months [32]. In the case of procedure conduction at an older age, its efficiency reduces.

We have experience of surgical correction of BA using Kasai procedure in 36 patients. To prevent cholangitis we use a modified Kasai procedure - invaginated hepatic porto-jejunostomy (creation of an invaginated valve in the biliary area of the intestinal tract).

COMPLICATIONS AFTER KASAI PROCEDURE

There exists a probability of the development of cholangitis, portal hypertension, hepatopulmonary syndrome or pulmonary hypertension, intrahepatic cysts and tumors after the Kasai procedure. The early detection and timely correction of these complications have a very significant meaning [1-3].

Direct connection of intrahepatic bile ducts with the intestinal tract leads to the infection and development of *cholangitis*, which may be observed both during the first weeks and months and in the distant future. Prophylaxis of cholangitis includes long-term use of bactrim for 3–6 months with a gradual transition to lower use under the condition of the absence of inflammatory changes in total and biochemical blood tests, 2–3 days per week for 3–6 months. Cholangitis treatment includes parenteral administration of antibacterial and/or antiviral medication and conduction of non-specific syndromic therapy. Development of cholangitis in a delayed post-surgery period may be associated with the formation of intestinal reflux, stoma obstruction, as well as the result of cyst infection. Detection of the surgical cause of this complication is the indication for repeated surgery, while in other situations antibacterial therapy is appointed.

Portal hypertension develops in approximately 2/3 of children after portoenterostomy.

In case of adequate bile passage, varicose veins treatment is conducted in the form of sclerotherapy or vein ligation.

Release of vasoactive substances from the intestine after surgery or a decrease in their clearance in porto-systemic shunts can lead to the development of pulmonary arteriovenous shunts or pulmonary hypertension.

Intrahepatic cysts may develop within several months or years after the Kasai procedure. Cysts of the liver may not have any clinical manifestations, however, there is the risk of their infection and portal vein compression. In such a situation, antibacterial therapy or surgical correction is recommended.

Hepatocarcinoma, hepatoblastoma and cholangiocarcinoma were described in children after the Kasai procedure. Due to this, the patients should undergo examination, allowing detection of a tumor at the early stages of its development.

Obstruction of bile outflow. In such situations, repeated surgical intervention with the revision of porta hepatis may be conducted.

INDICATIONS FOR LIVER TRANSPLANTATION

1. Portal hypertension, especially in the events of hemorrhages
2. Recurrent cholangitis
3. Recurrent jaundice and/or liver failure syndrome
4. Suspicion of hepatocellular carcinoma
5. Significant physical and mental development delay

Liver transplantation is the main method of treatment for children with BA due to life-threatening complications. Liver transplantation is considered to be more appropriate than Kasai portoenterostomy in the case of BA in many transplantation centers in the world [39, 40].

CONCLUSIONS

Thus, BA is the leading cause of neonatal cholestasis development. Early diagnostics of BA, based on the complex evaluation of clinical-laboratory, instrumental and morphological signs of the pathology, has a significant meaning. Surgical correction during the first 2 months of life – the Kasai procedure, as well as dynamic post-surgery follow-up significantly prolong the life of children and allow postponing liver transplantation. The highest patient survival both at the first stage of treatment - conduction of the Kasai procedure and the stage of liver transplantation may be achieved by joined work of surgeons and pediatricians, which allows considering the whole row of possible problems.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Olga M. Gorbatyuk
Shupyk National Healthcare University of Ukraine
9 Dorogozhitska St., 04112 Kyiv, Ukraine
E-mail: ol.gorbatyuk@ gmail.com

ORCID AND CONTRIBUTIONSHIP
Olga Gorbatyuk: 0000-0003-3970-8797 Ⓐ Ⓑ Ⓓ Ⓔ
Halyna Kurylo: 0009-0001-0863-4046 Ⓕ

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

REVIEW ARTICLE

Expanding the possibilities of using sodium-glucose cotransporter 2 inhibitors in patients with heart failure

Maryana M. Rosul, Myroslava M. Bletskan, Nataliya V. Ivano, Svitlana O. Rudakova
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT
Aim: To study the potential mechanisms of the beneficial cardiovascular effects of sodium-glucose cotransporter 2 (SGLT-2) inhibitors, the possibilities of improving the treatment and prognosis of patients with acute heart failure (HF) during their use.
Materials and Methods: The data analysis of literary sources has been conducted regarding the results of existing studies evaluating the clinical benefit and safety of SGLT-2 inhibitors in patients with acute heart failure.
Conclusions: The peculiarities of the pharmacological action of SGLT-2 inhibitors and the obtained research results expand the possibilities of using this group of drugs, demonstrating encouraging prospects in improving the prognosis of patients hospitalized with acute heart failure.

KEY WORDS: heart failure, acute heart failure, sodium-glucose cotransporter 2 (SGLT-2) inhibitors, empagliflozin, dapagliflozin

INTRODUCTION

Heart failure (HF) is a global pandemic with a worldwide prevalence of more than 60 million cases. After the diagnosis of chronic HF, a little more than 50% of patients survive within 5 years, and the prognosis of patients after decompensation of chronic HF, requiring hospitalization, is significantly worsening [1-3]. Improving the prognosis of patients with HF and the reduced left ventricular (LV) ejection fraction (EF) in the form of reduced mortality was until recently based on the use of three groups of drugs, the so-called "triple neurohumoral blockade": the therapy with angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker (ACEI/ARB), beta-blocker and mineralocorticoid receptor antagonist (MRA). It was revised for the first time after receiving positive results from studies on the reduction of mortality and the risk of re-hospitalization in this cohort of patients with the use of angiotensin receptor–neprilysin inhibitor (ANRI) [4]. However, a real breakthrough in the medical treatment of chronic heart failure was the addition of SGLT-2 inhibitors (empagliflozin and dapagliflozin) to the standard therapy. Two large randomized clinical trials (EMPEROR-Reduced and DAPA-HF) provided convincing evidence that empagliflozin and dapagliflozin reduce the combined endpoint in patients with chronic HF and reduced ejection fraction (HFrEF), the risk of cardiovascular death, recurrent hospitalizations

in patients with and without existing diabetes mellitus type 2 (T2DM), and also help to reduce the loss of renal function [5-7]. The European Society of Cardiology (ESC) 2021 Guidelines for the treatment of HFrEF have helped to establish the appointment of SGLT-2 inhibitors as the fourth drug in the first-line therapy of chronic HF. It remains unchanged in the presented 2023 Focused Update of the 2021 ESC Guidelines for the management of HF [8, 9].

According to the results of the EMPEROR-Preserved study, empagliflozin became the first drug with proven effectiveness in the treatment of chronic HF and LVEF >40%, regardless of the presence of type 2 diabetes [10]. This discovery was not a coincidence, as the DELIVER study was published the following year, which resembled EMPEROR-Preserved in terms of design and the results of using another SGLT-2 inhibitor, dapagliflozin [11]. The presented studies, supported by meta-analyses, convincingly indicated the positive effect of empagliflozin and dapagliflozin on the course of chronic HF regardless of LVEF values and the presence of T2DM [10, 11]. The results obtained were reflected in the 2023 Focused Update of the 2021 ESC Guidelines for the management of HF, where SGLT2 inhibitors and diuretics (if needed) are indicated as the only drugs for the treatment of HF with mildly reduced EF (HFmrEF) or HF with preserved EF (HFprEF) with class recommendations I and the level of evidence A [9].

AIM

The aim is to study the potential mechanisms of the beneficial cardiovascular effects of sodium-glucose cotransporter 2 inhibitors, the possibilities of improving the treatment and prognosis of patients with acute HF during their use.

MATERIALS AND METHODS

The data analysis of literary sources has been conducted regarding the results of existing studies evaluating the clinical benefit and safety of SGLT-2 inhibitors in patients with acute heart failure.

REVIEW AND DISCUSSION

The mechanisms by which SGLT-2 inhibitors reduce mortality from cardiovascular diseases and chronic heart failure are multifactorial. The "fast" mechanisms include hemodynamic ones, which are characterized by a reduction in preload and afterload and are due to a positive effect on blood pressure indicators and volume of circulating blood. SGLT-2 inhibitors block the reuptake of glucose and sodium in the proximal tubules of the kidneys, therefore causing glucosuria, mediated osmotic diuresis and transient natriuresis. In contrast to the action of traditional diuretics, there is a more significant decrease in the volume of interstitial (tissue) fluid, not the intravascular volume and the increase in diuresis observed in the treatment of SGLT-2 inhibitors, which is not accompanied by the activation of the renin-angiotensin-aldosterone and sympatho-adrenal systems [12, 13].

Experimental studies have shown that SGLT2 inhibitors, in particular, empagliflozin, can accelerate the processes of active relaxation and reduce inflammation in the myocardium, oxidative stress, hypertrophy and fibrosis of the myocardium [14-16] and thus can improve LV function. Despite the existence of a significant amount of experimental data, clinical data on the effect of SGLT-2 inhibitors on the structure and function of the LV are limited. The EMPA-HEART CardioLink-6 randomized clinical trial aimed at filling this gap by examining the effects of empagliflozin in patients with coronary heart disease and type 2 diabetes without HF or significant LV dysfunction. The study demonstrated a significant reduction in left ventricular myocardial mass index (LVMI), measured by cardiac magnetic resonance imaging (MRI), after a 6-month course of empagliflozin at a dose of 10 mg/day (-2.6 and -0.01 g/m^2 in empagliflozin and placebo groups, respectively, adjusted difference being -3.35 g/m^2; p=0.01) [17]. The decrease in the mass of the left ventricle (LVM) was not accompanied by a change in LV volumes, which in most patients at the beginning of the study had been within the normal range and indicates a decrease in the thickness of the LV wall under the influence of the studied therapy. The observed effect on LVM may be one of the factors explaining the cardioprotective effects of empagliflozin observed in larger studies evaluating cardiovascular outcomes, particularly in EMPA-REG OUTCOME, since LVM is a strong and independent predictor of cardiovascular events, including myocardial infarction, HF and cardiovascular death [17].

In 2 other studies, an effect on LV remodeling (decrease in the LV end-diastolic (EDV) and end-systolic (ESV) volume) was observed when using SGLT-2 inhibitors in patients with chronic HF and reduced LVEF. Thus, in the EMPA-TROPISM study (n=84) involving patients with HF without diabetes, 6-month use of empagliflozin led to a statistically significant decrease in LV EDV and ESV compared with placebo (p<0.001) [18]. Empagliflozin was also associated with a reduction in LVM (-17.8±31.9 g vs. 4.1±13.4 g in the empagliflozin and placebo groups, respectively; p<0.001) and LV sphericity (Δ sphericity index: -0.1 ± 0.08 g vs. 0.01 ± 0.08 g for empagliflozin vs. placebo; p < 0.001), as well as an improvement LVEF (6.0±4.2 % vs. -0.1±3.9 %; p<0.001) [18]. A similar decrease in LV EDV and ESV was reported in the SUGAR-DM-HF study (n=105, empagliflozin vs placebo, 36-week follow-up), which was conducted with the participation of patients with existing type 2 diabetes and HFrEF [19]. The results of these studies confirm the beneficial effect of SGLT-2 inhibitors on reverse LV remodeling in patients with HFrEF, regardless of their glycemic status. Reverse LV remodeling is associated with the improved prognosis [18, 19].

However, it should be noted that despite the relationship between reverse LV remodeling and reduced mortality and morbidity in chronic HFrEF, it is unlikely that reverse LV remodeling explains all the positive prognostic effects of SGLT-2 inhibitors, since these effects are already manifested in the early stages of treatment, long before noticeable changes in the LV volume and shape. At the same time, it is assumed that the potential impact of SGLT-2 inhibitors on the structure and function of the LV is multifactorial and mediated not only by systemic hemodynamic, but also metabolic effects.

In addition to free fatty acids and glucose, cardiomyocytes can also oxidize ketone bodies, which are the most energetically efficient substrate, since their oxidation is associated with the use of a minimal amount of oxygen and the formation of a large number of adenosine triphosphate (ATP) molecules. Since SGLT-2 inhibitors induce ketogenesis and hyperketonemia through the

reduction of glucose and insulin levels along with the increase of glucagon levels in plasma, as well as through the stimulation of lipolysis, it has been suggested that the beneficial cardiological effects of the drug may be related to its ability to improve energy metabolism in myocardium by switching myocardial metabolism from glucose consumption to ketone bodies with subsequent inhibition of adverse LV remodeling [20, 21]. Thus, SGLT-2 inhibitors can modify metabolism towards the utilization of more energy-efficient ketone bodies, which has a beneficial effect on the heart, increasing "energy efficiency". There is also an increase in the production of renal erythropoietin, an increase in hematocrit, as well as hemoglobin, which potentially reflects hemoconcentration and can further improve oxygen delivery to the myocardium [22].

The nephroprotective effect of SGLT-2 inhibitors is also known. This group of drugs contributes to the reduction of intraglomerular pressure, reduction of hyperfiltration, albuminuria and stabilization of kidney function. At the same time, the use of SGLT-2 inhibitors prevents the development of the terminal stage of chronic kidney disease or a persistent decrease in glomerular filtration rate (GFR) regardless of the presence of type 2 diabetes [23, 24]. This beneficial effect on renal function is particularly important because some other major drugs (e.g., MRA) often impair renal function in patients with HFrEF [25].

The effect of SGLT-2 inhibitors on increasing the sensitivity of muscle tissue to insulin cannot be neglected, since it reduces the level of uric acid and body weight, which occurs mainly due to the reduction of abdominal (visceral) fat [23]. Research studies from 2021 also show that the use of SGLT-2 inhibitors leads to a significant reduction in the volume of epicardial adipose tissue in a population of patients with type 2 diabetes [26]. In patients with obesity, metabolic syndrome, or coronary heart disease, epicardial adipocytes secrete less adiponectin and more leptin than in healthy individuals. Decrease in adiponectin expression impairs endothelial function and leads to increased production of tumor necrosis factor-α, which exacerbates inflammation and oxidative stress. An increased level of leptin promotes monocyte adhesion, macrophage transformation into foam cells, and adverse changes in the level of lipids and inflammatory cytokines in adipose tissue. Therefore, epicardial adipose tissue (as in the case of abdominal obesity) is one of the most important risk factors for the development of atherosclerosis, while epicardial adipose tissue volume and thickness are independently associated with cardiovascular events [27, 28].

The successful use of SGLT-2 inhibitors in chronic HF and the known mechanisms of their pharmacological action became the triggering factor for evaluating the impact of this group of drugs on the prognosis in patients with acute decompensation of HF, regardless of EF and diabetes status.

The results of treatment with SGLT-2 inhibitors in acute decompensated heart failure were analyzed in the EMPA-RESPONSE-AHF pilot study for the first time. 80 patients with acute decompensated chronic HF, a third of whom had T2DM, were included in the study within 24 hours from the moment of hospitalization and were divided equally into the group receiving empagliflozin 10 mg per day and placebo. The duration of taking the drugs was 30 days, the safety assessment was carried out for 60 days. According to the study, empagliflozin had a minor effect on symptoms, diuretic response (namely, body weight change with furosemide), natriuretic peptide levels, and the period of hospital stay, probably due to the size of the study group. However, empagliflozin reduced the occurrence of the composite endpoint, which included in-hospital worsening of HF requiring treatment intensification; repeated hospitalizations due to HF; overall mortality within 60 days compared with placebo (10% and 33%, respectively; p=0.014). Empagliflozin therapy was safe, well tolerated, and did not cause significant adverse events in terms of blood pressure and kidney function in accord with the findings of EMPA-RESPONSE-AHF study. In general, the frequency of serious side effects was comparable to the placebo group (p=0.54), which proved the high safety profile of SGLT-2 inhibitors in acute decompensated heart failure for the first time [29]. In the empagliflozin group, compared to placebo, a decrease in estimated glomerular filtration rate (eGFR) was more often observed during the first 24-72 hours of therapy, with subsequent recovery of kidney function after 96 hours and further up to 30 days [30].

The EMPULSE study, which was a logical continuation of the previous work, examined the early use of empagliflozin at a dose of 10 mg per day in patients hospitalized for acute HF (33% with de novo and 67% with acute decompensation of chronic HF) regardless of LVEF and T2DM. The study included 530 patients after stabilization of the condition (from 24 hours to 5 days after admission to the hospital). The following criteria for stabilizing the condition were used: systolic blood pressure level of 100 mm Hg. and more, the absence of clinical manifestations of arterial hypotension during the previous 6 hours; stable dose of intravenous diuretic for the last 6 hours; no intravenous administration of peripheral vasodilators and inotropic agents in the last 6 and 24 hours, respectively. The duration of observation was 90 days. According to the results of the EMPULSE study, patients receiving empagliflozin were 36% more

likely to experience clinical benefits of treatment, expressed in reductions in all-cause death, episodes of HF destabilization, and improved quality of life within 90 days (p=0.0054). In addition, a more significant decrease in body weight was noted in this group of patients. It is important that during the entire period of observation, ketoacidosis, hypotension or other problems with the safety of drug use were not noted in the patients. The level of the eGFR, which decreased in the initial period, was restored by the 15th day of empagliflozin use [31]. Data from the EMPULSE study show that, in addition to other clinical benefits, patients also feel better on SGLT-2 inhibitors after just 2 weeks. Notably, empagliflozin was equally effective among patients with acute de novo HF and with decompensated chronic HF regardless of LVEF and T2DM status. The presented results of EMPULSE extend and complement the results of EMPEROR-Reduced and EMPEROR-Preserved, and indicate the feasibility of early prescription of empagliflozin in patients with acute HF in hospital, provided that the patient has no contraindications.

In the EMPAG-HF study, the tactic of prescribing 25 mg of empagliflozin was chosen to achieve the maximum diuretic effect, and the drug was prescribed within the next 12 hours after admission to the hospital, without waiting for clinical stabilization, which ultimately did not lead to safety problems [32].

The results of the large study SOLOIST-WHF should also be mentioned. In this study 1222 patients with type 2 diabetes and chronic HF with both reduced and preserved LVEF were prescribed sotagliflozin during hospitalization for acute decompensated HF after stabilization of the condition and transition to oral administration diuretic drugs or within 3 days after discharge from the hospital in a dosage of 200 mg with an increase in the dose to 400 mg/day in the absence of contraindications. Sotagliflozin therapy reduced the incidence of the combined endpoint (cardiovascular mortality and the number of hospitalizations for acute decompensated heart failure) by 33% (p=0.0009) compared with placebo at a median follow-up of 9 months. Although the number of patients hospitalized at least 1 time did not differ between the study groups (38.5% in the sotagliflozin group and 41.4% in the placebo group), fewer patients were hospitalized more than 1 time in the sotagliflozin group (16.3 and 22.1% respectively). Improvement of the quality of life indicators was also emphasized within this study. In the SOLOIST-WHF study, there were significant differences between the drug and placebo groups in terms of hypotension (6.0% vs. 4.6%), urinary tract infections (4.8% vs. 5.1%); acute kidney disease (4.1% against 4.4%) was not observed. The results of the SOLOIST-WHF study demonstrated that initiation of therapy with SGLT2 inhibitors in patients with acute decompensation of chronic HF after stabilization of the condition is safe and effective regardless of LVEF [33].

A meta-analysis of randomized studies EMPA-RESPONSE-AHF, SOLOIST-WHF, EMPULSE (2022), which evaluated the effectiveness and safety of initiation of SGLT-2 inhibitors in 1831 patients with acute decompensated chronic heart failure regardless of LVEF and the presence of type 2 diabetes, confirmed the effectiveness of SGLT-2 inhibitors. This scheme of therapy reduced the risk of re-hospitalizations due to HF. According to the Kansas questionnaire (Kansas City Cardiomyopathy Questionnaire, KCCQ), an improvement in the quality of life was also observed. The conducted meta-analysis did not show an increase in the risk of acute kidney disease, hypotension, hypoglycemia against the background of treatment with SGLT-2 inhibitors [34]. However, the given results are only a preliminary stage in changing treatment tactics in acute de novo HF/acute decompensated HF. Summing up, it should be noted that according to the results of the conducted studies, empagliflozin currently has the largest evidence base for the safety use and improvement in the effectiveness of treatment for patients with acute heart failure. As attractive as it may seem, the results cannot necessarily be generalized to other SGLT-2 inhibitors without an evidence base. The question of the class effect of the use of SGLT-2 inhibitors in patients with acute heart failure remains open. The results of study on the effectiveness of dapagliflozin in such a clinical situation are expected in the nearest future (DAPA ACT HF-TIMI 68). The possibilities of early (up to 24 hours) appointment of SGLT-2 inhibitors in the category of patients with acute HF are also not sufficiently studied, leaving many unsolved problems and requiring further study in larger prospective controlled studies. The study of the effectiveness of early initiation of SGLT-2 inhibitors in acute myocardial infarction (EMPACT-MI) also seems interesting, taking into account the beneficial effect of SGLT-2 inhibitors on myocardial remodeling, fluid balance and cardiac function. We expect that an additional opportunity to improve the clinical status and prognosis of patients will appear in the arsenal of emergency cardiology doctors.

CONCLUSIONS

The peculiarities of the pharmacological action of SGLT-2 inhibitors and the obtained research results expand the possibilities of using this group of drugs, demonstrating encouraging prospects in improving the prognosis of patients hospitalized with acute heart failure.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Maryana M. Rosul
Uzhhorod National University
148 Sobranetska St., 88017 Uzhhorod, Ukraine
e-mail: maryana.rosul@uzhnu.edu.ua

ORCID AND CONTRIBUTIONSHIP
Maryana M. Rosul: 0000-0002-2106-5386 Ⓐ Ⓑ Ⓓ Ⓔ
Myroslava M. Bletskan: 0000-0002-8069-6145 Ⓐ Ⓑ Ⓓ
Nataliya V. Ivanio: 0000-0003-0147-2176 Ⓑ Ⓕ
Svitlana O. Rudakova: 0000-0001-9129-4523 Ⓑ Ⓕ

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

REVIEW ARTICLE

Legal conflicts in forensic dentistry: practice and methods of resolving them

Vasyl V. Tymchak, Maryana V. Tymchak, Roman M. Fridmanskyy, Viktoriia I. Fridmanska
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

Aim: To investigate and analyze legal conflicts in forensic dentistry of Ukraine.

Materials and Methods: A comparative research method for determining the common and distinctive features of the legal regulation of forensic dental expertise as a subcluster in the legislation of Ukraine. The descriptive (monographic) method reveals the problematic aspects of forensic dental examination through the lens of local normative and general normative connotations. The structural-functional research method made it possible to systematize the peculiarities of forensic medical and forensic dental expert activity.

Conclusions: The analysis of legal conflicts made it possible to come to the following conclusions, that in Ukraine today there is no specific legal act that would regulate the aspects of performing dental activities. Forensic dental examination in Ukraine, in accordance with the legislation, is an examination of the actions and inactions of the dentist. The adoption of normative legal acts in the field of dental activity and forensic dental examination in Ukraine would make it possible to determine the specifics of establishing facts and circumstances that indicate a violation of the patient's rights.

KEY WORDS: legal conflict, forensic dentistry, forensic expertise, legal regulation, dental clinic

INTRODUCTION

Dentistry is a separate branch of medicine that analyzes and investigates the etiological and pathogenetic features of diseases of the tooth, oral cavity and jaw area, focusing on the search for methods of diagnostic, therapeutic and preventive elimination of problems of an organic profile.

At the same time, dental activity is aimed at providing diagnosis, prevention and rehabilitation of the patient, subject to receiving informed consent from him. It is noteworthy that the dentist is entrusted with the duty of ensuring compliance of dental services with organizational, sanitary, anti-epidemic and technical standards. Currently, there is no specific regulatory legal act in Ukraine that would regulate the aspects of performing dental activities (only the Draft Law of Ukraine «On Dental Activities» No. 4736 dated 31.05.2016, rejected by the Verkhovna Rada of Ukraine in the first reading - it will be discussed later). Instead, the legal regulation of dental activity is carried out according to the legal-analogous paradigm, namely, the National Classifier of Professions DK 003:2010 (Derzhspozhivstandard), the Law of Ukraine «Basics of the Legislation of Ukraine on Health Care» No. 2801-XII, the Code of Ukraine on Administrative Offenses (KUpAP) No. 8073-X, Criminal Code of Ukraine No. 2341-III, Civil Code of Ukraine No. 435-IV, Law of Ukraine «On Protection of Consumer Rights» No. 1023-XII, Law of Ukraine «On Appeals of Citizens» No. 393/96-VR, etc.

The above-mentioned legislative uncertainty and simultaneous ramifications lead to a collision forensic dental investigation of the actions and inactions of the dentist (orthodontist, orthopedist, therapist, surgeon)/ dental clinic, which led to or could cause harm to the patient's health. Considering that the forensic dental examination in Ukraine is also carried out according to the analogy of the law (the Law of Ukraine «On Forensic Examination» No. 4038-XII of 02/25/1994 (ed. of 01/01/2024) acts as the guiding principle), analytical search acquires additional relevance of practical and methodological methods of eliminating legal conflicts in the field of forensic dentistry in Ukraine.

AIM

The aim was to investigate and analyze legal conflicts in the forensic dentistry of Ukraine through the prism of practical and methodological proposals for their solution, taking into account the trends of legal and legal analogies of the regulation of dental activities in Ukraine.

MATERIALS AND METHODS

The study of legal conflicts in forensic dentistry for the purposes of the specified theoretical and practical analysis requires a comprehensive approach. We will single out the work in forensic medicine (in general), stomatology (indirectly-specifically), forensic-dentistry (specifically) scientific paradigms.

For example, the forensic medical research cluster is represented by the works of V. Kikinchuk (features of conducting forensic medical research); N. Pasyuk (normative problems, legal (legal) grounds for conducting forensic medical examination); L. Holubovych (definition of framework models for conducting forensic medical expert examinations with the aim of making this process more effective); N. Ergard (theoretical and methodological features of forensic medicine as a separate research field).

The paradigm of scientific research from the point of view of dental theory is revealed in the works of I. Mazur (retrospective and perspective of the formation and development of domestic dentistry); Yu. Khvostivskyi (demand factors in the domestic market of dental services); E. Vishnevskaia (analysis of the domestic dental services market); V. Paliychuk (application of situational modeling methods in the process of professional training of dentists and dental workers), etc.

Forensic-dental scientific paradigm is the most unexplored theoretical branch of domestic science. Among the developments applicable to the topic of our analysis, we single out the work of M. Honcharuk-Homin (forensic dental examination and its implementation); V. Chernyaka (forensic medicine as a scientific discipline for students of medical universities); V. Bakinsky (specifics of teaching forensic medicine to students studying dentistry).

Taking into account the stated trends of understudied issues of forensic dental examination both within the normative and within the theoretical scientific field of Ukraine, it is advisable to use the following methodological toolkit for its complete and comprehensive analysis.

With the help of a comparative research method, the common and distinctive features between the implementation and legal regulation of forensic medical examination in general and forensic dental examination as its sub-cluster under the legislation of Ukraine were identified.

The use of the descriptive (monographic) method made it possible to reveal the problematic aspects of forensic dental examination in Ukraine through the prism of local normative (absence of a profile document-regulator of dental activity) and general normative (the conflict of the Law of Ukraine «On Forensic Expertise» No. 4038-XII) problematic connotations

The structural-functional research method provided an option to systematize the features of forensic medical and forensic dental expert activity in Ukraine in their organic common-distinctive aggregate.

The set of system-analytical methodological tools became the basis for the scientific substantiation of the specific features of forensic dental examination in Ukraine in the conditions of regulatory and legal uncertainty and regulatory deregulation.

REVIEW AND DISCUSSION

Defining the term «forensic dentistry» in accordance with domestic scientific and normative doctrines is of primary importance in the context of highlighting the specifics of conflict-of-law features of forensic dentistry in Ukraine.
We suggest that you pay attention to the scientific approach of M. Honcharuk-Homin [1], according to which forensic dentistry should be considered a separate branch of expert activity, which conducts research on the activities of dental specialists on the subject of appropriateness, qualification, professionalism and compliance with the official accreditation status as a medical specialist, as well as the company (clinic) he represents.

The author adds that dental interventions in their essence are an invariance of clinical intervention with a high risk of trauma, damage, etc., as a result of which the examination and research of not only the dentist, but also the patient acquires fundamental importance for the forensic dental examination of the unqualified activities of the dentist (history of dental research, interventions, etc.) [1]. Currently, however, this approach is not regulated at the level of the legislation of Ukraine, as a result of which the legal analogy refers to the use of the Law of Ukraine «On Forensic Expertise» No. 4038-XII, which has been transformed to the realities of dental activity, as a result of which the intended function of dental activity is lost and the possibility of qualified forensic-medical (forensic-dental) review of a precedent decision of a dentist.

The theoretical understanding of the phenomenon of forensic dentistry was also revealed by V. Paliychuk in his own works [2]. The researcher noted that the correlation between forensic medicine and dental practice, as a rule, is aimed at establishing the facts and circumstances of official incompetence or negligence of a dentist, orthodontist, orthopedist, dentist-therapist, and dentist-surgeon. The author calls the work with biological evidence samples, the description of the degree of traumatic severity and tendency of complexity in the process of the forensic dental examination of the injured patient and the ability to carry out a dental analytical and descriptive examination of the corpse as specific features of the forensic dental examination.

Normative qualification of forensic dental activity in Ukraine is carried out, as mentioned earlier, according to the analogue-legal spectrum. In the legislation of Ukraine, the concept of «dentistry» or «dental activity» is generally absent, as a result of which the forensic dental research profile is regulated by the Law of Ukraine «On Forensic Expertise» No. 4038-XII, according to which forensic dental expertise must be called a special study based on industry knowledge in the medical and dental scientific field, which is carried out on the principles of a legal, independent, objective and full research spectrum (Articles 1, 3 of the Law of Ukraine No. 4038-XII).

In view of the above, we further propose to consider the legal connotations of the Draft Law of Ukraine «On Stomatological Activities» No. 4736 dated 31.05.2016, which proposed definitive amendments regarding the content of dental activities and compliance of such activities with professional qualification standards (to the issue of the application of forensic dentistry in the process study of the results of the activity of a dentist).

In the Draft Law of Ukraine «On Stomatological Activity» No. 4736 dated 31.05.2016 [3], in particular, proposals were put forward to grant stomatological activity the status and form of a guaranteed medical activity, which is generated in proportion to the performance of a dentist of a different specialty and profile own professional duties. In addition, the People's Deputies of Ukraine, the initiators of this regulatory document, insisted on the potential use of this Law of Ukraine in organic unity with the Law of Ukraine «Basics of the Legislation of Ukraine on Health Care» No. 2801-XII. Such an approach, in the opinion of the subjects of the legislative initiative, would make it possible to direct dental activity to the field of compliance and responsibility with the general health and safety act of legislation, which would automatically equate dental activity and the conduct of potential forensic dental examinations to the regulations of the Law of Ukraine «On Forensic Expertise» No. 4038-XII.

In fact, this approach solved the issue of deregulation of dental and forensic dental cluster medical activities only partially, concentrating on the theorizing of the problem, rather than its practical regulation. In addition to the above-mentioned example, the Draft Law of Ukraine «On Stomatological Activity» No. 4736 dated 31.05.2016 [3] also proposed systematizing the professional responsibility of a dentist under Art. 36-45 of Section V of this normative legal act, where the lawmaker actually referred to the norms of civil, administrative and criminal legislation by legal analogy with the specified norms. Such a situation, once again, testified to the settlement of the «facade» rule-making part of the conflict between the legal status of the dentist and the legal responsibility of the latter, which results in conducting examinations, inspections

and forensic dental analysis of the professional actions of the specialist doctor. In addition, the normative document was characterized by non-contextual legal and technical interpretations, as a result of which it was withdrawn in the first reading [4].

The problems described above logically lead to the presence of the following aspect of conflicting dental-legislative regulation in Ukraine: forensic dentistry at the level of regulatory support (by analogy, because there is no branch legislation, as is known) is considered rather as a process of expert work with existing problems of dental treatment (injuries to soft tissues tissues, mechanical traumatization, thermal and (or) electrical radiation effects harmful to the health of the person), and not forensic dental examination of the corpse [5]. As a result of this interpretation of the «legality» of the dentist's actions, it takes on an evaluative form, because it requires the forensic dental expert to make an individualized decision based on an internal imperative (this reduces the impartiality and objectivity factor of making the appropriate decision regarding a specific case).

In the future, it is worth highlighting the legal (normative) and etymological conflicts that logically follow from the provisions of the National Classifier of Professions DK 003:2010 (Derzhspozhivstandard, hereinafter - DK 003:2010) [6] in the context of stomatological and, as a result, forensic dentistry activities in Ukraine .

According to DK 003:2010, a dentist is a person who provides qualified operative and professional assistance to patients at the level of consultations and direct interventions [6].

In turn, a dentist-orthodontist according to DK 003:2010 is a person who, on the basis of professional licensing, provides assistance to the patient with problems related to occlusion imperfections and dental architecture [6].

At the same time, according to DK 003:2010, a qualified specialist who detects dental problems, deals with prosthetics and implantation of missing teeth, and treatment of defects of adjacent tissues of the oral cavity is recognized as an orthopedic dentist.

According to DK 003:2010, a dentist-therapist is a person who provides universal care, current examination of teeth and oral cavity, and prevention of diseases associated with non-compliance with the patient's hygiene regime [6].

A dentist-surgeon (expert in the field of surgical dentistry), in accordance with DK 003:2010, is a specialist in the field of computer-tomographic, orthopantomographic, intraoral-camera examination of the oral cavity/teeth for the purpose of further intervention aimed at treating problem areas (according to conditions of ineffectiveness of conservative treatment methods) [6].

The defined definitions of profiles and types of dental activity according to the National Classifier DK 003:2010

(Derzhspozhivstandartu) allow us to highlight the following collisional transformations in the context of the forensic dental paradigm.

First of all, the absence in Ukraine of a specific normative legal act regulating dental activity and defining this activity in DK 003:2010 creates a precedent of an «empty legal field», which, in particular, can be used by dentists and dental clinics (state and private forms of ownership) to avoid liability for cases of official/professional negligence, etc.

Secondly, the correlation between DK 003:2010 and regulatory documents of the combined (analog-legal) jurisdiction, such as the Law of Ukraine «Basics of the Legislation of Ukraine on Health Care» No. 2801-XII has signs of difficult effectiveness, because it de facto defines the framework principles , on which the dental activities of certain sub-professions and sub-branches are based through the prism of dentistry directly (see the above proposed definitions of DK 003:2010) and health care in Ukraine (so, for example, Article 35-2 of the Law of Ukraine No. 2801-XII only refers to dental care as a subspecies specialized medical care, combined with other types of it, including pediatric, etc.).

The stated state of affairs is combined with the absence of a specific legal act not only in the field of dental activity, but also in the field of forensic dentistry (forensic dentistry, as mentioned, is regulated by the Law of Ukraine «On Forensic Expertise» No. 4038-XII dated 25.02.1994 (ed. dated 01.01.2024) leads to the actual leave of absence in this field, which, taking into account the specifics of the stomatological narrow profile, leads to deterioration in the accuracy of the qualification of professional misconduct of dentists, which led to injuries of the oral cavity, deterioration of the patient's teeth/false qualification of the conclusions of the forensic dental examination regarding the causes of death in the relevant cases.

Instead, the correlation-collision transformations associated with the definition of dental activity according to DK 003:2010 and, for example, the definition by normative legal acts establishing various types of responsibility of a dentist and conducting relevant forensic dental examinations regarding his competence (Code of Ukraine on Administrative offense (KupAP) No. 8073-X, Criminal Code of Ukraine No. 2341-III, Civil Code of Ukraine No. 435-IV) criteria of such responsibility allows to identify such trends.

First of all, the Code of Ukraine on Administrative Offenses No. 8073-X [7] does not have a rule that would directly regulate responsibility in the field of dental activity or the field of activity of a dentist as a specialized competent specialist. At the same time, suitable for dental work and conducting further forensic dental research is Art. 9 of the Code of Criminal Procedure, which establishes the presumption of responsibility of a person for a misdemeanor (commitment of illegal, including professionally illegal

actions that do not correlate with internal procedures) and Art. 42 of the Code of Administrative Offenses, which includes the option of administrative liability for violation of sanitary regulations. At the same time, for example, a violation of sanitary norms is usually a reason for conducting a forensic dental examination (if it is about causing damage, in particular, to the soft tissues of the injured patient in the process of providing operative dental care), but at the level of legal regulation of forensic medical activity The Law of Ukraine «On Forensic Expertise» No. 4038-XII dated 25.02.1994 (ed. dated 01.01.2024), which acts as a regulatory document of first choice regarding the provision of a legal field for forensic dental activity by legal analogy, does not contain algorithms for implementation inspections of the dentist's actions on the specified grounds.

In addition, in the Criminal Code of Ukraine No. 2341-III (hereinafter - CCU) [8] potential cases of responsibility of the dentist and the involvement of forensic dental expert assistance are revealed through the prism of the interpretation of «medical crimes» (Chapter II of the CCU - «Crimes against life and personal health»), namely, Art. 131 of the Civil Code (infection of a person with VILISIDS as a result of improper performance of professional duties); Art. 138 of the Code of Criminal Procedure (provision of unlicensed treatment services); Art. 139 of the Civil Code (failure to provide assistance to the patient); Art. 140 of the Code of Criminal Procedure (improper performance of medical duties/violation of the patient's rights - both individually and as part of an organic remedial complex). Again, similar provisions focus exclusively on responsibility for what has been done, but not on the methods, means and mechanisms by which the measure and degree of professional malpractice (negligence) of a dentist can be determined in the performance of his own duties.

At the same time, the Civil Code of Ukraine No. 435-IV [9] defines the norms and procedure for financial recovery for the benefit of the patient in case of mutilation due to the provision of professional (qualified) medical care (Chapter 82, Article 1166 of the Civil Code). This approach, as in previous cases, allows to act exclusively with the result (a proven fact of official negligence, incompetence, criminal carelessness) of professional dental activity, provided there are no clear criteria for establishing such facts at the level of legislation, which is hypothetically negative for the credibility/legal qualification - expert opinion in the field of forensic dentistry).

As a separate conflict narrative of forensic dental research under the legislation of Ukraine, it is worth highlighting the imperfection of the process of protecting the patient's rights, which in turn slows down the expert examination of unprofessional actions or inaction of the dentist. As you know, the direct responsibility of the dentist is not prescribed by the domestic legislator (similar proposals

can be found only in Section V of the rejected Draft Law of Ukraine «On Dental Activity» No. 4736 dated 31.05.2016), as a result of which the analogous legal regulation of this issue is carried out by the Law of Ukraine «On protection of consumer rights» No. 1023-XII (for notification of violations by private providers of medical and dental services) and the Law of Ukraine «On Appeals of Citizens» No. 393/96-BP (regarding notification of violations of dental, sanitary-dental, professional dental prescriptions in public hospitals). Let's consider each of the aspects in more detail.

In the Law of Ukraine «On the Protection of Consumer Rights» No. 1023-XII [10], the legislator, once again, indirectly means standardized prescriptions for the quality of services that may be applicable to dental activities, giving the patient the right to appeal illegal or incompetent, in his opinion, professional actions of a private dental clinic and a specialist in the person of a dentist (Article 1-1 of the Law of Ukraine No. 1023-XII). In addition, in Art. 16, 17 of the Law of Ukraine No. 1023-XII establishes the presumption of compensation for material/moral damage regardless of the fact of the latter's presence/absence in contractual relations with the service provider — a dental clinic, a dentist. However, for the purposes of a forensic dental examination, the fact of violation of professional duties by a private dental clinic must be confirmed by additional evidence/facts, in addition to the patient's subjective testimony. As a summary, the specified norms of the Law of Ukraine «On the Protection of Consumer Rights» No. 1023-XII, on the one hand, attest to the patient's right to apply for the protection of his own violated, unrecognized or disputed right to the competent authorities (State Consumer Services), but, on the other hand, they do not correlate with the process of proving/refuting similar evidence provided.

In turn, by the Law of Ukraine «On Appeals of Citizens» No. 393/96-VR [11], the legislator gives the patient the option of writing a written appeal against the unprofessional actions of the dentist to the head doctor of the state hospital where the person received dental services, or to the local health department/management or of regional significance. Such an approach can be considered projective towards the patient through the prism of the appointment of a forensic medical examination (establishment of facts and circumstances that may indicate intentional or careless harm to the life and health of a person due to various factors) [12], however, as in previous cases, not integrates research processes with

the analog-legal Law of Ukraine «On Forensic Expertise» No. 4038-XII and, in addition, is divided exclusively by the legal relationship «state clinic - patient».

CONCLUSIONS

The analysis of legal conflicts of the forensic dental profile in Ukraine made it possible to reach the following conclusions.

Firstly, there is currently no specific regulatory legal act in Ukraine that would regulate aspects of performing dental activities (there is only the Draft Law of Ukraine «On Dental Activities» No. 4736 dated 05/31/2016, rejected by the Verkhovna Rada of Ukraine in the first reading). Instead, the legal regulation of dental activity is carried out according to the legal-analogous paradigm, namely, the National Classifier of Professions DK 003:2010 (Derzhspozhivstandard), the Law of Ukraine «Basics of the Legislation of Ukraine on Health Care» No. 2801-XII, the Code of Ukraine on Administrative Offenses (KUpAP) No. 8073-X, Criminal Code of Ukraine No. 2341-III, Civil Code of Ukraine No. 435-IV, Law of Ukraine «On Protection of Consumer Rights» No. 1023-XII, Law of Ukraine «On Appeals of Citizens» No. 393/96-VR, etc.

Secondly, forensic dental examination in Ukraine is also carried out according to the law (the Law of Ukraine «On Forensic Examination» No. 4038-XII dated 25.02.1994 (ed. dated 01.01.2024) acts as the guiding principle), as a result of which the latter can be considered extensive regarding the forensic dental investigation of the actions and inactions of the dentist (orthodontist, orthopedist, therapist, surgeon)/dental clinic that led to or could cause harm to the patient's health.

Thirdly, in our opinion, the aspect of eliminating the above-mentioned conflicts is the adoption of normative legal acts in the field of dental activity and forensic dental examination in Ukraine, which would determine the specifics of establishing facts and circumstances that indicate a violation of the patient's rights under the substantive and legal appropriation instead of analog-legal. In addition, the role/place of forensic dental expert activity during the examination of the corpse (regarding the establishment of facts and circumstances that could indicate the occurrence of a violent death) needs to be regulated and clarified.

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ORCID AND CONTRIBUTIONSHIP
Vasyl V. Tymchak: 0000-0001-9739-1914 **A** **D**
Maryana V. Tymchak: 0000-0001-9135-3714 **B** **E**
Roman M. Fridmanskyy: 0000-0003-4213-8449 **F**
Viktoriia I. Fridmanska: 0000-0002-7689-2290 **E**

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The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Vasyl V. Tymchak
Uzhhorod National University
3 Narodna Square, 88000 Uzhhorod, Ukraine
e-mail: vasyl.tymchak@uzhnu.edu.ua

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

Peculiarities of treatment of maxillofacial polytrauma during wartime

Victor P. Korkishko[1], Natalia I. Zhero[1], Andrej Jenča[2], Andrej Jenča[2], Adriána Petrášová[2], Janka Jenčová[2]
[1]UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE
[2]KOŠICE MEDICAL UNIVERSITY, KOŠICE, SLOVAKIA

ABSTRACT

Aim: Analyzing of the last-time papers in this subject in organizing, diagnostic and surgery tactic is the aim of this work.

Materials and Methods: The study analyzed the experience of treating patients with maxillofacial polytrauma before and after February 24, 2022. Research methods: bibliographic, systematic, comparative, general clinical, radiological and retrospective analysis.

Conclusions: Tactic of the multidisciplinary team should based on the principles of damage control, which involves the initial performance of manipulations and surgical interventions that ensure the patient's survival. Modern strategies for infusion-transfusion therapy play a significant role in severe trauma cases. Choosing the rational management of this therapy for severe trauma remains an important issue. Reconstructive surgeries are recommended to be performed deferred, after surgical wound management, neurosurgical interventions and stabilization of the patient common status. Patients with maxillofacial polytrauma needs in specialized medical care at all the levels. Active wound management aimed at creating favorable conditions for healing, comprehensive medical treatment, prevention, early detection and timely treatment of complications.

KEY WORDS: maxillofacial polytrauma, wartime, surgery tactics, complications, rehabilitation

INTRODUCTION

Lately, there has been a growing trend in the proportion of maxillofacial injuries among all traumatic injuries sustained as a result of wounds during armed conflicts [1-5].

The cited sources were based on medical and statistical data obtained by the authors during the analysis of the structure of combat trauma in local conflicts (Iraq, Afghanistan). Combat injuries of the maxillofacial area comprise 75-82%, and up to 20% - non-combat injuries (road accidents, etc.) [6]. In the structure of injuries, local and combined damages of the head and neck comprise 21-25%. In 11% they were combined with damages of other parts of the body. The experience of military medics of the USSR during military operations in Afghanistan (1979-1989) is also summarized. Such data are given in the paper by T. Khersonska [7]. The methods of individual protection of servicemen of all armies against traumatic damage (body armor, modern helmets) were improved over time. However, the protection of the craniofacial area did not change significantly. Only the use of tactical glasses reduced the percentage of traumatic injuries of eye. Accordingly, the share of maxillofacial trauma in

the structure grew and in combination with closed or open craniocerebral trauma reached 25-30% in recent years [8, 9]. The nature of the injury changed in favor of mine-explosive damages by high-energy fragments. An analysis of the medical care provided to patients with maxillofacial polytrauma indicates significant qualitative changes in the organization of treatment for such injuries at all levels [6, 10-14]. However, the mortality rate as a result of explosive injuries to the maxillofacial area remains quite high in both military and civilian personnel. It should be noted that high-velocity ballistic gunshot lesions of the maxillofacial area with concomitant damages of the brain and main vessels also have a high mortality rate [15, 16]. There is no uniform treatment protocol for patients with combat maxillofacial polytrauma. The proposed protocols depend on the experience of surgeons and the nature of the injuries requiring treatment. Surgeons actively implement modern techniques in treating such patients [9, 17-20]. Concentration of maxillofacial war trauma victims in specialized centers (eg the Royal Center for Defense Medicine (RCDM) at Birmingham University Hospitals) has provided better immediate and long-term outcomes [21].

AIM

To study the features of specialized treatment of patients with polytraumatic injuries of the maxillofacial area in the conditions of full-scale war.

MATERIALS AND METHODS

The study analyzed the experience of treating patients with maxillofacial polytrauma before and after February 24, 2022. Research methods: bibliographic, systematic, comparative, general clinical, radiological and retrospective analysis.

REVIEW AND DISCUSSION

As a result of the full-scale Russian invasion of Ukraine, the number of patients with polytraumatic injuries of the maxillofacial area significantly increased. A characteristic feature of this war is that the majority of injuries were caused by mine-blast and high-velocity shrapnel wounds. Among the hospitalized patients, predominate severe injuries with bone defects of the lower and upper jaw and the maxillo-orbital complex. A significant number of combined injuries of the mid-facial area with damage to the eyes, brain, ENT organs, and limbs complicate the course of their treatment and postoperative care.

All patients with maxillofacial injuries undergo computed tomography, and if necessary, MRI and ultrasound as part of the diagnostic process. Based on the results of CT and ultrasound, specialists such as neurosurgeons, maxillofacial surgeons, general surgeons, thoracic surgeons, and traumatologists make final conclusions regarding the priority of providing primary surgical care. If a patient was diagnosed with combined injuries, primary surgical treatment is carried out simultaneously by several teams of surgeons of different specialties in the ward for the predominant pathology treatment.

Treatment for patients with polytrauma is carried out according to a staged diagnostic and therapeutic plan. In order to optimally provide assistance to those affected by polytrauma, five phases (periods) of trauma treatment are distinguished (according to M. Wolf, 1978, L. Schweiverer, A. Betz, 1985):

- the resuscitation period (acute period – up to 3 hours),
- the first operative period (operations for life-saving indications – up to 72 hours),
- the stabilization period (from several hours to several days),
- the second operative period (period of delayed operations),
- the rehabilitation period.

It is important to note that the basis of the strategy for the comprehensive treatment of combat injuries is surgical intervention and intensive therapy, based on the principles of damage control, which involves the initial performance of manipulations and surgical interventions that ensure the patient's survival. In this case, the staging of surgical operations and their content, as well as the timing (periods and stages), are necessarily subordinated to the pathogenetic course of traumatic and hemorrhagic shock, the complexity of traumatic disease, the defined criteria for assessing the condition of the victim, and his operability.

Patients undergo primary or repeat surgical wound treatment. The technique of primary surgical wound treatment has certain features: determining tissue viability, the extent of debridement, irrigation, fasciotomy, fracture synthesis with external fixation devices, shunting of major vessels, treatment of peripheral nerves, features of wound closure, VAC therapy. The question of effective antibacterial prophylaxis and therapy of infected wounds of the head and neck remains open [22]. The 10-year experience of military medics in Iraq and Afghanistan prompts not only to improve protocols for the treatment of combat head and neck injuries, but also to develop the latest protective equipment for soldiers [3].

Modern strategies for infusion-transfusion therapy play a significant role in severe trauma cases. Choosing the rational management of this therapy for severe trauma remains an important issue. Almost all shock conditions require a large volume of infusion due to serious depletion of the intravascular environment.

Fractures in the facial region, sustained during combat, usually differ significantly from those that occur in civilian life. Injuries from high-velocity projectiles and blast injuries are usually associated with complex tissue deficits, development of necrotic areas, bone comminution, and sequestration of ballistic fragments (Fig. 1). The interpretation of the optimal method of treating such injuries is problematic due to the lack of international consensus on the optimal tactics.

Mini and macro titanium plates with screw fixation are preferred for reliable fixation of bone fragments (Fig. 2). Titanium mesh is widely used for reconstructing defects of the maxillo-orbital complex. Classic jaw immobilization performed for additional immobilization (if possible).

For the treatment of complex high-energy or infected fractures of the mandible, external fixation recommended instead of immediate internal fixation, which is quite rare in "civilian" medicine.
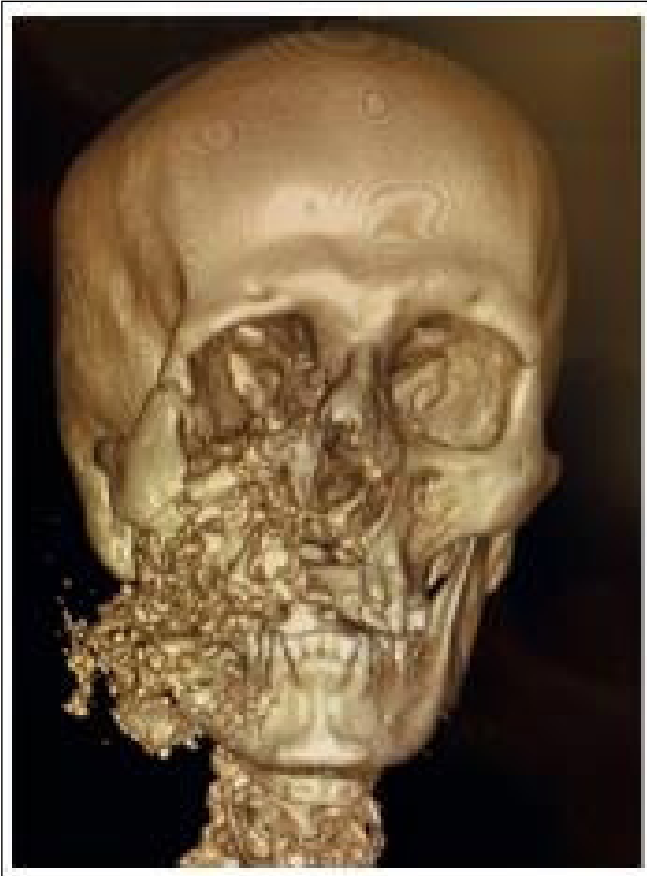
Fig. 1. CT (3D reconstruction) of a patient with maxillofacial injuries resulting from high-velocity projectile impact [17].

Fig. 2. Control CT (3D reconstruction) of a patient with injuries of the maxillofacial area after primary surgical wound management [17].

Facial injuries associated with combat require early diagnosis and treatment, internal fixation to prevent functional and sometimes life-threatening damage caused by these injuries. Immediate intervention improves functional and cosmetic outcomes.

Complications of the trauma in the postoperative period include:

1. Persistent facial muscle paralysis;
2. Dysfunction of the mandible (restricted mouth opening);
3. Necrosis of damaged oral cavity tissues;
4. Necrosis and suppuration of tissues around the wounds;
5. Formation of deforming scars and fistulas;
6. Sequestration.

It has been determined that the main causes of postoperative complications (suture dehiscence, wound infection, post-traumatic osteomyelitis, contracture) are the untimely provision of specialized medical care, underestimation of the severity of the injury, overestimation of one's capabilities in the absence of necessary technical equipment and materials, as well as insufficient experience in providing assistance to patients with modern combat trauma [23].

Reconstructive surgeries are recommended to be performed deferred, after surgical wound management, neurosurgical interventions (treatment of intracranial hematoma, decompressive craniotomy, etc.), stabilization of water-electrolyte balance and patient hemodynamics [24]. Maxillofacial injuries are associated with concomitant hard traumatic brain injury in 306 Israel military personell. Long-term follow-up demonstrated long-term disability included central nervous system disorders [25].

CONCLUSIONS

Specialized treatment of patients with maxillofacial polytrauma includes comprehensive surgical wound management with primary soft tissue reconstruction, rigid fixation of bone fragments with modern osteosynthesis devices, open irrigation and drainage of the wound and surrounding spaces, intensive postoperative care, active wound management aimed at creating favorable conditions for healing, comprehensive medical treatment, prevention, early detection and timely treatment of complications, ensuring proper nutrition, and provision of dental and prosthodontic care.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Natalia I. Zhero
Uzhhorod National University
3 Narodna sq., 88000 Uzhhorod, Ukraine
e-mail natalia.zhero@uzhnu.edu.ua

ORCID AND CONTRIBUTIONSHIP
Victor P. Korkishko: 0009-0006-9956-1490 Ⓓ
Natalia I. Zhero: 0000-0002-8083-9310 Ⓐ Ⓔ Ⓕ
Andrej Jenča: 0000-0002-9297-9134 Ⓑ Ⓔ
Andrej Jenča: 0000-0003-4501-7407 Ⓑ
Adriána Petrášová: 0000-0002-4289-7691 Ⓑ
Janka Jenčová: 0009-0007-4431-3227 Ⓑ

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

REVIEW ARTICLE

The orthodontic treatment and the periodontal status of patients

Zynoviya O. Bumbar, Khrystyna A. Sichkoriz, Oleg I. Bumbar, Lidiya Y. Minko
DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE

ABSTRACT
Aim: To review and analyze modern professional literature on the impact of orthodontic treatment of maxillofacial anomalies on the periodontal status of patients, in particular, the subsiqent pathogenetic mechanisms of the development of periodontal diseases in this category of patients.
Materials and Methods: Bibliometric and analytical methods were used data from international scientific sources in the field of studying the impact of orthodontic treatment on the state of periodontal tissues were used.
Conclusions: Patients undergoing orthodontic treatment need the increased attention to the state of periodontal tissues and regular high-quality both professional and individual oral hygiene. A comprehensive approach to treatment and preventive measures for periodontal diseases, taking into account all pathogenetic links of their development in this category of patients, aims not only to keep the periodontium healthy, but also to ensure successful completion of orthodontic treatment. Taking into account consideration the periodontal status of patients allows individual correction of the treatment plan to increase its clinical effectiveness both in the early and in the long term.

  KEY WORDS: periodontal disease, orthodontic appliences, maxillofacial anomalies, ligature and self-ligating bracket systems

INTRODUCTION
The analysis of scientific literature gives the grounds to systematize the peculiarities of periodontal health and periodontal desease in patiens during orthodontal treatment by modern appliences.

Considering the high prevalence of periodontal diseases in orthodontic patients on one side, spreading of new orthodontic technologies from another side, the approach has to be actualized.

AIM
The aim of the work is to review and analyze modern professional literature on the impact of orthodontic treatment of maxillofacial anomalies on the periodontal status of patients, in particular, the subsiqent pathogenetic mechanisms of the development of periodontal diseases in this category of patients.

MATERIALS AND METHODS
Bibliometric and analytical methods were used data from international scientific sources in the field of studying the impact of orthodontic treatment on the state of periodontal tissues were used.

REVIEW AND DISCUSSION
According to modern classifications of periodontal diseases and the understanding the concept of «periodontal health», leading scientists assign a special role in the occurrence and development of lesions of the tooth-holding apparatus to the presence of orthodontic pathology in patients and the process of orthodontic treatment [1-8]. However, completed successful orthodontic treatment significantly improves the patient's quality of life, which is directly related to the health of his oral cavity [9-11].

Modern epidemiological studies confirm that the frequency of periodontal tissue damage is extremely high in the population, is the second after the spread of caries, and is one of the main socio-economic problems of dentistry and directly affects the quality of life of patients [1-5].

Dento-jaw anomalies, in the aspect of studying the etiopathogenetic mechanisms of the development of periodontal diseases and the creation of a new classification of diseases of periodontal and peri-implant tissues (EFP&AAP World Workshop, 2017, World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions) were enrolled by leading specialists to local factors in the patient's body

that determine and affect the state of his periodontal health. Periodontal diseases occur 2 times more often in people with abnormalities of the dental and jaw system, than in people without orthodontic pathology. Among them, the position and crowding of teeth are of great importance [7, 12]. The prevalence of crowded teeth can reach 80% or more, and the frequency of detection of periodontal diseases is, on average, 1.2 times higher than in patients without orthodontic pathology [10].

The work of Ph. Meyer-Marcotty and co-authors, who had studed correlations between periodontal diseases and orthodontic parameters in patients older than 40 years, has showed that the higher the degree of periodontal damage was, the worse the orthodontic parameters were. At the same time, 90% of patients with severe periodontitis had a moderate or very high need for orthodontic treatment [13].

The most common periodontal diseases are etiologically related to the accumulation of dental biofilm, which is the main factor that launch the beginning of the inflammatory process in the periodont. According accepted contemporary views, periodontal tissue inflammation is caused by a red-orange complex of bacteria that is associated with periodontal disease. Bacteria of the red complex Porphyromonas gingivalis, Treponema denticola and Tannerella forysthia are considered the main causative agents of these periodontal diseases [14]. These three bacteria have symbiotic relationships and associations within a highly ordered biofilm system, which serves as a factor promoting adhesion to the oral environment. The presence of these microorganisms is associated with the development of generalized periodontitis in adults, while orthodontic patients are predominantly younger. However, studies have confirmed their appearance and significant role in the development of periodontal tissue pathology in young people compromised by orthodontic treatment [15-34].

In the presence of orthodontic appliances in the oral cavity, local resistance to these anaerobic pathogens decreases and the beginning of inflammation is initiated. The reseaches by use of deoxyribonucleic acid (DNA) probes and the BANA test (The benzoyl-DL-arginine-naphthylamide, Knowell Periodontal Tech, Toronto) were carried on by many researchers for rapid and accurate detection of periodontal pathogens of the red complex in dental plaque [16]. Numerous scientific works have described the data on the accumulation of dental plaque as a result of a significant decrease in oral hygiene in dental patients with orthodontic pathology and during orthodontic treatment [9, 10, 14-18]. All of them have confirmed the leading role of periodontopathogens of the red complex and the

increased risk of developing gingivitis and generalized periodontitis [16, 18]. Violation of the microbiocenosis of the oral cavity is accompanied by a significant accumulation of both periodontal pathogens and cariogenic species of microorganisms, among which Prevotella, Bacteroides, Fusobacteria and Lactobacillus, as well as T. denticola, Eikenella corrodens, T. forsythia and Aggregatibacter actionmycetemcomitans are most often found [14].

At the same time, the microbiome of the oral cavity changes quantitatively and qualitatively, and the composition of both the dental biofilm and saliva undergoes changes. On average, the treatment of an incorrect bite with fixed appliances lasts from 18 to 36 months and depends on the previous diagnosis and the chosen type of construction. Longer duration of treatment with the presence of a bracket system, as an additional retention point for biofilm, increases risks for periodontal health due to long-term difficulties in maintaining oral hygiene [19-21].

In reseaches by Moolya and co-authors it was shown that the average plaque index and gingival index were increasing on days 3 and 7 - there was a shift from aerobic to anaerobic species; increase in the number of anaerobic species by 3- 7th day (P<0.05), the number of colony-forming units on the 7th day is greater compared to the 3rd day. Also, this observation confirms that the design of braces can have a significant effect on the bacterial load and periodontal status of patients [17].

Along with the study of the influence of the type of bracket system the relationship between the material of the brackets, the presence of metal ligatures, arches, elastics and composite materials for attachment was reseached. Also the accumulation of dental plaque, which can also affect bacterial adhesion. Bollen and co-authors concluded that an increase in the roughness of both the supra- and subgingival surface of the arch leads to a higher rate of colonization of the surface by bacteria, maturation of dental plaque; it had been shown in vivo and in vitro that the surface roughness of archwires increases over time due to intraoral wear, changes in the mechanochemical properties of the wire due to the influence of saliva, abrasion due to brushing teeth and food, as well as the interaction between archwires and ligatures [22, 33].

The presence of orthodontic appliances in the oral cavity was shown to cause the increase in stimulated salivation, buffer capacity and pH 1-3 months after the start of orthodontic treatment, what indicates the adaptive capacity of the oral cavity. In the reseaches of Zogakis and co-authors a significant decrease in pH was observed immediately after the installation of

fixed orthodontic appliances, which returned to normal levels after 4–6 weeks; what was explained by the use of 37% orthophosphoric acid to etch the enamel before pasting the orthodontic appliances [22].

The researches of braces, arches and retainers coated with various materials (nano copper oxide, nano zinc oxide, zinc oxide, silver-platinum, silver ions, titanium oxide) have demonstrated favorable antimicrobial activity, which allows to reduce the number of bacterial colonies in the oral cavity, compared to braces without coating [19, 22, 23].

Assessment of the state of oral hygiene of patients with fixed orthodontic appliances shows different percentages in different studies, which is due to the age and gender of the researched individuals, their awareness and ability to apply individual hygiene skills, the variability of culture, the availability of oral care for different categories of the population [8, 24].

After removing the equipment, the papillary-marginal-alveolar index, as a clinical marker of inflammation, was significantly higher in 91.2% of patients than before the start of treatment. Almost all patients were diagnosed with bleeding gums of the first degree, and 41% - of the second degree [25]. Periodontal pocket depth was bigger in the diagnosed patients who used a fixed appliance for more than 18 months.

Along with dysbiotic changes in the microbiological composition of dental plaque, changes in the immune system also stimulate the occurrence and progression of periodontal tissue damage, which is manifested by an increase in the level of inflammatory cytokines, such as tumor necrosis factor (TNF-α) and interleukins (IL-1α, IL-1β and IL -6), their increased concentration was determined in the gingival cervical fluid. Pro-inflammatory mediators, released during the inflammatory reaction, participate in the initiation of the process of periodontal collagen destruction and the loss of tooth-epithelial attachment with the rapid progression of the disease [14].

Hormonal adjustment during puberty, which leads to a decrease in the barrier function of the periodontium, explains the high prevalence (55-90%) of periodontal diseases in adolescence, the most common of which is diagnosed as chronic catarrhal gingivitis. The last one in patients aged 13-16 occurs in almost 90% of the examined, which is explained by the peculiarities of sexual development in this age category. If the brace system is fixed, the frequency of gum inflammation increases even more: catarrhal in 57%, hypertrophic in 27% [9, 26, 35].

Pinto A. S. and co-authors evaluated the impact of the duration of permanent orthodontic treatment on the development of hypertrophic gingivitis in adolescents and young people (age 10-30 years). Patients were divided into 4 groups: patients without fixed orthodontic appliances and patients undergoing orthodontic treatment for 1, 2, and 3 years. Adjusted Poisson regression analysis showed that patients who were getting orthodontic treatment had a 20- to 28-times increased risk of developing hypertrophic gingivitis compared with those who did not have orthodontic appliances. The duration of orthodontic treatment significantly affects the occurrence of hypertrophic gingivitis [28] and the development and course of periodontal diseases in general [29].

When treated with a bracket system, such diseases as catarrhal and hypertrophic gingivitis are most often detected. Their prevalence is 55-62% [27]. This research indicates more positive dynamics of complex treatment of chronic hypertrophic gingivitis against the background of the use of a bracket system, which involved the use of drugs with an immunocorrective effect for 3 months. With such a treatment scheme, periodontal indices and oral hygiene indices improved on average by 60%.

A separate, extremely important link in the pathogenesis of periodontal diseases under the influence of orthodontic treatment are hemodynamic and microcirculatory disorders. They progress significantly during tooth movement and dynamic impact on the supporting tissues of fixed orthodontic structures. In the area of crowded teeth, the vessels of the adjacent tissues are in a state of spasm, with vasoconstriction, the walls of the vessels are tense and practically do not undergo additional stretching by the pulse wave of blood filling. As a result, this provokes an increase in the peripheral tone in these vessels, the contractility of the vascular wall is suppressed and the passage of the pulse wave becomes difficult. This determines the development of stagnant phenomena in the periodontium. A change in the microcirculation and hemodynamics of periodontal tissues leads to a decrease in their tolerance, which provokes increased gum bleeding and deepens periodontal damage [25].

Traumatic occlusion, formed by premature occlusal contacts in case of maxillofacial anomalies, due to excessive parafunctional load leads to loss of tooth-gingival attachment and damage to the tooth-retaining apparatus. Overloading of the periodontium is accompanied by a violation of blood circulation by squeezing the vessels, which, together with the deterioration of hygienic parameters, causes the development of inflammatory processes [30].

The investigations on the effect on periodontal tissues of different types of bracket systems, namely conventional ligature brackets (CB) and self-ligating

brackets (SLB) present the particular interest. Research data are quite contradictory. The absence of ligatures to hold the orthodontic wire in the gap, which was present in CB, was expected to significantly reduce bacterial aggregation. However, practical experience has shown that SLBs also have areas of increased plaque accumulation, but at the same time, the features of the fixation made it easier to move the teeth and significantly reduced the overall treatment time. Reducing the duration of orthodontic treatment allows better level of oral hygiene and has a positive effect on periodontal health [20].

Mester A. and co-authors analyzed 453 cases comparing the periodontal health effects of SLB and CB. An analysis of scientific works in which periodontal indexes were studied showed the absence of significant differences between the objective values of indices [31, 32].

The effect of orthodontic treatment on bone tissue, which is part of supporting tissues, is undeniable. This is explained by the action of orthodontic appliances aimed at moving teeth and remodeling the surrounding bone tissue. R. Guo and co-authors analyzed the literature on the influence of tooth movement on changes in the alveolar bone of the front teeth of the upper and lower jaws [36]. There are studies aimed at determining the density of bone tissue during treatment and after it has been compled. Data on the effect of orthodontic tooth movement on bone density are contradictory and describe both a decrease in bone density around orthodontically treated teeth and an increase in it. Such differences are probably explained by the action of different orthodontic appliances with different types and amounts of tooth movement, as well

as the initial state of the bone tissue before the start of orthodontic treatment [37-40].

CONCLUSIONS

The analysis of numerous scientific studies made it possible to significantly expand the understanding of the influence of orthodontic appliances on the periodontal status and had demonstrated the multi-vector nature of the pathogenetic mechanisms of periodontal tissue damage, which includes the deterioration of the hygienic status, dysbiotic changes, a decrease in the local immune response, hemodynamic and microcirculatory disorders and changes in bone tissue. However, despite to the negative impact on the periodontium on the early stages, under the conditions of carrying out treatment and preventive measures for periodontal diseases, orthodontic treatment is able to stabilize the state of the supporting apparatus due to the normalization of periodontal parameters in the long term.

Patients undergoing orthodontic treatment need the increased attention to the state of periodontal tissues and regular high-quality both professional and individual oral hygiene. A comprehensive approach to treatment and preventive measures for periodontal diseases, taking into account all pathogenetic links of their development in this category of patients, aims not only to keep the periodontium healthy, but also to ensure successful completion of orthodontic treatment. Taking into account consideration the periodontal status of patients allows individual correction of the treatment plan to increase its clinical effectiveness both in the early and in the long term.

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CONFLICT OF INTEREST

CORRESPONDING AUTHOR

Zynoviya O. Bumbar
Danylo Halytsky Lviv National Medical University
69 Pekarska st, 79010 Lviv, Ukraine
e-mail: zenysja@gmail.com

ORCID AND CONTRIBUTIONSHIP

Zynoviya O. Bumbar: 0009-0007-7696-1181 **A** **D**
Khrystyna A. Sichkoriz: 0000-0002-5534-8173 **B**
Oleg I. Bumbar: 0009-0000-6260-3956 **E** **F**
Lidiya Y. Minko: 0000-0002-1631-478X **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

CASE STUDY   CONTENTS ↗

A case of leptospirosis in transcarpathia complicated with Jarish -Herxheimer reaction

Vlasta V. Vysochanska[1,2], Myroslava S. Babych[2], Anton I. Kohutych[1,2], Andriana A. Halamba[1]
[1]UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE
[2]REGIONAL CLINICAL INFECTIOUS DISEASES HOSPITAL, UZHHOROD, UKRAINE

ABSTRACT
A case report of Jarisch-Herxheimer (JHR) reaction on a 10th day of Leptospirosis caused by *Leptospira Pomona*. JHR occurs as a complication of an antibiotic treatment of various spirochetes and may lead to respiratory distress syndrome, renal failure, hepatic insufficiency, and multiple organ failure. This case represents a skin and cardio-vascular form of JHR with no lung involvement. The patient was treated with benzylpenicillin and low dexamethasone doses for 5th day of the disease with a shift to ceftriaxone and high doses of methylprednisolone. The fastest diagnosis of a sporadic zoonotic disease, early start of antibiotic therapy, and adequate doses of corticosteroids are key to the successful treatment of leptospirosis.

KEY WORDS: Leptospirosis, Jarisch-Herxheimer reaction

INTRODUCTION

Leptospirosis is not a widespread disease in Ukraine, but some areas may become endemic. Disease is more common in rural and semi-urban areas that are exposed to populations of rodents due to close farming activity [1]. Any mammal may become a source of leptospirosis by excreting *Leptospira* from the proximal tubules of the kidneys [2]. The majority of incidents come from contact of a damaged human skin, or mucosa in oral and respiratory tract with contaminated dust, water, or food [3]. The disease severity may vary from a non-icteric fever with a muscular ache to hemorrhagic fever with multiple organ failure resulting in death in 15-50% of cases [4]. Typical treatment requires penicillin, ceftriaxone, or doxycycline. However, the use of high corticosteroid doses is not supported by high-quality evidence and routine use is not recommended [5].

Jarisch-Herxheimer reaction (JHR) is an acute, self-limiting condition that occurs after antibiotic treatment of spirochetal infections. It is accompanied by the following symptoms: chills, febrile fever, drop of a blood pressure, skin rashes [6], but may result in acute respiratory distress syndrome [7] and disseminated intravascular coagulation syndrome [8], and severe pulmonary hemorrhagic syndrome [9].

JHR is a result of exposure to antigens released from lysis during a high-quantity spirochetemic phase of infection and a massive increase of interleukin-6 (IL-6), IL-8, IL-1β, and tumor necrosis factor (TNF-α) [10]. Corticosteroid infusions before the antibiotic administration as a routine prevention of JHR have shown limited efficacy [11]. The rate of JHR incidents is reported from 19% to 82% of all leptospirosis cases [12,13]. Further studies are required to propose a predictive and clinically efficient protocol for leptospirosis treatment.

CASE REPORT

The patient was informed about the research plan, developed within the framework of the Helsinki Declaration of the World Medical Association "Ethical Principles of Medical Research with the Participation of a Person as an Object of Research," the Convention of the Council of Europe on Human Rights and Biomedicine, and the legislation of Ukraine, and signed the informed consent to use his medical records and photos. Complete blood count, coagulogram, liver and kidney biochemistry tests, c-reactive protein, procalcitonin were analyzed on 1, 5, 7, 10, 15 days of treatment in Transcarpathia regional infectious disease hospital.

A 72-year-old male was admitted to a Transcarpathian regional Infectious hospital with complaints of headache, loss of appetite, frequent urination, thirstiness, pain in the joints, and yellowing of the skin and eyes. The patient considered himself sick for 5 days. The sickness started with a rise in body temperature to 38°C,
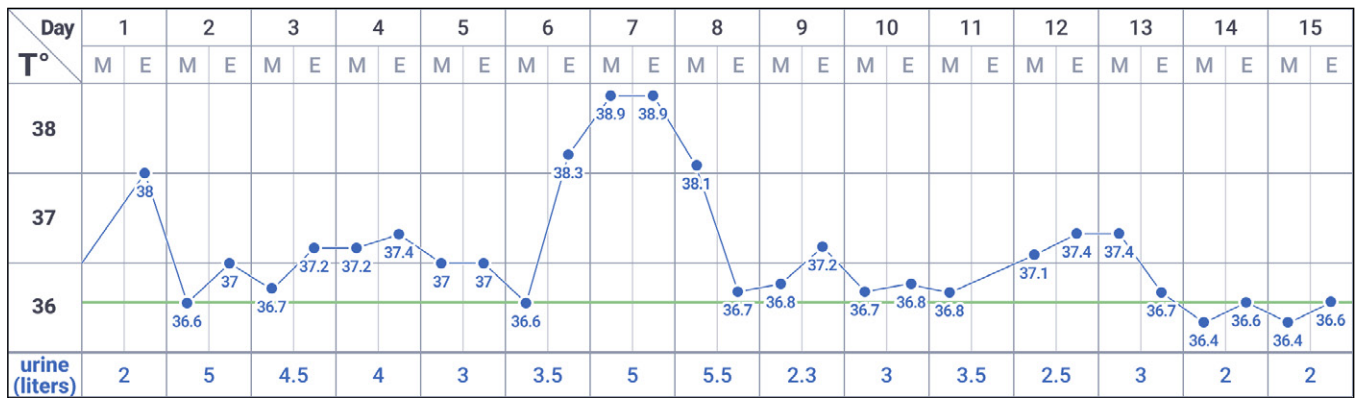
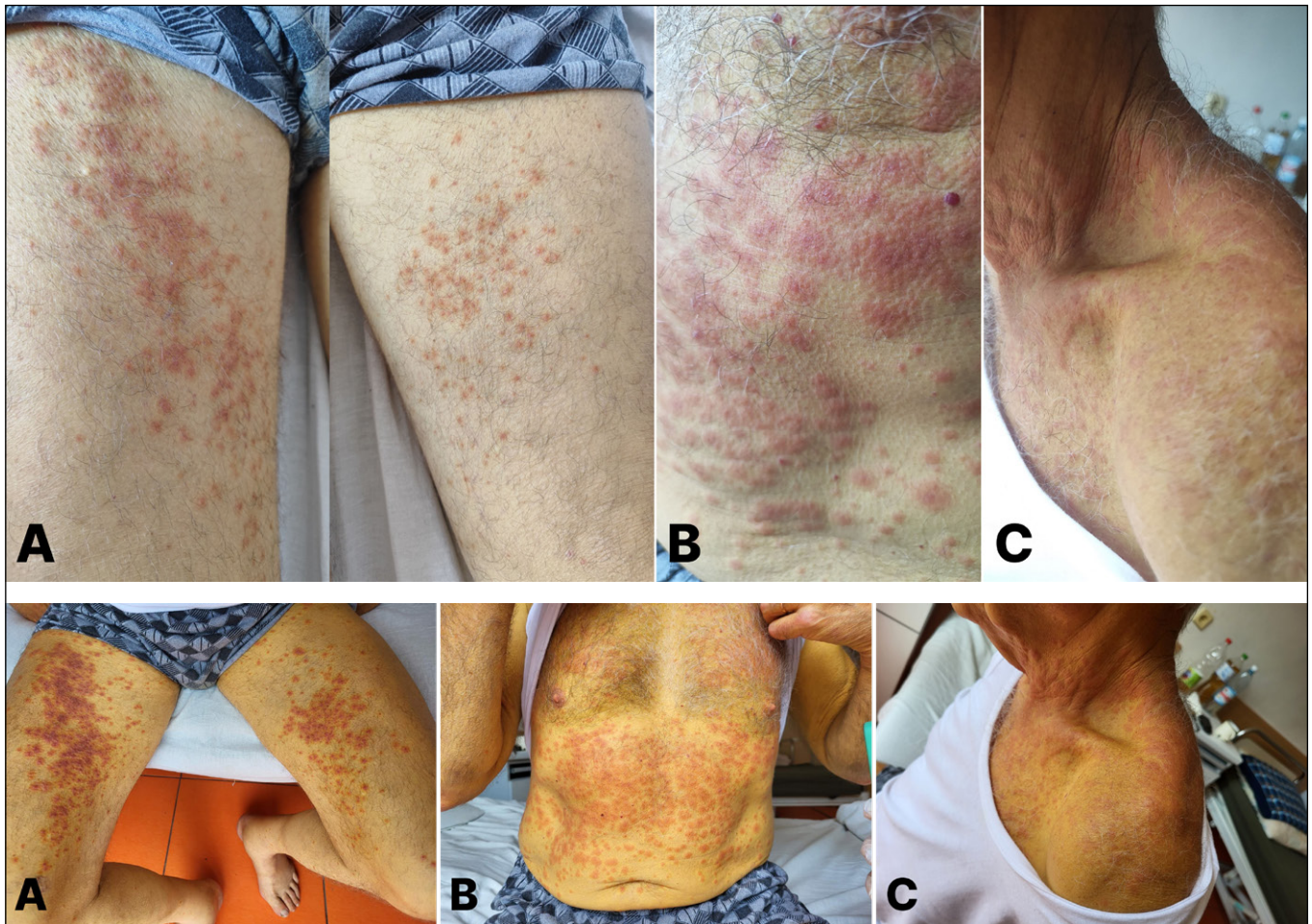**Fig.1.** Dynamics of body temperature and volume of urination.

Fig. 2. Jarisch-Herxheimer reaction on a 5th day of antibiotic therapy (8-hour difference). A. thigh area; B. trunk area; C. neck and shoulders.

muscle aches, and sudden weakness. The patient took paracetamol with no improvement in the condition. In a few days, the man noticed urine became darker. On the night before hospitalization, the patient was suffering from intense pain in muscles and first seen eyes turned yellow. The patient lives in a rural area and has a grain storage next to his house.

Primary examination in hospital: low-grade fever (37.9℃), breathing rate 14/min, and blood pressure of 155/90 mmHg. Skin is pale with a yellowish tone and dry. Sclera are subicteric. The tongue is covered with a white coating. Lymph nodes are not enlarged. Auscultation: hard bronchial breathing in the lungs, arrhythmic tones of the heart with a systolic murmur. Moderate swelling of the lower extremities (feet to ankle-foot joint). Urination: 2 liters per day of dark-colored urine (Fig. 1).

Indirect agglutination test was negative for *Leptospira* on 6h day of disease. Based on clinical examination, anamnesis, and laboratory tests a previous diagnosis of leptospirosis was put and the patient was treated with

Table 1. Laboratory examination and etiological treatment during treatment period in hospital

Day in hospital	1th	5th	7th	10th	15th
Treatment	Benzylpenicillin 16 M/day Dexamethasone 8 mg		Ceftriaxone 2 g, Methylprednisolone 500/250 mg		-
WBC (*10⁹/l)	18.03	17.24	19.16	11.59	6.39
LYM (*10⁹/l)	0.34	0.58	0.79	1.14	1.28
MID (*10⁹/l)	0.06	0.05	0.11	0.72	0.41
GRA (*10⁹/l)	17.64	16.6	18.26	9.73	4.7
LY %	1.9	3.4	4.1	9.8	20
MI %	0.3	0.3	0.6	6.2	6.5
GR %	97.8	96.3	95.3	83.9	73.5
RBC (*10¹²/l)	4.03	3.45	3.5	3.31	3.46
HBG (g/l)	105	100	99	91	99
HCT %	33.92	27.97	29.3	28.38	32.97
PLT (*10⁹/l)	36	141	348	537	244
TP (g/l)	53.8	52.1	54.4	52.1	48.5
ALB (g/l)	33.3	27.7	28.6	28.4	28
UCB (umol/l)	194.4	251.7	163.4	64.7	41.9
TBil (umol/l)	256.6	366.8	264.3	94.4	74.2
ALT (U/l)	136.8	72.1	72	108.2	98.5
AST (U/l)	185	104.9	96	101.4	74.2
GGT (U/l)	38.9	35.2	57.5	55.3	58.1
ALP (U/l)	101	421.6	420	-	-
Cr (umol/l)	437	186.6	204.8	140.7	132.5
Urea (mmol/l)	29.15	17.08	14.93	11.23	8.31
GLU	7.96	8.65	6.7	5.2	4.18
PT (sec)	14.44	16.84	15.44	14.32	-
PA %	100.9	86.01	94.13	101.8	-
INR	1.09	1.24	1.15	1.08	-
PTI %	94	81	88	95	-
APTT (sec)	34.12	36.96	33.8	27.4	-
Fibrinogen (g/l)	6.09	5.39	5.61	4.12	-

*WBC: White blood cell count; LYM: absolute lymphocyte count; MID: cells include less frequently occurring and rare cells correlating to monocytes, eosinophils, basophils, blasts and other precursor white cells that fall in a particular size range; GRA: absolute neutrophils, monocytes, eosinophils, and basophils count; RBC: red blood cells; HGB: Hemoglobin; HCT: hematocrit; PLT: Platelets; TP: total protein; ALB: albumin; UCB: unconjugated bilirubin; TBil: total bilirubin; ALT: alanine transaminase; AST: aspartate transaminase; GGT: gamma-glutamyl transferase; ALP: alkaline phosphatase; Cr: creatinine; GLU: glucose; PT: prothrombin time; PA: prothrombin activity according to Kwik; INR: international normalized ratio; PTI: prothrombin index; APTT: activated partial thromboplastin time.

2 000000 units of benzylpenicillin 8 times per day, 8 mg of dexamethasone, disintoxication therapy, furosemide, metoclopramide and oral lactulose.

On the 5th-day patient's condition worsened: fever of 39,5°C, tachycardia, tachypnoea (20/min.), blood pressure 90/60 mmHg, SpO2 - 98%. Skin and sclera became intensively icteric. The rash appeared on the trunk and spread to the lower and upper limbs, abdomen, neck, and trunk, on the thighs it became bluish, confluent in nature; on the front surface of the lower legs – petechiae (Fig. 2). Patient complained to a horrible itch and pain in the rash's sites. Auscultation: hard bronchial breathing with wet rales in the lungs (Fig. 3 chest X-ray, Table 1). The abdomen was soft on palpation and somewhat sensitive in the right hypochondrium. On percussion, the liver was slightly enlarged by 1 cm. The spleen was not palpable. Urinary output was about 5 liters, urine was dark yellow and foamy. Excretions 1 time/day formed, acholic.

The antibiotic was changed to 2 grams of ceftriaxone per day. Instead of dexamethasone, 500 mg of methylprednisolone was prescribed for the first infusion and 250 mg during the next days. Indirect agglutination
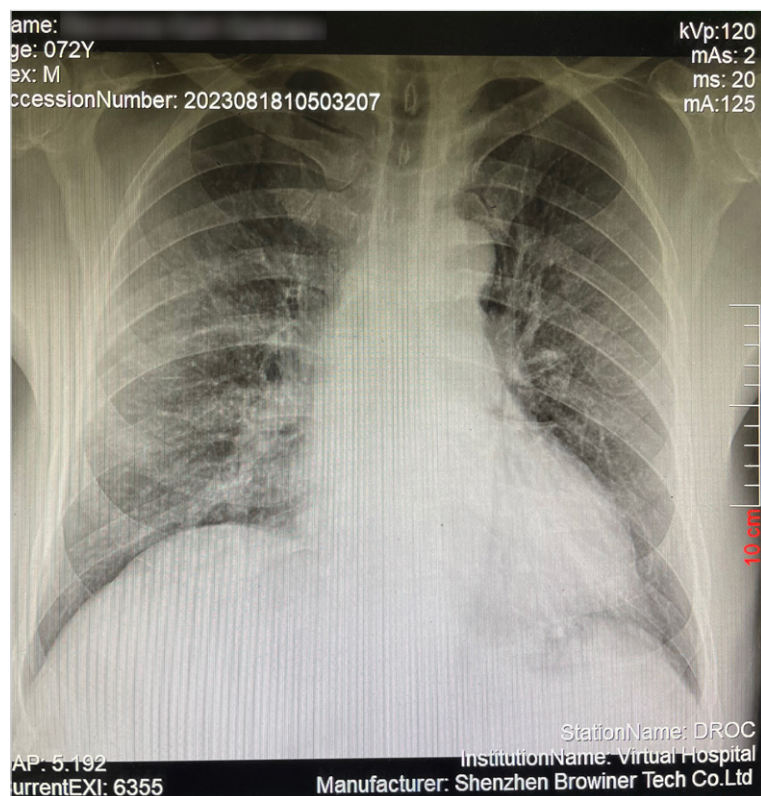
Fig. 3. X-ray of the chest in the frontal direct projection on a 5th day in hospital.
The sinuses are free. The domes are not relaxed. The roots are structural. Lung parenchyma has no focal infiltrative changes. Shadow of the heart of the usual configuration. Conclusion: There are no focal infiltrative changes in the lung parenchyma.

test came positive for *Leptospira Pomona* in 1:400 titer. Procalcitonin 1.16 ng/ml, CRP 72.36 mg/l.

In 2 days, body temperature normalized and the patient's condition began to improve. The intensity of itching and pain on the skin has dramatically decreased. The patient was discharged on the 15th day.

The main contributors of JHR are serogroup *interrogans* of the *Leptospira* genus, delay of antibiotic therapy for more than 3 days after the onset of symptoms, and treatment with amoxicillin [12]. Also, it was reported that JHR occurs within 24 hours of antibiotic therapy [14]. Comparison of symptom severity, and complication rate showed no statistical difference between the patients who developed JHR and those who did not. Renal failure, severe thrombocytopenia, and a lung hemorrhage were reported not to be statistically associated with JHR. Our case represents a typical symptom of JHR with an itchy and painful skin rash and a short-term cardio-vascular insufficiency with a delay in the onset of reaction to a 5th day of antibiotic therapy. The described case was caused by an *L. interrogans* serovar *Pomona* which serves in favor of the theory described above [15].

CONCLUSIONS

Leptospirosis is an underestimated disease despite a high mortality rate. The earliest antimicrobial therapy is crucial for the successful treatment and prevention of bacteria and secondary immune-associated complications. Corticosteroids can reduce the mortality of patients with severe leptospirosis but routine administration requires further investigation.

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CONFLICT OF INTEREST
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CORRESPONDING AUTHOR
Vlasta V. Vysochanska
Uzhhorod National University
1 sq. Narodna, 88000 Uzhhorod, Ukraine
e-mail: lachupakabramail@gmail.com

ORCID AND CONTRIBUTIONSHIP
Vlasta V. Vysochanska: 0000-0003-0998-554 Ⓐ Ⓓ Ⓕ
Myroslava S. Babych: 0009-0005-1123-9855 Ⓑ
Anton I. Kohutych: 0000-0003-3838-9106 Ⓔ
Andriana A. Halamba: 0000-0003-0175-9943 Ⓔ

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

Dear colleagues,

On May 30, 2024, a scientific and practical conference with international participation *"Organisational and Clinical Aspects of Patient-Centred Approach to Treatment and Rehabilitation in Modern Conditions"* will be held. The organisers of the conference are the State Institution of Science "Scientific and Practical Center of Preventive and Clinical Medicine" State Administrative Department, the National Academy of Medical Sciences of Ukraine, the Ukrainian Military Medical Academy and the Public Organisation "Ukrainian Association of Healthcare Management".

Publication of articles and abstracts in the professional journal **"Clinical and Preventive Medicine"** (SCOPUS) (Kyiv, Ukraine) (http://cp-medical.com/index.php/journal).

The programme issues of the conference:

1. Interdisciplinary aspects of medical and non-medical methods of rehabilitation of military personnel-combatants.
2. Topical issues of rehabilitation of civilians who have suffered as a result of the impact of stress and physical destructive factors of war.
3. Organisational and clinical aspects of the use of various rehabilitation types in modern practice of internal medicine.
4. Comorbidity in military personnel: the current state of the problem (topical issues of diagnosis, treatment and rehabilitation).
5. Theoretical and applied aspects of reflexotherapy in the complex rehabilitation of military personnel and civilians who have suffered as a result of war.
6. Features of the application of complex rehabilitation programmes in patients with acute cerebrovascular accident.
7. Modern approaches to the treatment and rehabilitation of patients with pathological changes caused by coronavirus infection (COVID-19).
8. Features of medical rehabilitation of patients with pain syndromes of different localisation.
9. Organisational and clinical aspects and perspective directions of medical rehabilitation after surgical interventions.
10. Clinical approaches to the treatment and rehabilitation of patients with mine-blast trauma.
11. Modern principles of perioperative management of patients and rational anaesthetic accompaniment in surgical practice.
12. Topical questions of rehabilitation at different stages and levels of medical care.
13. Problematic issues of interprofessional and long-term rehabilitation in primary health care.
14. The current state of integration of rehabilitation into primary health care.
15. Experience in implementing European training standards in the system of training medical personnel in the field of health care.

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