Polski Merkuriusz Lekarski

POLISH MEDICAL JOURNAL

ISSN 1426-9686

VOLUME LII, ISSUE 2, MAR-APR, 2024

Polski Merkuriusz Lekarski

POLISH MEDICAL JOURNAL

ISSN 1426-9686

VOLUME LII, ISSUE 2, MAR-APR, 2024

EDITORIAL BOARD

Polski Merkuriusz Lekarski cited by PUBMED/MEDLINE, SCOPUS, INDEX COPERNICUS, EBSCO, POLISH MEDICAL BIBLIOGRAPHY, Ministry of Science and Higher Education.

© **ALUNA PUBLISHING**
Z.M. Przesmyckiego 29
05-510 Konstancin-Jeziorna, Poland
tel. +48 604 776 311
a.luczynska@wydawnictwo-aluna.pl

www.polskimerkuriuszlekarski.pl

Distribution and subscription
Bartosz Guterman
tel. +48 22 245 10 55
prenumerata@wydawnictwo-aluna.pl

CONTENTS

ORIGINAL ARTICLES

REVIEW ARTICLES

VARIA

ORIGINAL ARTICLE

Healthcare-associated infections in postoperative patients with intracranial aneurysm in Ukraine

Aidyn G. Salmanov[1,2], Dmytro V. Shchehlov[2], Maryna Mamonova[2,3], Oleh E. Svyrydiuk[2], Ihor M. Bortnik[2], Nadiia B. Chabanovych[2], Yaroslav E. Kudelskyi[2], Oleksandr P.Kovalenko[2], Daria Chekhunova[2]

[1]SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE
[2]SCIENTIFIC-PRACTICAL CENTER OF ENDOVASCULAR NEURORADIOLOGY AND SURGERY OF NATIONAL ACADEMY OF MEDICAL SCIENCES OF UKRAINE, KYIV, UKRAINE
[3]BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

ABSTRACT

Aim: to investigate the epidemiology, microbiology, and risk factors for healthcare-associated infections (HAIs) in postoperative patients with intracranial aneurysm in Ukraine.

Materials and Methods: Retrospective cohort study was conducted from January 2018 to December 2022 in four tertiary care hospitals of Ukraine. The diagnostic criteria were based on specific HAI site were adapted from the CDC/NHSN case definitions.

Results: Of 1,084 postoperative patients with intracranial aneurysm, 128 (11.4%) HAIs were observed. The most common of HAI type was possible ventilator-associated pneumonia (38.2%) followed by central line-associated bloodstream infections (33.8%), catheter -associated urinary tract infection (18.5%), and surgical site infection (9.6%). Inpatient mortality from HAI was 5.1%. Emergency admission, mechanical ventilation, taking antiplatelet aggregation drugs, albumin reduction, hyperglycaemia, hyponatremia, surgical procedure, operation time > 4 h, mechanical ventilation, urinary catheter, and central venous catheterization were risk factors associated with HAI in patients with intracranial aneurysm surgery. A total of 26% cases of HAIs by MDROs were notified over the study period. *Klebsiella* spp. – essentially *K. pneumoniae* – were the most frequent, followed by *Enterobacter* spp. and *Escherichia coli.* Carbapenemase production in Enterobacterales constituted the most frequent mechanism of resistance, while ESBL-production in Enterobacterales and meticillin-resistance in *Staphylococcus aureus* (MRSA) were detected in 65,7% 62,3% and 20% of cases, respectively.

Conclusions: The present study showed that HAIs is a common complication in postoperative patients with intracranial aneurysm in Ukraine and multidrug-resistant organisms the major pathogen causing infection.

KEY WORDS: neurosurgery, intracranial aneurysm, healthcare-associated infection, risk factor, pathogens, antimicrobial resistance, Ukraine

Pol Merkur Lek,2024;52(1):137-144. doi: 10.36740/Merkur202402101

INTRODUCTION

Healthcare-associated infections (HAIs) in postoperative patients with intracranial aneurysm are a serious complication of neurosurgery. In recent years, more and more attention has been paid to this issue in the medical field. An intracranial aneurysm is a bulged, weakened area in the wall of an artery in the brain. Postoperative intracranial infection after intracranial aneurysm is relatively common in clinical setting. Surgical intervention before aneurysm rupture is the main method used to reduce the risk of bleeding and mortality, however surgical and surgery-related factors increase the risk of HAIs [1,2].

The patients in the neurosurgical ward are exposed to many risk factors causing HAIs. These factors are related to operations, invasive diagnosing and monitoring of the nervous system and mechanical support of vital functions [3-5]. According to literature, intracranial aneurysms are the main cause of subarachnoid hemorrhage (SAH), accounting for about 70% of SAH [6]. In all cerebrovascular accidents, aneurysm rupture and hemorrhage is second only to cerebral thrombosis and hypertensive cerebral hemorrhage, and the mortality is very high [7].

In the process of patients with intracranial aneurysm undergoing craniotomy, the blood-brain barrier is vulnerable to severe damage, which greatly increases the risk of intracranial infection [8]. Intracranial infection is one of the most serious clinical diseases of the central nervous system, and most common intracranial infections are bacterial infections [9, 10]. Craniotomy aneurysm clipping is still the first choice for surgical treatment. However, due to the difficulty of the operation and the long operation time, the incidence of intracranial infection after craniotomy for intracranial aneurysms ranges from 2.6% to 30.0%, and the mortality is over 30.0% [11, 12].

Intracranial infections are more complicated and difficult to treat. Long-term infections may affect normal brain nerve function and seriously affect the prognosis of patients. Timely and accurate prediction of intracranial infection is of positive significance for the clinical treatment of patients. Therefore, the early control of intracranial infection and the

improvement of patient prognosis are of positive significance, and the early indicators of intracranial infection should be comprehensively analyzed in clinical treatment.

The prevalence of HAIs and mortality among postoperative patients with intracranial aneurysm in Ukraine is unknown. In Ukraine the impact of risk factors for HAIs in postoperative patients with intracranial aneurysm and antimicrobial resistance responsible pathogens has not been previously investigated.

AIM

The aim of the study was to investigate the epidemiology, microbiology, and risk factors for HAIs in postoperative patients with intracranial aneurysm in Ukraine.

MATERIALS AND METHODS

STUDY DESIGN AND POPULATION

This was a retrospective cohort study. We selected patients with intracranial aneurysm admitted in four tertiary care hospitals for adult patients from January 1, 2018, to December 31, 2022, as the study population. The inclusion criteria of the patients were as follows: (a) all patients met the diagnostic criteria for aneurysms based on the "Expert Consensus on the Diagnosis and Treatment of Neurosurgery Aneurysms in Ukraine; (b) age ≥ 20years; (c) postoperative survival time > 1week. The exclusion criteria for patients were patients with intracranial infections such as meningitis and brain abscess; patients with intracranial vascular malformations and cerebral hemorrhage; patients with immune dysfunction.

DEFINITION

The diagnostic criteria were based on specific HAI site were adapted from the Centers for Disease Control and Prevention's (CDC) and National Healthcare Safety Network's (NHSN) case definitions. This study included diagnoses of HAIs involving the respiratory system, blood system, central nervous system, and urinary system. In this study, intraoperative Cerebrospinal fluid (CSF) leakage was defined as the communication between the CSF cavity and the outside of the skull. It was judged by the presence of clear liquid or light red bloody fluid in the surgical incision.

DATA COLLECTION

The central line-associated bloodstream infections (CLABSIs), catheter-associated urinary tract infections (CAUTIs), select ventilator-associated events (VAEs), and SSIs that occurred between 2018–2022 were included in this study. Unless otherwise noted, CLABSI data included events classified as mucosal barrier injury laboratory-confirmed bloodstream infection (MBI-LCBI). VAE data were limited to events classified as possible ventilator-associated pneumonia (PVAP) because this is the only subtype of VAE for which a pathogen can be reported. Asymptomatic bacteremic urinary tract infections and outpatient SSIs were excluded.

In this study according to the selection criteria, the retrospective medical information survey method was used to collect patient medical data using a self-designed form, which included the patient's basic information, diagnosis and treatment data, disease-related symptoms and signs, as well as test and imaging results. Basic data (age, gender, diagnosis, history, etc.), laboratory data (leucocyte, albumin, blood glucose, liver function, renal function, electrolytes, etc.), and treatment information (length of stay in ICU, mechanical ventilation, surgery, central venous catheterization, etc.) were collected.

ETHICS

In this study, all methods were performed in accordance with the relevant guidelines and regulations. Our study had been verified and approved by the ethical committee of the Shupyk National Healthcare University of Ukraine. For this type of study, formal consent is not required.

MICROBIOLOGICAL METHODS

In this study when the drainage volume reached 30mL (before the intracranial infection was confirmed) in all patients, 3mL of CSF specimens were collected and submitted for testing the bacterial composition of CSF culture. For the microbiological diagnosis of HAIs, appropriate clinical material (blood, swabs, urine samples and others) was collected following doctor's orders. Only the first isolate from each patient was selected for microbiological analysis. In this study species identification was performed with standard microbial methods. Antibiotic susceptibility testing of bacteria was determined using by protocol of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://eucast.org). *Staphylococcus aureus* was defined as methicillin-resistant (MRSA) if the isolate was reported as R to oxacillin, cefoxitin, or methicillin. *Enterococcus* spp. isolates were defined as vancomycin-resistant (VRE) if they tested R to vancomycin. Carbapenem-resistant Enterobacteriaceae (CRE) were defined as *Klebsiella* spp., *Escherichia coli*, or *Enterobacter* spp. that tested R to imipenem, meropenem, doripenem, or ertapenem. All other pathogen-antimicrobial combinations (phenotypes) were described using a metric for nonsusceptibility, which included pathogens that tested I or R to the applicable drugs.

STATISTICAL ANALYSIS

In this study statistical analysis was performed using SPSS 22.0. The count data is presented as frequency (percentage) and the differences between groups were assessed using the $\chi 2$ test. The normally distributed continuous data is presented as mean ± standard deviation, and the non-normally distributed continuous data is presented as median. When the data was normally distributed and the between-group variance was equal, differences between groups were assessed using t-tests; the rank sum test was used when the data showed a biased distribution. Univariate analysis was used to perform preliminary variable selection, using an α level of 0.1. On the other hand, logistic stepwise regression analysis was used to analyse the independent predictors of HAI in patients with intracranial aneurysms; differences with $P < 0.05$ were considered statistically significant

RESULTS

PREVALENCE OF HAI

In the study period, a total of 1,084 patients with neurosurgical intracranial aneurysms were enrolled. A total of 154 cases of healthcare-associated infections (HAIs) we detected in 128 patients. Of all HAI cases, 11.4% were detected after hospital discharge. The prevalence of HAIs among postoperative patients with intracranial aneurysm in in Ukrainian hospitals was 11.8% (95% CI: 10.8-12.8). The most common type was possible ventilator-associated pneumonia (PVAP), with 38.2% (95% CI:37.2-39.6) cases, followed by central line-associated bloodstream infections (CLABSIs), 33.8% (33.0-35.4) cases, catheter-associated urinary tract infections (CAUTIs), 18.5% (95% CI:17.1-19.1) cases, and surgical site infection (SSI), 9.6% (95% CI:8.6-10.0) cases. HAIs occurred 1 to 18 days after surgery procedure, with an average occurrence of 5 days after surgery. A total this study including 41.5% males and 58.5% females, aged 28–81 years, with an average age of 54.5 ± 11.8 years. Patient characteristics and risk factors for HAI are shown in Table 1.

RISK FACTORS FOR HAI

Univariate analysis indicated that lower activities of daily living score on admission, emergency admission, mechanical ventilation, taking antiplatelet aggregation drugs, albumin reduction, hyperglycaemia, hyponatremia, surgical procedure, operation time > 4 h, mechanical ventilation, urinary catheter, and central venous catheterization were risk factors associated with HAI in patients with intracranial aneurysm surgery ($P < 0.05$) (Table 1). Multivariate analysis was performed for the variables for which $P < 0.0001$ in the univariate analysis. From the logistic regression results, it appeared that mechanical ventilation (odds ratio = 4.261, 95% confidence interval 1.102–16.476), hyperglycaemia (OR 2.759, 95% CI 1.159–6.564), urinary catheterization (OR 6.557, 95% CI 2.244–19.159), and CVC (OR 8.853, 95% CI 2.860–27.398) were independent risk factors for HAI in patients with intracranial aneurysm surgery.

MORTALITY FROM HAI

In this study of the cases of HAI identified, 5.1% died before discharge. The most mortality risk was observed in postoperative patients with possible ventilator-associated pneumonia (PVAP) and in patients with CLABSIs. There were no deaths in patients with CAUTI and SSI.

PATHOGENS AND ANTIMICROBIAL RESISTANCE

During 2018–2022, a total of 154 HAIs and 196 pathogens were detected (Tables 2 and 3). PVAPs contributed the highest proportion of pathogens (31.1%) followed by CLABSIs (27%), SSI (23%) and CAUTIs (18,5%). *Escherichia coli* was the most common pathogen across all HAIs, constituting almost 17.3% of reported pathogens. *Staphylococcus aureus* (12.8%) and *Klebsiella* spp. (11.2%) were the second and third most commonly reported pathogens, respectively. Distribution and rank order of the most frequently isolated pathogens across all types of HAIs in postoperative patients with intracranial aneurysm in Ukraine, 2018-2022 is shown in Table 3.

A total of 40 cases of HAIs by multidrug-resistant organisms (MDROs) were notified over the period 2018–2022. Their characteristics are shown in Table 4. Concerning the MDROs, *Klebsiella* spp. (50%) – essentially *K. pneumoniae* – were the most frequent, followed by *Enterobacter* spp. (25%) and *E. coli* (20,5%) as presented in Table IV. Carbapenemase production in Enterobacterales constituted the most frequent mechanism of resistance (62,3%), while ESBL-production in Enterobacterales and meticillin-resistance in *S. aureus* (MRSA) were detected in 65,7% 62,3% and 20% of cases, respectively.

DISCUSSION

This is the first study focused on epidemiology and microbiology of HAIs in postoperative patients with intracranial aneurysm in Ukraine. In the study period, a total of 1,084 patients with neurosurgical intracranial aneurysms were enrolled. A total of 154 cases of HAIs we detected in 128 patients. Of all HAI cases, 11.4% were detected after hospital discharge. Inpatient mortality from HAI was 5.1%. A previous study in Ukraine conducted found that mortality rate in neurosurgical patients with HAIs was 11.3% [4]. In other countries mortality in neurosurgical patients with HAIs was from 3.5% to 12% [1, 13-15]. The prevalence of HAIs among postoperative patients with intracranial aneurysm in in Ukrainian hospitals was 11.8%. Previous studies found that the incidence of HAI in non-Ukrainian neurosurgical hospitals was 3.1–11.8% [1, 16, 17], while the incidence in Ukrainian hospitals is 5.6–20.9% [3, 4, 18].

In this study the most common of HAI type in postoperative patients with intracranial aneurysm was possible ventilator-associated pneumonia (38.2%) followed by central line-associated bloodstream infections (33.8%), catheter -associated urinary tract infection (18.5%), and surgical site infection (9.6%). Our results are consistent with previous studies [5, 16, 17].

HAIs in postoperative patients with intracranial aneurysms can be affected by a variety of factors. This study shows that emergency admission, mechanical ventilation, taking antiplatelet aggregation drugs, albumin reduction, hyperglycaemia, hyponatremia, surgical procedure, operation time > 4 h, mechanical ventilation, urinary catheter, and central venous catheterization were risk factors associated with HAI in patients with intracranial aneurysm surgery.

The techniques used for treatment of intracranial aneurysms have progressed dramatically over the decades. The introduction of modern endovascular techniques and the continued refinement of progressively less invasive neurosurgical approaches have contributed to steadily improving clinical outcomes. Moreover, innovations such as flow-diverting stents have achieved dramatic success and have gained rapid widespread adoption. Particularly in lesions for which the application of conventional treatment techniques is difficult, flow diversion technology has revolutionized aneurysm management. Patients with coma manifest disturbances of consciousness with inhibition of normal physiological reflexes such as swallowing and coughing, resulting in increased respiratory tract secretions

Table 1. Characteristics and risk factors for healthcare-associated infections (HAIs) in postoperative patients with intracranial aneurysm in Ukraine, 2018-2022

Variable	All patients (n=1084)	HAI		P value
		Yes (n = 128)	No (n = 956)	
Gender, n (%)				
Male	450 (41.5)	62 (48.4)	388 (40.6)	0.231
Female	634 (58.5)	66 (51.6)	568 (59.4)	
Age, n (%)				
≤ 40 years	174 (16.1)	16 (12.5)	158 (16.5)	0.255
41–59 years	534 (49.3)	56 (43.8)	478 (50.0)	
≥ 60 years	376 (34.7)	56 (43.8)	320 (33.5)	
Admission route, n (%)				
Emergency	476 (43.9)	94 (73.4)	382 (40.0)	<0.000
Elective	608 (56.1)	34 (26.6)	574 (60.0)	
Hospitalization time, n (%)				
6–12	658 (60.7)	38 (29.7)	620 (64.9)	<0.000
13-24	426 (39.3)	90 (70.3)	336 (35.1)	
Diabetes, n (%)				
Yes	156 (14.1)	18 (14.1)	138 (14.4)	0.936
No	928 (85.6)	110 (85.9)	818 (85.6)	
Steroids, n (%)				
Yes	254 (23.4)	24 (18.8)	230 (24.1)	0.346
No	830 (76.6)	104 (81.3)	726 (75.9)	
Antiplatelet aggregate, n (%)				
Yes	464 (42.8)	22 (17.2)	442 (46.2)	<0.000
No	620 (57.2	106 (82.8)	514 (53.8)	
Albumin reduction, n (%)				
Yes	100 (9.2)	28 (21.9)	72 (7.5)	<0.000
No	984 (90.8)	100 (78.1)	884 (92.5)	
Hyperglycaemia, n (%)				
Yes	422 (38.9)	90 (70.3)	332 (34.7)	<0.000
No	662 (61.1)	38 (29.7)	624 (65.3)	
Hyperkalaemia, n (%)				
Yes	4 (0.4)	2 (1.6)	2 (0.2)	0.562
No	1080 (99.6)	126 (98.4)	954 (98.8)	
Surgical approach, n (%)				
Intervention	684 (63.1)	44 (34.4)	640 (66.9)	<0.000
Craniotomy	400 (36.9)	84 (65.6)	316 (33.1)	
Surgery duration, n (%)				
≤ 4 h	790 (72.9)	60 (46.9)	730 (76.4)	<0.000
> 4 h	294 (27.1)	68 (53.1)	226 (23.6)	
Hyponatremia, n (%)				
Yes	76 (7.0)	24 (18.8)	52 (5.4)	<0.000
No	1008 (93.0)	104 (81.3)	904 (94.6)	
Mechanical ventilation, n (%)				
Yes	248 (22.9)	96 (75.0)	152 (15.9)	<0.000
No	836 (77.1)	32 (25.0)	804 (84.1)	
Urinary catheter, n (%)				
Yes	100 (9.2)	28 (21.9)	72 (7.5)	<0.000
No	984 (90.8)	100 (78.1)	884 (92.5)	
Central venous catheter, n (%)				
Yes	354 (32.7)	116 (90.6)	238 (24.9)	<0.000
No	730 (67.3)	12 (9.4)	718 (75.1)	

Table 2. Frequency of healthcare-associated infection (HAI) events and pathogens, by HAI type in postoperative patients with intracranial aneurysm in Ukraine, 2018-2022

HAI types	HAI events (n=157)		Pathogens (n=196)	
	n	%	n	%
SSI	15	9.6	45	23.0
CAUTI	29	18.5	36	18.4
CLABSI	53	33.8	53	27.0
PVAP	60	38.2	62	31.6

SSI, surgical site infection; CAUTI, catheter-associated urinary tract infection; CLABSI, central line-associated bloodstream infection; PVAP, possible ventilator-associated pneumonia.

Table 3. Distribution and rank order of the most frequently isolated pathogens across all types of healthcare-associated infections (HAIs) in postoperative patients with intracranial aneurysm in Ukraine, 2018-2022

Pathogen	All HAI Types, Pathogens (n=196)			PVAP, Pathogens (n=62)		CABSI, Pathogens (n=53)		CAUTI, Pathogens (n=36)		SSI, Pathogens (n=45)	
	n	%	Rank	%	Rank	%	Rank	%	Rank	%	Rank
Escherichia coli	34	17.3	1	4.9	7	6.6	8	34.3	1	19.5	1
Staphylococcus aureus	25	12.8	2	15.1	3	9.2	3	2.1	10	14.1	2
Klebsiella spp.	22	11.2	3	18.7	2	8.9	4	14.2	2	6.1	8
Pseudomonas aeruginosa	19	9.7	4	21.8	1	5.9	9	12.8	3	8.6	4
Enterococcus spp.	16	8.2	5	2.1	12	7.7	7	11.5	4	8.7	3
Coagulase-negative staphylococci	14	7.1	6	0.4	13	13.8	1	2.7	7	7.2	6
Acinetobacter baumannii	13	6.6	7	7.9	4	7.9	6	0.5	12	2.9	13
Enterobacter spp.	12	6.1	8	6.2	5	4.1	12	5.7	6	7.7	5
Proteus mirabilis	11	5.6	9	2.4	10	0	0	9.6	5	3.8	11
Stenotrophomonas maltophilia	9	4.6	10	5.9	6	4.7	11	2.4	8	4.9	9
Serratia marcescens	7	3.6	11	5.8	8	5.8	10	2.3	9	4.6	10
Candida albicans	6	3.1	12	5.7	9	12.9	2	1.9	11	3.1	12
Other Candida spp.	5	2.6	13	3.1	11	8.8	5	0	0	2.1	14
Bacteroides spp.	3	1.5	14	0	0	3.7	13	0	0	6.7	7

SSI, surgical site infection; CAUTI, catheter-associated urinary tract infection; CLABSI, central line-associated bloodstream infection; PVAP, possible ventilator-associated pneumonia.

and susceptibility to bacterial reproduction. In addition, patients with coma tend to suffer from severe diseases, with the need for tracheal intubation, tracheotomy, mechanical ventilation, nasogastric stomach tube, or urinary tract intubation-assisted treatment. Such operations can damage the mucosa and decrease its barrier function; this is an important risk factor for HAI. Therefore, patients with coma should be actively treated and their early waking encouraged, as well as strictly controlling the indications for invasive operations, performing operations under strict aseptic conditions, promoting gentle movement, keeping the airway open, and ensuring the removal of deep sputum to reduce the incidence of infection [1]. Despite these advances, there has been no reduction in HAIs in postoperative patients with intracranial aneurysm.

Consistent with previous studies, the results indicate that hyperglycaemia is another risk factor for HAI [19]. Hyperglycaemia increases plasma osmotic pressure of patients, inhibits the chemotaxis activity, adhesion ability, phagocytic ability and intracellular killing effect of leucocyte, and reduces the body's resistance to infection. In addition, long-term hyperglycaemia favours bacterial reproduction, especially in the respiratory tract, urinary tract, skin, and female patients' genital area, which increases the risk of infection [20]. Consequently, attention should be paid to the monitoring and management of blood sugar and control of glucose intake, as well as whether diabetic patients develop a further increase in blood sugar after trauma, or non-diabetic patients experience stress hyperglycaemia as a result of trauma, surgery, or other stress conditions.

Prevention and control of HAIs, encountered when treating with open surgery and various endovascular techniques is the cornerstone of patient safety procedures. The identification of the risk factors associated with HAI and early prevention and control play important parts in reducing its incidence. A few studies on HAI in patients with intracranial aneurysms have been conducted. Previous studies found that the risk factors for HAI in postoperative

Table 4. Characteristics of multidrug-resistant bacteria caused healthcare-associated infections (HAIs) in postoperative patients with intracranial aneurysm in Ukraine, 2018-2022

Mechanisms of resistance and causative agent	Values	
	Number	Percentage
ESBL-producing *Enterobacterales*	14	62.3
Klebsiella spp.	6	27.3
Escherichia coli	6	17.6
Enterobacter spp.	1	8.3
Proteus mirabilis	1	9.1
Carbapenemase-producing *Enterobacterales*	10	65.7
Klebsiella spp.	5	22.7
Enterobacter spp.	2	16.7
Escherichia coli	1	2.9
Proteus mirabilis	1	9.1
Serratia marcescens	1	14.3
Meticillin-resistant Staphylococcus aureus	5	20.0
Vancomycin-resistant Enterococcus spp.	2	12.5
Multidrug-resistant Acinetobacter baumannii	2	15.4
Multidrug-resistant Pseudomonas aeruginosa	2	10.5
Carbapenemase-producing P. aeruginosa	5	26.3

ESBL: extended-spectrum β-lactamase.

patients with intracranial aneurysm included age, sex, poor clinical condition upon hospital admission, ICU admission, oral intubation, tracheotomy, and urethral/ventricular drainage [21, 22]. Wang J. et al [1] found that the being comatose upon hospital admission, having hyperglycaemia or hypothermia, and indwelling central venous catheterization are major risk factors for HAIs in patients undergoing surgery for intracranial aneurysms. Other authors analysed the risk factors for respiratory infection in patients with aneurysmal subarachnoid haemorrhage clipping. Postoperative tracheal intubation of more than 48 h, tracheotomy, and stay in the intensive care unit of more than 5 days were independent risk factors for respiratory infection [23].

In present study PVAPs contributed the highest proportion of pathogens followed by CLABSIs, SSI and CAUTIs. *E. coli* was the most common pathogen across all HAIs, constituting almost 17.3% of reported pathogens. *S. aureus* and *Klebsiella* spp. were the second and third most commonly reported pathogens, respectively. A total of 26% cases of HAIs by MDROs were notified over the study period. *Klebsiella* spp. – essentially *K. pneumoniae* – were the most frequent, followed by *Enterobacter* spp. and *E. coli*. Carbapenemase production in Enterobacterales constituted the most frequent mechanism of resistance, while ESBL-production in Enterobacterales and meticillin-resistance in *S. aureus* (MRSA) were detected in 65,7% 62,3% and 20% of cases, respectively. Our results are consistent with previous studies [3, 4, 18].

STRENGTH AND LIMITATION

Strength: This is the first study focused on epidemiology and microbiology of HAIs in postoperative patients with intracranial aneurysm in Ukraine. Limitation: This was a retrospective study, although its results were confirmed by a multicentre prospective study. Furthermore, this study did not provide long-term follow-up of patients. The long-term impact of HAI on patients with intracranial aneurysms and their prognosis is not yet clear and needs to be further studied.

CONCLUSIONS

The present study showed that HAIs is a common complication in postoperative patients with intracranial aneurysm in Ukraine and multidrug-resistant organisms (MDROs) the many pathogens causing infection. Also, MDROs were a major contributor to HAI-caused deaths in neurosurgical patients. These data underscore the importance of tracking antimicrobial resistance, particularly in vulnerable populations such as neurosurgical patients. Emergency admission, mechanical ventilation, taking antiplatelet aggregation drugs, albumin reduction, hyperglycaemia, hyponatremia, surgical procedure, operation time > 4 h, mechanical ventilation, urinary catheter, and central venous catheterization were risk factors associated with HAI in patients with intracranial aneurysm surgery. Understanding the importance of HAIs in postoperative patients with intracranial aneurysm is helpful for surgeons to deal with the unexpected situation and improve the curative effect. However, it must be emphasised that since this study is retrospective, the significant correlation of findings must be evaluated very carefully, and the causal relationship between risk factors and the incidence of infection must be further studied in prospective randomised clinical trials. Similarly, the non-significant relationship between

some factors and infection rate does not exclude the possibility of correlation. These risk factors may lead to changes in infection rate. Improvement of care among patients with intracranial aneurysm requires an accurate assessment of postoperative complications. Although neurosurgical procedure is associated with low morbidity, adverse events do occur that are potentially preventable. Perioperative complications are increasingly identified through administrative data, comprising information from discharge summaries. The epidemiological and risk factors for HAIs in patients with intracranial aneurysms need to be further explored.

REFERENCES

1. Wang J, Ji Y, Jiang L et al. Zhang H. Analysis of factors influencing hospital-acquired infection in postoperative patients with intracranial aneurysm. BMC Neurol. 2019;19(1):332. doi: 10.1186/s12883-019-1565-2.
2. Guo X, Fang J, Wu Y. Risk factors of intracranial infection in patients after intracranial aneurysm surgery: Implication for treatment strategies. Medicine (Baltimore). 2021;100(48):e27946. doi: 10.1097/MD.0000000000027946.
3. Salmanov AG, Shchehlov DV, Svyrydiuk O et al. Surgical site infections after neurosurgical procedures in Ukraine: results of a multicenter studv (2018-2020). Wiad Lek. 2022;75(1):27-33. doi: 10.36740/WLek202201105.
4. Salmanov AG, Shchehlov DV, Svyrydiuk O et. al. Healthcare-associated infection in neurosurgical patients in Ukraine: results of a multicenter study (2017-2019). Wiad Lek. 2021;74(8):1945-1951. dol: 10.36740/WLek202108129.
5. Wałaszek M. The analysis of the occurrence of nosocomial infections in the neurosurgical ward in the District Hospital from 2003-2012. Przegl Epidemiol. 2015;69(3):507-14, 619-23.
6. Andrade L, Hoskoppal A, Hunt Martin M et al. Intracranial aneurysm and coarctation of the aorta: prevalence in the current era. Cardiol Young 2021;31(2):229-32. doi: 10.1017/S1047951120003716.
7. Dandapat S, Mendez-Ruiz A, Martinez-Galdamez M et al. Review of current intracranial aneurysm flow diversion technology and clinical use. J Neurointerv Surg. 2021;13(1):54-62. doi: 10.1136/neurintsurg-2020-015877.
8. Han P, Jin D, Wei W et al. The prognostic effects of hemodynamic parameters on rupture of intracranial aneurysm: a systematic review and meta-analysis. Int J Surg. 2021;86:15-23. doi: 10.1016/j.ijsu.2020.12.012.
9. Ko H, Kim G, Lee HD et al. Ruptured intracranial aneurysm in an adolescent with infective endocarditis. Pediatr Int. 2018;60(4):376-7. doi: 10.1111/ped.13513.
10. Shiba M, Toma N, Ikezawa M et al. Intracranial-intracranial bypass using a Y-shaped artery graft for growing unruptured Gemella morbillorum infectious aneurysm on artery supplying sensory cortex. World Neurosurg. 2020;142:328-33. doi: 10.1016/j.wneu.2020.08.076.
11. Ryu J, Lee KM. Intracranial mycotic aneurysm in a patient with abdominal actinomycosis. World Neurosurg. 2021;147:161-3. doi: 10.1016/j.wneu.2020.11.069.
12. Ravina K, Rennert RC, Brandel MG et al. Comparative assessment of extracranial-to-intracranial and intracranial-to-intracranial in situ bypass for complex intracranial aneurysm treatment based on rupture status: a case series. World Neurosurg. 2021;146:e122-38. doi: 10.1016/j.wneu.2020.10.056.
13. Hammers R, Anzalone S, Sinacore J et al. Neurosurgical mortality rates: what variables affect mortality within a single institution and within a national database? J Neurosurg. 2010;112(2):257-64. doi:10.3171/2009.6.JNS081235.
14. Elsamadicy AA, Sergesketter A, Sampson JH et al. Institutional Review of Mortality in 5434 Consecutive Neurosurgery Patients: Are We Improving? Neurosurgery. 2018 6(83):1269-1276. doi: 10.1093/neuros/nyx603.
15. Tomlinson SB, Piper K, Kimmell KT et al. Preoperative Frailty Score for 30-Day Morbidity and Mortality After Cranial Neurosurgery. World Neurosurg. 2017;107:959-965. doi: 10.1016/j.wneu.2017.07.081.
16. Agarwal R, Mohapatra S, Rath GP et al. Active Surveillance of Health Care Associated Infections in Neurosurgical Patients. J Clin Diagn Res. 2017;11(7):DC01--DC04. doi: 10.7860/JCDR/2017/26681.
17. Rafa E, Kołpa M, Wałaszek MZ et al. Healthcare-Acquired Infection Surveillance in Neurosurgery Patients, Incidence and Microbiology, Five Years of Experience in Two Polish Units. Int J Environ Res Public Health. 2022;19(12):7544. doi: 10.3390/ijerph19127544.
18. Salmanov A, Shchehlov D, Svyrydiuk O et al. Epidemiology of healthcare-associated infections and mechanisms of antimicrobial resistance of responsible pathogens in Ukraine: Results of a multicentre study (2019-2021). J Hosp Infect. 2023;131:129-138. doi: 10.1016/j.jhin.2022.10.007.
19. Diringer MN, Bleck TP, Claude Hemphill J et al. Critical care management of patients following aneurysmal subarachnoid hemorrhage: recommendations from the Neurocritical Care Society's Multidisciplinary Consensus Conference. Neurocrit Care. 2011;15(2):211-40. doi: 10.1007/s12028-011-9605-9.
20. Casqueiro J, Casqueiro J, Alves C. Infections in patients with diabetes mellitus: A review of pathogenesis. Indian J Endocrinol Metab. 2012;16(1):S27-36. doi: 10.4103/2230-8210.94253.
21. Frontera JA, Fernandez A, Schmidt JM et al. Impact of nosocomial infectious complications after subarachnoid hemorrhage. Neurosurgery. 2008;62:80-7. doi: 10.1227/01.NEU.0000311064.18368.EA.
22. Cinotti R, Dordonnat-Moynard A, Feuillet F et al. Risk factors and pathogens involved in early ventilator-acquired pneumonia in patients with severe subarachnoid hemorrhage. Eur J Clin Microbiol Infect Dis. 2014;33:823-30. doi: 10.1007/s10096-013-2020-8.
23. Savardekar A, Gyurmey T, Agarwal R et al. Incidence, risk factors, and outcome of postoperative pneumonia after microsurgical clipping of ruptured intracranial aneurysms. Surg Neurol Int. 2013;4:24. doi: 10.4103/2152-7806.107894.

We would like to thank all members of Ukrainian association of infection control and antimicrobial resistance who contributed to the prevalence surveys. The authors received no financial support for the research, authorship, and/or publication of this article.

CONFLICT OF INTEREST
The Authors declare no conflict of interest

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 A, C-F
Dmytro V. Shchehlov: 0000-0003-1465-8738 B, C, D, F
Maryna Mamonova: 0000-0002-0697-4864 B, C, D, F
Oleh E. Svyrydiuk: 0000-0001-7455-0396 B, C, D, F
Ihor M. Bortnik: 0000-0001-8072-6570 B, C, D, F
Nadiia B. Chabanovych: 0000-0002-5113-5082 B, C, D, F
Yaroslav E. Kudelskyi: 0000-0002-4345-5959 B, C, D, F
Oleksandr P. Kovalenko: 0000-0002-8422-1753 B, C, D, F
Daria Chekhunova: 0009-0002-4474-5533 B, C, D, F

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

RECEIVED: 10.11.2023
ACCEPTED: 18.02.2024

ORIGINAL ARTICLE

Effects of exercise training after acute coronary syndromes in octogenarians – prospective 12-month evaluation

Hanna Rymuza[1], Justyna Mączyńska[2], Natasza Krauze[3], Ilona Kowalik[2], Edyta Smolis-Bąk[2], Andrzej Ciszewski[4], Hanna Szwed[2], Rafał Dąbrowski[2]

[1]CARDIOLOGY CLINIC, NATIONAL INSTITUTE OF CARDIOLOGY, WARSAW, POLAND
[2]DEPARTMENT OF CORONARY DISEASE AND CARDIOLOGICAL REHABILITATION, NATIONAL INSTITUTE OF CARDIOLOGY, WARSAW, POLAND
[3]DEPARTMENT OF CARDIOLOGY, MEDICAL UNIVERSITY OF WARSAW, WARSAW, POLAND
[4]DEPARTMENT OF INTERVENTIONAL CARDIOLOGY AND ANGIOLOGY, NATIONAL INSTITUTE OF CARDIOLOGY, WARSAW, POLAND

ABSTRACT

Aim: To demonstrate the impact of individual exercise training on the course of the disease, exercise tolerance and quality of life (QoL) in patients over 75 years after acute coronary syndrome (ACS).

Materials and methods: Study included octogenarians after ACS randomly assigned into two groups: a training group (ExT) subjected to individualized physical training and a control group (CG) with standard recommendations for activity. Patients underwent exercise tolerance test (ETT), 6-minute walk test (6-MWT), NHP and QoL questionnaires evaluation, lab tests, ECG, echocardiographic examination at the beginning and after 2, 6 and 12 months.

Results: Study included 51 patients, mean age 80 years, 50% men, all patients completed the study. Initial physical capacity was comparable in both groups. After 2-month training the average ETT exercise time increased by 12.5% (p=0.0004), the load increased by 13% (p=0.0005) and the 6-MWT results improved by 8.3% (p=0.0114). Among CG these changes were not significant. But 6 and 12 months after training cessation 6-MWT results returned to the initial values (p=0.069, p=0.062 respecitvely). Average ETT exercise time and average load decreased significantly after 12 months (p=0.0009, p=0.0006). Level of pain was significantly lower at the end of the training in ExT group (p=0.007), but it returned to initial 12 months later (p=0.48). QoL deteriorated significantly in the ExT group 12 months after training cessation (p=0.04).

Conclusions: Cardiac rehabilitation in octogenarians after ACS was safe and improved physical performance in a short period of time. Cessation of training resulted in a loss of achieved effects and deterioration of the QoL.

KEY WORDS: acute coronary syndromes, octogenarians, exercise training

Pol Merkur Lek,2024;52(1):145-152. doi: 10.36740/Merkur202402102

INTRODUCTION

Needless to mention that exercise training (ExT) has been used for years in patients after acute coronary syndrome (ACS). It effectively reduces overall mortality and the risk of re-infarction, improves physical fitness and quality of life [1]. ExT increases perfusion of the myocardium, development of microcirculation vessels and improves endothelial function. It has an additional anticoagulant effect by increasing the volume of plasma, thrombolytic activity, also reducing blood viscosity and platelet aggregation [2]. ExT has been shown to reduce myocardial ischemia [3]. In addition, ExT also has a beneficial influence on modulation of the autonomic nervous system and reducing the burden of cardiac arrhythmias [4]. Physical activity also reduces the impact of cardiovascular risk factors: it facilitates weight control, prevents development of hypertension and diabetes [5]. The ESC guidelines for the management of acute myocardial infarction with persistent ST-segment elevation strongly recommend exercise rehabilitation, class IA [6]. Due to effective revascularization and optimal medical therapy, the risk of cardiovascular complications in this group has been reduced. While the RAMIT study failed to show the beneficial effects of rehabilitation on clinical outcomes and quality of life, another study (GOSPEL) showed that long-term multifactorial educational and behavioral intervention has a beneficial effect on the incidence of reinfarction, reduces cardiovascular risk, and facilitates proper doses of drugs selection in secondary prevention [7, 8]. However the percentage of people over 75-years-old in the studied populations was low.

AIM

The main hypothesis of the current study assumed that individualized cardiac rehabilitation in people over 75 years of age after ACS treated invasively or non-invasively will have a beneficial effect on exercise tolerance and exercise capacity assessed in the ECG exercise test (ETT) and 6-minute walk test (6-MWT), also on quality of life (QoL), and that it will have a positive effect on longer-term prognosis.

MATERIALS AND METHODS

The study was performed between Jan, 01 2015 and Nov 30, 2017 and it was supported by the State Committee for Scientific Research Grant. The design and protocol of the study was accepted by the Institutional Review Board and Ethics Committee (registration number 2.3 / I / 15). It was a randomized prospective controlled clinical trial including patients over 75 years of age after ACS, who underwent PCI or were treated non-invasively. Patients were enrolled after signing in the informed consent to participate in the study. Total of 20% from screened patients were eligible to participate. Exclusion criteria included inability to perform ExT due to dyspnoea or anginal pain, coexistence of comorbidities affecting the long-term prognosis or ExT performance (i.e. cancer, musculoskeletal diseases, senile dementia, severe chronic obstructive pulmonary disease), advanced structural heart disease, planned cardiac surgery, uncontrolled hypertension, complex ventricular arrhythmias, low left ventricular ejection fraction (i.e. LVEF< 40%) or resting refractory tachycardia above 100 BPM. Recruitment period lasted 24 months. Patients were randomly assigned into two groups. The training group (ExT) underwent individualized physical training 3 times a week for 10 weeks. Exercise and load times were individually adjusted to the capabilities of the patients according to model B or C of the second stage of rehabilitation. The second stage of rehabilitation was carried out in a hospital or on an outpatient basis, with duration of 4-12 weeks. It included exercises on 3-5 days a week, with 3 days a week of medically supervised workouts and daily walks lasting at least 30-60 minutes. Workouts included a warm-up lasting 5-10 minutes, the main part of training lasting 20-60 minutes and a cool-down phase lasting 5-15 minutes, consisting of continuation of exercises with low intensity. The exercise program consisted of a set of general exercises improving the strength of muscles, endurance training, resistance training elements and improving balance exercises. The control group (CG) received standard recommendations for activity. In addition, laboratory tests, ECG, echocardiography, 6-MWT, ETT on a cycloergometer according to the RAMP protocol, NHP and QoL questionnaire evaluations were performed. Both groups were regularly supervised by a cardiologist.

Statistical analysis was performed with SAS 9.2 software (SAS Institute Inc, Cary, NC, USA). Normally distributed continuous variables were reported as means and SD, and qualitative variables were presented as counts and percentages. The Pearson's chi^2 test or the Fisher's exact test (in case of minimum expected count less than 5) for qualitative variables and Student's t-test for numerical variables were performed to compare baseline characteristic in the two groups of patients. The impact of individual training on the level of physical capacity was assessed by Two-way repeated analysis of variance ANOVA. The study concerns two main effects (between subject: R and NR groups, within groups: repeated measures) and their interaction (impact method of rehabilitation on repeated measures). If results of analysis were statistically significant, preplanned comparison was performed. The simple contrast method was used, where the first measures were the reference level. All hypotheses testing were two-tailed with a $p < 0.05$ type I error.

RESULTS

Finally 51 patients with average age 80.3±3.7 years, 55% men were included in our study. The ExT group consisted of 25 patients and the CG group – of 26. Study groups did not differ in terms of demographics, medical history and treatment (Table 1). Physical capacity before the beginning of the training cycle assessed in ETT and 6-MWT was comparable in both groups, ns (Table 2). Rehabilitation procedures were safe. The results are shown in Figures 1, 2 and 3. In the ExT group, after the 10-week training cycle, a statistically significant improvement in physical performance was found. The average ETT duration increased by 12.5% in ExT group, from 416±152 to 468±153 s, p=0.0004 (Fig. 1). The workload in the ETT increased by 13.0%, from 69±25.2 watts to 78±25.4 watts, p=0.0005, (Fig. 2). The average distance in 6-MWT increased by 8.3% in comparison to the initial measurement: from 446±90m to 483±60 m, p=0.0114 (Fig. 3). However 6 and 12 months after rehabilitation cessation values of the analyzed physical performance indexes decreased. The mean distances after 6 months and after 12 months returned to the initial values: 474±73 and 476±80 m, p=0.069, p=0.062 respectively. Similarly 12 months after training cessation the average duration of the exercise test (424±147 s) and the average load obtained (70±24.4 wat) decreased significantly compared to the results right after rehabilitation course, p=0.0009, p=0.0006, respectively. The results were equal as in initial tests (p=0.481, p=0.593). In CG group distance prolongation was not observed in 6-MWT after 6 and 12 months (p=0.123 and p=0.147, respectively). Also in ETT there was no increase in the duration after 12 months of observation, p=0.179. Two-way repeated analysis of variance showed statistically significant interactions between the type of the group and the measurements observed at different times of observation for two parameters: duration of ETT, p=0.0163, and maximum load, p=0.023. The results of the NHP questionnaire showed a significantly lower level of pain at the end of rehabilitation course among ExT group compared to initial measurements, p=0.007, but it got significantly worse over time: on the 12-month follow-up it was worse than after 6 months (p=0.029), reaching the baseline level (0.48), means respectively: 27.7±5.7, 18.6±5.3 and 22.0±5.3. Changes were not found in the CG group. There was a statistically significant deterioration in the area of QoL and emotions in the ExT group after 12 months in relation to the measurement right after completing the rehabilitation course, means respectively: 20.6±4.1 and 25.4±4.2, p=0.040. Interestingly we observed an improvement trend in the corresponding period of the CG group (mean after 12 months and after 2 months: 14.3±4.3 and 17.2±4.5, p=0.07), resulting in a significant differences between the groups after 12 months, p=0.006.

Table 1. Demographic and clinical characteristics of the study patients, CG – control group, ExT – training group

	Total N=51	CG N=26	ExT N=25	p
Age, years	80.3±3.7	80.7±4.3	79.8±2.9	0.3896
Male gender, n(%)	28 (54.9)	17 (65.4)	11 (44)	0.1250
BMI, kg/m^2	26.2±3.4	25.6±3.2	26.9±3.5	0.1797
STEMI, n(%)	16 (31.4)	6 (23.1)	10 (40)	0.1929
History of infarction, n(%)	16 (32.0)	9 (36.0)	7 (28)	0.5443
PCI, n(%)	14 (27.4)	7 (26.9)	7 (28)	0.9313
CABG, n(%)	7 (13.7)	2 (7.7)	5 (20)	0.2485
Hypertension, n(%)	40 (78.4)	20 (76.9)	20 (80)	0.7894
Diabetes, type 2, n(%)	13 (25.5)	7 (26.9)	6 (24)	0.8108
Insulin, n(%)	2 (3.9%)	0	2 (8)	0.2353
Dyslipidemia, n(%)	42 (82.3)	20 (76.9)	22 (88)	0.4654
Smoking, n(%)	9 (17.7)	6 (23.1)	3 (12)	0.4654
History of stroke, n(%)	1 (2.0)	0	1 (4)	0.4902
ASO, n(%)	5 (9.8)	3 (11.5)	2 (8)	1.0000
Depression, n(%)	3 (5.9)	2 (7.7)	1 (4)	1,000
Heart failure, NYHA II, n(%)	51 (100)	26 (100)	25 (100)	1.000
AF, n(%)	11 (21.6)	4 (15.4)	7 (28)	0.2735
Pacemaker, n(%)	2 (3.9)	1 (3.9)	1 (4)	1.000
Hemoglobin, g/dL	13.1±1.1	13.1±1.2	13.0±1.0	0.7876
GFR, ml/min/m^2	73.2±15.2	73.3±16.2	73.2±14.4	0.9779
Glucose, mg/dL	106.0±16.7	106.7±16.8	105.3± 16.9	0.7720
Cholesterol, mmol/L	4.4±1.1	4.3±1.3	4.6 ± 0.9	0.3640
LDL, mmol/L	2.6±0.9	2.5±1.1	2.7± 0.7	0.3510
Ejection fraction, n(%)	50.3±7.9	50.4±6.5	50.2±9.3	0.9080
LVSD, mm	46.9±5.6	47.6±5.3	46.1±6.0	0.3595
SBP, mmHg	126.9±17.8	124.0±18.2	129.8±17.3	0.2520
DBP, mmHg	73.7±7.6	73.3±7.9	74.2±7.5	0.6667
HR, x/per minute	66.4±9.2	67.2±10.9	65.6±7.2	0.5495

BMI: body mass index, STEMI: myocardial infarction STEMI, PCI: percutaneous coronary intervention, CABG: coronary artery bypass grafting, NYHA: New York Heart Association, AF: atrial fibrillation, GFR: glomerular filtration rate, LDL: low density lipoprotein, LVSD: left ventricular systolic dimension, SBP: systolic blood pressure, DBP: diastolic blood pressure, HR: heart rate.

DISCUSSION

Currently there are no studies assessing optimal model of rehabilitation in elderly people after ACS. The World Health Organization recommends 2.5 hours a week of moderate effort or 1.5 hours a week of intensive training for healthy, older people [9]. However in our research only a very small group of patients was able to follow these recommendations due to the inability to overcome such burdens [10]. In fact, for elderly patients after ACS, with existing comorbidities, such recommendations do not exist. The average age of both groups was 80 years and what is worth emphasizing, women accounted for 45% of the studied groups. In the current study a significant improvement in physical performance and QoL was achieved in the group subjected to regular exercise. These effects were observed in a short time interval – immediately after the end of training. All subjects completed the study. The trainings were safe. Cooperation with patients was satisfactory. As expected, immediately after the end of the training cycle, a significant improvement in physical fitness was assessed in objective tests: ETT and 6-MWT. However this effect was short-lived and just 4 months after the end of rehabilitation the differences between the examined groups were leveled out. In the RAMIT trial patients after ACS (mean age 64.2±11.2 years), have not demonstrated the beneficial effect of rehabilitation on clinical outcomes and QoL [7]. The GOSPEL study showed that long-term multifactorial educational and behavioral intervention had a beneficial effect on reducing the risk of re-infarction, reduced global cardiovascular risk, and improved the quality of optimal medical therapy after ACS. However the percentage of people over 75 years of

Table 2. Results of 6-minute walk tests (6-MWT) and ECG exercise tolerance test (ETT)

	Control group (CG)				Training group (ExT)				P –effect	P – effect				
	Baseline	After 10 weeks	After 12 months	P*	Baseline	After 10 weeks	After 12 months	P*	BG **	Baseline	After 10 weeks	After 12 months	Time	Inter
Distance in 6-MWT, m	452±86	457±103	473±103	0.699 0.147 0.170	446±90	483±60	476±80	0.011 0.062 0.56	0.83	0.83	0.36	0.92	0.016	0.19
SBP Baseline, mm Hg	126±21	124±15	128±21	0.72 0.63 0.29	130±19	123±19	134±20	0.18 0.45 0.01	0.68	0.48	0.90	0.34	0.12	0.44
DBP Baseline, mm Hg	68±9	71±7	71±8	0.04 0.23 0.76	72±10	66±10	72±8	0.01 1.00 0.79	1.00	0.18	0.06	0.71	0.34	0.04
SBP max, mm Hg	142±21	142±16	143±22	0.92 0.86 0.76	137±27	142±20	143±26	0.38 0.32 0.77	0.66	0.49	0.92	0.95	0.22	0.84
DBP max, mm Hg	73±8	71 ±7	73 ±9	0.16 1.00 0.26	73±9	72 ±11	74±7	0.52 0.54 0.25	0.76	0.87	0.82	0.70	0.20	0.45
HR max, x/min.	81±12	81±13	79±11	0.96 0.43 0.51	82±12	80±19	80±10	0.46 0.47 0.83	0.80	0.78	0.82	0.77	0.70	0.50
ECG exercise tolerance test														
Time, sec	394 ±112	404 ±111	411 ±110	0.461 0.179 0.559	416 ±152	468 ±153	424 ±147	0.0004 0.481 0.0009	0.38	0.57	0.11	0.72	0.004	0.016
Watts	65±19	67±19	68±18	0.411 0.252 0.822	69±25	78±25	70±24	0.0005 0.593 0.0006	0.38	0.58	0.12	0.72	0.003	0.023
HR limit	74±11	74±11	75±13	1.00 0.58 0.64	76±14	76±14	79±15	1.00 0.22 0.29	0.47	0.63	0.62	0.42	0.41	0.88

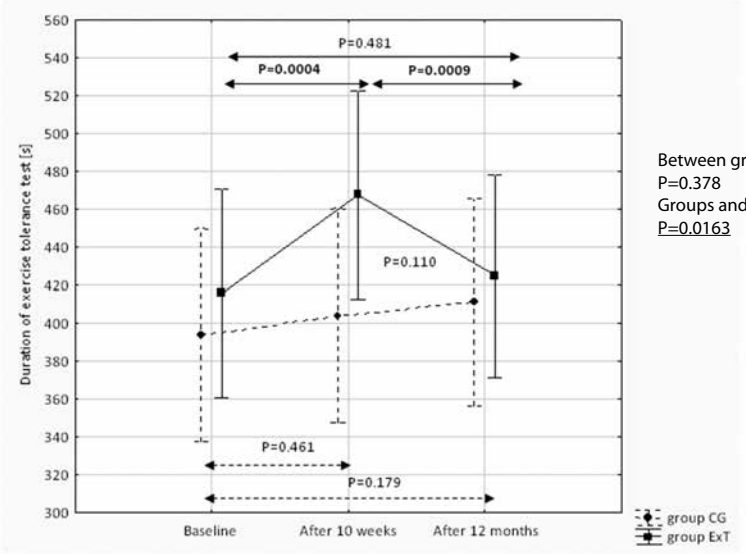
**level of statistical significance respectively in rows: result of the overall test: baseline vs afer 10 weeks, baseline after 12 months and after 10 weeks vs. after 12 months from randomization.*

***general comparison between groups, in subsequent columns - for following time points.*

SBP – systolic blood pressure, DBP – diastolic blood pressure, HR – heart rate, Watts – workload unit.

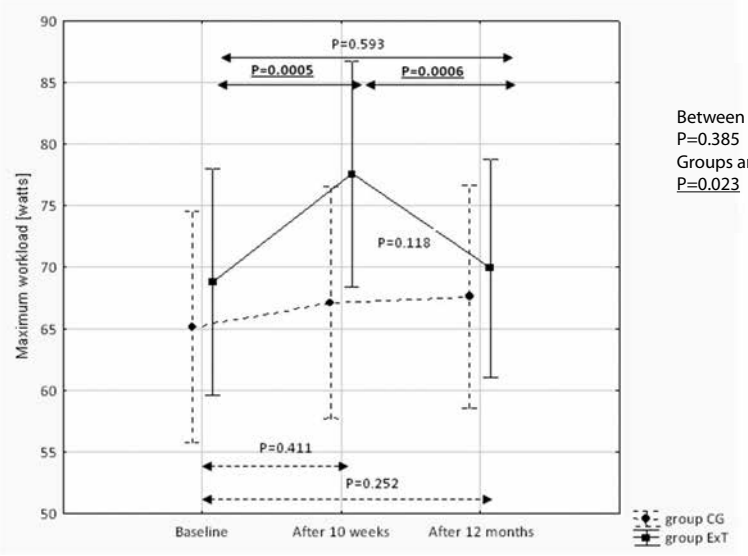
Table 3. The Nottingham Health Profile (NHP) questionnaire results

	Control group (CG)				Training group (ExT)				P-effect	P-effect				
NHP	0	10 weeks	12 months	P	0	10 weeks	12 months	p	Both groups	0	10 weeks	12 months	time	Inter
Energy	25.9 ±7.4	30.8 ±7.4	34.9 ±9.0	0.49 0.21 0.57	50.7 ±6.6	41.5 ±8.6	49.4 ±7.3	0.18 0.84 0.26	0.09	0.02	0.36	0.28	0.48	0.34
Pain	19.6 ±5.6	18.8 ±5.9	15.5 ±5.7	0.40 0.09 0.41	27.7 ±5.7	18.6 ±5.3	27.0 ±5.3	0.007 0.48 0.029	0.18	0.16	0.74	0.06	0.003	0.103
Emotional reactions	17.2 ±4.5	17.3 ±3.9	14.3 ±4.3	0.37 0.44 0.07	20.6 ±4.1	16.3 ±3.4	25.4 ±4.2	0.79 0.10 0.04	0.11	0.32	0.81	0.006	0.81	0.03
Sleep disturbances	30.8 ±7.8	34.9 ±6.9	26.1 ±6.2	0.47 0.44 0.11	51.7 ±8.6	49.7 ±8.3	51.0 ±7.8	0.71 0.90 0.80	0.047	0.08	0.18	0.02	0.62	0.44
Social isolation	15.1 ±4.2	11.1 ±3.7	5.5 ±2.7	0.39 0.02 0.06	5.2 ±1.9	6.9 ±2.3	12.6 ±3.9	0.56 0.14 0.24	0.79	0.15	0.53	0.15	0.74	0.009
Physical limitation	27.1 ±6.0	26.5 ±5.3	24.1 ±5.5	0.85 0.31 0.46	29.4 ±4.3	24.0 ±4.0	29.5 ±5.3	0.06 0.98 0.08	0.80	0.75	0.71	0.49	0.36	0.17
Beck depression scale	3.9 ±2.8	3.8 ±3.2	2.9 ±1.9	0.93 0.18 0.17	3.4 ±2.5	4.4 ±2.6	3.7 ±2.1	0.13 0.65 0.28	0.63	0.58	0.54	0.23	0.24	0.35



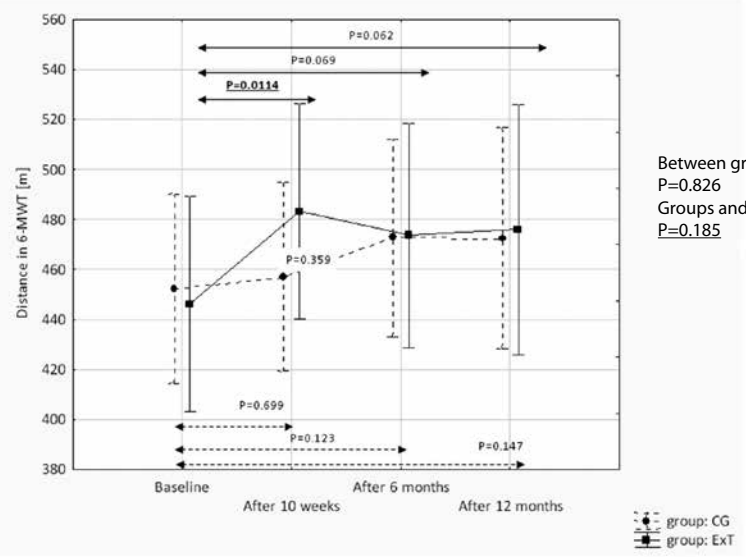
Between groups:
P=0.378
Groups and time interaction:
P=0.0163

Fig. 1. The durations (seconds) of exercise tolerance stress tests (ETT): at baseline, after 10 weeks and 12 months.



Between groups:
P=0.385
Groups and time interaction:
P=0.023

Fig. 2. Maximum workload (watts) achieved in the exercise tolerance stress tests (ETT): at baseline, after 10 weeks and 12 months.



Between groups:
P=0.826
Groups and time interaction:
P=0.185

Fig. 3. Distances (meters) in 6-minute walk tests (6-MWT): at baseline, after 10 weeks, 6 and 12 months.

age in the studied populations was low: only 8.1% [8]. Due to the aging of the population, more and more patients after ACS are in the older age. But people over 75 years of age are rarely included in the clinical trials. In the RAMIT study, the average age of the exercised group was 64 years, with 20% aged over 70 years. In the GOSPEL study, the average age was 58 years, only 8% over 70 years old. Therefore we are still lacking data assessing the impact of rehabilitation after ACS in older people. The results of OPACH study showed a linear relationship between all-cause mortality and the number of minutes devoted to physical effort of a low and moderate intensity during the day. The study involved women aged 63-99, average follow-up was 3.3 years. Although moderate physical exercise had significant impact on mortality reduction, low physical activity such as a slow walk (0.6-0.8 m/sec), or performing everyday activities in a standing position (washing up, cooking) had also a positive effect on the survival time. A 12%-reduction in mortality risk was found for every 30 minutes daily spent in motion relative to a sedentary lifestyle. These results also concerned women over 80 years of age with comorbidities. Authors concluded that people who are unable to exercise moderate physical efforts should be advised to change the sedentary lifestyle performing even small efforts, e.g. slow walks [11]. A reluctance of older people to participate in cardiological rehabilitation programs, especially women, is an important problem [12]. Results of a single-center study showed that after coronary surgery or ACS the number of patients with the declared intention to participate in rehabilitation increases from 2 to 66% if the patient obtains clear recommendations from the attending physician [13, 14]. Recently, several papers have been published documenting the beneficial effect of pedometers on physical activity of adults and improvement of their health [15, 16]. We should point that results of the present study were obtained in conditions of close supervision by cardiologists and *physiotherapists*. Participation of patients in exercises was often associated with the need to travel and required family involvement, which was a significant inconvenience. Taking into account the presented results and the results of the OPACH study, a different model of rehabilitation should be considered, emphasizing the small but daily physical activity recommended regularly by the physician, assisted by close relatives. The activity level can be monitored, e.g. with the use of pedometers. The future is in telemonitoring guided home-based training programs especially for older people with heart failure, after ACS and with comorbidities [17].

LIMITATIONS OF THE STUDY

It was a single center study. The studied groups' numbers were small, but on the other hand recruitment to the study was difficult due to advanced age and limited possibility of cooperation. None of the patients was lost during the study. The studied groups were homogenous and presented numerical sizes were sufficient for statistical evaluation.

CONCLUSIONS

In octogenarians after ACS, cardiac rehabilitation is safe and it improves physical performance in a short period of time. Cessation of the 2-month training results in the loss of achieved effects and quality of life deterioration after 12 month from ACS.

REFERENCES

1. Lawler PR, Filion KB, Eisenberg MJ. Efficacy of exercise-based cardiac rehabilitation post-myocardial infarction: a systematic review and meta-analysis of randomized controlled trials. Am Heart J. 2011;162:571–584.
2. Di Francescomarino S, Sciartilli A, Di Valerio V, Di Baldassarre A, Gallina S. The effect of physical exercise on endothelial function. Sports Med. 2009;39:797–812.
3. Kavazis AN. Exercise preconditioning of the myocardium. Sports Med. 2009;39:923–935.
4. Billman GE. Cardiac autonomic neural remodeling and susceptibility to sudden cardiac death: effect of endurance exercise training. Am J Physiol Heart Circ Physiol. 2009;297:H1171–H1193.
5. Graham I, Atar D, Borch-Johnsen K, et al. European guidelines on cardiovascular disease prevention in clinical practice: full text. Fourth Joint Task Force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts). Eur J Cardiovasc Prev Rehabil. 2007;14 Suppl 2:S1–113.
6. Ibanez B, James S, Agewall S, et al. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). Eur Heart J. 2018;39(2):119–177.
7. West RR, Jones DA, Henderson AH. Rehabilitation after myocardial infarction trial (RAMIT): multi-centre randomised controlled trial of comprehensive cardiac rehabilitation in patients following acute myocardial infarction. Heart 2011;98:637–644.
8. Giannuzzi P, Temporelli PL, Marchioli R, et al. Global secondary prevention strategies to limit event recurrence after myocardial infarction: results of the GOSPEL study, a multicenter, randomized controlled trial from the Italian Cardiac Rehabilitation Network. Arch Intern Med. 2008;168:2194–2204.
9. World Health Organization. Global Recommendations on Physical Activity for Health. Geneva, Switzerland: World Health Organization. https://www.who.int/publications-detail-redirect/9789241599979 [Access: January 2023]
10. Barnes PM, Schoenborn CA. Physical Activity Among Adults: United States. National Center for Health Statistics 2000. Report No.333. https://www.cdc.gov/nchs/data/ad/ad333.pdf [Access: January 2023]

12. LaMonte MJ, Buchner DM, et al. Accelerometer-Measured Physical Activity and Mortality in Women Aged 63 to 99. J Am Geriatr Soc. 2018;66(5):886-894.
13. Ades PA, Waldmann ML, McCann WJ, et al. Predictors of cardiac rehabilitation participation in older coronary patients. Arch Intern Med. 1992; 152, 5: 1033-1035.
14. Martin BJ, Hauer T, Arena R, et al. Cardiac rehabilitation attendance and outcomes in coronary artery disease patients. Circulation 2012;126(6):677-87.
15. Arem H, Moore SC, Patel A, et al. Leisure time physical activity and mortality: a detailed pooled analysis of the dose-response relationship. JAMA Intern Med. 2015;175(6):959-67.
16. Ekelund U, Steene-Johannessen J, Brown WJ. Does physical activity attenuate, or even eliminate, the detrimental association of sitting time with mortality? A harmonised meta-analysis of data from more than 1 million men and women. Lancet 2016;388:1302-1310.
17. Smolis-Bąk E, Dąbrowski R, Piotrowicz E, et al. Hospital-based and telemonitoring guided home-based training programs: effects on exercise tolerance and quality of life in patients with heart failure (NYHA class III) and cardiac resynchronization therapy. A randomized, prospective observation. Int J Cardiol. 2015 Nov 15;199:442-7.

The results of the study presented at the ESC Congress 2019 together with World Congress of Cardiology 31 August – 4 September 2019, Paris - France.
Poster Session 5: Acute coronary syndrome treatment.
P4603. Exercise training after acute coronary syndromes in the octogenarians has beneficial effect on the course of the disease and the exercise tolerance - single-center, prospective 12-months evaluation.
European Heart Journal, Volume 40, Issue Supplement_1, October 2019, ehz745.0986.
https://doi.org/10.1093/eurheartj/ehz745.0986.

ORCID AND CONTRIBUTIONSHIP

Hanna Rymuza: 0009-0004-9832-4640 A-C, F
Justyna Mączyńska: 0000-0001-7995-8489 D-F
Natasza Krauze: 0000-0002-6067-5881 A-B, F
Ilona Kowalik: 0000-0002-8040-5109 C, E-F
Edyta Smolis-Bąk: 0000-0002-8706-4247 A-B, E-F
Andrzej Ciszewski: 0000-0002-5905-1342 B, E-F,
Hanna Szwed: 0000-0001-7888-5708 A, C, E-F
Rafał Dąbrowski: 0000-0002-5602-0313 A, C-F

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESOPNDING AUTHOR

Justyna Mączyńska
Department of Coronary Disease
and Cardiological Rehabilitation
National Institute of Cardiology, Poland
email: just.maczynska@gmail.com

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

RECEIVED: 10.12.2023
ACCEPTED: 20.03.2024

ORIGINAL ARTICLE

Histological and immunohistochemical characteristic of the gingival stroma in the portion of the third molars in children of various age

Oksana I. Godovanets[1], Igor S. Davydenko[1], Taras I. Muryniuk[1], Larysa Ya. Fedoniuk[2]

[1]BUKOVINIAN STATE MEDICAL UNIVERSITY, CHERNIVTSI, UKRAINE
[2]I. HORBACHEVSKY TERNOPIL NATIONAL MEDICAL UNIVERSITY, TERNOPIL, UKRAINE

ABSTRACT

Aim: To evaluate the state of the gingival stromal elements in the portion of the third molars requiring extraction of these teeth due to orthodontic indications considering the stage of tooth germ formation.

Materials and Methods: The surgery to extract third molars due to orthodontic indications was performed on 95 children aged 11 to 18 years. The three groups of observation were isolated according to clinical-radiological signs: I (n=30) – children aged 11-13 years; II (n=35) – children aged 13-16 years, and III (n=30) – children aged 16-18 years. During surgery, the samples of gums were taken from the adjacent areas for examination. The samples were fixed, dehydrated, paraffinized for further histological processing. Immunohistochemical methods were used according to the protocols supplied by a producer. In particular, by means of immunohistochemical method, Ki-67, CD-34 antigens and vimentin with primary antibodies against them were determined. The primary antibodies were visualized by the polymeric visualization system with diaminobenzidine giving a brown color to the places of location of the antigens examined. The data obtained were statistically processed.

Results: The results of the study showed that specific gravity of the vascular bed in the gingival papillary layer of children was the most variable. It ranges from (12,7±0,09) % at the stage of "D" root formation to (54,8±0,17) % at the "H" stage. Lower concentrations of CD-34 antigens and vimentin are found in the endotheliocytes of children aged 13-16 and 16-18 years, compared to the children aged 11-13 years ($p<0,05$). No changes were found in the specific volume of the blood vessels, CD-34 antigens and vimentin in the reticular gingival layer of children from the groups of observation.

Conclusions: Therefore, the conducted histological and immunohistochemical study of the connective gingival tissues in the portion of the third molars in children enables to draw a conclusion that in the process of formation of the root of this tooth a number of changes occur in the gingival stroma. They include an increase of the blood flow volume in the papillary gingival layer on the background of a decreased concentration of CD-34 genes and vimentin, a longer stage of development of the third molar root. The specific volume of the islets of neoangiogenesis of the papillary gingival layer is the largest in children aged 13-16 years.

KEY WORDS: children, extraction of the third molars, gingival stroma, immunohistochemical study

Pol Merkur Lek,2024;53(1):153-160. doi: 10.36740/Merkur202402103

INTRODUCTION

Extraction of the third molar is one of the most common surgeries in the practical work of a dental surgeon and in high demand in orthodontics since it provides stability of orthodontic treatment [1-3].

An important stage of any surgery is a post-operative period characterized by a certain duration and complications available which is stipulated by the regeneration potential of the tissues. Extraction of impacted and dislocated third molars is rather traumatic manipulation. It is often associated with a large defect of the osseous tissue and wound infection [4, 5].

Therefore, practical dentistry is looking for optimal solutions concerning the time and mechanism of surgery on the third molars. Many scientists consider that [6, 7] germectomy would be reasonable, since it has a number of advantages over typical and atypical tooth extraction. Moreover, germs tissues of the third molar are a valuable source of odontogenic stem cells [8, 9]. They can be used both in dentistry and medicine in general [10, 11]. This opens up great opportunities for regenerative dentistry [12-14]. The issue of restoration of intraosseous tissue at various stages of dental care is particularly relevant [15, 16].

AIM

To evaluate the state of the gingival stromal elements in the portion of the third molars requiring extraction of these teeth due to orthodontic indications considering the stage of tooth germ formation.

MATERIALS AND METHODS

The surgery to extract third molars due to orthodontic indications was performed on 95 children aged 11 to 18 years. The state of germs of the third molars was estimated by means of radiological Demirjian method [17]. The three groups of observation were isolated according to clinical-radiological signs. Group I (n=30) included children aged 11-13 years with the third molar on the

"D" stage of formation. Group II (n=35) included children aged 13-16 years with the third molar on the "E" and "F" stages of formation. Group III (n=30) included children aged 16-18 years with the third molar on the "G" та "H" stages of formation.

During surgery, the samples of gums were taken from the adjacent areas for examination. The samples were fixed, dehydrated, paraffinized for further histological processing. Immunohistochemical methods were used according to the protocols supplied by a producer. In particular, by means of immunohistochemical method, Ki-67, CD-34 antigens and vimentin with primary antibodies against them were determined. The primary antibodies were visualized by the polymeric visualization system with diaminobenzidine giving a brown color to the places of location of the antigens examined [18].

To obtain digital copies of optic microscopic images of the tissues, the microscope Delta Optical Evolution 100 and camera Olympus SP550UZ were used. Digital copies of images were analyzed by means of the specialized computer program ImageJ v1.52 (США) [19]. The data obtained were statistically processed [20].

RESULTS

The blood vessels of the gingival connective tissue were in the focus of our attention, since they mostly provide one of the major aspects – trophic of both epithelial and stromal components. In its turn, it ensures a complete course of the postoperative period and regeneration processes.

Histological and metric indices of the blood vessels in the gingival papillary layer in children from the groups of observation are presented in Table 1.

It should be noted that the vessels of the microcirculation prevailed, that is, capillaries, venules, and arterioles. Capillaries dominated. The volume of the blood vessels increased with an increased stage of development of the third molar root, which is illustrated in the Fig. 1-3.

Table 1. Characteristic of the vascular bed in the gingival papillary layer in the portion of the third molars in children depending on age

	Observation group	I group (11-13 years)	II group (13-16 years)		III group (16-18 years)	
	The stage of formation of the third molar germ according to Demirjian method	D	E	F	G	H
1.	Specific volume of the vascular bed (%)	12,7±0,09	12,9±0,08	14,0±0,14	28,5±0,16	54,8±0,17
2.	Optic density of staining on CD-34 in the vascular endotheliocytes (units of optic density)	0,392± 0,0015	0,385± 0,0016	0,370± 0,004	0,338± 0,0014	0,310± 0,0014
3.	Optic density of staining on vimentin in the vascular endotheliocytes (units of optic density)	0,316± 0,0018	0,312± 0,0017	0,309± 0,0016	0,306± 0,0014	0,287± 0,0016

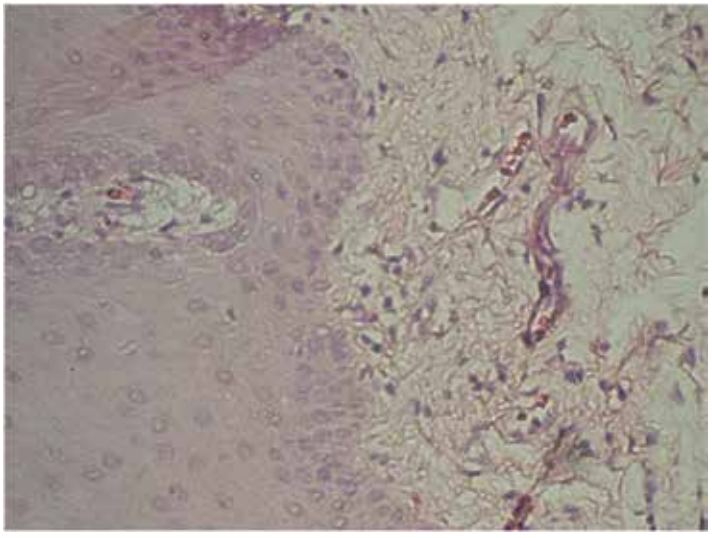


Fig. 1. Histological image of the gingival papillary layer at the "D" stage of formation of the dental root in an 11-year-old child. Staining with hematoxylin and eosin. Optic magnification 200^{x} (oc.20^{x}, vol.10^{x}).

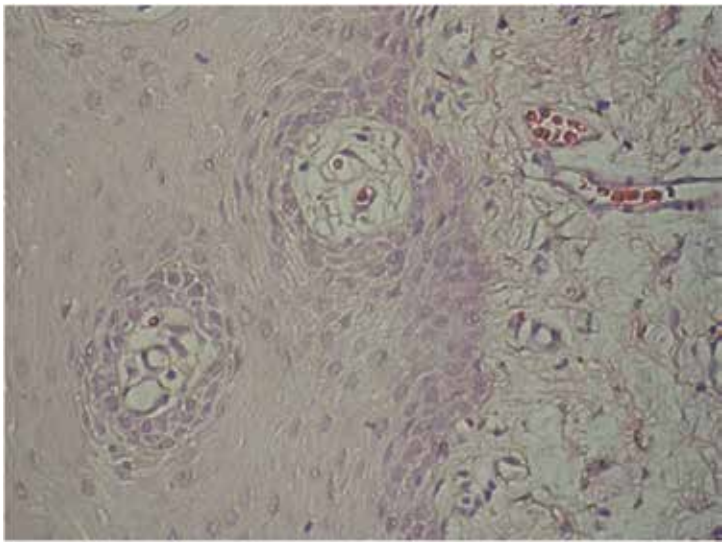


Fig. 2. Histological image of the gingival papillary layer at the "F" stage of formation of the dental root in a 15-year-old child. Staining with hematoxylin and eosin. Optic magnification 200^{x} (oc.20^{x}, vol.10^{x}).

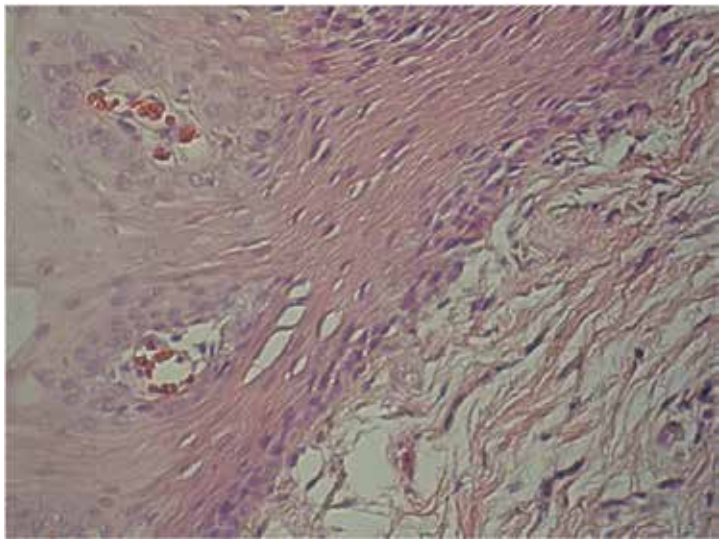


Fig. 3. Histological image of the gingival papillary layer at the "H" stage of formation of the dental root in an 18-year-old child "H". Staining with hematoxylin and eosin. Optic magnification 200^{x} (oc.20^{x}, vol.10^{x}).

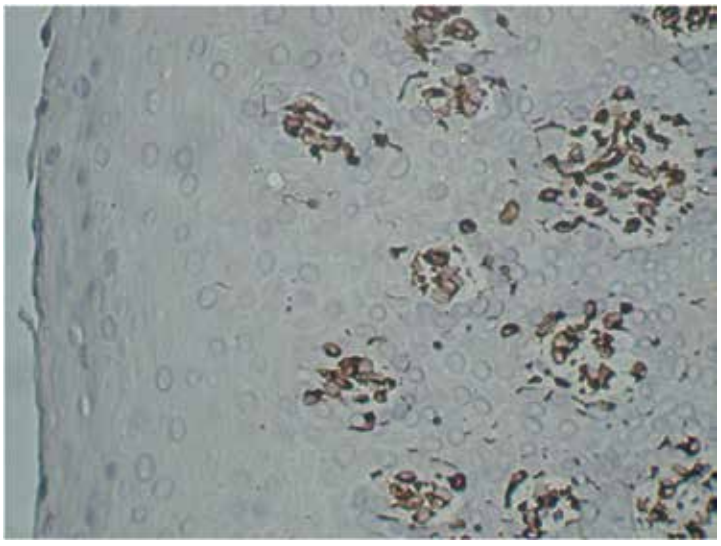


Fig. 4. The gingival papillary layer at the "D" stage of formation of a dental root in a 12-year-old child. CD-34-positive endothelium of the microcirculation. Immunohistochemical method with primary antibodies to CD-34, polymeric detection system and visualization by means of diaminobenzidine with additional hematoxylin staining of nuclei. Optic magnification 200^{x} (oc.20^{x}, vol.10^{x}).

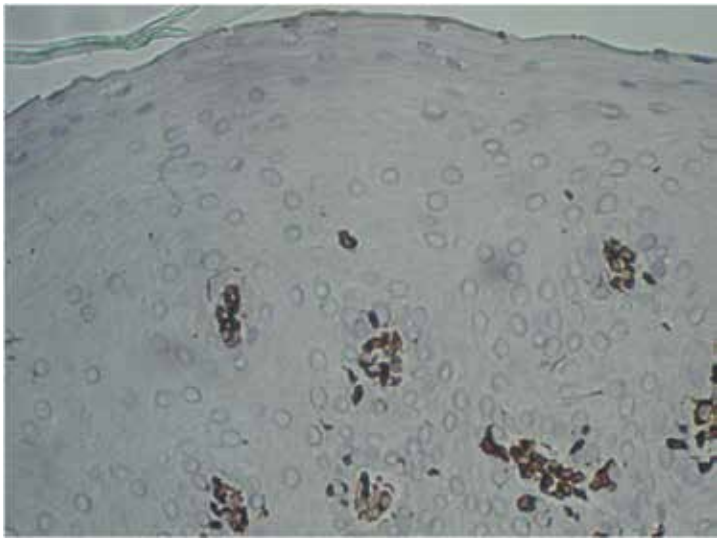


Fig. 5. The gingival papillary layer at the "H" stage of formation of a dental root in an 18-year-old child. CD-34-positive endothelium of the microcirculation. Immunohistochemical method with primary antibodies to CD-34, polymeric detection system and visualization by means of diaminobenzidine with additional hematoxylin staining of nuclei. Optic magnification 200^{x} (oc.20^{x}, vol.10^{x}).

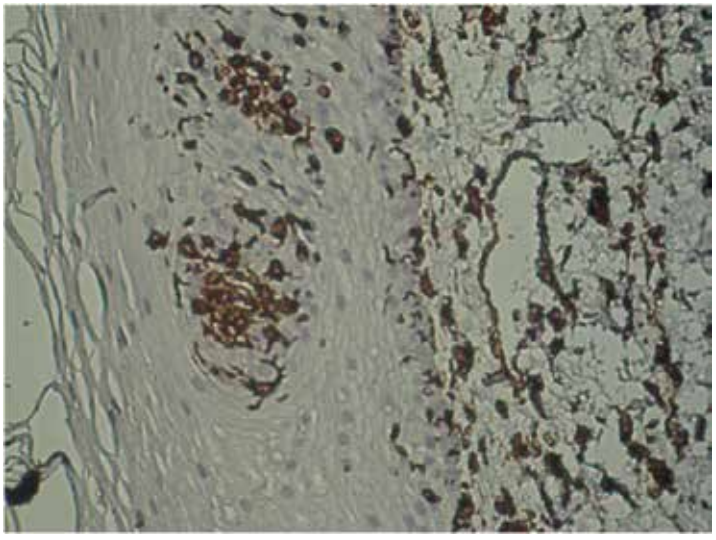


Fig. 6. The gingival papillary layer at the "D" stage of formation of a dental root in an 11-year-old child. Vimentin-positive endothelium of the microcirculation. Immunohistochemical method with primary antibodies to vimentin, polymeric detection system and visualization by means of diaminobenzidine with additional hematoxylin staining of nuclei. Optic magnification 200^{x} (oc.20^{x}, vol.10^{x}).

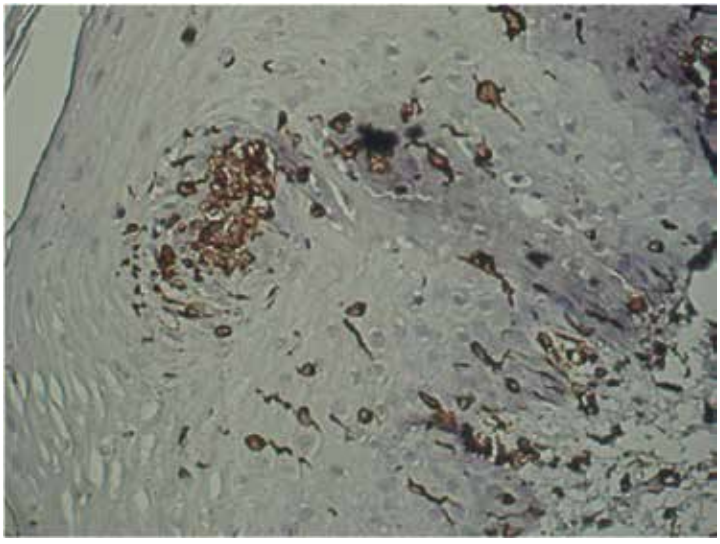


Fig. 7. The gingival papillary layer at the "H" stage of formation of a dental root in a 17-year-old child. Vimentin-positive endothelium of the microcirculation. Immunohistochemical method with primary antibodies to vimentin, polymeric detection system and visualization by means of diaminobenzidine with additional hematoxylin staining of nuclei. Optic magnification 200^{x} (oc.20^{x}, vol.10^{x}).

At the same time, when the volume of blood supply increased, the concentration of CD-34 antigen decreased. It might be due to the completion of its function concerning the vascular bed formation (Fig. 4-5).

The decrease in the optical density of immunohistochemical staining for vimentin indicates it indirectly. At the same time, its considerable decrease is found only at the "H" stage of the dental root formation (Fig. 6-7).

Concerning the age aspect, it should be noted that the specific volume of the blood vessels did not show differences between the age categories. Nevertheless, certain data were obtained about lower concentrations of CD-34 antigens and vimentin in the endotheliocytes of children from II and III groups compared to the children from I group ($p<0,05$).

No changes were found in the reticular layer of the gums from the side of the specific volume of the blood vessels, CD-34 antigens and vimentin in children from the observation groups (Table 2).

The peculiarities of morphological changes of the vascular bed and immunohistochemical endothelial changes of the blood vessels in the papillary layer determined the area of further investigations of the gingival stroma. This includes the study of neoangiogenesis islets of the gingival papillary layer. For this purpose, immunohistochemical method was used to determine CD-34 antigen. Quantitative results of neoangiogenesis islets of the gingival papillary layer are presented in Table 3.

The results contained in the Table show that the index of optic density of staining on CD-34 in the endotheliocytes of neoangiogenesis islets underwent fluctuations at the stages of dental root formation. Thus, the largest specific volume of neoangiogenesis islets in the papillary layer was found in children aged 13-16 years. Then this index dropped sharply to minimum values at the "H" stage, which is indicative of neoangiogenesis at the last stage of the tooth formation, although its intensity decreases considerably (Fig. 8-9).

DISCUSSION

The neoangiogenesis islets mentioned above can be associated with pluripotent (stem) cells. Theoretically, any kinds of cells can form including endotheliocytes.

Our research results are unique regarding the choice of biomaterial and the conditions of its collection. There are

Table 2. Characteristic of the vascular bed in the gingival reticular layer in the portion of the third molars in children depending on age

Observation group		I group (11-13 years)	II group (13-16 years)		III group (16-18 years)	
The stage of formation of the third molar germ according to Demirjian method		D	E	F	G	H
1.	Specific volume of the vascular bed (%)	10,4±0,16	11,0±0,18	11,3±0,17	11,6±0,17	12,0±0,18
2.	Optic density of staining on CD-34 in the vascular endotheliocytes (units of optic density)	0,319± 0,0014	0,318± 0,0016	0,318± 0,0018	0,316± 0,0018	0,315± 0,0019
3.	Optic density of staining on vimentin in the vascular endotheliocytes (units of optic density)	0,297± 0,0018	0,297± 0,0014	0,295± 0,0017	0,295± 0,0017	0,293± 0,0021

Table 3. Characteristic of neoangiogenesis islets of the gingival papillary layer in the portion of the third molars in children depending on age

Observation group		I group (11-13 years)	II group (13-16 years)		III group (16-18 years)	
The stage of formation of the third molar germ according to Demirjian method		D	E	F	G	H
1.	Specific volume of neoangiogenesis islets according to the data of CD-34 expression (%)	5,2±0,13	7,5±0,10	8,1±0,09	5,1±0,10	2,5±0,07
2.	Optic density of staining on CD-34 in the endotheliocytes of neoangiogenesis islets (units of optic density)	0,457± 0,0020	0,454± 0,0019	0,453± 0,0018	0,453± 0,0020	0,451± 0,0021

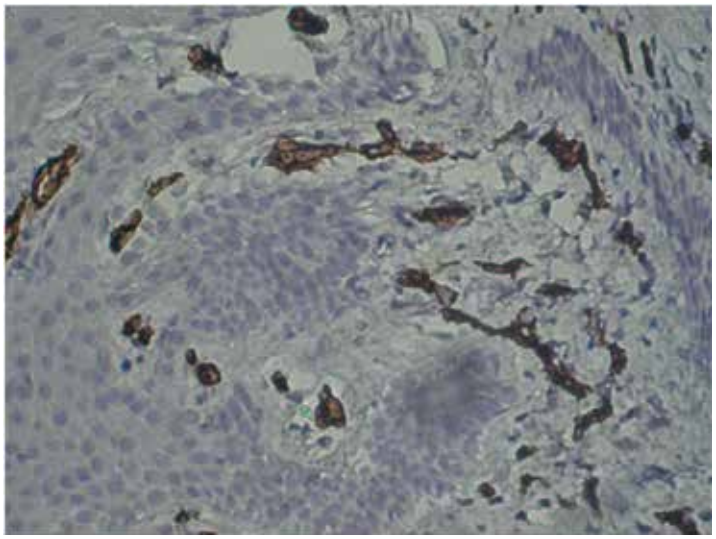


Fig. 8. The gingival papillary layer at the "E" stage of formation of a dental root in a 14-year-old child. Neoangiogenesis islets with CD-34-positive endothelium of the microcirculation. Immunohistochemical method with primary antibodies to CD-34, polymeric detection system and visualization by means of diaminobenzidine with additional hematoxylin staining of nuclei. Optic magnification 200^{x} (oc.20^{x}, vol.10^{x}).

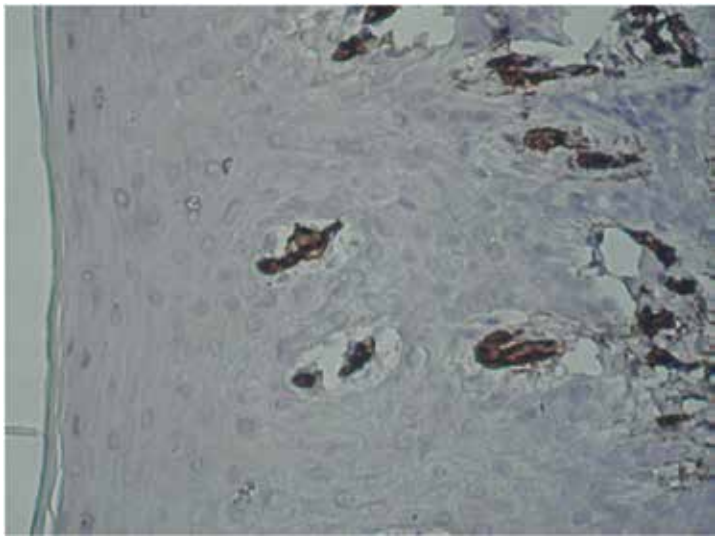


Fig. 9. The gingival papillary layer at the "H" stage of formation of a dental root in a 17-year-old child. Neoangiogenesis islets with CD-34-positive endothelium of the microcirculation. Immunohistochemical method with primary antibodies to CD-34, polymeric detection system and visualization by means of diaminobenzidine with additional hematoxylin staining of nuclei. Optic magnification 200^{x} (oc.20^{x}, vol.10^{x}).

many studies of the gums and their connective tissue under experimental conditions, moreover, the parameters of the epithelial layer and the relative capillaries' volume in normal and pathological conditions were already investigated [21, 22]. There are studies of gums in the context of orthodontic pathology [23-25]. However, our goal was to study the structure and regenerative potential of healthy tissues around the third molars germs under conditions of planned operative interventions, taking into account the age factor in the possibility of further use in practice.

CONCLUSIONS

Therefore, the conducted histological and immunohistochemical study of the connective gingival tissues in the portion of the third molars in children enables to draw a conclusion that in the process of formation of the root of this tooth a number of changes occur in the gingival stroma. They include an increase of the blood flow volume in the papillary gingival layer on the background of a decreased concentration of CD-34 genes and vimentin, a longer stage of development of the third molar root. The specific volume of the islets of neoangiogenesis of the papillary gingival layer is the largest in children at the "E" and "F" stages of the root formation. The data obtained are indicative of a reduced regeneration potential of the tissues of the surgical field with the increase of the age of a child and the degree of formation of the third molar. It will be considered in further clinical work.

REFERENCES

1. Pankevych AI, Kolisnyk IA, Hohol AM. Dyferentsiyovanyy pidkhid do operatsiyi atypovoho vydalennya zubiv mudrosti [Differentiated approach to surgery for atypical removal of wisdom teeth]. Ukrainian dental almanac. 2019;4:24-8. (Ukrainian).
2. Kuroyedova VD, Vyzhenko YEYE. Pozytsiya tretikh molyariv ta yikhnikh zachatkiv za danymy ortopantomohram u patsiyentiv iz dystal'nym prykusom [The position of third molars and their rudiments according to orthopantomograms in patients with a distal bite]. Ukrainian dental almanac. 2015;2:60-4. (Ukrainian).
3. Hohol AM, Pankevych AI, Kolisnyk IA. Vybir metodu atypovoho vydalennya tretikh nyzhnikh molyariv u zalezhnosti vid yikh polozhennya v aspekti profilaktyky pislyaoperatsiynykh sensornykh porushen [The choice of the method of atypical removal of third lower molars depending on their position in the aspect of prevention of postoperative sensory disturbances]. Herald of problems of biology and medicine. 2016;2-1(128):179-183. (Ukrainian).
4. Brunello G, Biagi M, Crepaldi G et al. An Observational Cohort Study on Delayed-Onset Infections after Mandibular Int J Dent. 2017:2017:1435348. doi: 10.1155/2017/1435348.
5. Agrawal A, Yadav A, Chandel S et al. Wisdom tooth – complications in extraction. J Contemp Dent Pract. 2014;15(1):34-6. doi: 10.5005/jp-journals-10024-1484.
6. Tkachenko PI, Hohol AM, Pankevych AI. et al. Koronektomiya yak sposib khirurhichnoho likuvannya retenovanykh tretikh nyzhnikh molyariv [Coronectomy as a method of surgical treatment of retained lower third molars]. The world of medicine and biology. 2019;2(68):117-121. (Ukrainian)
7. Frenkel B, Givol N, Shoshani Y. Coronectomy of the mandibular third molar: a retrospective study of 185 procedures and the decision to repeat the coronectomy in cases of failure. J Oral Maxillofac Surg. 2015;73(4):587-94. doi: 10.1016/j.joms.2014.10.011.
8. Hodovanets OI, Kitsak T-S, Halchuk KL, Sauka OE. Odontogenic stem cells and prospects for their use in practice (Literature review). Bukovyna Medical Herald. 2021;25(4((100)):117-22. doi: 10.24061/2413-0737.xxv.4.100.2021.20.
9. Lopes Limaa R, Carvalho Holanda-Afonsob R, Moura-Netob V et al, Margareth Maria Souzaa Human dental follicle cells express embryonic, mesenchymal and neural stem cells markers. Arch Oral Biol. 2017;73:121-128. doi: 10.1016/j.archoralbio.2016.10.003.
10. Dzobo K, Thomford NE, Senthebane DA et al. Advances in Regenerative Medicine and Tissue Engineering: Innovation and Transformation of Medicine. Stem Cells Int. 2018;2018:2495848. doi: 10.1155/2018/2495848.
11. Tatullo M. About stem cell research in dentistry: many doubts and too many pitfalls still affect the regenerative dentistry. Int. J. Med. Sci. 2018;15(14):1616-18. doi: 10.7150/ijms.27908.
12. Bakopolou A. Prospects of Advanced Therapy Medicinal Products-based Therapies in Regenerative Dentistry: Current Status, Comparison with Global Trends in Medicine, and Future Perspectives. JOE. 2020;46(9S):175-188. doi: 10.1016/j.joen.2020.06.026.
13. Mozaffari MS, Emami G, Khodadadi H, Baban B. Stem cells and tooth regeneration: prospects for personalized dentistry. EPMA J. 2019;10(1):31-42. doi: 10.1007/s13167-018-0156-4.
14. Amrollahi P, Shah B, Seifi A, Tayebi L. Recent advancements in regenerative dentistry: a review. Materials Science and Engineering. 2016;69:1383-90. doi: 10.1016/j.msec.2016.08.045.
15. Hu L, Liu Y, Wang S. Stem cell-based tooth and periodontal regeneration. Oral Diseases. 2018;24(5):696-705. doi: 10.1111/odi.12703.
16. Enezei HH, Qabbani AA, Ahmad A et al. The Effect of Strontium on Osteoblastogenesis and Osteoclastogenesis in Dental Stem Cells-induced Epidermal Growth Factor at Molecular Level: In Vitro Study. Journal of Hard Tissue Biology. 2020;29(1):1-8.
17. Jain V, Kapoor P, Miglani R. Demirjian approach of dental age estimation: Abridged for operator ease. J Forensic Dent Sci. 2016;8(3):177. doi: 10.4103/0975-1475.195103.
18. Bahriy MM, Dibrova VA. Metodyky morfolohichnykh doslidzhen [Methods of morphological research] Monograph. Vinnytsia: New book; 2016, p.328. (Ukrainian).
19. Ferreira T, Rasband W. Image J. User Guide. New York: National Institute of Health. 2012, p.187.
20. Hammer O. PAST: Paleontological Statistics, Version 4.14. Reference manual. Oslo: Natural History Museum University of Oslo. 2023, p.311.
21. Tverdokhlib NO. Slyzova obolonka porozhnyny rota ta yiyi hemo- ta limfotsyrkulyatorne ruslo yak vidobrazhennya stanu posteksperymentalnoyi mekhanichnoyi zhovtyanytsi u shchuriv [Mucous membrane of the oral cavity and its hemo- and lymphocirculatory channel as a reflection of the state of post-experimental mechanical jaundice in rats]. Ukrainian scientific and medical youth magazine. 2014;2(78):91. (Ukrainian).
22. Bedenyuk OS, Korda MM. Patomorfolohichni osoblyvosti rozvytku lipopolisakharydnoho parodontytu na foni khronichnoho atrofichnoho hastrytu [Pathomorphological features of the development of lipopolysaccharide periodontitis against the background of chronic atrophic gastritis]. Achievements of clinical and experimental medicine. 2019;4:27-35. (Ukrainian).
23. Godovanets OI, Kitsak TS, Vitkovsky OO et al. The Influence of Diffuse Nontoxic Goiter on the State of Protective Mechanisms of the Oral Cavity in Children. Journal of Medicine and Life. 2020;13(1):21–25. doi: 10.25122/jml-2020-0013.
24. Malanchuk V, Volovar O, Oblap M et al. Clinical and morphological correlation dependencies and their significance in patients with complications of labored eruption of the lower third molars. Wiad Lek. 2021;3(1):441-9.
25. Tkachenko PI, Starchenko II, Dmytrenko MI et al. Morfolohichni osoblyvosti slyzovoyi obolonky nad retenovanymy zubamy zalezhno vid umov yikh roztashuvannya [Morphological features of the mucous membrane above retained teeth depending on the conditions of their location]. Ukrainian dental almanac. 2020;1:31-6. (Ukrainian).

The study was conducted as a fragment of the complex scientific projects of the Department of Pediatric Dentistry (Bukovinian State Medical University) «Working out of methods for prevention and treatment of main dental diseases among children, taking into account risk factors for their development» (state registration number : 0121U110122).

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Larysa Fedoniuk
I. Horbachevsky Ternopil National Medical University
9 Valova St., 46000 Ternopil, Ukraine
e-mail: Fedonyuk22Larisa@gmail.com

ORCID AND CONTRIBUTIONSHIP
Oksana Godovanets: 0000-0002-1889-3893 A, F
Igor Davydenko: 0000-0001-6712-3396 C, E
Taras Muryniuk: 0000-0002-8367-7580 A, B, C, D
Larysa Fedoniuk: 0000-0003-4910-6888 E, F

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

RECEIVED: 18.10.2023
ACCEPTED: 08.02.2024

ORIGINAL ARTICLE

Features of D-vitamin status in young children in the Kazakh population

Akmaral Zhumalina, Irina Kim, Balash Tusupkaliev, Mairamkul Zharlykasinova, Botagoz Zhekeyeva
WEST KAZAKHSTAN MARAT OSPANOV MEDICAL UNIVERSITY, AKTOBE, REPUBLIC OF KAZAKHSTAN

ABSTRACT

Aim: The purpose of the article is to form the parameters of vitamin D status in young children in the ethnic group of Kazakh nationality with the factor of highlighting the necessary recommendations for the prevention of hypovitaminosis D.

Materials and Methods: Methods for the study of the highlighted problem are the diagnosis of young children in the parameter of clinical and anamnestic research, which includes the collection of anamnestic data of children of Kazakh nationality within the framework of the identified data based on a questionnaire of parents, an evaluation component in the child's health factor at the level of his initial state, and laboratory analysis to determine 25(OH)D to identify the content of vitamin D using the method of electrochemiluminescent immunoassay.

Results: Analysis of vitamin D levels revealed significant differences among age groups. In the 0-28-day group, average vitamin D was 13.35 ng/ml, with 92.8% deficient. In the 1-6-month group, it was 21.47 ng/ml, with 84% deficient. In the over 6-month group, it was 33.58 ng/ml, with 40% sufficient. Formula-fed children had the lowest levels (average 15.21 ng/ml), while breastfed children had insufficiency (average 23.91 ng/ml). Children with vitamin D supplementation averaged 25.9 ng/ml, compared to 19.01 ng/ml without supplementation.

Conclusions: The results point to a widespread deficiency of vitamin D and offer practical recommendations for its prevention, such as creating a unified system of timely diagnosis, implementing preventive measures in pregnant women and young children, including a balanced diet enriched with vitamin D, staying outdoors in the bright hours of the day.

KEY WORDS: Vitamin D, hypovitaminosis, prevention, paediatrics, Kazakh nationality

Pol Merkur Lek,2024;53(1):161-170. doi: 10.36740/Merkur202402104

INTRODUCTION

Currently, one of their urgent problems in the field of healthcare is vitamin deficiency, which disrupts the natural biochemical balance in the body, conditioned upon which violations occur that lead to the development of various pathological conditions. One of the most important for the growth and development of children is vitamin D, and according to various studies, if a deficiency of this vitamin is detected, in fact the development of hypovitaminosis D, manifestations in the form of disorders in the development of the skeletal system of the musculoskeletal system, as in other life systems, will be revealed [1]. The definition of vitamin D is currently understood as a group of substances that, based on their influence in the human body, have all the characteristic characteristics in the criteria for manifestation within the framework of full-fledged activity, as sterols, which in turn belong to the pharmacological group of steroids, therefore, in the classification of accepted as synthetic analogues in medicinal dosage forms, vitamin D refers to secosteroids [2]. The existing analogues of vitamin D in the manifestation of natural factors in its natural distribution are represented by a group that are synthesized in the human body at the skin level using a photochemical reaction that is observed with direct exposure to sunlight, as well as the replenishment of the body of vitamins occurs when taking a number of foods that carry a high content of vitamin D [3]. Thus, in considering the highlighted problem of this research work, it should be noted that the data identified during the research allow us to talk about the apparent importance of vitamin D in the manifestation of its action in the body, so new functions were discovered that it performs at the level of endocrine, autocrine and paracrine effect. Vitamin D binds to vitamin D receptors, which are located in the human body in various organs of the immune system, bones, intestines, mammary gland and kidneys, and in this regard, its influence on the development of various diseases at this organ level, and within the framework of the formation of immunity [4].

Based on this, the deficiency of this vitamin in young children is one of the important tasks at the medical level in different regions, including Kazakhstan, as part of solving this direction in its correction at the level of restoring the natural balance of vitamin D in the child's body. This problem in certain regions reaches large values in the detection factor of hypovitaminosis D in the majority of the world's population, and in the prevailing majority it is observed in children, and in the course of research, the figures of this in some regions reach up to 98% of residents in the position of detecting insufficient amounts of vitamin D [5].

Considering the particular relevance of the importance of maintaining the quantitative ratio in vitamin D for children, the importance of considering this issue with an emphasis on the intrauterine development of the child in the parameter of studying the features of possible pathology of pregnancy, in the manifestation of various conditions in the course of pregnancy, nutrition of a pregnant or nursing woman, and the nature of infant feeding, since it is in children up to a year that there is often vitamin D deficiency [6]. Also in this review, it should be noted that there are aspects in the spread of pleiotropic effects of vitamin D, which may also be reflected either in the development of pregnancy pathology, or in the reduction of this fact. After all, even pregnancy, proceeding in the parameters of the physiological norm, is accompanied by an increase in phosphorus-calcium metabolism, affecting, among other things, the processes of vitamin D metabolism [7]. Thus, a pregnancy proceeding from an insufficient amount of vitamin D and calcium, pregnancy pathology or concomitant diseases of a woman will relate to risk factors for the development of hypovitaminosis D in a child.

The results of the medical clinical and anamnestic study were analysed in detail with an activity-semantic analysis, which made it possible to determine the parameters of the sphere within the use of vitamin D at the level of its epidemiological importance in maintaining the preservation of various health parameters based on the metabolic properties of vitamin D [8]. In recent years, a laboratory research method has become widespread to identify the content of vitamin D in the human body based on the importance of its presence in the required amount in the body, which has become a separate indicator of the state of health in laboratory studies. Its parameter is also determined isolated, with the exception of changes in phosphorus-calcium metabolism [9]. After the introduction of the study of a separate component, such as 25(OH)D, there was an intensification of his research in the study of the polyclinic line in the factor of one of the mandatory examinations, which allows us to effectively study the presence of a quantitative factor of vitamin D in the human body [10].

In this case, in order to maintain the child's health, prevention of this condition is necessary, which will be carried out as on the food line in the intake of the necessary components to compensate for the necessary amount of important trace elements and vitamin D, which, with existing studies, can positively affect the concentration of 25(OH)D in serum, and in the parameter of exposure to the natural factor of sunlight, which is the main source of 25(OH)D in circulating serum [11]. But there are also difficulties in determining the necessary parameters of hypovitaminosis D, since there are no clearly defined norms at the international level in the content of vitamin D in the body, and despite the common knowledge about the norms of physiology and health for any person, but each country has its own values within the definition of vitamin D deficiency [12]. Universal screening for the level of 25(OH) D has not yet been carried out in Kazakhstan, so there is no data on the prevalence of vitamin D deficiency and insufficiency in young children of the Kazakh population [13]. But there is a scientific and practical interest in the framework of an unexplored topic, but necessary based on the existing need for vitamin D in children, and scientifically based preventive technologies and corrective measures in different periods of childhood.

AIM

The purpose of the study is to form the parameters of vitamin D status in young children in the ethnic group of Kazakh nationality with the factor of highlighting the necessary recommendations for the prevention of hypovitaminosis D.

MATERIALS AND METHODS

The diagnostic parameters of this scientific and medical study were determined by the methods of clinical and anamnestic research, which included the collection of anamnestic data of children in an isolated parameter of the ethnic population at the level of Kazakh nationality, which was identified by questioning their both parents, who filled out data on belonging to the genus, and the selection of children for the study occurred when choosing the absence of a conflict of interests in this one. The components in the assessment factor of the initial health of the child and his satisfactory condition were also determined at the level of this study, where groups of infants were identified in the parameters practically healthy for further investigation of their vitamin D content at the level of further determination of their vitamin status. For this purpose, the method of electrochemiluminescent immunoassay (ECLIA) was used, which allows to determine the level of the amount of 25(OH)D in blood plasma taken from a vein in the morning [14]. Diagnostics of the selected parameter 25(OH)D was determined with the existing criteria recognised at the level of the International Society of Endocrinologists. Thus, the effective values in the vitamin D supply factor in the recommended level parameter were diagnosed at a value of 25(OH)D equal to 30-80 ng/ml, its insufficiency was considered in the parameters of 20-30 ng/ml, and deficiency was observed at 10-19 ng/ml, severe deficiency was determined at a value of 25(OH)D less than 10 ng/ml [15].

An individual registration card was created for each child included in this medical study, where all the identified indicators were recorded. The resultant data obtained during the study were processed using Statistica 10 and SPSS 25 programmes. The analysis of quantitative indicators was carried out with the calculation of the median (Me) and the indication of the 25th and 75th percentiles (Me (Q25%; Q75%)). The Kraskel-Wallis criterion was used to statistically assess the confidence level (p) of 3 independent groups. The results obtained are also displayed as a graphic image in diagrams and tables. The medical study was conducted in the city of Aktobe, Republic of Kazakhstan. 104 children aged 0-12 months in the Kazakh population who were born full-term and were in the 1st or 2nd health group based on their health status, which corresponds to

practically healthy children, were examined. At the time of the study, all the examined infants were healthy, they had no genetic syndromes, and they did not take vitamin D in dosage form. The medical examination of the children took place within the framework of an informed consent signed by the parents or legal representatives.

Scientific and medical research of the highlighted problem was carried out in three stages. At the first stage, an analytical and theoretical study of scientific, research and methodological literature was carried out on aspects related to normal or deficient vitamin D content in the body and various factors in the sphere of their influence on health, during which the actual problem, purpose, research methods were determined and a work plan was drawn up. At the second stage, a clinical and anamnestic study of a selected group of healthy children for their inherent vitamin D level and a questionnaire of their parents was conducted, then an analysis of the results was made and conclusions were formulated. At the third stage, the results obtained, conclusions were clarified and the results obtained were systematised.

RESULTS

It should be noted that in the form of providing vitamin D, there are accents in its replenishment against the background of feeding a child, so with natural feeding, the importance of providing this comes from a woman who must observe a balanced diet and adequate daily presence in the sunny period of time on the street. With artificial feeding, this is carried out in the factor of the internationally accepted processing of baby food and various milk mixtures, which are already enriched at the level of the presence of vitamin D product. In the absence of this, a child may develop hypovitaminosis D [16]. So, considering the above, all the elements and parameters identified in this study were taken into account, and also analysed in detail separately as its effective components, which together opens the process of forming a model for determining aspects of vitamin D status in young children of isolated Kazakh nationality with further preparation of recommendations for the prevention of vitamin D deficiency in them, which can be further applied in practice in increasing high efficiency in this. The result of the study under consideration is the degree of formation of the model in question in the process of its implementation in the field of healthcare.

Thus, this medical research work with the created model for determining the level of vitamin D in healthy children living in Kazakhstan and belonging to the Kazakh nationality with practical recommendations for the prevention of hypovitaminosis D was introduced into the field of medical control in several stages, which included the study of aspects related to the origin and effect of vitamin D on the body, including in the parameter of determining its quantitative component at the level of laboratory research in young children, such as using the diagnostic method to determine electrochemiluminescent immunoassay, which allows obtaining results satisfying the necessary parameters, and clarifications at the level of conducting a questionnaire of parents in a selected group of children. The data obtained were further statistically processed in the selection of the software described above, and then at the next stage, a model for determining the parameters of vitamin D status was developed and tested, and then practical recommendations for the prevention of hypovitaminosis D were formed at the level of the identified parameters, for the subsequent implementation of this in the field of practical education, which will further control the formation of deficiency vitamin D and will create conditions for preventing the development of rickets and other diseases associated with insufficient vitamin D factor. This allowed determining the level of its importance at a high and important level of medical support in solving the problem of prevention of hypovitaminosis D in Kazakhstan. Thus, 104 children in the age category up to 1 year were covered by scientific and medical research, of which there were 64 boys and 40 girls. The distribution of the group of selected children in the age criteria is shown in Fig. 1.

All children selected for the study were divided into 3 groups depending on their age criteria. The 1st group included newborn children from 0 to 28 days, the 2nd group consisted of children in the age parameter from 1 to 6 months, the 3rd group included infants who were older than 6 months. The expediency of distributing children into groups depending on their age was within the framework of the features of possible exogenous intake of vitamin D. Thus, in the manifestation of the first group in children, the intake of vitamin D is based on their indicative dependence on the revealed figures of this in relation to similar indicators in a woman. In the second group, children are on the line of natural feeding, and the factor of their vitamin D is outside the scope of the exogenous pathway of vitamin intake into their body, since this category of children does not receive complementary foods. In group 3, represented by children older than 6 months, additional intake of vitamin D is expected as a reflector of the use of various complementary foods, which is within the approved recommendations of the National Programme for Optimising Child Feeding at the level of the first year of life in Kazakhstan.

The survey of the mothers of the selected children identified data reflecting the manifested development of pathological syndromes during their pregnancy, which were detected in almost every third woman, and they were within the framework of various manifestations, such as anemia (35; 43.2%), gestosis of the 2nd half of pregnancy (26; 32.1%), infectious pathology of the mother (25; 30.9%), the threat of termination of pregnancy (24; 29.6%), gestosis of the 1st half of pregnancy (23; 28.4%), somatic pathology of the mother (9; 11.1%), as shown in Fig. 2.

Also, the data revealed during the questionnaire made it possible to identify the level and characteristics of feeding children, which were in the parameters of only breastfeeding (BF) in 54 children, artificial feeding (AF) in 21 children, in the factor of the combination of breastfeeding with complementary feeding (BFCF), feeding was in 21 infants, and 8 children were on artificial feeding with complementary

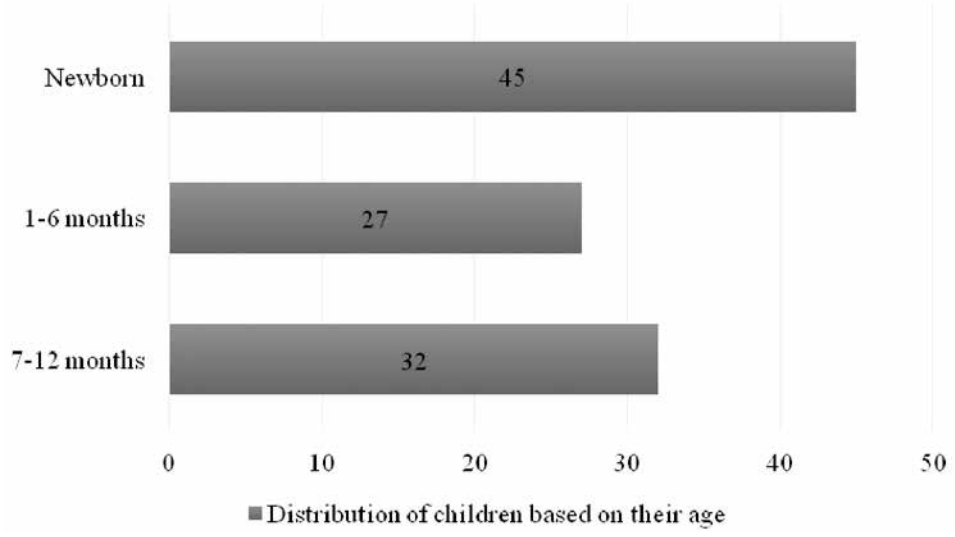


Fig. 1. Distribution of the group of selected children based on their age criteria.

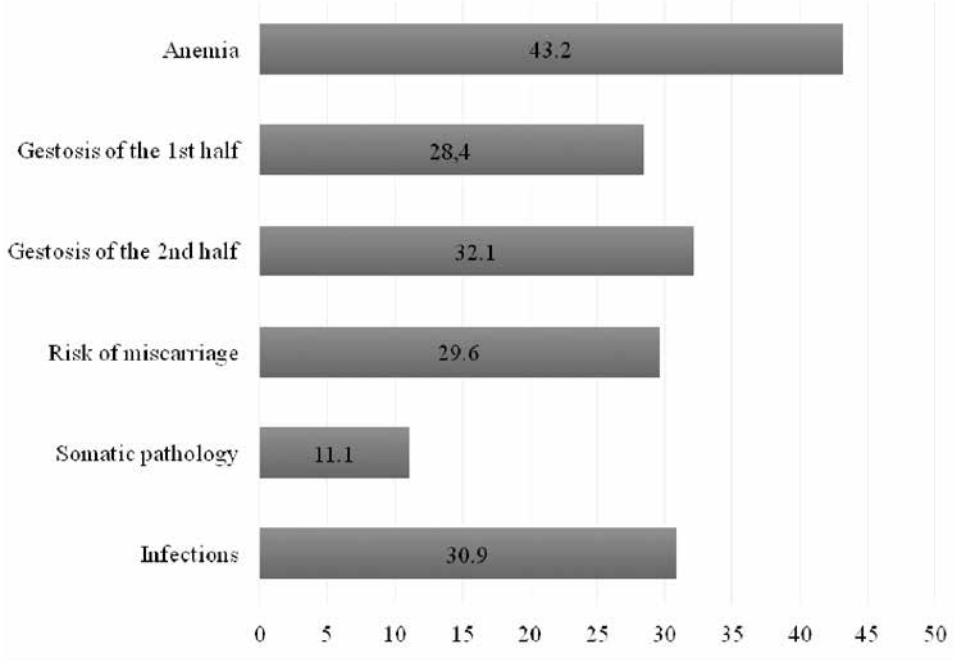


Fig. 2. Distribution of mothers of selected children depending on the pathologies detected in them during the course of their pregnancy.

foods (AFCF), which is reflected in Fig. 3.

Based on the allocation of the necessary parameters for the level of vitamin D availability in children of Kazakh nationality, it is important to clarify when taking vitamin D by women during pregnancy, which provided antenatal prevention in this review. Thus, a detailed analysis of the relevant questionnaire questions reveal, that 38 women took a complex version of vitamin within the vitamin-mineral complex, which included vitamin D, where it was in the quantitative characteristic of 400 IU, and 125-200 mg of calcium was present in it, and these women made up the first group in the form of the identified form of vitamin intake D. The second group consisted of women who took only calcium, in its isolated form, of which there were 17 women. The third group consisted of 49 women who did not take additional non-mineral, non-vitamin complexes. These indicators are shown in Fig. 4.

Also, all mothers of the studied children were analysed for the determination of vitamin D, and the data obtained were analyzed on the basis of a certain comparable analysis of their antenatal prophylaxis and the quantitative content of vitamin D in them, the revealed indicators are reflected in Table 1.

This observation suggests that there is a need to deepen the study of the highlighted problem in this direction. The above-described facts of the importance of the influence of the vitamin factor in the presence of vitamin D at a certain level in the child's body and the data identified in the survey criteria during the study suggest that it is necessary to investigate the data at the level of Kazakh

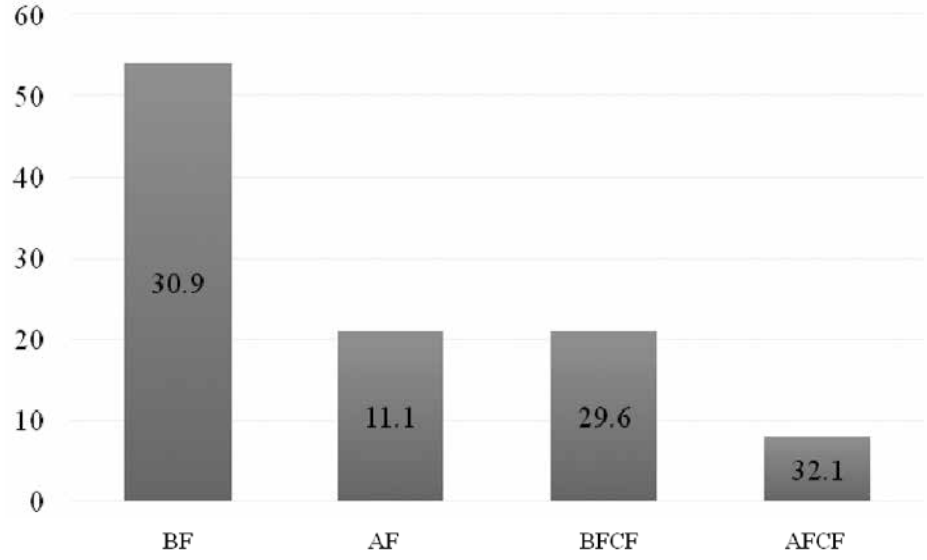


Fig. 3. Distribution of children depending on the type of their feeding.

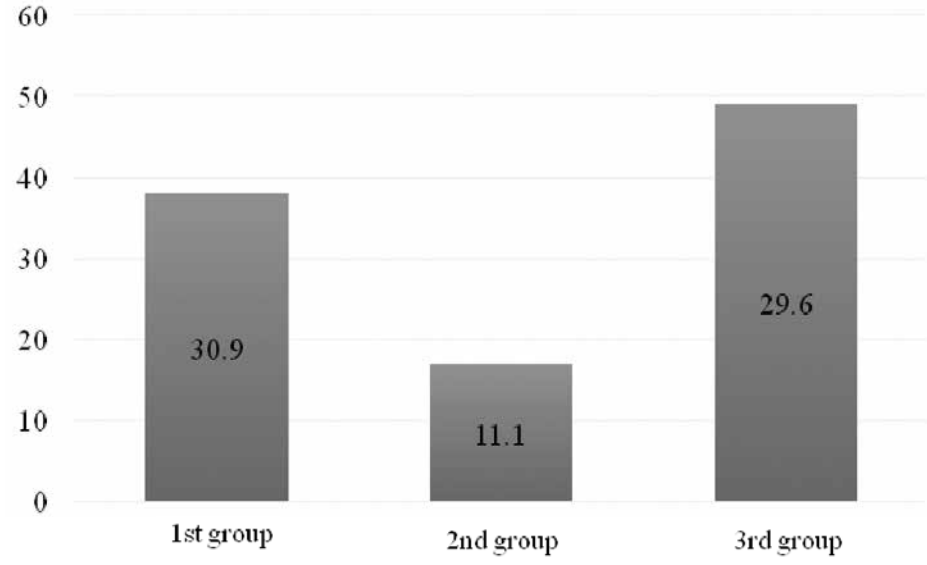


Fig. 4. Distribution of women depending on their intake of vitamins and minerals during pregnancy.

Table 1. Vitamin D indicators in women depending on antenatal prophylaxis

Group No.	Quantity	Vitamin D levels	Insufficiency
		Deficiency	
1st group	38	22 (11.16 ng/ml)	7 (25.39 ng/ml)
2nd group	17	13 (10.57 ng/ml)	-
3rd group	49	37 (11.3 ng/ml)	5 (24.3 ng/ml)

nationality in the factor of proper development of children and creating a preventive set of measures that will be aimed at stabilising the situation at the level of solving the problem of hypovitaminosis D in children of the younger generation in the face of young children, so that their further and growth would be compensated at a satisfactory level within the framework of the creation of an official health care program. Thus, the implementation of the conditions of scientific and medical research required methodological analysis and allowed us to identify its main parameters, which, based on a generalised approach based on the creation and retention of a prosperous component of healthy development of children in Kazakhstan, allowed forming a high-level data system in the manifestation of average indicators in the factor of shortage and shortage on a national basis in the fact of living in this region in

children, which will allow developing a programme for the prevention of this problem in the future at a qualitative level.

Based on the position under consideration, parameters were formed in the criteria for determining vitamin D status in the factor of nationality, as an isolated Kazakh origin and residence in this area, which allows determining the general condition regarding the lack of a vital indicator in the presence of vitamin D in the body, which affects the healthy development of the musculoskeletal system in children, and also, based on the maintenance of their immune system and others, based on the important influence of this vitamin in the manifestation of the influence in various life systems in the factor that will further create a system in the field of healthcare that will timely diagnose the condition in the parameter of determining hypovitaminosis D, and further therapeutic correction when it is detected, and prevention at different periods affecting both the stage of pregnancy and in the factor of periodic intake of vitamin D preparations in children starting from their early age, in the situation of creating an official health programme in the Republic of Kazakhstan for the prevention of deficiency conditions based on vitamin D deficiency, which will include the intake of vitamin D-enriched foods in the form of a balanced nutrition, staying outdoors in the bright hours of the day, additional intake of vitamin D in various available and approved forms by the healthcare sector. It is also necessary to cancel that, given the importance of the problem under consideration, which carries components at the level of maintaining the health of the nation, this study should deepen in the factor of developing new ways to diagnose and stabilise vitamin status in children in different age categories at the level of the population of Kazakh nationality.

Further, at the control stage of the study, to determine the indicators of the total vitamin D status of children of Kazakh nationality in the age criteria up to one year, based on the identified criteria in the questionnaire factor of their mothers in the described parameters, data on the quantitative content of vitamin D in the child's body were investigated based on various external components of the process of the type of pregnancy, the type of breastfeeding or the factor of antenatal prevention, including other related elements. Thus, using the method of electrochemiluminescent immunoassay, when examining 104 children, the level of the general indicator of vitamin D status revealed that in the majority of children - 81 people (78%) it was at the level of reference values with reduced levels of vitamin D in the body. Deficiency was detected in 69 children (85.1%), and insufficiency in 12 infants (14.8%). Table 2 presents detailed data on the level of vitamin D in the blood serum of children within the framework formed earlier during the study groups, and it should also be noted that in the parameter of average statistical significance, vitamin D is determined in the values of 21.44 ± 1.7 ng/ml, which shows its insufficiency.

Analysis of the data in Table 2 allowed us to determine that in the first group of children the lowest indicators in terms of reduced vitamin D indicators are identified, so its average value was revealed within 13.35, almost all children (39; 92.8%) had a deficiency in vitamin D. The second group showed the results of the analysis in the fact of vitamin D deficiency, also in the prevailing majority of children (84%), and only 16% of infants have vitamin D indicators within the normal range. The third group has average indicators (33.57), and 40% of children in this group have a sufficient level of vitamin D status. In group 3, the average vitamin D levels correspond to the norm (33.57). Further, a comparative analysis of the identified indicators on the content of vitamin D in the factor of consideration of three groups allowed us to establish significant differences between groups 1 and 3 ($p \leq 0.001$), groups 2 and 3 ($p \leq 0.05$). Fig. 5. shows the data on the vitamin D content in the studied children.

Further analysis of the data obtained revealed the level of vitamin D content from the characteristics of their feeding, which is shown in Table 3.

The analysed data presented in Table 3 reflect that children who were artificially fed have the smallest indicators of vitamin D content ($p<0.001$) at the level of its deficiency, which allows establishing a certain contradiction with the content of modern literary sources, along with the reasoning that perhaps not all infant formulas have in the necessary amount of vitamin D, as an additive that makes the milk mixture a balanced diet for the baby. And the low values of vitamin D content in children identified in this study are due to the fact that artificially fed children apparently received mixtures at the level of initial vitamin deficiency status. Although the currently existing infant formula is mostly fortified with various forms of vitamin D, which fully meet the physiological needs of children in this age group in it [17]. Table 3 also shows that the group of naturally fed children also has a reduced vitamin D content, but which already falls under the criteria of its insufficiency ($p<0.001$). This can be explained from the position of not high bioavailability in the fact of absorption of vitamin D with its content in maternal breast milk [18]. The data obtained from infants who were at the level of natural feeding with the addition of complementary foods to their diet show that there was no significantly prevailing increase in vitamin D content. Moreover, in

Table 2. The content of vitamin D in the blood serum of the examined children

Group No.	Quantity of children	Trust. -95.000%	Trust. 95.000%	Median	Minimum	Maximum	Lower Quartile	Upper Quartile	Dev. degree	p
1st group	45	13.35378	10.38201	16.32555	11.00000	3.800000	50.4400	6.670000	14.30000	9.89162
2nd group	27	21.47080	12.10205	30.83955	15.74000	1.500000	94.40000	8.400000	21.91000	22.69674
3rd group	32	33.57600	24.40865	42.74335	25.50000	6.000000	105.2400	17.90000	42.40000	24.55063

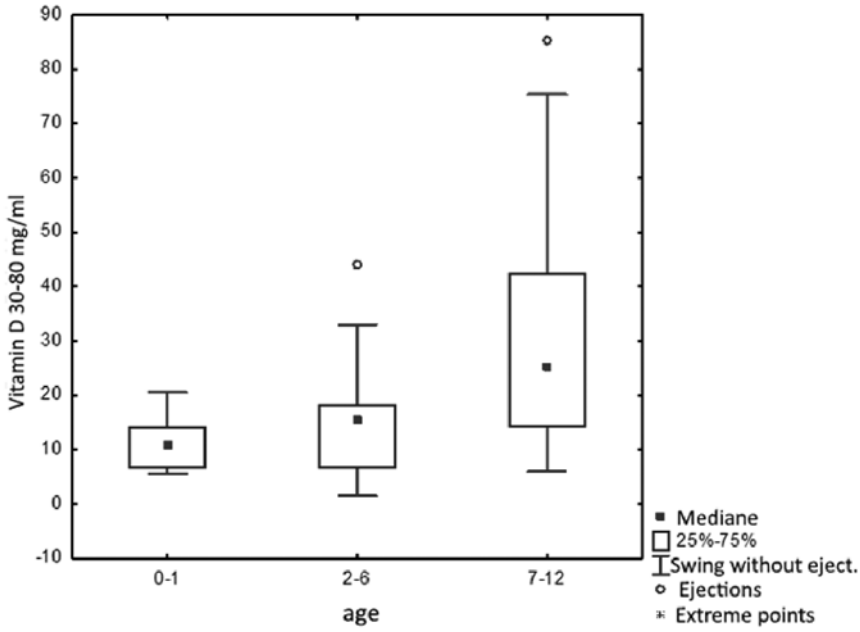


Fig. 5. Vitamin D content in children based on age criteria.

Table 3. The content of vitamin D in children, depending on the type of their feeding

Feeding type	Quantity of children	Average	Trust. -95.000%	Trust. 95.000%	Median	Minimum	Maximum	Lower Quartile	Upper Quartile	Dev. degree	P
BF	54	23.90556	17.84212	29.96899	16.29500	3.800000	105.2400	10.96000	25.80000	22.21464	0.010777
AF	21	15.20952	10.51181	19.90724	14.20000	1.500000	42.30000	10.42000	18.20000	10.32024	0.001408
BFCF	21	25.90714	9.239955	22.57433	8.500000	4.800000	61.58000	6.670000	19.10000	14.64690	
AFCF	8	50.15500	-6.83671	107.1467	51.73000	11.69000	85.47000	19.89500	80.41500	35.81629	0.001734

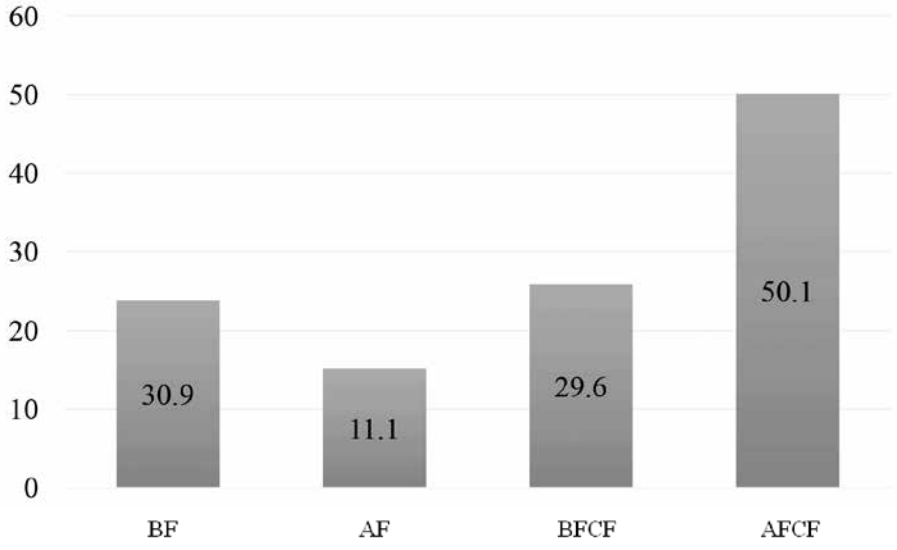


Fig. 6. Distribution of children depending on their vitamin D level in the factor of the type of feeding they receive.

group 4, where children are artificially fed in combination with complementary foods, the data obtained suggest that the detected level of vitamin D content in their body is in indicators that correspond to the norm ($p<0.001$). This fact can be explained by natural phenomena at the level of enhancing the function of the gastrointestinal tract at the level of a greater variety in food intake, which stimulate the development and exchange of bile, which is an important component for improving the absorption of vitamin D [19]. Data on the presence of vitamin D in the body of children, depending on the type of their feeding, are shown in Fig. 6.

The influence of postnatal prophylaxis was also studied based on its effect on the quantitative content of vitamin D in the body of young children. It should be noted that the data obtained suggest that children who received prevention of hypovitaminosis by taking a dosage form of vitamin D at a dose of 500 IU showed a decrease in the parameter within 25.9 ng/ml, and this value reflects vitamin D deficiency, and in children who did not take preventive doses of vitamin D, the revealed values of the presence of vitamin in the body were in terms of its deficiency (19.01ng/ml).

DISCUSSION

Vitamin D is an important constituent element that supports the level of health and allows for the healthy development of the bone system of children. It is known that at the level of biochemical processes, it undergoes several transformations in the body and against this background becomes an activator within a biologically active element that takes part in many processes that ensure the functional operation of many vital activity systems in the body [20]. So, in general, the homeostasis of the body depends on the amount of vitamin D in the body, and in general, maintaining the level of functional health, both in children and adults, and it is based on this that the issues of eliminating the factors of the onset of vitamin D are an important task at the level of its international solution in the field of healthcare.

Cashman [21] discusses the concern raised internationally about the little notable progress in the collection, analysis, and use of population micronutrient status and deficiency data. The author reviews the available data on vitamin D status and dietary intake from different regions of the world, including the Americas, Europe, Oceania, and Asia. In general, the prevalence of serum 25(OH)D <30 nmol/L, indicating increased risk of deficiency, appears lowest in South America, Oceania, and North America (median around 5-7%) and moderate in Europe (~13%) and parts of Asia (~18%). However, the prevalence may be as high as ~19% in Africa based on meta-analysis, though representative data are lacking. Overall, vitamin D deficiency (<30 nmol/L) likely affects at least hundreds of millions globally, increasing to billions with inadequacy (<50 nmol/L). The results of this study are consistent with Cashman [21], confirming the high prevalence of vitamin D deficiency across age groups, highlighting the positive impact of supplementation on vitamin D levels, and recognising age-related changes in vitamin D status. However, differences in units of measurement, focus on age groups and regional variations, and inclusion of data on breastfeeding and artificial feeding were noted.

Wei et al. [22] analysed the serum vitamin D levels of children aged 0-12 years in the First Affiliated Hospital of Harbin Medical University, China. The researchers found that the mean serum 25(OH)D levels in boys and girls were 25.18 and 24.34 ng/mL, respectively. Vitamin D deficiency (VDD) was more prevalent in girls (44.2%) than in boys (41.3%). Among school-age children, the prevalence of VDD was less among girls (63.2%) than boys (69.2%). The results of Wei et al. [22] are consistent with the authors' observations of gender differences in the prevalence of VDD, with girls showing a higher prevalence. In addition, the more extensive age-specific analysis provides valuable information on how vitamin D levels change with age, which complements the authors' understanding of this topic. Finally, a common theme in both studies is the effect of diet and supplementation on vitamin D levels, which highlights the importance of these factors in assessing vitamin D status in children.

In turn, the study by Xu eta al. [23] aimed to determine the vitamin D status of children with short stature and accurately determine serum vitamin D components using high-performance liquid chromatography-tandem mass spectrometry. The study found that both serum levels of 25(OH)D3 and f-25(OH)D in subgroups 2 and 3 of short-stature patients were significantly lower compared with those of healthy controls, whereas C3-epi levels and C3-epi/25(OH)D3 ratios were significantly higher. Similarly, the authors' study found age differences in vitamin D levels, with the youngest age group having the lowest levels and a high proportion of deficiency. However, the authors' study extended these findings by examining the impact of feeding practices and vitamin D supplementation on children's vitamin D status, finding differences between artificially and breastfed children, as well as a positive effect of supplementation on vitamin D levels.

Since Kazakhstan is a country of the Central Asian region, the authors decided to compare the results of their work with the findings presented by Oktaria et al. [24]. They identified 21 studies from 5 different countries (Thailand, Indonesia, Vietnam, Malaysia, and Cambodia) that were included in forest plots. The prevalence of vitamin D deficiency (VDD) (<50 nmol/L) ranged from 0.9% to 96.4%, with more than 50% of new-borns having VDD, and severe VDD (<30 nmol/L) ranged from 0% to 55.8%. Female sex and urban living were the most common determinants of VDD. The common determinants of vitamin D deficiency in the Oktaria et al. [24] and the authors' study were female gender and urban residence. However, the authors' study provided additional insights by analysing vitamin D levels in different age groups and highlighted the evolving nature of deficiency, with the highest prevalence in the youngest age group.

The study by Adnan et al. [25] investigated the vitamin D status of very low birth weight infants and their response to vitamin D intake during their NICU stays. The primary outcome was serum 25(OH)D concentrations of infants at birth and during the NICU stays. The secondary outcome measurements were daily vitamin D intake, the response of 25(OH)D concentrations to vitamin D intake, and the relationship between serum calcium, phosphorus, ALP, and TRP with serum 25(OH)D concentrations. Comparing also the results of this study, there is a significant prevalence of vitamin D deficiency or insufficiency and similar observations regarding the potential benefit of supplementation to increase 25(OH)D concentrations.

In summary, the studies underscore the global importance of addressing vitamin D deficiency, particularly among children. They reveal a consistent prevalence of deficiency or insufficiency across various age groups and regions, with notable gender differences. These findings emphasize the role of diet, supplementation, and feeding practices in influencing vitamin D levels. Additionally, insights from regional studies in Central Asia highlight the widespread nature of the issue. Overall, the research underscores the need for comprehensive strategies to improve vitamin D status globally.

CONCLUSIONS

The results of this study indicate the need to introduce into the health care system a method that would allow timely determination of vitamin D levels at the stage of laboratory diagnosis, and for its further correction at the level of providing it to young children of Kazakh nationality, which is of particular importance, since according to the results of this scientific and medical study, the majority (78%) of the surveyed children have vitamin D deficiency, and this fact may be reflected in a negative parameter on the well-being and health of children.

It is also important to develop practical recommendations for the prevention of hypovitaminosis D, which will be presented in the factor of balanced and rich consumption of vitamin D infant formula and complementary foods, walks in the fresh air during daylight hours and periodic intake of vitamin supplements. The data obtained allow us to speak about the success of this study, since the selected criteria and parameters obtained in the process of determining the general vitamin status in young children of Kazakh nationality are an important component for the development of the healthcare sector in the areas of improving children's health.

The present study ensured its correctness, as the characteristics, parameters of the questionnaire, research, data obtained and recommendations developed were compared in the study correctly, and the analysis of its results made it possible to determine that the results of the study allow solving issues in the field of medical care at an effective level.

REFERENCES

1. Bilici ME, Erdeve ŞS, Çetinkaya S et al. The effect of 2000 ıu/day vitamin D supplementation on insulin resistance and cardiovascular risk parameters in vitamin D deficient obese adolescents. Turk J Pediatr. 2019;61(5):723-732. doi:10.24953/turkjped.2019.05.011.
2. Hanel A, Carlberg C. Skin colour and vitamin D: An update. Exp Dermatol. 2020;29(9):864-875. doi:10.1111/exd.14142.
3. Young AR, Morgan KA, Ho TW et al. Melanin has a small inhibitory effect on cutaneous Vitamin D synthesis: A comparison of extreme phenotypes. J Invest Dermatol. 2020;140(7):1418-1426.e1. doi:10.1016/j.jid.2019.11.019.
4. Minisola S, Colangelo L, Pepe J et al. Osteomalacia and Vitamin D status: A clinical update 2020. JBMR Plus. 2020;5(1):e10447. doi:10.1002/jbm4.10447.
5. Griffin G, Hewison M, Hopkin J et al. Preventing vitamin D deficiency during the COVID-19 pandemic: UK definitions of vitamin D sufficiency and recommended supplement dose are set too low. Clin Med (Lond). 2021;21(1):e48-e51. doi:10.7861/clinmed.2020-0858.
6. Bischoff-Ferrari HA, Dawson-Hughes B, Orav EJ et al. Monthly high-dose Vitamin D treatment for the prevention of functional decline: A randomized clinical trial. JAMA Intern Med. 2016;176(2):175-183. doi:10.1001/jamainternmed.2015.7148.
7. Bouillon R. Comparative analysis of nutritional guidelines for vitamin D. Nat Rev Endocrinol. 2017;13(8):466-479. doi:10.1038/nrendo.2017.31.
8. O'Neill CM, Kazantzidis A, Ryan MJ et al. Seasonal changes in Vitamin D-Effective UVB availability in Europe and associations with population serum 25-hydroxyvitamin D. Nutrients. 2016;8(9):533. doi:10.3390/nu8090533.
9. Calame W, Street L, Hulshof T. Vitamin D serum levels in the UK Population, including a mathematical approach to evaluate the impact of Vitamin D fortified ready-to-eat breakfast cereals: Application of the NDNS Database. Nutrients. 2020;12(6):1868. doi:10.3390/nu12061868.
10. Boucher BJ. Why do so many trials of vitamin D supplementation fail? Endocr Connect. 2020;9(9):R195-R206. doi:10.1530/EC-20-0274.
11. Giustina A, Adler RA, Binkley N et al. Consensus statement from 2nd International Conference on Controversies in Vitamin D. Rev Endocr Metab Disord. 2020;21(1):89-116. doi:10.1007/s11154-019-09532-w.
12. Cashman KD, Dowling KG, Škrabáková Z et al. Vitamin D deficiency in Europe: Pandemic? Am J Clin Nutr. 2016;103(4):1033-1044. doi:10.3945/ajcn.115.120873.
13. Gaksch M, Jorde R, Grimnes G et al. Vitamin D and mortality: Individual participant data meta-analysis of standardized 25-hydroxyvitamin D in 26916 individuals from a European consortium. PLoS One. 2017;12(2):e0170791. doi:10.1371/journal.pone.0170791.
14. Manson JE, Cook NR, Lee IM et al. Vitamin D supplements and prevention of cancer and cardiovascular disease. N Engl J Med. 2019;380(1):33-44. doi:10.1056/NEJMoa1809944.
15. Kiess W, Bae YJ, Penke M et al. Vitamin D in health and disease: The global threat of vitamin D deficient rickets. J Pediatr Endocrinol Metab. 2016;29(4):391-393. doi:10.1515/jpem-2016-0054.
16. Pludowski P, Holick MF, Grant WB et al. Vitamin D supplementation guidelines. J Steroid Biochem Mol Biol. 2018;175:125-135. doi:10.1016/j.jsbmb.2017.01.021.
17. Cashman KD, Sheehy T, O'Neill CM. Is vitamin D deficiency a public health concern for low middle income countries? A systematic literature review. Eur J Nutr. 2019;58(1):433-453. doi:10.1007/s00394-018-1607-3.
18. Kahwati LC, Weber RP, Pan H et al. Vitamin D, calcium, or combined supplementation for the primary prevention of fractures in community-dwelling adults: Evidence report and systematic review for the US Preventive Services Task Force. JAMA. 2018;319(15):1600-1612. doi:10.1001/jama.2017.21640.
19. Bassatne A, Chakhtoura M, Saad R et al. Vitamin D supplementation in obesity and during weight loss: A review of randomized controlled trials. Metabolism. 2019;92:193-205. doi:10.1016/j.metabol.2018.12.010.

20. Karras S, Paschou SA, Kandaraki E et al. Hypovitaminosis D in pregnancy in the Mediterranean region: a systematic review. Eur J Clin Nutr. 2016;70(9):979-986. doi:10.1038/ejcn.2016.12.
21. Cashman KD. 100 years of Vitamin D: Global differences in vitamin D status and dietary intake: a review of the data. Endocr Connect. 2022;11(1):e210282. doi:10.1530/EC-21-0282.
22. Wei F, Wang Z, Wang J et al. Serum vitamin D levels among children aged 0-12 years in the First Affiliated Hospital of Harbin Medical University, China. J Public Health (Oxf). 2018;40(4):721-726. doi:10.1093/pubmed/fdy055.
23. Xu B, Feng Y, Gan L et al. Vitamin D status in children with short stature: Accurate determination of serum Vitamin D components using high-performance liquid chromatography-tandem mass spectrometry. Front Endocrinol (Lausanne). 2021;12:707283. doi:10.3389/fendo.2021.707283.
24. Oktaria V, Putri DAD, Ihyauddin Z et al. Vitamin D deficiency in South-East Asian children: A systematic review. Arch Dis Child. 2022;107:980-987. doi:10.1136/archdischild-2021-323765.
25. Adnan M, Wu SY, Khilfeh M et al. Vitamin D status in very low birth weight infants and response to vitamin D intake during their NICU stays: A prospective cohort study. J Perinatol. 2022;42(2):209-216. doi:10.1038/s41372-021-01238-9.

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Akmaral Zhumalina
West Kazakhstan Marat Ospanov Medical University
Maresyeva st., 030012 Aktobe, Kazakhstan
e-mail: akmar.zhumalina@aol.com

ORCID AND CONTRIBUTIONSHIP
Akmaral Zhumalina: 0000-0002-4583-5779 A, F
Irina Kim: 0000-0003-0304-3156 B, D, F
Balash Tusupkaliev: 0000-0001-5693-8346 C, F
Mairamkul Zharlykasinova: 0000-0002-0825-1862 C, E, F
Botagoz Zhekeyeva: 0000-0002-6185-729X C, E-F

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

RECEIVED: 21.07.2023
ACCEPTED: 19.03.2024

ORIGINAL ARTICLE

Expression features of T-lymphocytes, B-lymphocytes and macrophages in the post-traumatic regenerate of the mandible rats under conditions of filling a bone defect with hydroxyapatite-containing osteotropic material and thymalin injecting the surrounding soft tissues

Andrii A. Boiko[1], Vladislav A. Malanchuk[1], Mykhailo S. Myroshnychenko[2], Olena V. Markovska[2], Anton S. Shapkin[2], Dmytro I. Marakushyn[2]

[1]BOHOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE
[2]KHARKIV NATIONAL MEDICAL UNIVERSITY, KHARKIV, UKRAINE

ABSTRACT

Aim: The purpose of the study was to determine the features of the expression of T-lymphocytes, B-lymphocytes, macrophages in the post-traumatic regenerate of the mandible rats under conditions of filling a bone defect with hydroxyapatite-containing osteotropic material and thymalin injecting the surrounding soft tissues.

Materials and Methods: An experiment was conducted on 48 mature rats of the WAG population weighing 160-180 grams. Four groups were formed. Group 1 included 12 rats with a simulated holey defect in the lower jaw. Group 2 included 12 rats with a simulated holey defect in the lower jaw followed by its closure with hydroxyapatite-containing osteotropic material (bone graft "Biomin GT"). Group 3 included 12 rats with a simulated holey defect in the lower jaw with injecting the surrounding soft tissues with thymalin. Group 4 included 12 rats with a simulated holey defect in the lower jaw followed by its closure with hydroxyapatite-containing osteotropic material (bone graft "Biomin GT") and injecting the surrounding soft tissues with thymalin. The material for the morphological study was a fragment of the lower jaw from the area of the simulated holey defect. An immunohistochemical study was performed using monoclonal antibodies to CD68, CD20, CD163, CD86, CD3.

Results: A comprehensive experimental and morphological study conducted by the authors revealed that thymalin injection of the soft tissues surrounding the bone defect of the lower jaw, filled with hydroxyapatite-containing osteotropic material "Biomin GT", stimulates local immune reactions in the post-traumatic regenerate, which is manifested, firstly, by an increase in the number T-lymphocytes on the 3rd day of the experiment and their increase up to the 28th day; secondly, by increasing the number of B-lymphocytes on the 14th day of the experiment with their further increase up to the 28th day; thirdly, by increasing the number of macrophages on the 3rd day of the experiment and their growth up to the 28th day; fourth, changes in macrophages phenotypes (decrease in the number of M1-macrophages and increase in the number of M2-macrophages).

Conclusions: Stimulation of local immune reactions in the post-traumatic regenerate can be one of the mechanisms that activate reparative osteogenesis in the lower jaw of rats under the conditions of filling bone defects with hydroxyapatite-containing osteotropic material "Biomin GT" and thymalin injecting the surrounding soft tissues.

KEY WORDS: T-lymphocytes, B-lymphocytes, macrophages, post-traumatic regenerate, hydroxyapatite-containing osteotropic material

Pol Merkur Lek,2024;53(1):171-177. doi: 10.36740/Merkur202402105

INTRODUCTION

In recent years a rapidly increasing number of publications are focusing on the topic of relationship between bone and the immune system, and this research field has been termed "osteoimmunology" to emphasize the close and complex communication between bone and the immune system [1, 2]. The immune and skeletal systems are found to be closely related, sharing a number of cytokines, chemokines, hormones, receptors, signaling molecules and transcription factors [3]. Bone cells interact with immune cells under physiological and pathological conditions [4].

The immune cells play an important role in reparative osteogenesis [5, 6]. Immune cells serve as the initial responders at the site of injury, mending vasculature, and initiating cascades of signals to recruit cells to carry out the repair processes [7]. Bone tissue damage initial immune response is mainly composed of the innate immune system which includes neutrophils, macrophages, dendritic cells. The later immune response is mainly composed of an adaptive immune system which includes T- and B-lymphocytes [5, 8]. Experimental studies have shown that decrease in morphofunctional activity of T- and B-lymphocytes is associated with impairment in bone mineralization and maturation of osteoblasts with delayed repair and remodeling phases [9]. HIV-positive patients display slower bone fracture healing [7].

Regulating the immune microenvironment is a promising therapeutic target to promote bone tissue regeneration [5]. Our previous morphological study showed stimulation of reparative osteogenesis in the lower jaw of rats under conditions of filling a bone defect with hydroxyapatite-containing osteotropic material and injecting the surrounding soft tissues with thymalin [10]. The features of local immune reactions in the regenerate filling the mandible bone defect under conditions of the above-mentioned therapeutic measures remain unknown, which requires further research.

AIM

The purpose of the study was to determine the features of the expression of T-lymphocytes, B-lymphocytes, macrophages in the post-traumatic regenerate of the mandible rats under conditions of filling a bone defect with hydroxyapatite-containing osteotropic material and thymalin injecting the surrounding soft tissues.

MATERIALS AND METHODS

An experiment was conducted on 48 mature rats of the WAG population, which were divided into four groups (12 animals in each group).

Group 1 included rats that underwent an incision of the skin, subcutaneous tissue and superficial fascia in the left submandibular area with a length of 1-1.2 cm and skeletonized a fragment of the outer surface of the branch and body of the lower jaw under ketamine intraperitoneal anesthesia and ultracaine infiltration anesthesia. A ball-shaped drill bit for a straight tip with a diameter of 3 mm with a rotation frequency up to 1000 revolutions per minute was used to form a transcortical hole defect of the body of the lower jaw in the form of a channel departing from the lower edge of the lower jaw upwards by 2 mm (until the feeling of the bur falling through). The wound was sutured layer by layer with polyamide after the formation of a holey defect.

Group 2 included rats that were modeled with a lower jaw defect similar to group 1. The formed defect was filled with synthetic bone graft "Biomin GT" (RAPID, Ukraine) which included hydroxyapatite and β-tricalcium phosphate. The wound was sutured layer by layer with polyamide.

Group 3 included rats that were modeled with a lower jaw defect similar to groups 1 and 2. The wound was sutured layer by layer with polyamide. Thymalin (LLC PP BIOPHARMA, Ukraine) was injected into the soft tissues around the defect for 10 days (0.01 mg/ml per 100 grams of animal weight).

Group 4 included rats that were modeled with a lower jaw defect similar to groups 1-3, which was filled with synthetic bone graft "Biomin GT" (RAPID, Ukraine). The wound was sutured layer by layer with polyamide. Thymalin (0.01 mg/ml per 100 grams of animal weight) was injected into the soft tissues around the defect for 10 days.

In each groups the animals were removed from the experiment on 3, 7, 14 and 28 days (3 animals for each experimental period).

The material for the study was a fragment of the lower jaw from the area of the simulated holey defect. It was fixed in a 10% solution of neutral formalin (pH 7.4) for 24-48 hours, decalcified and carried out according to the generally accepted method and embedded in paraffin. From paraffin blocks serial sections with a thickness of 4-5 μm were made, which were stained with hematoxylin and eosin.

Immunohistochemical study was performed on Super Frost Plus adhesive slides (Menzel, Germany). The Master Polymer Plus Detection System (Peroxidase, DAB chromogen) (Master Diagnostica, Spain) was used. The citrate buffer (pH 6.0), EDTA buffer (pH 8.0) were used for high-temperature processing of antigen epitopes. An immunohistochemical study was performed using mouse monoclonal antibodies to CD68 (clone KP-1, Master Diagnostica, Spain) (marker of cells of macrophage lineage), CD20 (clone L26, Thermo Fisher Scientific, Great Britain) (marker of B-lymphocytes), rabbit monoclonal antibodies to CD163 (clone EP324, Master Diagnostica, Spain) (marker of M2-macrophages), CD86 (clone B7-2, Thermo Fisher Scientific, Great Britain) (marker of M1-macrophages), CD3 (clone EP41, Master Diagnostica, Spain) (marker of T-lymphocytes). Immunohistochemical reactions with antibodies to CD68, CD20 and CD3 were evaluated by counting the absolute number of immunopositive cells in the field of view of a microscope ×400, and reactions with antibodies to CD86, CD163 – by counting the relative number (%) of immunopositive cells in the field of view of a microscope ×400. Examination of the microslides was carried out using a laboratory microscope ZEISS Primostar 3 (Carl Zeiss, Germany) with a built-in color digital camera.

The indicators in the groups were processed statistically using the PAST program (version 4.15, Natural History Museum, University of Oslo, Norway). Mean values of indicators in groups were compared using the Student's t-test and Mann-Whitney U-test. Differences were considered significant at $p<0.05$.

RESULTS

During an immunohistochemical study in groups 1-4 at all experiment days, $CD20^+$-, $CD3^+$-, $CD68^+$-, $CD86^+$-, $CD163^+$-cells were visualized among the previously described polymorphic cellular infiltration in the regenerate filling the cavity of the bone defect of the rats lower jaw (Fig. 1-3).

The analysis of the mean value of the absolute number of $CD20^+$-cells is shown in Table 1. As indicated in this table, the absolute number of $CD20^+$-cells did not differ ($p>0.05$) in group 2 compared to group 1 at all experiment terms. The absolute number of $CD20^+$-cells in groups 3 and 4 compared to the corresponding indicators in groups 1 and 2 did not differ ($p>0.05$) on days 3 and 7, and on days 14 and 28 had a greater ($p<0.05$) value. At all experiment days, the indicators of groups 3 and 4 did not differ ($p>0.05$).

During the period from the 3rd to the 28th day of the experiment, the absolute number of $CD20^+$-cells in the regenerate did not change ($p>0.05$) in groups 1 and 2. In groups 3 and 4, this indicator also did not change ($p>0.05$) from the 3rd to the 7th day and increased ($p<0.05$) from the 7th to the 28th day.

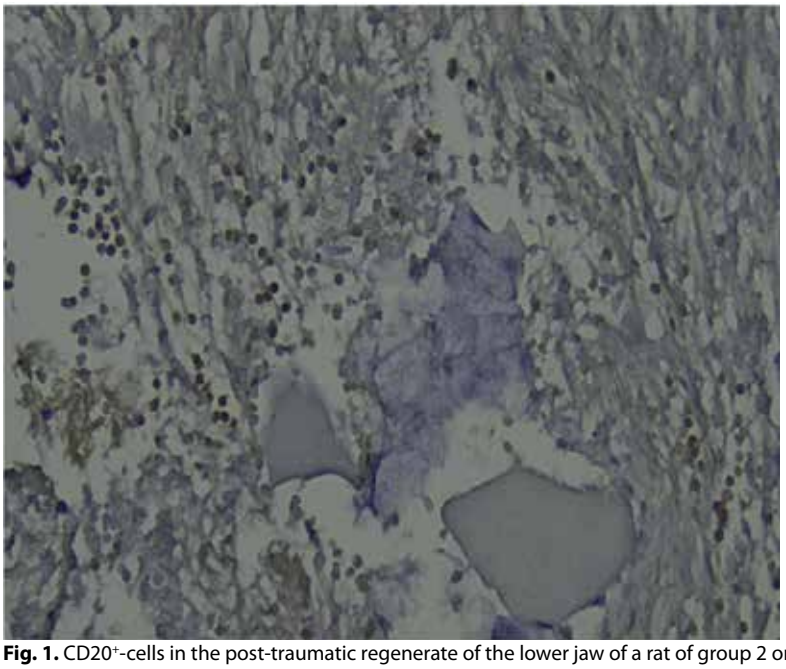


Fig. 1. $CD20^+$-cells in the post-traumatic regenerate of the lower jaw of a rat of group 2 on the 3rd day of the experiment. Immunohistochemical study with a monoclonal antibody to CD20, ×400.

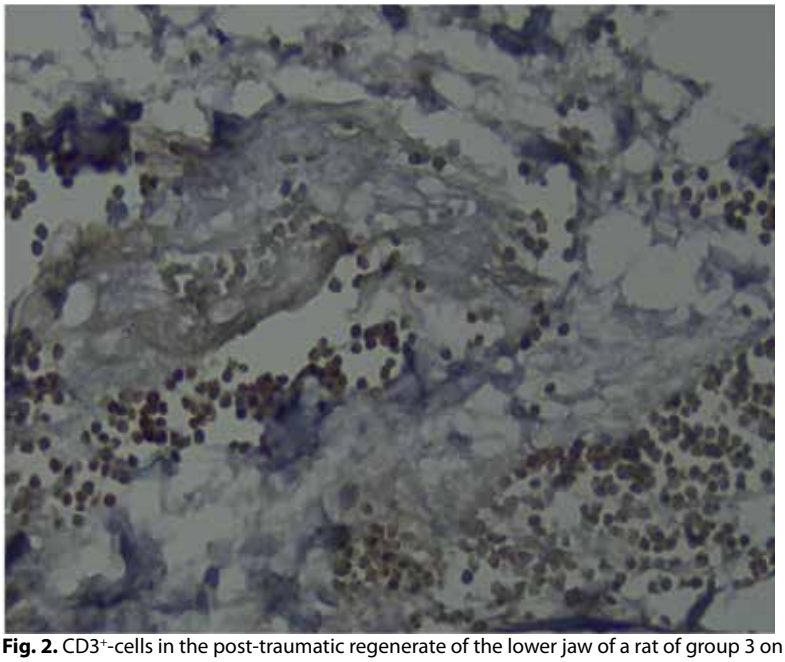


Fig. 2. $CD3^+$-cells in the post-traumatic regenerate of the lower jaw of a rat of group 3 on the 3rd day of the experiment. Immunohistochemical study with a monoclonal antibody to CD3, ×400.

The analysis of the average value of the absolute number of $CD3^+$-cells in the regenerate in groups 1-4 is shown in Table 2. Compared to group 1, in group 2 this indicator had a greater value ($p<0.05$) at all experimental days. From the 3rd to the 28th day of the experiment, the number of $CD3^+$-cells in groups 3 and 4 was higher ($p<0.05$) compared to the indicators of groups 1 and 2. At all days of the experiment, the indicator of group 4 did not differ ($p>0.05$) from the corresponding indicator of group 3. From the 3rd to the 28th day of the experiment, an increase ($p<0.05$) in the number of $CD3^+$-cells was registered in all groups.

The results of counting the number of $CD68^+$-, $CD86^+$-, $CD163^+$-cells in groups 1-4 are shown in Table 3. In group 2, compared to group 1, the absolute number of $CD68^+$-cells did not differ ($p>0.05$) on day 3 and had more ($p<0.05$) values from the 7th to the 28th day. At all days of the experiment, the absolute number of $CD68^+$-cells did not differ ($p>0.05$) in groups 3 and 4, however, in the latter, this indicator had a greater value ($p<0.05$) compared to the indicators of groups 1 and 2.

Analyzing the absolute number of $CD68^+$-cells in groups 1-4 in dynamics (from the 3rd to the 28th day), it was noted

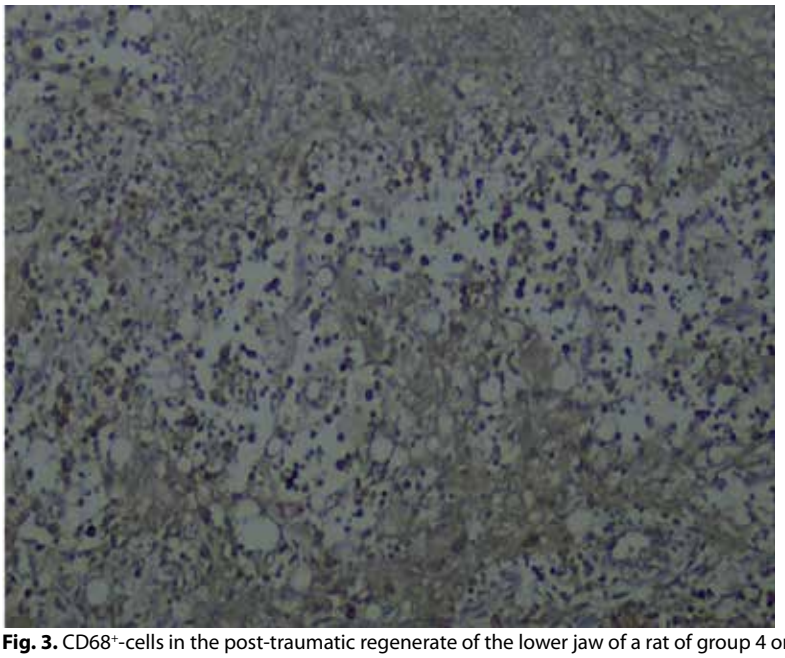


Fig. 3. CD68^{+}-cells in the post-traumatic regenerate of the lower jaw of a rat of group 4 on the 3rd day of the experiment. Immunohistochemical study with a monoclonal antibody to CD68, ×100.

Table 1. Mean values of the absolute number of CD20^{+}-cells

Group number	Day of experiment			
	3	7	14	28
Group 1 (holey defect)	77.3±2.38	81.2±1.96	83.2±1.87	82.2±1.35
Group 2 (holey defect+bone graft «Biomin GT»)	80.2±1.92	82.2±1.33	83.7±1.02	84.3±0.88
Group 3 (holey defect+thymalin)	79.3±1.84	80.7±0.71	100.8±2.24[1, 2, 5]	127.8±1.58[1, 2, 6]
Group 4 (holey defect+bone graft «Biomin GT»+ thymalin)	82.2±0.98	83.2±1.08	108.2±2.41[1, 2, 5]	133.3±1.65[1, 2, 6]

Note: [1] – significance of the differences compared to the indicator of group 1;
[2] – significance of the differences compared to the indicator of group 2;
[3] – significance of the differences compared to the indicator of group 3;
[4] – significance of the differences compared to the indicator on the 3rd day of the experiment;
[5] – significance of the differences compared to the indicator on the 7th day of the experiment;
[6] – significance of the differences compared to the indicator on the 14th day of the experiment.

Table 2. Mean values of the absolute number of CD3^{+}-cells

Group number	Day of experiment			
	3	7	14	28
Group 1 (holey defect)	190.7±4.42	241.2±4.48[4]	300.5±4.04[5]	334.8±3.23[6]
Group 2 (holey defect+bone graft «Biomin GT»)	239.3±3.07[1]	285.2±3.00[1, 4]	336.5±8.12[1, 5]	374.2±6.57[1, 6]
Group 3 (holey defect+thymalin)	339.3±10.66[1, 2]	385.7±10.73[1, 2, 4]	428.2±6.61[1, 2, 5]	470.3±4.15[1, 2, 6]
Group 4 (holey defect+bone graft «Biomin GT»+ thymalin)	344.2±2.43[1, 2]	393.8±5.17[1, 2, 4]	435.2±6.39[1, 2, 5]	473.7±7.33[1, 2, 6]

Note: [1] – significance of the differences compared to the indicator of group 1;
[2] – significance of the differences compared to the indicator of group 2;
[3] – significance of the differences compared to the indicator of group 3;
[4] – significance of the differences compared to the indicator on the 3rd day of the experiment;
[5] – significance of the differences compared to the indicator on the 7th day of the experiment;
[6] – significance of the differences compared to the indicator on the 14th day of the experiment.

Table 3. Mean values of the absolute number of CD68$^+$-cells and the relative number of CD86$^+$-, CD163$^+$-cells

Group number	Cell name	Day of experiment			
		3	7	14	28
Group 1 (holey defect)	CD68$^+$-cells	185.7±12.7	200.7±4.97	240.5±14.43[2]	268.3±26.44[3]
	CD86$^+$-cells	82.8±9.02	83.0±9.90	67.5±12.14[2]	56.0±8.60[3]
	CD163$^+$-cells	17.2±9.02[4]	17.0±9.90[4]	32.5±12.14[2,4]	44.0±8.60[3,4]
Group 2 (holey defect+bone graft «Biomin GT»)	CD68$^+$-cells	201.7±39.37	227.8±19.57[5]	279.8±15.60[2,5]	311.8±13.04[3,5]
	CD86$^+$-cells	81.5±8.46	82.3±7.66	70.8±11.58	56.3±2.94[3]
	CD163$^+$-cells	18.5±8.46[4]	17.7±7.66[4]	29.2±11.58[4]	43.7±2.94[3,4]
Group 3 (holey defect+thymalin)	CD68$^+$-cells	295.2±25.34[5,6]	334.5±9.79[1,5,6]	357.2±17.38[2,5,6]	387.3±12.75[3,5,6]
	CD86$^+$-cells	57.8±6.49[5,6]	49.67±5.82[1,5,6]	33.3±6.06[2,5,6]	18.3±5.16[3,5,6]
	CD163$^+$-cells	42.2±6.49[4,5,6]	50.3±5.82[1,5,6]	66.7±6.06[2,4,5,6]	81.7±5.16[3,4,5,6]
Group 4 (holey defect+bone graft «Biomin GT»+ thymalin)	CD68$^+$-cells	288.7±41.41[5,6]	338.5±10.82[1,5,6]	359.7±8.50[2,5,6]	383.7±17.14[3,5,6]
	CD86$^+$-cells	58.3±6.25[5,6]	49.8±5.49[1,5,6]	34.5±11.81[2,5,6]	16.8±5.31[3,5,6]
	CD163$^+$-cells	41.7±6.25[4,5,6]	50.2±5.49[1,5,6]	65.5±11.81[2,4,5,6]	83.2±5.31[3,4,5,6]

Note: [1] – significance of the differences compared to the indicator on day 3;
[2] – significance of the differences compared to the indicator on the 7th day;
[3] – significance of the differences compared to the indicator on the 14th day;
[4] – significance of the differences compared to the relative number of CD86$^+$-cells;
[5] – significance of the differences compared to the indicator of group 1;
[6] – significance of the differences compared to the indicator of group 2;
[7] – significance of the differences compared to the indicator of group 3.

that in groups 1 and 2 this indicator did not change ($p>0.05$) from the 3rd to the 7th day and increased ($p<0.05$) from the 7th to the 28th day. In groups 3 and 4, the absolute number of these immunopositive cells increased ($p<0.05$) from the 3rd to the 28th day.

When analyzing the average value of the relative number of macrophage phenotypes (M1-macrophages (CD86$^+$-cells) and M2-macrophages (CD163$^+$-cells)) in groups 1 and 2, the relative number of CD86$^+$-cells prevailed ($p<0.05$) at all days of the experiment. In groups 3 and 4, on the 3rd day, there was a predominance ($p<0.05$) of the relative number of CD86$^+$-cells, on the 7th day, no difference was found ($p>0.05$) in the relative numbers of CD86$^+$-cells and CD163$^+$-cells, on the 14th and 28th day, the relative number of CD163$^+$-cells prevailed ($p<0.05$).

During the period from the 3rd to the 28th day in group 1, the relative number of M1- and M2-macrophages did not change ($p>0.05$) from the 3rd to the 7th day, however, from the 7th to 28th day the relative number of M1-macrophages decreased ($p<0.05$), and the relative number of M2-macrophages increased ($p<0.05$) (table 3). In group 2, the relative number of CD86$^+$- and CD163+ cells did not change ($p>0.05$) from the 3rd to the 7th day, the relative number of CD86$^+$- and CD163$^+$-cells tended ($p>0.05$) to decrease and increase, respectively on the 14th day compared to the 7th day, and on the 28th day compared to the 14th day, the relative number of M1-macrophages decreased ($p<0.05$) against the background of an increase ($p<0.05$) in the relative number of M2-macrophages. In groups 3 and 4, from the 3rd to the 28th day, a decrease ($p<0.05$) in the relative number of M1-macrophages and an increase ($p<0.05$) in the relative number of M2-macrophages were recorded.

Intergroup analysis of the relative number of CD86$^+$-, CD163$^+$-cells showed no differences ($p>0.05$) in indicators in group 2 compared to group 1. The relative number of CD86$^+$-, CD163$^+$-cells did not differ ($p>0.05$) in groups 4 and 3, but in the latter, compared to groups 1 and 2, the indicator of the relative number of CD86$^+$-cells had a smaller ($p<0.05$) value, and the indicator of the relative number of CD163$^+$-cells had a greater ($p<0.05$) value.

DISCUSSION

The authors conducted a complex immunohistochemical study, which revealed the features of the expression of markers of the general population of T-lymphocytes (CD3), B-lymphocytes (CD20), macrophages (CD68) and their phenotypes (CD86 and CD163) in the post-traumatic regenerate of the lower jaw of rats under the conditions of bone filling defect with hydroxyapatite-containing osteotropic material "Biomin GT" and encircling the surrounding soft tissues with thymalin.

Thymalin, as is known, is a polypeptide complex isolated from the thymus that regulates the number and ratio of T- and B-lymphocytes, as well as their subpopulations, stimulates cellular immune responses, and enhances phagocytosis [11]. In cases of circumcision with thymalin of the soft tissues surrounding the bone defect of the lower jaw, the authors found an increase in the content of B-lymphocytes in the post-traumatic regenerate on the 14th and 28th day of the experiment.

B-lymphocytes regulate bone formation and have a significant role in the risk of bone metabolism disruption. B-lymphocytes ensure bone homeostasis by producing various cytokines and chemokines (TNFα, TNF-β, IL-6, IL-10)

[1]. Recent studies have disclosed a regulatory effect of B cells, indicating that B cells affect osteoclasts [12]. B cells antagonistically block the effect of RANKL (receptor activator of nuclear factor ligand) by secreting osteoprotegerin and promoting bone tissue regeneration [5].

Thymalin injection of the soft tissues surrounding the bone defect of the lower jaw, filled with hydroxyapatite-containing osteotropic material, led to an increase in the number of T-lymphocytes in the post-traumatic regenerate on the 3rd day of the experiment. Moreover, the absolute number of T-lymphocytes increased from the 3rd to the 28th day.

Activated T cells indirectly or directly regulate bone health and bone remodeling by secreting various cytokines, growth factors [13]. To date, it has been proven that T-lymphocytes affect the activity of bone tissue cells [14]. T cells are divided into αβ T cells and γδ T cells. αβ T cells are further subcategorised into $CD4^+$ and $CD8^+$ T cells, which have dual functions of promoting and inhibiting regeneration. γδ T cells are a small subset of T cells, which are considered to promote regeneration [12]. T cells affect macrophages and fibroblasts, which play important roles in bone tissue regeneration [15].

Deficiency of T- and B-lymphocytes is the cause of suppression of reparative osteogenesis, violations of the processes of mineralization and differentiation of osteoblasts [16].

The complex treatment measures carried out by the authors of the article also led to an increase in the total population of macrophages in the post-traumatic regenerate from the 3rd to the 28th day of the experiment. Against the background of an increase in the number of the total population of macrophages, changes in the content of their phenotypes were recorded: the number of M1-macrophages decreased and the number of M2-macrophages increased.

Macrophages act as phagocytes to prevent pathogens from invading remove necrotic tissue. Macrophages also secrete various cytokines, chemokines, growth factors to initiate the recruitment of fibroblasts, mesenchymal stem cells, and osteoprogenitor cells from their local niches [5]. It's a well-known fact that macrophages may convert into osteoclasts capable of resorbing bone [17].

Macrophages are highly plastic and dynamic cell populations that are capable of changing their phenotype. Today, most scientists distinguish two phenotypes of macrophages: "classically activated" pro-inflammatory M1 phenotype and "alternatively activated" anti-inflammatory M2 phenotype [18]. M1-macrophages secrete pro-inflammatory cytokines, which affect osteoblasts by inhibiting their differentiation and promoting apoptosis. M2-macrophages produce anti-inflammatory cytokines, transforming growth factor-β, vascular endothelial growth factors, bone morphogenetic proteins, which enhance the differentiation and function of bone-healing cells [17].

The correct balance between M1- and M2-macrophages is the key to successful reparative osteogenesis [17].

CONCLUSIONS

A comprehensive experimental and morphological study conducted by the authors revealed that thymalin injection of the soft tissues surrounding the bone defect of the lower jaw, filled with hydroxyapatite-containing osteotropic material "Biomin GT", stimulates local immune reactions in the post-traumatic regenerate, which is manifested, firstly, by an increase in the number T-lymphocytes on the 3rd day of the experiment and their increase up to the 28th day; secondly, by increasing the number of B-lymphocytes on the 14th day of the experiment with their further increase up to the 28th day; thirdly, by increasing the number of macrophages on the 3rd day of the experiment and their growth up to the 28th day; fourth, changes in macrophages phenotypes (decrease in the number of M1-macrophages and increase in the number of M2-macrophages). Stimulation of local immune reactions in the post-traumatic regenerate can be one of the mechanisms that activate reparative osteogenesis in the lower jaw of rats under the conditions of filling bone defects with hydroxyapatite-containing osteotropic material "Biomin GT" and thymalin injecting the surrounding soft tissues.

REFERENCES

1. Zhao T, Chu Z, Ma J, Ouyang L. Immunomodulation Effect of Biomaterials on Bone Formation. J Funct Biomater. 2022;13(3):103. doi: 10.3390/jfb13030103.
2. Cai L, Lv Y, Yan Q, Guo W. Cytokines: the links between bone and the immune system. Injury. 2024;55(2):111203. doi: 10.1016/j.injury.2023.111203.
3. Xiao Y, Chen Z, Chang J, Wu C. Osteoimmunomodulation for the development of advanced bone biomaterials. Front. Bioeng. Biotechnol. Conference Abstract: 10th World Biomaterials Congress (10th World Biomaterials Congress, Montréal, Canada, 17 May - 22 May, 2016): doi: 10.3389/conf.FBIOE.2016.01.00215.
4. Okamoto K, Nakashima T, Shinohara M, Negishi-Koga T, Komatsu N, Terashima A, Sawa S, Nitta T, Takayanagi H. Osteoimmunology: The Conceptual Framework Unifying the Immune and Skeletal Systems. Physiol Rev. 2017;97(4):1295-1349. doi: 10.1152/physrev.00036.2016.
5. Yang N, Liu Y. The Role of the Immune Microenvironment in Bone Regeneration. Int J Med Sci. 2021;18(16):3697-3707. doi: 10.7150/ijms.61080.
6. Markovska OV, Tovazhnyanska OL, Myroshnychenko MS, Shapkin AS, Nekrasova NO, Samoilova HP, Lapshyna IO. Features of local immune reactions in skin with underlying soft tissues in patients with multiple sclerosis. Wiad Lek. 2020;73(10):2109-2115.
7. Baht GS, Vi L, Alman BA. The Role of the Immune Cells in Fracture Healing. Curr Osteoporos Rep. 2018;16(2):138-145. doi: 10.1007/s11914-018-0423-2.
8. Su N, Villicana C, Yang F. Immunomodulatory strategies for bone regeneration: a review from the perspective of disease types. Biomaterials. 2022;286:121604. doi: 10.1016/j.biomaterials.2022.121604.
9. El-Jawhari JJ, Jones E, Giannoudis PV. The roles of immune cells in bone healing; what we know, do not know and future perspectives. Injury. 2016;47(11):2399-2406. doi: 10.1016/j.injury.2016.10.008.

10. Boiko AA, Malanchuk VA, Myroshnychenko MS. Reparative osteogenesis in mandible in cases of filling a bone defect with hydroxyapatite-containing osteotropic material and injecting the surrounding soft tissues with thymalin: experimental and morphological study. Wiad Lek. 2024;77(1):68-76. doi: 10.36740/WLek202401110.
11. Kozlovsky MM. Efektyvnist imunomoduljatora tymalinu pry eksperymentalnij koronavirusnij infekcii. Effectiveness of the immunomodulator thymalin in experimental coronavirus infection. Emergency Medicine. 2022;18(7):62-64. doi: 10.22141/2224-0586.18.7.2022.1534. in Ukrainian.
12. Li N, Fu L, Li Z, Ke Y, Wang Y, Wu J, Yu J. The Role of Immune Microenvironment in Maxillofacial Bone Homeostasis. Front. Dent. Med. 2021. 2:780973. doi: 10.3389/fdmed.2021.780973
13. Srivastava RK, Dar HY, Mishra PK. Immunoporosis: Immunology of Osteoporosis-Role of T Cells. Front Immunol. 2018;9:657. doi: 10.3389/fimmu.2018.00657.
14. Zhang W, Dang K, Huai Y, Qian A. Osteoimmunology: The Regulatory Roles of T Lymphocytes in Osteoporosis. Front. Endocrinol. 2020; 11:465. doi: 10.3389/fendo.2020.00465
15. Xiong Y, Mi BB, Lin Z, Hu YQ, Yu L, Zha KK, Panayi AC, Yu T, Chen L, Liu ZP, Patel A, Feng Q, Zhou SH, Liu GH. The role of the immune microenvironment in bone, cartilage, and soft tissue regeneration: from mechanism to therapeutic opportunity. Mil Med Res. 2022;9(1):65. doi: 10.1186/s40779-022-00426-8.
16. Deng Z, Zhang Q, Zhao Z, Li Y, Chen X, Lin Z, Deng Z, Liu J, Duan L, Wang D, Li W. Crosstalk between immune cells and bone cells or chondrocytes. Int Immunopharmacol. 2021; 101 (Pt A): 108179. doi: 10.1016/j.intimp.2021.108179.
17. Hu Y, Huang J, Chen C, Wang Y, Hao Z, Chen T, Wang J, Li J. Strategies of Macrophages to Maintain Bone Homeostasis and Promote Bone Repair: A Narrative Review. J Funct Biomater. 2022;14(1):18. doi: 10.3390/jfb14010018.
18. Hu K, Shang Z, Yang X, Zhang Y, Cao L. Macrophage Polarization and the Regulation of Bone Immunity in Bone Homeostasis. J Inflamm Res. 2023;16:3563-3580. doi: 10.2147/JIR.S423819.

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Mykhailo S. Myroshnychenko
Department of General and Clinical Pathological Physiology
named after D.O. Alpern, Kharkiv National Medical University
4 Nauky avenue, Kharkiv, 61022, Ukraine
e-mail: msmyroshnychenko@ukr.net

ORCID AND CONTRIBUTIONSHIP
Andrii A. Boiko: 0000-0003-0432-5091 (A, D)
Vladislav A. Malanchuk: 0000-0001-8111-0436 (A, E)
Mykhailo S. Myroshnychenko: 0000-0002-6920-8374 (B)
Olena V. Markovska: 0000-0002-8759-4272 (F)
Anton S. Shapkin: 0000-0002-6437-4840 (E)
Dmytro I. Marakushyn: 0000-0002-0956-9776 (C)

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

RECEIVED: 20.10.2023
ACCEPTED: 08.02.2024

ORIGINAL ARTICLE

Design, synthesis, insilco study and biological evaluation of new isatin-sulfonamide derivatives by using mono amide linker as possible as histone deacetylase inhibitors

Ammar Abdul Aziz Alibeg, Mohammed Hassan Mohammed
UNIVERSITY OF KUFA, NAJAF, IRAQ

ABSTRACT

Aim: To evaluate the cytotoxic activity of newly synthesized a series of novel HDAC inhibitors comprising sulfonamide as zinc binding group and Isatin derivatives as cap group joined by mono amide linker as required to act as HDAC inhibitors.

Materials and Methods: The utilization of sulfonamide as zinc binding group joined by N-alkylation reaction with ethyl-bromo hexanoate as linker group that joined by amide reaction with Isatin derivatives as cap groups which 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 compounds were synthesized and characterized by successfully by ART-FTIR, NMR and ESI- Ms. Assessed for their cytotoxic activity against human colon adenocarcinoma MCF-7 (IC50, I=105.15, II=60.00, III=54.11, IV=56.57, vorinostat=28.41) and hepatoblastoma HepG2 (IC50, I=63.91, II=135.18, III=118.85, IV=51.46, vorinostat=37.50). Most of them exhibited potent HDAC inhibitory activity and significant cytotoxicity.

Conclusions: The synthesized compounds (I, II, III and IV) showed cytotoxicity toward MCF-7 and HepG2 cancer cell lines and their docking analysis provided a preliminary indication that they are viable [HDAC6] candidates.

KEY WORDS: Sulfonamide, Isatin derivatives, Histone deacetylase; cytotoxic activity; Histone deacetylase inhibitors

Pol Merkur Lek,2024;53(1):178-187. doi: 10.36740/Merkur202402106

INTRODUCTION

One of the leading causes of mortality in the globe is cancer. Significant side effects and drug resistance remain the main drawbacks of new chemotherapeutic drugs, which restrict out of uncontrolled cell cycle processes for the treatment of various cancers. Various studies are being carried out to find and create potent anticancer medications [1-3]. Epigenetic gene silencing may increase the risk of cancer development by promoting unchecked cell proliferation [4]. Unwanted epigenetic changes, including improper DNA methylation, histone modifications, irregular nucleosome structure, and non-coding RNAs, have all been related to the development and spread of cancer, according to recent study [5]. An improper histone modification is one of the fundamental pathways, and the main one being studied is acetylation [6]. Histone acetyltransferase (HATs) and histone deacetylase (HDACs), which catalyze the removal of acetyl groups from histone amino-terminal lysine residues and are likely to cause chromatin compaction and transcriptional suppression, respectively, are the two groups of enzymes that regulate acetylation [7].

Numerous studies demonstrate that various histone deacetylase (HDAC) family members exhibit aberrant expression in a range of malignancies and participate in the non-redundant regulation of malignant cell characteristics. As a result, there is a substantial relationship between chromatin architecture, histone tail deacetylation, and overexpression of histone deacetylase (HDAC) activity. Transcription factors may lose access to some genes' transcription, which would repress those genes. This could be a method to silence genes involved in cyclin-dependent kinase inhibition and tumor suppression, such as p21 and p53 [8]. HDACs have a role in several biological processes, such as chaperone activity, DNA repair, senescence, autophagy, metabolism, and cell cycle regulation. The 18 distinct isoforms of human HDAC are grouped into four classes: class I HDACs (HDACs 1, 2, 3, and 8) are linked to RPD3 deacetylase [9]; class II HDACs are subdivided into class IIa (HDACs 4, 5, 7, and 9) and class IIb (HDACs 6 and 10) [10]. The class III HDACs (SIRT1 through SIRT7) and yeast silent information regulator 2 (Sir2) are related biologically [11]. As a method of treating cancer, the zinc-dependent HDAC classes have been thoroughly investigated and tested for inhibition [12].

Despite the enormous structural diversity, traditional HDAC inhibitors share a three-part universal pharmacophore model [13-16]:

1) a zinc binding group (ZBG), typically a hydroxyl amine, thiourea, thiol, amide, or biguanide, at the bottom of the active site of HDACs

2) a cap group, often a hydrophobic ring, engaging with the amino acids at the entrance of HDACs;

3) a linker linking the ZBG and the cap group, such as aliphatic hydrocarbon [17-20].

The inhibitors should have group have the ability for binding with zinc ion such as hydroxyl amine, thiourea, thiol, amide

or biguanide, dithiocarbonate near the base of the HDACs' active site [21-22]. A linker chain is similar to an acetylated histone lysine and cap group to recognize and interact with amino acid residues at the rim outer surface of the enzyme. The cap group can adopt broad structural variation, such as Isatin making it feasible to build HDAC inhibitors with a vast range of configurations. These various inhibitors' selectivity and efficacy depend on variations in one or more of the three domains. In light of this, new zinc-dependent HDAC inhibitors should have complied with these three criteria [23]. Lysine deacetylase 6 (HDAC6) is a class IIB Zn2+ deacetylase and is the only HDAC to contain two deacetylase domains of distinct specificities. The first domain specifically deacetylates acetylated C-terminal lysine residues, while the second shows a particularly broad substrate selectivity [24]. There is evidence that HDAC6 catalyzes deacetylation of several proteins involved in a variety of cellular processes. Among them, HDAC6-mediated deacetylation of α-tubulin regulates microtubule stability and cell motility [25, 26]. Another characterized substrate, cortactin, binds to deacetylated actin filaments and participates in the fusion of lysosomes and autophagosomes [27]. The enzyme also plays a role in protein folding by regulating the activity of the Hsp90 chaperone protein via deacetylation [28]. In addition, HDAC6 is an important player in innate immunity, regulating the detection of pathogen genomic material via deacetylation of retinoic acid inducible Gene-I protein [29]. While the broad specificity of HDAC6 has been reported, a full understanding of the selectivity determinants is still lacking, as is a proper understanding of the underlying structural basis that makes this particular HDAC more promiscuous than others, such as HDAC8 [30] (Fig. 1).

In this research four novel substituted sulfonamide derivatives (compounds I, II, III and IV) were synthesized as enzyme inhibitors for zinc-dependent histone deacetylases (Fig. 2). They consist of an isatin cap group, a 5-carbon atom linker, and a sulfonamide group (ZBG). An in vitro cytotoxicity experiment was conducted against HepG2 hepatoblastoma and MCF7 human colon cancer to show whether these compounds could have cytotoxic action. Additionally, a docking study against HDAC6 were carried out using the generated molecules.

AIM

The aim of study to synthesis and evaluate the biological activity of new series of sulfonamide derivatives as HDAC inhibitors to treat cancer disease.

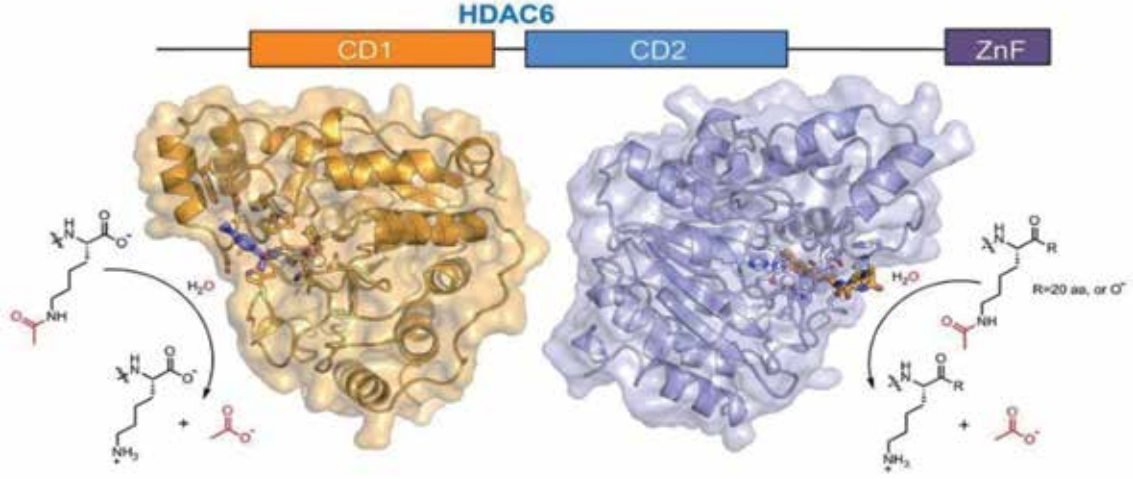
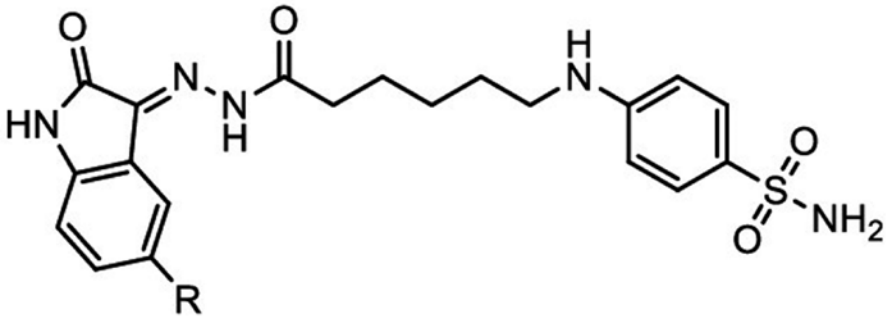


Fig. 1. The catalytic domain structure of HDAC6 isoform [31].



I-R=H
II-R=F
III-R=OCH3
IV-R=CH3

Fig. 2. The structure of designed compounds with a zinc binding group with sulfonamide functionality and isatin derivatives as cap groups.

MATERIALS AND METHODS

Amino benzene sulfonamide, monoethyl pimelate, Isatin, 5-Flouro Isatin, 5-methoxy Isatin, 5-methyl Isatin and ethyl 6-bromohexanoate, were purchased from BLD pharm, China, we bought 80% hydrazine hydrate from Alpha Chemika in India. All of the solvents were purchased commercially and didn't require any additional purification. The Stuart SMP30 Electronic Melting Point Apparatus was used to determine melting points (uncorrected). Thin-layer chromatography (TLC) using Merck silica gel 60F254 and UV-light vision were used to monitor the reactions, the solvent system was Ethyl acetate: Hexane (3:7) [32].

The FT-IR spectra were recorded on FTIR spectrophotometer/ Shimadzu, Japan, supplied by Specac® Quest ATR (diamond)- UK (College of Pharmacy, University of Baghdad), ^{1}H-NMR (^{1}HNMR 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. Were MCF7 (a human breast cancer cell line) and HepG2 (human liver cancer) were bought from the Iranian Pasteur Institute's National Cell Bank. Gibco's RPMI-1640 and DMEM: F12 media, each containing 10% FBS and antibiotics (100 U/ml penicillin and 100 g/ml streptomycin), were used to culture the cells. Cells were passaged using trypsin/EDTA (Gibco) and phosphate- buffered saline (PBS) solution and kept at 37°C in humidified air containing 5% CO_2.

CHEMICAL SYNTHESIS

Synthesis of ethyl 7-oxo-7-((4-sulfamoylphenyl) amino) heptanoate, Compound Ia [32]

Sulfanilamide (0.01 mole) was dissolved in (50ml) of absolute ethanol containing (0.01 mole) of pyridine. Ethyl 6-bromohexanoate (0.01 mole) was added slowly with stirring, the resultant solution was reflux for (24 hours). After that concentrating the final solution to a half and crushed ice was added. After (24 hours), the crude material was removed by filtering, washed in ice water, and then crystallized again from water to produce white crystals; Yield: (57%); m.p. 193-195 C^{0}; FTIR (ú, cm- 1): 3394,3361 (N-H, amine), 3269 (N-H, 2nd amine), 3093 (C-H, aromatic ring), 1730 (C=O, ester), 1595 (C=C, aromatic ring).

Synthesis of hydrazineylidene-5-(H, F, OCH3, CH3) methoxyindolin-2-one Compound Ib1-4 [33]

Isatin derivatives (20.4 mmol) was dissolved in 30 ml of absolute methanol then add 30 ml of 80% hydrazine hydrate. The finished mixture was refluxed for two hours. TLC (hexane: ethyl acetate (3:2 v/v), Rf value = 0.64, 0.68, 0.72, 0.78) respectively was used to monitor the reaction's progress. The final mixture was poured over ice water that had been crushed. The separated precipitate was filtered, repeatedly washed with hot distilled water and twice with methanol, dried, and recrystallized from ethanol. light brown solid; Yield: (81%, 81%, 85%, 83%) respectively; m.p. (229-231, 226-228, 234-236, 230-231 C^{0}) respectively; FTIR (ú,

Table 1. Docking results of compounds, I, II, III, IV and SAHA against HDAC6

Compound	μM	
	MCF-7 cell line	HepG2 cell line
I	0.244	0.148
II	0.134	0.302
III	0.117	0.258
IV	0.127	0.116
SAHA	0.107	0.141

Table 2. IC50 values of target compounds I, II III and IV

Ligand	Energy of binding ΔG (KJ/mol)	Amino acid
SAHA	-7.07	Cys 584 (3.68 A^{0})
I	-7.85	Cys 584 (4.36A^{0}), His 573 (2.81A^{0}), Phe 643 (3.09A^{0})
II	-9.43	His 574 (2.65A^{0}), His 573 (3.26A^{0}), Gly 582 (3.43A^{0})
III	-8.09	His 574 (2.1A^{0}), His 573 (2.19A^{0}), Gly 582 (3.12A^{0})
IV	-8.25	His 574 (2.17A^{0}), His 573 (2.39A^{0}), Gly 582

cm-1): 3236,3149 (N-H, amine), 3088 (N-H, amide),3057 (C-H, aromatic ring), 1689 (C=O, amide), 1587 (C=C, aromatic ring).

Synthesis of Compounds I - IV [34]

To compound Ia (1mmol) dissolved in (30 ml) of [DMF: Ethanol] the addition of compound Ib1-Ib4 (1mmol) was added and the reaction mixture was reflux for (12 hrs.). The solution was concentrated to a half and crushed ice was added. After 24 hours, the crude material was filtered off, washed with cold water and recrystallized from water, giving pale brown crystals, faint brown solid, yellowish white, pale yellow. Yield (51%, 57%, 61%, 59%) respectively; m.p. (209-211, 212-214, 211-213, 217-219 C^0) respectively.

4-((6-oxo-6-(2-(2-oxoindolin-3-ylidene)hydrazinyl) hexyl)amino)benzenesulfonamide, Compound I

FT-IR (ATR; υ, cm-1): 3286 (NH2 Str. Vib. of amine), 3165, 3103 (NH Str. Vib. of amide), 2995 (C-H Asymmetric str. Vib. Of CH3), 2895 (C-H str. Vibration of CH2), 1705,1688 (C=O Str. vibration band of amide), 1627 (str. Vibration of C=N); 1HNMR (400 MHz, DMSO-d6), ppm : 1.46 (m, 2H, CH2), 1.68 (m, 4H, CH2), 2.38 (t, 2H, CH2CO), 3.15 (m, 2H, CH2NH), 6.66 (t, 1H, NHCH2), 6.78 (s, 2H, SO2NH2), 7.07 (dd, 2H of Ar of sulfanilamide), 7.13-7.69 (m, m, d, 6H, H6-8 of isatin, 2H of Ar of sulfanilamide and H5 of isatin), 7.29 (td, 1H, H-6 of isatin), 10.86 (d, 2H of NH of isatin and =N-NHCO); 13CNMR (100 MHz, DMSO-d6), ppm : 24.37, 25.90, 28.68, 33.65, 43.27, 111.85, 111.91, 119.70, 121.50, 122.33, 128.02, 129.31, 135.81, 138.67, 139.23, 143.15, 153.11, 168.87, 170.25; and the ESI-MS for $C_{20}H_{23}N_5O_4S$ calculated 429.15; found 430.2[M+1]+.

4-((6-(2-(5-fluoro-2-oxoindolin-3-ylidene) hydrazinyl)-6-oxohexyl)amino)benzene sulfonamide, Compound II

FT-IR (ATR; υ, cm-1): 3239, 3216 (NH2 Str. Vib. of amine), 3115 (NH Str. Vib. of amide), 2947 (C-H Asymmetric str. Vib. Of CH3), 2872 (C-H str. Vibration of CH2), 1699,1665 (C=O

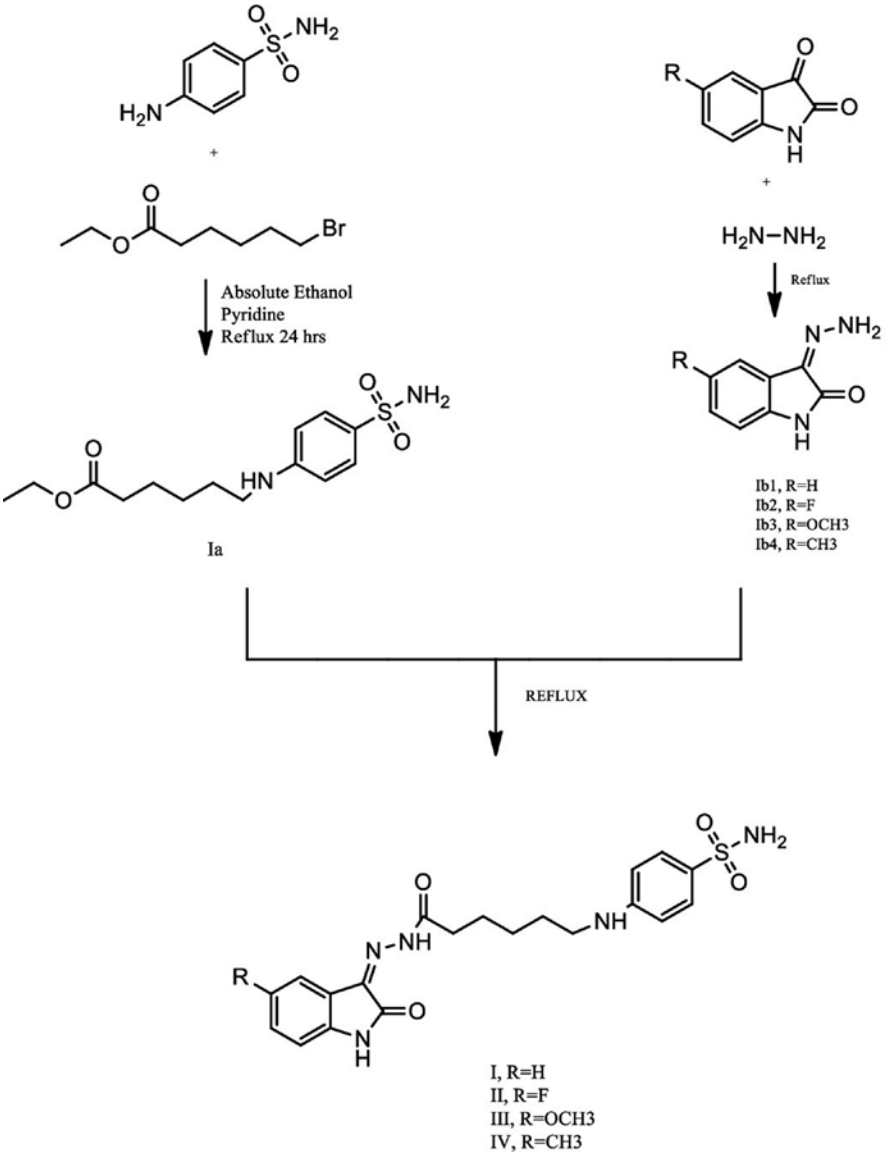


Fig. 3. Exemplify of the synthesis of the target substances (I - IV).

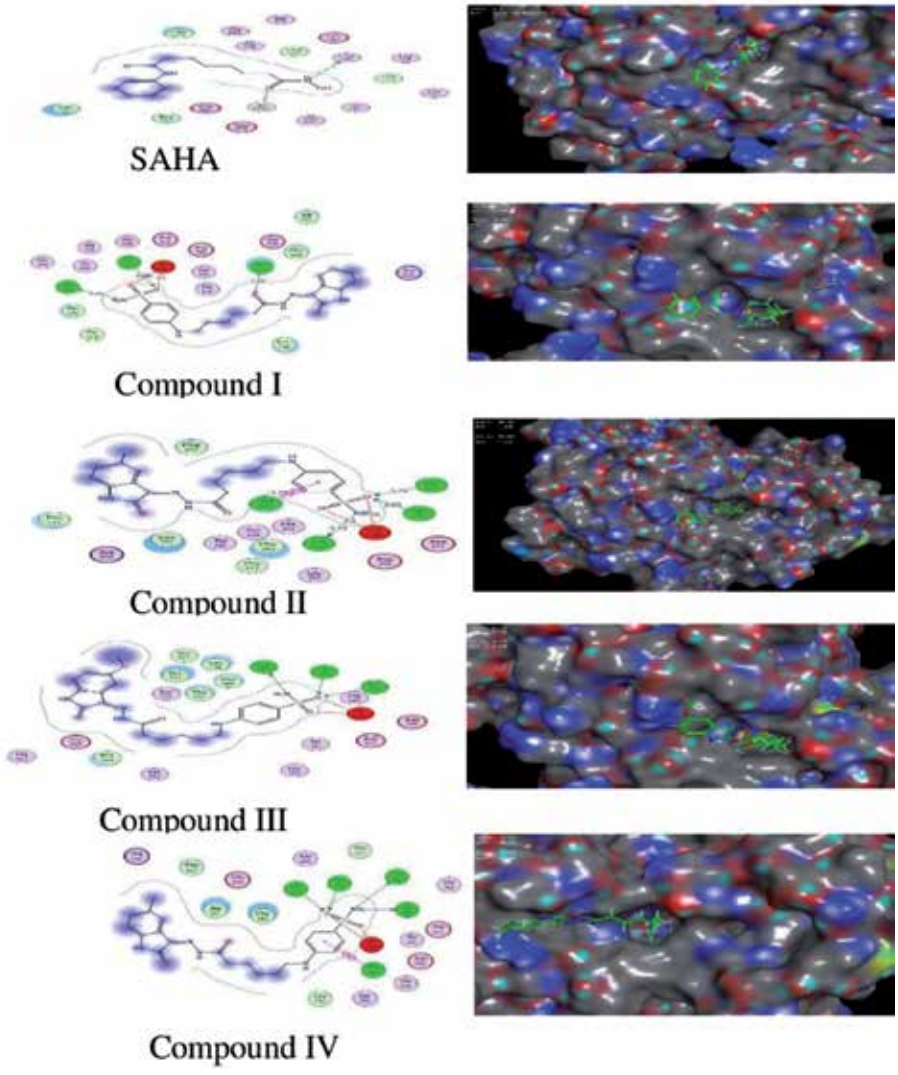


Fig. 4. Docking orientation and binding of synthesized compounds.

Str. vibration band of amide), 1627 (str. Vibration of C=N); 1HNMR (400 MHz, DMSO-d6), ppm: 1.49 (m, 2H, CH2), 1.62 (m, 4H, CH2), 2.42 (t, 2H, CH2CO), 3.15 (m, 2H, CH2NH), 6.42 (t, 1H, NHCH2), 6.65 (s, 2H, SO2NH2), 7.11 (dd, 2H, of Ar of sulfanilamide), 7.42 (s, 1H, H4 of isatin), 7.67 (m, dd, 4H, H6 and H7of isatin and 2H of Ar of sulfanilamide), 10.95 (d, 2H of NH of isatin and =N-NHCO); 13CNMR (100 MHz, DMSO-d6), ppm : 24.00, 26.13, 28.53, 33.11, 43.83, 105.88, 111.46, 111.49, 113.15, 119.45, 122.32, 122.35, 128.05, 133.42, 137.10, 153.61, 159.16, 160.69, 167.70; and the ESI-MS for $C_{20}H_{22}FN_5O_4S$ calculated 447.5; found 448.1[M+1]+.

4-((6-(2-(5-methoxy-2-oxoindolin-3-ylidene) hydrazinyl)-6-oxohexyl)amino)benzene sulfonamide, Compound III

FT-IR (ATR; υ, cm-1): 3377, 3298 (NH2 Str. Vib. of amine), 3240 (NH Str. Vib. of amide), 2929, 2868 (C-H Asymmetric, Symmetric str. Vib. Of CH3), 2852 (C-H str. Vibration of CH2), 1646,1620 (C=O Str. vibration band of amide), 1614 (str. Vibration of C=N); 1HNMR (400 MHz, DMSO-d6), ppm : 1.46 (m, 2H, CH2), 1.71 (m, 4H, CH2), 2.42 (t, 2H, CH2CO), 3.19 (m, 2H, CH2NH), 3.79 (s, 3H, OCH3), 6.44 (t, 1H, NHCH2), 6.77 (s, 2H, SO2NH2), 7.01 (m, 3H, H6 of isatin and 2H of Ar of sulfanilamide), 7.12 (s, 1H, H4 of isatin), 7.63 (m, 3H, H-7 of isatin and 2H of Ar of sulfanilamide), 10.96 (d, 2H of NH of isatin and =N-NHCO); 13CNMR (100 MHz, DMSO-d6), ppm : 24.38, 25.90, 28.68, 33.68, 43.27, 55.82, 111.82, 111.85, 116.38, 118.62, 120.96, 120.99, 128.02, 130.58, 133.80, 135.82, 138.21, 139.59, 153.12, 162.97, 170.27; and the ESI-MS for $C_{21}H_{25}N_5O_5S$ calculated 459.5; found 460.2[M+1]+.

4-((6-(2-(5-methyl-2-oxoindolin-3-ylidene) hydrazinyl)-6-oxohexyl)amino)benzene sulfonamide, Compound IV

FT-IR (ATR; υ, cm-1): 3286, 3199 (NH2 Str. Vib. of amine), 3167 (NH Str. Vib. of amide), 2995, 2895 (C-H Asymmetric, Symmetric str. Vib. Of CH3), 2846 (C-H str. Vibration of CH2), 1705,1629 (C=O Str. vibration band of amide), 1614 (str. Vibration of C=N); 1HNMR (400 MHz, DMSO-d6), ppm : 1.49 (m, 2H, CH2), 1.62 (m, 4H, CH2), 2.46 (m, 5H, CH2,CH3), 3.16 (m, 2H, CH2NH), 6.49 (t, d, 2H, NHCH2 and H7 of isatin), 6.67 (dd, 3H, H6 of isatin and SO2NH2), 7.08 (m, 2H, of

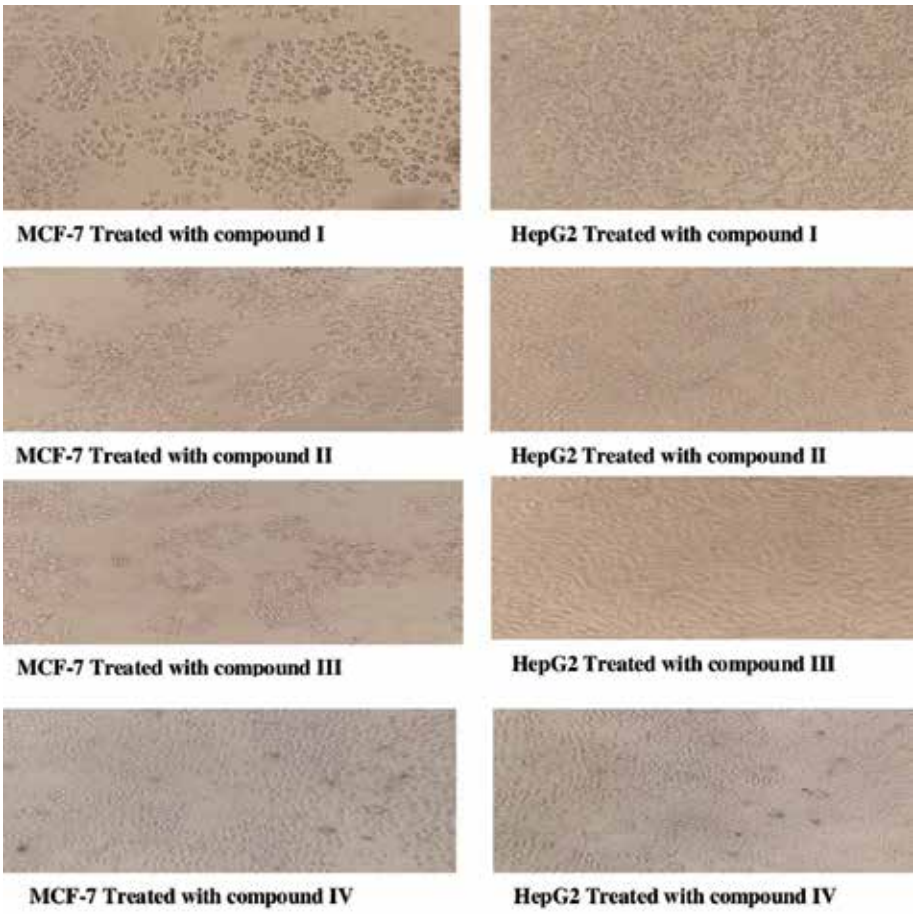


Fig. 5. The cell lines' morphology after being treated with chemicals I, II, III and IV.

Ar of sulfanilamide), 7.12 (m, 2H, of Ar of sulfanilamide), 7.61 (s, 1H, H4 of isatin), 10.93 (d, 2H of NH of isatin and =N-NHCO); 13CNMR (100 MHz, DMSO-d6), ppm: 24.36, 25.92, 28.66, 33.67, 43.28, 55.63, 111.58, 111.85, 115.83, 120.53, 123.41, 123.69, 127.97, 135.50, 135.92, 138.38, 141.95, 153.14, 155.58, 163.02, 170.14; and the ESI-MS for $C_{21}H_{25}N_5O_4S$ calculated 443.53; found 444.1[M+1]+.

IN VITRO CYTOTOXICITY STUDY MAINTENANCE OF CELL CULTURES

The cell lines were purchased from Pasteur Institute, Iran, the cells used are HepG2 [human liver cancer] and MCF7 [human breast cancer cell line]. Cells were grown and kept alive in (Gibco) RPMI-1640 and (Gibco) DMEM: F12 media, with (Gibco) 10% fetal bovine serum and antibiotics (100 g/ml streptomycin and 100 U/ml penicillin), respectively. The growing cells were passaged using [trypsin/EDTA] (Gibco) and (PBS) phosphate- buffered saline solution while being kept at 37°C in humidified air that contained (5% CO2) [35].

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 different concentration of the synthesized compounds which are (600, 200, 66.66, 22.22 and 7.4 µg/ml). 24 hours after the treatment ended, the supernatant was removed from the mono layer culture and then add 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 [32]. 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 [33].

FLUORESCENT STAINING

Fluorescent staining ethidium bromide (EB)/acridine orange (AO) (Sigma-Aldrich) was performed to assess rates of cellular viability (Live/Dead). Initially MCF-7 and HepG2. In a 12 well cell culture plate, cells were seeded and given a 24-hour treatment with 120 µg/ml of the compounds. A solution containing EB/AO was added after the cells had been rinsed with PBS, and the stained cells were then immediately seen and captured on camera using a fluorescent microscope (Axioskop 2 plus, Ziess, Germany).

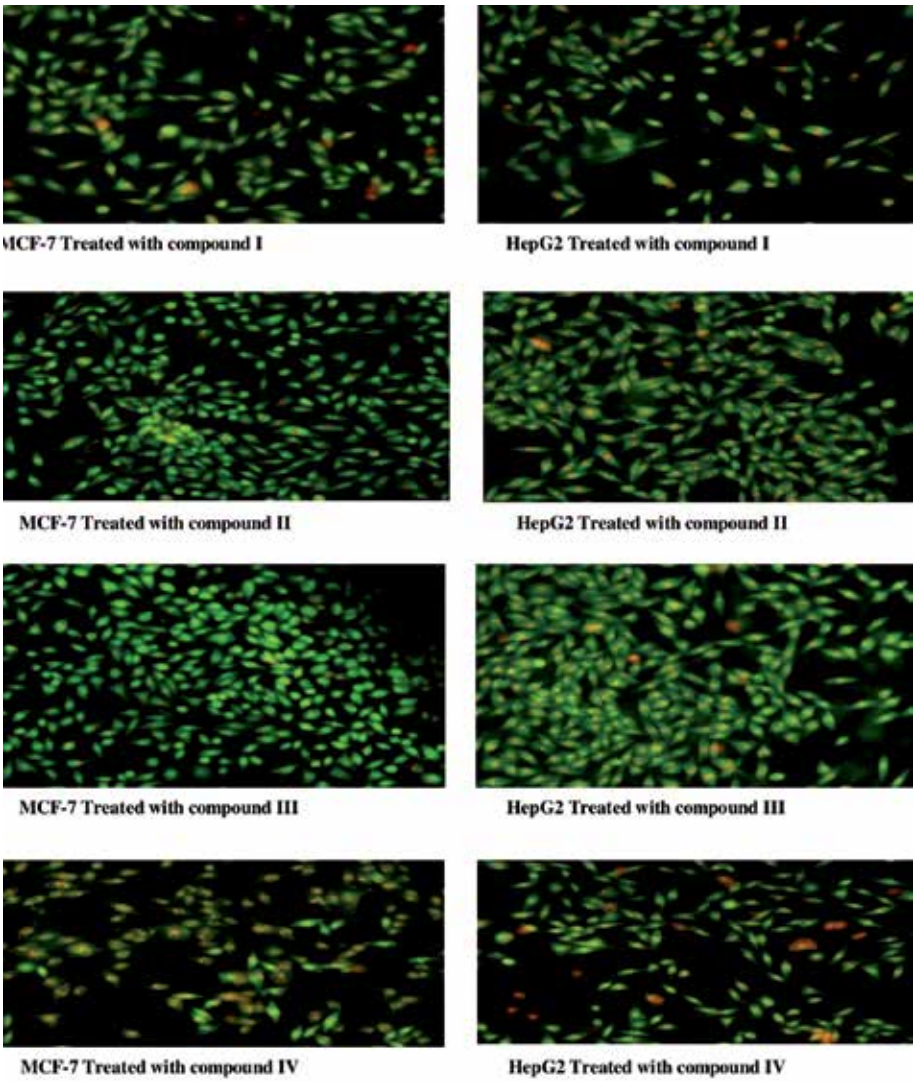


Fig. 6. Fluorescent images of selected cell lines.

MOLECULAR DOCKING

The software used for molecular docking studies was Molecular Operating Environment, version 2015.10 (Chemical Computing Group, Montreal, Canada). HDAC6 (5EDU in the PDB). The Protein Data Base (PDB) was used to extract X-ray crystal structures. Hydrogen atoms were then added to the protein after elimination all the water molecules. The chemical's optimized form was then docked into the binding site using the MOE-Dock technique. The Rigid Receptor was chosen as the final scoring technique after the London G was chosen as the first scoring system. Each ligand's top 5 stances were kept and scored. Finally, MOE's pose viewer utility examined the geometry of the docked complex [36, 37].

RESULTS

CHEMICAL SYNTHESIS

Fig. 3 illustrates the synthesis of the target compounds I-IV. 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. The synthesized compound gave the characterized amid band at 1688. Schiff base compound Ib1 – Ib4 were prepared from refluxing Isatin derivatives with hydrazine. The resulted compounds give the characterized imine band at 1537, 1587, 1598, 1577 and 3310, 3236 band for NH2.Compounds I – IV were obtaining from the reaction of compound Ia with compounds Ib1 – Ib4 respectively through nucleophilic substitution reaction. The IR, 1HNMR, 13CNMR and ESI mass spectrum for synthesized compounds show the disappearance of band and appearance new bands belong the newly formed functional groups (Fig. 3).

DOCKING STUDY RESULTS

For comparison purposes, Compounds I-IV together with SAHA were docked against [HDAC6] which obtained from protein data bank under the code (5EDU), the study of how these compounds docked with the desired enzyme was done by using MOE software version 2015.10. In vitro cytotoxicity results agreed with molecular docking studies, which provided a rationale for the greatest inhibitory activity. Table 1 lists the outcomes of docking with this isoform. Fig. 4 shows pictures showing how HDAC6's binding pocket is occupied. by target compounds and SAHA.

The docking study show data that are very good indicators that these compounds are good candidate as HDAC inhibitors when compared with FDA approved drug (SAHA).

THE CYTOTOXICITY ASSAY RESULTS

In vitro cytotoxicity of the compounds (I, II, III and IV) were assessed at micro concentrations multiplied by 3 times concentration according to the standard drug used (SAHA) (7.4, 22.22, 66.66, 200, 600 µg/ml) using the MTT assay. Two cell lines that overexpress HDAC6, a breast cancer cell line MCF-7, and human hepatocellular carcinoma HepG2 were employed. The cell proliferation and cytotoxic effect of the synthesized compounds was measured by MTT assay method [31]. 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 growth of the two cell lines (Table 2). Fig. 5 show the cell viability of compounds I, II, III and IV comparable to SAHA against selected cell lines.

FLUORESCENT STAINING

It is feasible to differentiate between living, apoptotic, and necrotic cells based on how differently a dye permeates an intact cell membrane. Figure 6 illustrates how necrotic cells are orange and stained with EB. Green cells, which are living cells, can only be stained with AO. Green and orange cells with condensed chromatin are indicative of early and late apoptotic cells; these cells are stained with AO and EB (with a slight decrease in membrane permeability) (Fig. 6).

DISCUSSION

The analysis result of the synthesized compound shows the following results for compound **I** show the disappearance of ester band and appearance of new bands belong to the newly formed functional groups.3286 (NH2 Str. Vib. of amine), 3165, 3103 (NH Str. Vib. of amide), 2995 (C-H Asymmetric str. Vib. Of CH3), 2895 (C-H str. Vibration of CH2), 1705,1688 (C=O Str. vibration band of amide), 1627 (str. Vibration of C=N); 1HNMR shows the presence of 2.38 (t, 2H, CH2CO), 3.15 (m, 2H, CH2NH), 6.66 (t, 1H, NHCH2), 6.78 (s, 2H, SO2NH2), 7.07 (dd, 2H of Ar of sulfanilamide), 7.13-7.69 (m, m, d, 6H, H6-8 of Isatin, 2H of Ar of sulfanilamide and H5 of Isatin), 7.29 (td, 1H, H-6 of Isatin), 10.86 (d, 2H of NH of Isatin and =N-NHCO); 13CNMR shows 43.27 (CH2NHAr), 139.23 (C=N), 153.11 (ArNHCH2), 168.87 (=N-NH-COCH2), 170.25 (CO of Isatin) and ESI-MS for $C_{20}H_{23}N_5O_4S$ calculated 429.15; found 430.2[M+1]+, while for compound II also the IR spectrum show disappearance of ester band and appearance of 3239, 3216 (NH2 Str. Vib. of amine), 3115 (NH Str. Vib. of amide), 2947 (C-H Asymmetric str. Vib. Of CH3), 2872 (C-H str. Vibration of CH2), 1699,1665 (C=O Str. vibration band of amide), 1627 (str. Vibration of C=N) ; 1HNMR shows the presence of 1.49 (m, 2H, CH2), 1.62 (m, 4H, CH2), 2.42 (t, 2H, CH2CO), 3.15 (m, 2H, CH2NH), 6.42 (t, 1H, NHCH2), 6.65 (s, 2H, SO2NH2), 7.11 (dd, 2H, of Ar of sulfanilamide), 7.42 (s, 1H, H4 of Isatin), 7.67 (m, dd, 4H, H6 and H7of Isatin and 2H of Ar of sulfanilamide), 10.95 (d, 2H of NH of Isatin and =N-NHCO); 13CNMR shows 43.83 (CH2NHAr), 137.10 (C=N), 153.16 (ArNHCH2), 160.69 (CO of Isatin), 167.70 (=N-NH-COCH2) and ESI-MS for $C_{20}H_{22}FN_5O_4S$ calculated 447.5; found 448.1[M+1]+. For compound III the IR spectrum show disappearance of ester band and appearance of 3377, 3298 (NH2 Str. Vib. of amine), 3240 (NH Str. Vib. of amide), 2929, 2868 (C-H Asymmetric Symmetric str. Vib. of CH3), 2852 (C-H str. Vibration of CH2), 1646, 1620 (C=O Str. vibration band of amide), 1614 (str. Vibration of C=N); 1HNMR shows the presence of 1.46 (m, 2H, CH2), 1.71 (m, 4H, CH2), 2.42 (t, 2H, CH2CO), 3.19 (m, 2H, CH2NH), 3.79 (s, 3H, OCH3), 6.44 (t, 1H, NHCH2), 6.77 (s, 2H, SO2NH2), 7.01 (m, 3H, H6 of Isatin and 2H of Ar of sulfanilamide), 7.12 (s, 1H, H4 of Isatin), 7.63 (m, 3H, H-7 of Isatin and 2H of Ar of sulfanilamide), 10.96 (d, 2H of NH of Isatin and =N-NHCO); 13CNMR shows 43.27 (CH2NHAr), 55.82 (OCH3), 138.21 (C=N), 139.59 (ArNHCH2), 162.97 (=N-NH-COCH2), 170.27 (CO of Isatin); and the ESI-MS for $C_{21}H_{25}N_5O_5S$ calculated 459.5; found 460.2[M+1]+. For final compound IV the IR spectrum show disappearance of ester band and appearance of 3286, 3199 (NH2 Str. Vib. of amine), 3167 (NH Str. Vib. of amide), 2995, 2895 (C-H Asymmetric, Symmetric str. Vib. Of CH3), 2846 (C-H str. Vibration of CH2), 1705, 1629 (C=O Str. vibration band of amide), 1614 (str. Vibration of C=N); 1HNMR shows the presence of 1.49 (m, 2H, CH2), 1.62 (m, 4H, CH2), 2.46 (m, 5H, CH2,CH3), 3.16 (m, 2H, CH2NH), 6.49 (t, d, 2H, NHCH2 and H7 of Isatin), 6.67 (dd, 3H, H6 of Isatin and SO2NH2), 7.08 (m, 2H, of Ar of sulfanilamide), 7.12 (m, 2H, of Ar of sulfanilamide), 7.61 (s, 1H, H4 of Isatin), 10.93 (d, 2H of NH of Isatin and =N-NHCO); 13CNMR shows 24.36 (CH3Ar), 55.63 (CH2NHAr), 141.95 (C=N), 155.58 (ArNHCH2), 163.02 (=N-NH-COCH2), 170.14 (CO of Isatin); and the ESI-MS for $C_{21}H_{25}N_5O_4S$ calculated 443.53; found 444.1[M+1]+.

While the docking and cytotoxic assay show that these molecules in compares with the FDA approved show good results in the binding free energy with active site of enzyme according to docking study and the cytotoxicity results show good values of IC50 as showed by cell pictures and florescent pictures

CONCLUSIONS

Synthesis of the target compounds (I, II, III and IV) was achieved successfully starting from 4- aminobenzene sulfonamide, ethyl-6-bromo hexanote and Isatin. 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 Isatin as the cap group and sulfonamide functionality as a ZBG. As a result, we are motivated to broaden the scope of our ongoing research to examine the cytotoxicity assay to numerous cancer cell lines, investigate in vivo toxicity, and determine their pharmacokinetic properties.

REFERENCES

1. Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. Nat Rev Drug Discov. 2006;5(9):769-784. doi:10.1038/nrd2133.
2. Alibeg AAA, Abdulsada AH, Nasser NH et al. Design and synthesis of possible mutual prodrugs of (nsaid) etodolac and tolmetin with (cytotoxic) gemcitabine. Sys Rev Pharm 2020;11(11):315-318.
3. Alibeg AAA, Hasan SA, Hussein AK et al. Design, synthesis and preliminary pharmacological evaluation of mutual prodrug of non steroidal anti-inflammatory drugs coupling with natural anti-oxidants. International Journal of Pharmaceutical Research (09752366). 2020;12(2):3707-3713. doi:10.31838/ijpr/2020.SP2.450.
4. Baylin SB, Ohm JE. Epigenetic gene silencing in cancer - a mechanism for early oncogenic pathway addiction? Nat Rev Cancer. 2006;6(2):107-116. doi:10.1038/nrc1799.
5. Giannini G, Cabri W, Fattorusso C et al. Histone deacetylase inhibitors in the treatment of cancer: overview and perspectives. Future Med Chem. 2012;4(11):1439-1460. doi:10.4155/fmc.12.80.
6. Micelli C, Rastelli G. Histone deacetylases: structural determinants of inhibitor selectivity. Drug Discov Today. 2015;20(6):718-735. doi:10.1016/j.drudis.2015.01.007.
7. Shinsky SA, Christianson DW. Polyamine deacetylase structure and catalysis: prokaryotic acetylpolyamine amidohydrolase and eukaryotic HDAC10. Biochemistry. 2018;57(22):3105-3114. doi:10.1021/acs.biochem.8b00079.
8. Delehanty LL, Bullock GC, Goldfarb AN. Protein kinase D-HDAC5 signaling regulates erythropoiesis and contributes to erythropoietin cross-talk with GATA1. Blood. 2012;120(20):4219-4228. doi:10.1182/blood-2011-10-387050.
9. Gao HM, Jiang J, Wilson B et al. Microglial activation-mediated delayed and progressive degeneration of rat nigral dopaminergic neurons: relevance to Parkinson's disease. J Neurochem. 2002;81(6):1285-1297. doi:10.1046/j.1471-4159.2002.00928.x.
10. Gharia BK, Upadhyay J, Suhagia BN et al. Current perspective of histone deacetylase inhibitors: A review. International Journal of Pharmaceutical Chemistry and Analysis (IJPCA). 2018;5(3):108-122. doi: 10.18231/2394-2797.2018.0018.
11. Tillekeratne LM, Al-Hamashi AA, Dlamini S et al. In search of selectivity: design, synthesis, and biological evaluation of new classes of HDAC inhibitors. Multidisciplinary Digital Publishing Institute Proceedings. 2019;22(1):63. doi:10.3390/proceedings2019022063.
12. Dokmanovic M, Clarke C, Marks PA. Histone deacetylase inhibitors: overview and perspectives. Mol Cancer Res. 2007;5(10):981-989. doi:10.1158/1541-7786.MCR-07-0324.
13. Koutsounas I, Giaginis C, Patsouris E et al. Current evidence for histone deacetylase inhibitors in pancreatic cancer. World J Gastroenterol. 2013;19(6):813-828. doi:10.3748/wjg.v19.i6.813.
14. Marks PA, Xu WS. Histone deacetylase inhibitors: Potential in cancer therapy. J Cell Biochem. 2009;107(4):600-608. doi:10.1002/jcb.22185.
15. Al-Amily DH, Mohammed MH. Design, synthesis and cytotoxicity study of primary amides as histone deacetylase inhibitors. Iraqi J. Pharm. Sci. 2019;28(2):151-158. doi:10.31351/vol28iss2pp151-158.
16. Al-Amily DH, Mohammed MH. Design, synthesis, and docking study of acyl thiourea derivatives as possible histone deacetylase inhibitors with a novel zinc binding group. Sci. Pharm. 2019;87(4):1-15. doi: 10.3390/scipharm87040028.
17. Sagheer OM, Mohammed MH, Wadi JS et al. Studying the cytotoxic activity of newly designed and synthesized HDAC inhibitors derivatives of pentanoyl anilide-5-biguanide. Macromol. Symp. 2022;401(1):1-10. doi:10.1002/masy.202100346.
18. Yang XJ, Grégoire S. Class II histone deacetylases: from sequence to function, regulation, and clinical implication. Mol Cell Biol. 2005;25(8):2873-2884. doi:10.1128/MCB.25.8.2873-2884.2005.
19. Seidel C, Schnekenburger M, Zwergel C et al. Novel inhibitors of human histone deacetylases: design, synthesis and bioactivity of 3-alkenoylcoumarines. Bioorg Med Chem Lett. 2014;24(16):3797-3801. doi:10.1016/j.bmcl.2014.06.067.
20. Luan Y, Li J, Bernatchez JA et al. Kinase and histone deacetylase hybrid inhibitors for cancer therapy. J Med Chem. 2019;62(7):3171-3183. doi:10.1021/acs.jmedchem.8b00189.
21. Jabbar SS, Mohammed HM. Coumarin based-histone deactylace HADC inhibitors. Egypt. J. Chem. 2022;65(7):379-384. doi:10.21608/EJCHEM.2021.104398.4821.
22. Sagheer OM, Mohammed HM, Ibraheem ZO et al. Synthesis of gamma biguanides butyric acid analogues as HDAC inhibitors and studying their cytotoxic activity. Materials today: Proceedings. 2021;47(17):5983-5991. doi:10.1016/j.matpr.2021.04.539.
23. Xu WS, Parmigiani RB, Marks PA. Histone deacetylase inhibitors: molecular mechanisms of action. Oncogene. 2007;26(37):5541-5552. doi:10.1038/sj.onc.1210620.
24. Inoue A, Fujimoto D. Enzymatic deacetylation of histone. Biochem Biophys Res Commun. 1969;36:146-150. doi: 10.1016/0006-291x(69)90661-5.
25. Hubbert C, Guardiola A, Shao R et al. HDAC6 is a microtubule-associated deacetylase. Nature. 2002;417(6887):455-458. doi:10.1038/417455a.
26. Varga JK, Diffley K, Welker Leng KR et al. Structure-based prediction of HDAC6 substrates validated by enzymatic assay reveals determinants of promiscuity and detects new potential substrates. Sci Rep. 2022;12(1):1788. doi:10.1038/s41598-022-05681-2.
27. Yan J. Interplay between HDAC6 and its interacting partners: essential roles in the aggresome-autophagy pathway and neurodegenerative diseases. DNA Cell Biol. 2014;33(9):567-580. doi:10.1089/dna.2013.2300.
28. Bali P, Pranpat M, Bradner J et al. Inhibition of histone deacetylase 6 acetylates and disrupts the chaperone function of heat shock protein 90: a novel basis for antileukemia activity of histone deacetylase inhibitors. J Biol Chem. 2005;280(29):26729-26734. doi:10.1074/jbc.C500186200.
29. Moreno-Gonzalo O, Ramírez-Huesca M, Blas-Rus N et al. HDAC6 controls innate immune and autophagy responses to TLR-mediated signalling by the intracellular bacteria Listeria monocytogenes. PLoS Pathog. 2017;13(12):e1006799. doi:10.1371/journal.ppat.1006799.
30. Lin HY, Chen CS, Lin SP et al. Targeting histone deacetylase in cancer therapy. Med Res Rev. 2006;26(4):397-413. doi:10.1002/med.20056.
31. Dadashpour S, Tuylu Kucukkilinc T, Unsal Tan O et al. Design, synthesis and in vitro study of 5,6-diaryl-1,2,4-triazine-3-ylthioacetate derivatives as COX-2 and β-amyloid aggregation inhibitors. Arch Pharm (Weinheim). 2015;348(3):179-187. doi:10.1002/ardp.201400400.

32. Hussain HY. Synthesis of some new sulfanilamide derivatives. J Edu Sci. 2007;19(1):9-17.
33. Shakir TH, Al-Mudhafar MMJ. Synthesis and preliminary antimicrobial evaluation of Schiff bases of N-Benzyl Isatin derivatives. Sys Rev Pharm. 2020;11(12):1950-1955. doi: 10.5530/srp.2020.1.01.
34. Aliabadi A, Shamsa F, Ostad SN et al. Synthesis and biological evaluation of 2-phenylthiazole-4-carboxamide derivatives as anticancer agents. Eur J Med Chem. 2010;45(11):5384-5389. doi:10.1016/j.ejmech.2010.08.063.
35. Jabir MS, Taha AA, Sahib UI et al. Novel of nano delivery system for Linalool loaded on gold nanoparticles conjugated with CALNN peptide for application in drug uptake and induction of cell death on breast cancer cell line. Mater Sci Eng C Mater Biol Appl. 2019;94:949-964. doi:10.1016/j.msec.2018.10.014.
36. Sulaiman GM, Jabir MS, Hameed AH. Nanoscale modification of chrysin for improved of therapeutic efficiency and cytotoxicity. Artif Cells Nanomed Biotechnol. 2018;46(1):708-720. doi:10.1080/21691401.2018.1434661.
37. Ferreira LG, Dos Santos RN, Oliva G et al. Molecular docking and structure-based drug design strategies. Molecules. 2015;20(7):13384-13421. doi:10.3390/molecules200713384.

We would like to express my gratitude to my supervisor, Dr. Mohammed Hasan Mohammed, chairman of the pharmacology department/ college of pharmacy/ University of Baghdad, for his unwavering support.

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Ammar Abdul Aziz Alibeg
University of Kufa
299G+HPX, Najaf 54003, Iraq
e-mail: sgahmed1331962@outlook.com

ORCID AND CONTRIBUTIONSHIP
Ammar Abdul Aziz Alibeg: 0009-0009-4975-7462 A - C
Mohammed Hassan Mohammed: 0000-0001-9125-1611 D - F

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

RECEIVED: 22.08.2023
ACCEPTED: 08.02.2024

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.

Media partners of the conference:

1. ALUNA Publishing House (Warsaw, Poland).

2. Journal "Clinical and Preventive Medicine" (SCOPUS) (State Institution of Science "Scientific and Practical Center of Preventive and Clinical Medicine" State Administrative Department, Kyiv, Ukraine).

ORIGINAL ARTICLE

Gender and age-related features of anxiety and depressive symptoms in patients in the acute phase of COVID-19

Iryna Mudrenko, Vitalii Mudrenko, Oksana Kolenko, Anastasia Kotkova, Ludmyla Kiptenko
SUMY STATE UNIVERSITY, SUMY, UKRAINE

ABSTRACT

Aim: To investigate the gender and age characteristics of psychopathological symptoms in patients in the acute phase of respiratory infection SARS-CoV-2.

Materials and Methods: A total of 66 patients aged 8 to 92 years, sick with COVID-19 during inpatient treatment with lung damage from 30 to 70%, confirmed radiologically, in the period up to 20 days from the onset of the disease, were examined. To assess the mental status of patients with COVID-19, we used a clinical, clinical-psychopathological, psychometric method, which included the following questionnaires as part of psychiatric screening for studying psychopathological symptoms upon admission to the hospital. The Patient Health Questionnaire – PHQ-9, The Generalised Anxiety Disorder – GAD-7, Hamilton Test were used. The research data collection period is from 2020 to 2022.

Results: Acute respiratory infection COVID-19 affects the psycho-emotional state. During inpatient treatment, 60.6% of patients have symptoms of depression, 66.1% have anxiety symptoms. Most women with COVID-19 are characterized by moderate depression according to PHQ-9, mild anxiety according to GAD-7, and moderate anxiety according to Hamilton. Mild depression characterizes most men according to PHQ-9, absence of anxiety according to GAD-7, and mild anxiety according to Hamilton. Anxiety symptoms were less pronounced among men with COVID-19, with insomnia and respiratory symptoms being the most significant. Among women, anxiety ($p \leq 0.001$), tension, depression ($p \leq 0.001$), somatic muscle ($p \leq 0.001$) and respiratory symptoms ($p \leq 0.001$) predominate in the structure of anxiety during the active phase of COVID-19.

Conclusions: The data obtained will be useful in the development of psychoprophylactic measures in patients with COVID-19.

KEY WORDS: COVID-19, depression, anxiety, behavioural disorders

Pol Merkur Lek,2024;53(1):189-196. doi: 10.36740/Merkur202402107

INTRODUCTION

WHO emphasizes that the COVID-19 pandemic harms mental health. A study by researchers at the University of Oxford shows that about 20% of patients with COVID-19 have mental disorders. A survey of college students in China showed that acute stress, anxiety and depressive symptoms were everyday during the COVID-19 pandemic, with 45% of respondents experiencing some symptoms of mental disorders [1]. Other studies show an increase in the prevalence of depression in the population during the COVID-19 pandemic by 27.6%, and anxiety disorders by 25.6% [1, 2]. Literature data on risk factors for mental disorders during the pandemic: students who watched media ≥3 hours per day, low social support, advanced age, mental disorders in the anamnesis [3].

Medical workers are at risk of developing mental disorders during the pandemic. According to a large-scale single-center cross-sectional survey of 5062 healthcare workers at a specialized hospital for severe patients with COVID-19 (Wuhan), 29.8% reported stress symptoms, 13.5% reported depression, 24.1% reported anxiety. Moreover, female gender, work experience of more than ten years, concomitant chronic diseases, personal history of mental disorders, and the presence of family members or relatives of patients diagnosed with COVID-19 or likely infected persons were risk factors for stress [4]. A study by American scientists revealed a direct relationship between the number of working hours of medical workers treating patients with COVID-19 and the level of depression, anxiety, and post-traumatic stress disorders (PTSD) [4, 5].

The spectrum of psychological consequences of the pandemic for humanity is significant, from the experience of negative emotions (fear of death, anger, anxiety, panic, insomnia), distress in caregivers and medical workers, cases of auto-aggression and completed suicides [6, 7, 8]. Delirium, delusions, and agitation often complicate a severe course of COVID-19. About a third of patients, after discharge from the hospital, experience anxiety, asthenia, sleep problems, and depressed mood for a long time [7-9]. And patients who suffer from long-term respiratory distress syndrome and receive artificial lung ventilation have disorders of concentration of attention, inhibition of thinking, neurocognitive deficit and "brain fog" [2, 6].

All of the above determines the relevance of the topic and requires further research into the gender and age characteristics of psychopathological manifestations in patients with COVID-19.

AIM

To investigate the gender and age characteristics of psychopathological symptoms in patients in the acute phase of respiratory infection SARS-CoV-2.

MATERIALS AND METHODS

A total of 66 patients aged 8 to 92 years with COVID-19 during inpatient treatment with lung involvement of 30 to 70%, confirmed by radiology, were examined within 20 days of the onset of the disease. An analysis of the patients' socio-demographic and clinical anamnestic data showed the following. By gender, the patients were equally distributed at 50 % – 33 women and 33 men. The age distribution was as follows: from 8 to 18 years – (3.03 ± 2.13)% (n=2), from 19 to 30 years – (18.2 ± 4.78)% (n=12), from 31 to 50 years (24.2 ± 5.32)% (n=16), from 51 to 70 years – (25.8 ± 5.42)% (n=17), from 71 to 92 years – (28.8 ± 5.62)% (n=19). At the same time, more than half (54.5%) (n=36) were patients older than 50 years. The average age of patients was 53±5.18 years. Among the examined patients, (54.5±6.18)% (n=36) had concomitant diseases. Somatic pathology was represented by hypertension in (30.6±4.62)% (n=11) of patients, diabetes mellitus in (22.2±4.05)% (n=8), chronic peptic ulcer disease in (8.33±2.58)% (n=3), osteochondrosis in (8.33±2.58)% (n=3), and other diseases in (30.6±4.62)% (n=11). Marital status of patients: (42.4±6.13)% (n=28) were single, respectively (57.6±6.13)% (n=38) had a partner.

To assess the mental status of patients with COVID-19, we used a clinical, clinical-psychopathological, psychometric method, which included the following questionnaires as part of psychiatric screening for the study of psychopathological symptoms upon admission to the hospital.

The Patient Health Questionnaire – PHQ-9 (Robert Spitzer, Janet Williams, Kurt Kronke, 2001) is a 9-item depression self-administered questionnaire designed to identify possible cases of depression and assess the severity of symptoms over the past two weeks. The Depression Rating Scale has been shown to be reliable in a variety of medical settings. The total score ranges from 0 to 27, with higher scores indicating more severe symptoms of depression. The scores were classified as follows: no depression (0-4), mild depression (5-9), moderate depression (10-14), moderately severe depression (15-19), and extremely severe depression (20-27).

The Generalised Anxiety Disorder – GAD-7 (Robert Spitzer, Janet Williams, Kurt Kroenke, 2006) is a 7-item self-report anxiety questionnaire with high reliability for primary care patients and the general population. All items are scored on a 4-point scale, and the total score ranges from 0 to 21 and is interpreted as follows: no anxiety (0-4), mild anxiety (5-9), moderate anxiety (10-14), and severe anxiety (15-21).

The Hamilton Anxiety Rating Scale (Hamilton, 1959) assesses the level of anxiety based on clinical questions, which allows to quantify the severity of anxiety syndrome. Scores of ≤17 indicate a mild degree of anxiety, 18 to 24 indicate a moderate degree of anxiety, 25-30 indicate a severe degree of anxiety, and scores >30 indicate very severe anxiety.

The statistical processing of the study results was carried out on a personal computer using Microsoft Excel software (Microsoft Office 2016 Professional Plus, Open License 67528927). Was calculated the mean and its error, calculated the percentage and its error, calculated the Student's criterion (t) and the significance of differences (p), and calculated the correlation between the parameters and their reliability (r).

Meta-analysis was to investigate gender and age-related peculiarities of psychopathological symptoms in patients in the acute phase of SARS-CoV-2 respiratory infection. The study was conducted at the Regional Hospitals during inpatient treatment of acute respiratory infection COVID-19, confirmed by laboratory tests in compliance with the principles of bioethics and medical deontology. The survey data collection period is from 2020 to 2022. All survey participants agreed to participate and provided verbal informed consent prior to participating in the study.

RESULTS

The study of the severity of depression using the PHQ-9 questionnaire showed that among all respondents with COVID-19, 39.4% (n=26) did not have depression (0-4 points), 18, 2% (n=12) – with mild depression (5-9 points), 31.8% (n=21) – with moderate depression (10-14 points), 9.09% (n=6) – with moderate-severe depression (15-19 points), 1.52% (n=1) – with severe depression (20-27 points) (Fig.1). The average severity of depression according to the PHQ-9 test was 7.93±0.68 points, which corresponds to the level of mild depression, the average severity of depressive symptoms among women was 10±0.96 points, which corresponds to a moderate level of depression, and among men – 6.27±0.91 points, which corresponds to a mild level of depression, with differences being significant at the level of p≤0.001.

When studying gender specifics of depressive symptoms, it was found that among women with COVID-19, 24.2% (n=8) did not have depression, 21.2% (n=7) had signs of mild depression, 39.4% (n=13) had moderate depression, 12.1% (n=4) had moderate-severe depression, and 3.03% (n=1) had severe depression (see Fig.2). In contrast, among men with COVID-19, 54.5% (n=18) did not have depression, 15.2% (n=5) had mild depression, 24.2% (n=8) had moderate depression, 6.06% (n=2) had moderate-severe depression, and no man had severe depression.

Statistical analysis of the data showed that women are prone to a depressive type of response in the form of various degrees of depression (from mild to severe) (p≤0.05) Most men (54.5%) did not have depressive symptoms, compared to women (24.2%) (p≤0.001) (see Fig.2).

In the clinical picture of depressive symptoms among women, the most pronounced symptoms were "problems with falling asleep, insomnia, sleeping a lot" (1.27±1.98) points, "feeling tired or losing strength" (1.58±2.22) points, "lack of appetite or overeating" (1.09±1.84) points, "anxious anticipation of terrible events" (1.09±1.84) points. In men, the peculiarities of depressive symptoms were the prevalence of such symptoms as "indifference, oppression" (1.12±1.86)

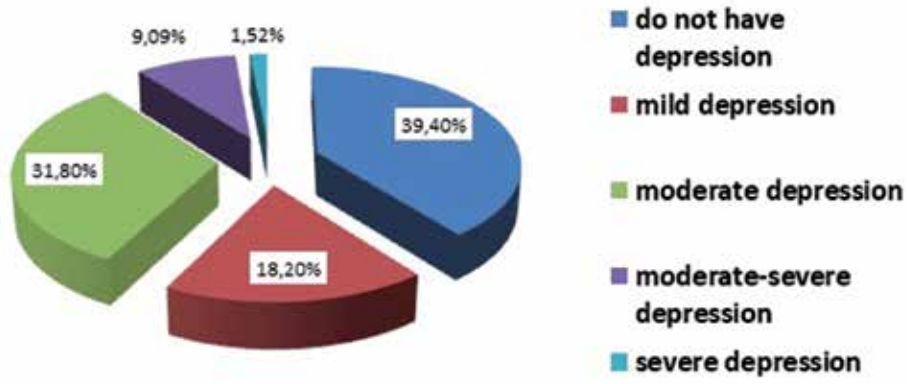


Fig. 1. Features of depressive symptoms according to the PHQ-9 questionnaire among patients with COVID-19.

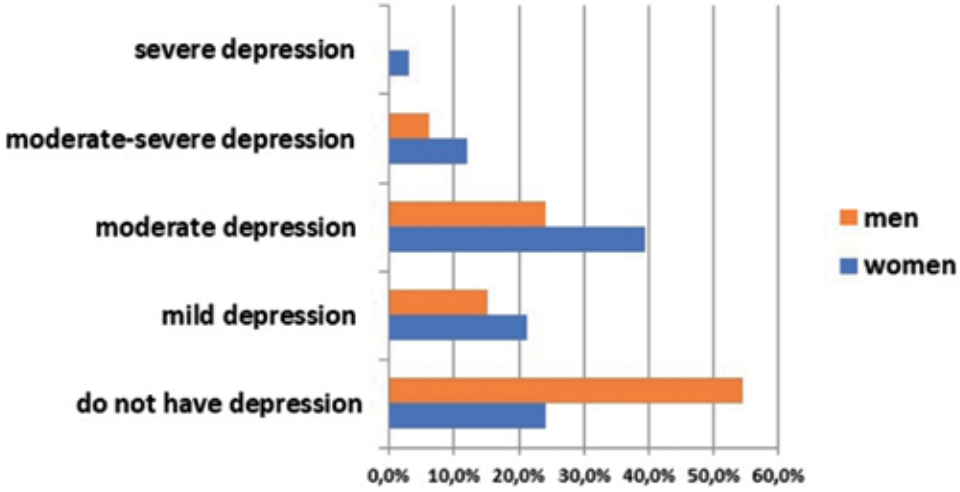


Fig. 2. Gender characteristics of depressive symptoms according to the PHQ-9 questionnaire among patients with COVID-19.

points, "problems with falling asleep, insomnia, sleeping a lot" (1.61±2.22) points, "feeling tired or losing strength" (1.18±1.91) points, "lack of appetite or overeating" (1.48±2.14) points.

Also, based on the results of the PHQ-9, a comparative analysis of the peculiarities of depressive symptoms among men and women was conducted. It was found that indifference and depression were more pronounced among men ((1.12±1.86) points) than among women ((0.88±1.65) points) ($p \leq 0.01$); problems with falling asleep, insomnia, sleeping a lot, and lack of appetite or overeating were also more prevalent among men than women ($p \leq 0.1$); the presence and severity of fatigue or loss of energy, anxious anticipation of terrible events, and feeling like a failure distinguished women from men with COVID-19 ($p \leq 0.1$) (see Table 1).

Statistical analysis of the correlations between depressive symptoms and age shows a greater affinity between all depressive symptoms (according to the PHQ-9) and age among women. A strong direct correlation was found among women between symptoms of sleep disturbance and age r=0.518, slowed speech rate and age r=0.591, and suicidal thoughts and intentions and age r=0.647; moderate direct correlations were found between the symptoms "lack of interest in events" r=0.412, "indifference, oppression" r=0.311, "lack of appetite or overeating" r=0.426, "anxious anticipation of terrible events" r=0.466, "do you move or speak unusually slowly or vice versa? " r=0, 591. Among men, there was a direct strong correlation between age and feeling tired or losing energy r=0.521, moderate direct correlations between indifference, oppression r=0.303, lack of appetite or overeating r=0.332 and age.

Additionally, we studied the age-related features of the formation of depressive symptoms. We found a direct linear relationship between age and total PHQ-9 score in the form of an average correlation of r=0.436, with a strong relationship among women r=0.728 and a weak relationship among men r=0.186 (see Fig. 3). Thus, the risk of depressive symptoms in hospitalised patients with COVID-19 increases proportionally with age.

The study of patients' anxiety levels using the GAD-7 questionnaire showed that among the respondents with COVID-19, 36.4% (n=24) had anxiety, 43.9% (n=29) did not, 25.8% (n=17) had mild anxiety, 13.6% (n=9) had

Table 1. Gender peculiarities of depressive symptoms (according to PHQ-9) among patients with COVID-19

Symptoms	Patients			P
	All patients	Women	Men	
1	2	3	4	5
Lack of interest in events (0-3 points)	0,91±1,18	0,15±1,89	0,67±1,44	0,2
Indifference, oppression (0-3 points)	1,02 ±1,24	0,88±1,65**	1,12±1,86**	0,01
Problems falling asleep, insomnia, sleeping a lot (0-3 points)	1,45 ±1,48	1,27±1,98*	1,61±2,22*	0,1
Feeling tired or losing energy (0-3 points)	1,38 ±1,45	1,58±2,22*	1,18±1,91*	0,1
Lack of appetite or overeating (0-3 points)	1,31±1,41	1,09±1,84*	1,48±2,14*	0,1
You feel like a failure (0-3 points)	0,49±0,87	0,64±1,41*	0,33±1,02*	0,1
Anxious anticipation of terrible events (0-3 points)	0,91±1,18	1,09±1,84*	0,7±1,47*	0,1
Do you move or speak unusually slowly or vice versa? (0-3 points)	0,82±1,12	0,58±1,34	1,03±1,79	0,2
Thoughts of suicide or self-harm? (0-3 points)	0,32±0,7	0,45±1,19	0,18±0,75	0,2
Average level of depression	7,93±0,68	10±0,96*	6,27±0,91*	0,1

*Notation: probability of differences at the level of *p≤0.1, **p≤0.01*

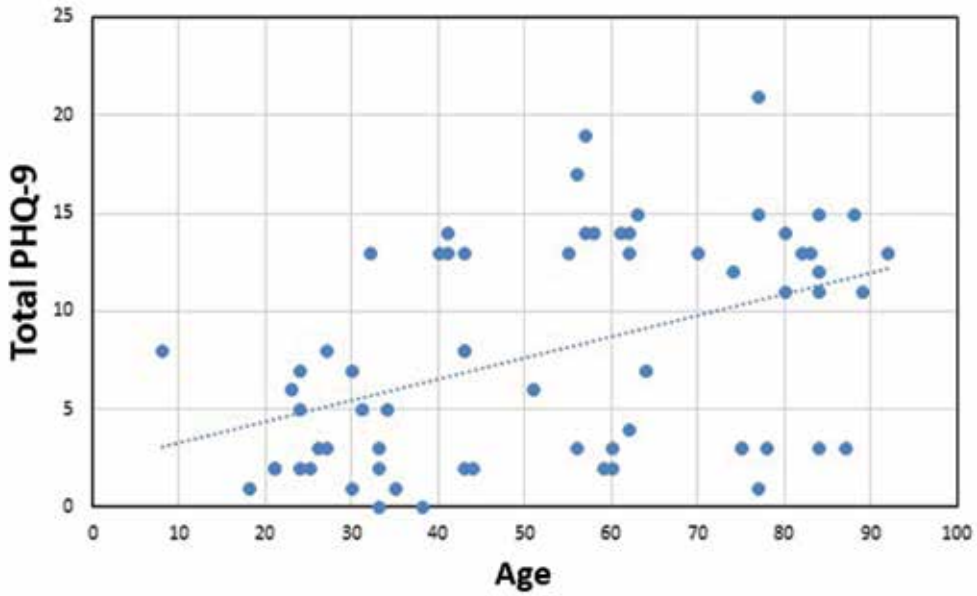


Fig. 3. Dot plot of the relationship between the severity of depressive symptoms according to the PHQ-9 questionnaire and age.

moderate anxiety, and 16.7% (n=11) had severe anxiety. The mean anxiety level (M) on the GAD-7 was 6.484±0.667 points (see Fig.3).

The study of anxiety severity by gender showed that, according to the GAD-7 questionnaire, among women with COVID-19, 24.2% (n=8) of women had mild anxiety, 24.2% (n=8) had moderate anxiety, 24.2% (n=8) had severe anxiety and 27.3% (n=9) had no anxiety. Among men, 27.3% (n=9) had mild anxiety, 3.03% (n=1) had moderate anxiety, 9.09% had severe anxiety, and 60.6% (n=20) had no anxiety (see Fig.5).

At the same time, probable differences between the compared groups were found: among women, there were more patients with moderate (p≤0.001) and severe anxiety (p≤0.01), and among men, there were more patients with no anxiety (p≤0.01).

The study of patients' anxiety level using the Hamilton Anxiety Inventory showed that among the respondents with COVID-19, 57.6% (n=38) have mild anxiety, 21.2% (n=14) have moderate anxiety, 19.7% (n=13) have severe anxiety, and 1.52% (n=1) have very severe anxiety. The average Hamilton score (M) = 17.621, moderate anxiety, m=1.314, among men this score was 14 points, which corresponds to a mild level of anxiety, among women 21 points, which corresponds to a moderate level of anxiety (significance of differences p≤0.001).

The study of the level of anxiety among women showed that 39.4% (n=13) had mild anxiety, 36.4% (n=12) – moderate anxiety, 21.2% (n=7) – severe anxiety, 3.03% (n=1) – very severe anxiety. In contrast, 75.8% of men (n=25) had mild anxiety, 6.06 (n=2) had moderate anxiety, and 18.2% (n=6) had severe anxiety.

Thus, the results demonstrated that women were characterised by moderate ($p \leq 0.01$) and severe anxiety ($p \leq 0.001$), while most men were characterised by mild anxiety ($p \leq 0.001$) (Fig. 6).

The next stage of our work was to study the age and gender characteristics of anxiety symptoms in COVID-19. We conducted a detailed analysis of anxiety symptoms.

Statistical analysis of the structure of anxiety symptoms among men and women showed a significant prevalence of the overall level of anxiety and all its components among women ($p \leq 0.001$). It was also found that the following anxiety symptoms predominated in women anxious mood (1.7±2.28) points, tension (1.64±2.24) points, insomnia (1.21±1.93) points, depressed mood (1.03±1.79) points, somatic muscle symptoms (1.58±2.2) points, somatic sensory symptoms (1.27±1.98) points, cardiovascular symptoms (1.33±2.03) points, respiratory symptoms (2.05±2.5) points, and autonomic symptoms (1.18±1.91) points.

Most of the anxiety symptoms were mild among men, with the highest severity in insomnia (1±1.76) and respiratory symptoms (1.09±1.84).

Uncharacteristic for patients in both groups was a violation of behaviour during the examination among women (0.48±1.23) points and (0.03±0.31) points among men and the presence of genitourinary symptoms, respectively (0.42±1.15) and (0.18±0.75) points.

The study revealed significant differences in the severity of the following components of anxiety among women compared to men anxious mood ((1.7±2.28) points, vs. (0.45±1.19) points) ($p \leq 0.001$), tension ((1.64±2.24) points, vs, (0.64±1.41)) points, depressive mood (1.03±1.79) points and (0.03±0.31) respectively) ($p \leq 0.001$), somatic muscle (1.27±1.98 and 0.73±1.5 points, respectively) ($p \leq 0.001$) and respiratory symptoms (2.05±2.5 and 1.09±1.84 points, respectively) ($p \leq 0.001$) (see Table 2, Table 3).

A moderate correlation between age and anxiety severity was found, according to the Hamilton test, at the level of r=0.5, with a weak direct correlation in women (r=0.382)

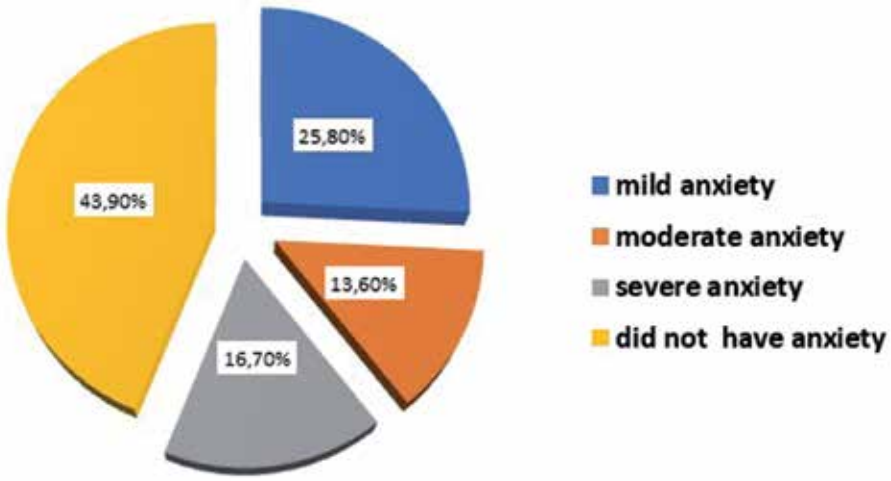


Fig. 4. Level of anxiety according to the GAD-7 questionnaire among patients with COVID-19.

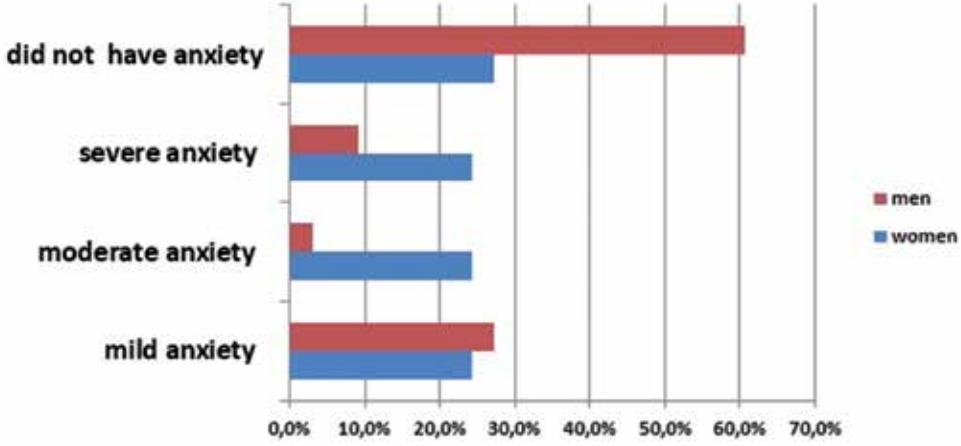


Fig. 5. Gender peculiarities of anxiety severity according to the GAD-7 questionnaire among patients with COVID-19.

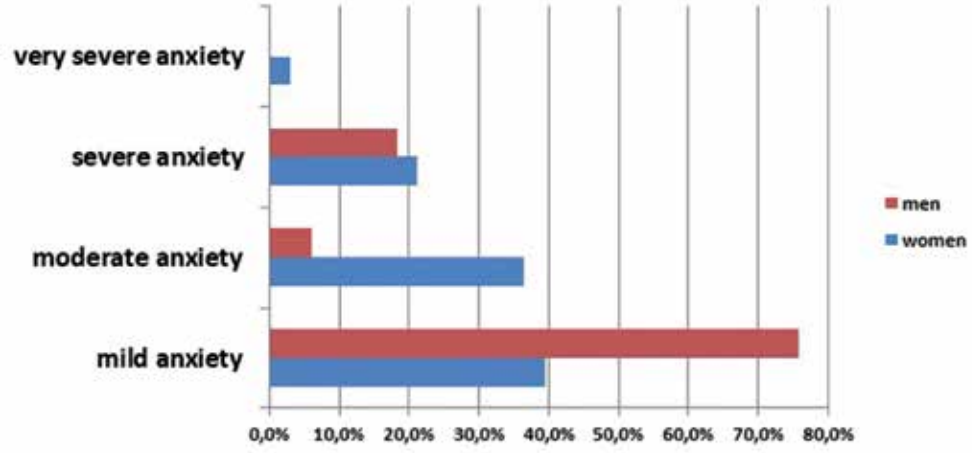


Fig. 6. Level of anxiety according to the Hamilton Anxiety Inventory among patients with COVID-19.

Table 2. Correlations between depressive symptoms and age in patients with COVID-19

Name of indicators			
According to the PHQ-9 depression scale	**All patients**	**men**	**women**
Lack of interest in events	0,334	0,275	0,412
Indifference, oppression	0,297	0,303	0,311
Problems falling asleep, insomnia, sleeping a lot	0,335	0,241	0,518
Feeling tired or losing energy	0,335	0,521	0,053
Lack of appetite or overeating	0,362	0,332	0,426
You feel like a failure	0,325	-0,096	0,693
Anxious anticipation of terrible events	0,351	0,231	0,466
Do you move or speak unusually slowly or vice versa?	0,11	-0,285	0,591
Thoughts of suicide or self-harm?	0,38	-0,025	0,647

r=0,1-0,3 – weak connection, r=0,3-0,5 – medium connection, r=0,5-1,0 – strong connection.

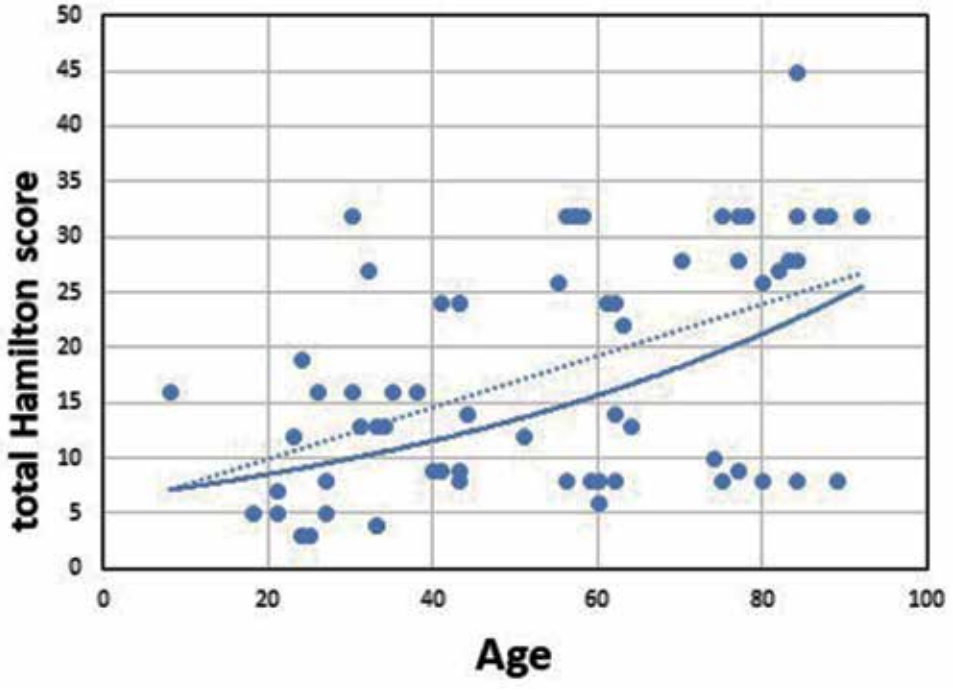


Fig. 7. Scatter plot of the relationship between anxiety severity according to the Hamilton Anxiety Inventory and age among patients with COVID-19.

Table 3. Gender peculiarities of anxiety symptoms (according to the Hamilton test) among patients with COVID-19

Symptoms	Average score	women	men	P
Anxious mood	1,08±1,28	1,7±2,28*	0,45±1,19*	0,001
Tension	1,14±1,31	1,64±2,24*	0,64±1,41*	0,001
Fears	0,65±1	0,94±1,71	0,36±1,06	0,607
Insomnia	1,11±1,3	1,21±1,93	1±1,76	0,22
Intellectual disability	0,61±0,96	0,85±1,62	0,36±1,06	0,513
Depressed mood	0,53±0,9	1,03±1,79*	0,03±0,31*	0,001
Somatic muscle symptoms	1,03±1,25	1,58±2,2*	0,48±1,23*	0,001
Somatic sensory symptoms	1±1,23	1,27±1,98	0,73±1,5	0,565
Cardiovascular symptoms	0,98±1,22	1,33±2,03	0,64±1,41	0,726
Respiratory symptoms	1,24±1,37	2,05±2,5*	1,09±1,84*	0,001
Gastrointestinal symptoms	0,61±0,96	0,85±1,62	0,36±1,06	0,513
Genitourinary symptoms	0,3±0,68	0,42±1,15	0,18±0,75	0,251
Vegetative symptoms	0,91±1,18	1,18±1,91	0,64±1,41	0,567
Behaviour during the examination	0,26±0,63	0,48±1,23	0,03±0,31	0,471
Average level of anxiety	17,6±4,73	20,9±7,19*	14,3±6,19*	0,001

*Notation: probability of differences at the level of *p≤0.001*

and a strong correlation in men (r=0.717). Thus, with age, the frequency and severity of anxiety manifestations in the psycho-emotional state of patients with COVID-19 increases proportionally (Fig. 7).

Gender analysis showed significant differences in the correlation of anxiety symptoms and age. We found a weak positive correlation in women between age and cardiovascular symptoms (r=0.332), age and respiratory symptoms (r=0.376). Statistical analysis of men's responses revealed a weak negative correlation between age and fear (r=-0.363), a weak negative correlation between age and intellectual disability (r=-0.349), and a weak negative correlation between age and examination behavioural disorders (r=-0.355).

DISCUSSION

Acute respiratory infection COVID-19 affects a person's psycho-emotional state. After the implementation of restrictive measures aimed at reducing COVID-19 cases in many countries, a great number of people had to face significant changes in their daily lives: remote work, temporary unemployment, homeschooling for children, and a lack of physical contact with other family members, friends, and colleagues. Adapting to this new reality proved to be challenging. Changes in our regular routines, the fear of contracting the virus, and concerns about ones who are at risk can cause psychological difficulties for all of us. This can be especially challenging for individuals with pre-existing mental health conditions [1, 3, 5].

According to research results during inpatient treatment, 60.6% of patients had symptoms of depression, and 66.1% had symptoms of anxiety. For most women with COVID-19, there is a presence of moderate depression according to PHQ-9, mild anxiety according to GAD-7, and moderate anxiety according to Hamilton. For most men, there is the presence of mild depression according to PHQ-9, an absence of anxiety according to GAD-7, and mild anxiety according to Hamilton. These facts correlate with the data of other studies [4, 6].

The presence of depression correlates with female gender, age (p≤0.001; r=0.728) (mostly occurring in individuals over the age of 50), and the presence of comorbid conditions (p≤0.001). Most men, 54.5%, did not exhibit depressive symptoms, in contrast to women at 24.2% (p≤0.001). For women, the strength and expression of all depression symptoms increase with age, supported by correlation links. Among women with COVID-19, there is a proportional increase in the frequency of suicidal thoughts and intentions with age range (r=0.647), sleep disturbances (insomnia, interrupted sleep, early awakening) (r=0.518), and a slowed speech pace (r=0.591). Among men, there is a strong direct correlation between age and feelings of fatigue or weakness (r=0.521).

The presence of anxiety according to GAD-7 and the degree of anxiety according to Hamilton correlates with female gender (p≤0.001), the presence of depression (p≤0.001), the presence of comorbid conditions (p≤0.05), and women without a partner. With age, the frequency and intensity of anxious manifestations in the psychoemotional state of men with COVID-19 proportionally increase (r=0.717), while in women, the risk of depression increases with age (r=0.728). Anxiety symptoms are less pronounced in men with COVID-19, with insomnia and respiratory symptoms being the most

significant. Among women, during the active phase of COVID-19, the prevailing anxiety symptoms include an anxious mood ($p \leq 0.001$), tension, a depressive mood ($p \leq 0.001$), somatic muscle ($p \leq 0.001$), and respiratory symptoms ($p \leq 0.001$). Similar correlations can be found in other reviews [6, 8].

CONCLUSIONS

Any large-scale outbreak of a dangerous infection will have a negative impact on individuals and society.

Researchers still do not fully understand why COVID-19 affects people differently and how this infection can impact the brain. However, studying the risk factors for developing depression can help explain how COVID-19 or circumstances related to coronavirus infection can contribute to the development of depression in some individuals.

After the implementation of restrictive measures aimed at reducing COVID-19 cases in many countries, a great number of people had to face significant changes in their daily lives: remote work, temporary unemployment, homeschooling for children, and a lack of physical contact with other family members, friends, and colleagues. Adapting to this new reality proved to be challenging. Changes in our regular routines, the fear of contracting the virus, and concerns about ones who are at risk can cause psychological difficulties for all of us. This can be especially challenging for individuals with pre-existing mental health conditions.

Unfortunately, our study lacks data on how many patients had depression and anxiety before being diagnosed with COVID-19, as well as the duration of such disorders in each patient.

Determining the likelihood of COVID-19 in individuals with pre-existing mental health disorders and studying the clinical characteristics of these disorders in healthcare workers can be a promising direction for future research.

The data obtained will be useful in developing psych prophylactic measures for patients with COVID-19.

REFERENCES

1. Ma Z, Zhao J, Li Y et al. Mental health problems and correlates among 746 217 college students during the coronavirus disease 2019 outbreak in China. Epidemiol Psychiatr Sci 2020;29:1-10. doi: 10.1017/S2045796020000931.
2. Khaustova OO, Omelyanovich VY, Assonov DO, Asanova AE. Therapy of post-covid depression: a proactive psychosomatic approach. Pol Merkur Lekarski. 2023;51(1):5-13. doi: 10.36740/Merkur202301101.
3. Xiang YT, Yang Y, Li W, Zhang L et al. Timely mental health care for the 2019 novel coronavirus outbreak is urgently needed. Lancet Psychiatry. 2020;7(3):228-229. doi: 10.1016/S2215-0366(20)30046-8.
4. Gainer DM, Nahhas RW, Bhatt NV et al. Association Between Proportion of Workday Treating COVID-19 and Depression, Anxiety, and PTSD Outcomes in US Physicians. J Occup Environ Med. 2021;63(2):89-97. doi: 10.1097/JOM.0000000000002086.
5. Santomauro DF, Whiteford HA, Ferrari AJ. Depression and anxiety during COVID-19 – Authors' reply. Lancet. 2022;399(10324):518-519. doi: 10.1016/S0140-6736(21)02143-7.
6. COVID-19 Mental Disorders Collaborators. Global prevalence and burden of depressive and anxiety disorders in 204 countries and territories in 2020 due to the COVID-19 pandemic. Lancet. 2021;398(10312):1700-1712. doi: 10.1016/S0140-6736(21)02143-7.
7. Global Burden of Disease Long COVID Collaborators; Estimated Global Proportions of Individuals With Persistent Fatigue, Cognitive, and Respiratory Symptom Clusters Following Symptomatic COVID-19 in 2020 and 2021. JAMA. 2022;328(16):1604-1615. doi: 10.1001/jama.2022.18931.
8. Daly M, Robinson E. Depression and anxiety during COVID-19. Lancet. 2022;399(10324):518. doi: 10.1016/S0140-6736(22)00187-8.
9. Helms J, Kremer S, Merdji H et al. Neurologic Features in Severe SARS-CoV-2 Infection. N Engl J Med. 2020;382(23):2268-2270. doi: 10.1056/NEJMc2008597.

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Oksana I. Kolenko
Sumy State University
2 Rymskogo-Korsakova st., 40007 Sumy, Ukraine
e-mail: o.kolenko@med.sumdu.edu.ua

ORCID AND CONTRIBUTIONSHIP

Iryna Mudrenko: 0000-0002-1849-338X D, F
Vitalii Mudrenko: 0000-0003-2235-3838 A, C-E
Oksana Kolenko: 0000-0002-3209-377X B, C
Oleksandra Kotkova: 0009-0008-7182-1603 B, C
Liudmyla I. Kiptenko: 0000-0003-4886-3342 D, E

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

RECEIVED: 21.08.2023
ACCEPTED: 05.03.2024

ORIGINAL ARTICLE

Physicochemical properties as a function of lomefloxacin biological activity

Noor H. Naser[1], Falah S.A. Suhail[2], Sahar A. Hussein[3], Shakir Shinawa Salih[2]

[1]AL-ZAHRAA UNIVERSITY FOR WOMEN, KARBALA, IRAQ
[2]THE ISLAMIC UNIVERSITY, NAJAF, IRAQ
[3]KUFA UNIVERSITY, NAJAF, IRAQ

ABSTRACT

Aim: The goal is to discover QSAR of Lomefloxacin as antibacterial activity.

Materials and Methods: A number of lomefloxacins analogs activities were studied by program Windows Chem SW. The analogues were obtained and energy minimization was carried out through Molecular Modeling Program, the calculations were performed using General Atomic and Molecular Electronic Structure System (GAMESS) software.

Results: There were six descriptions (N-quinoline more (-) ev charge, Kinetic Energy, Potential Energy, Log p, Log S, F6 charge) results have highly compatible of physicochemical properties with lomefloxacin analogs activities. It can be used to estimate the activities depending on QSAR equation of lomefloxacin analogs.

Conclusions: The parameters used for calculation were depending on the quantum chemical was employed in deriving from computational study of properties and can used to predict the activities of certain analogs of Lomefloxacins as antibacterial compounds.

KEY WORDS: antibacterial activity, MIC, QSAR, lomefloxacins, quantum chemistry, molecular information, multiple linear regression

Pol Merkur Lek,2024;53(1):197-202. doi: 10.36740/Merkur202402108

INTRODUCTION

The creation of novel antibacterial agents is crucial due to the growing health danger. This entails finding new antibacterial scaffolds as well as making enhancements to the ones that already exist. A widespread issue, antibiotic resistance is restricting the ability to treat microbiological infections. Rapid development of antibiotic resistance generates danger for humans worldwide [1]. As a result of the ongoing emergence of new forms of antibiotic resistance, many first-generation antibiotic drugs are no longer effective, putting patients at risk for infection. Furthermore, it takes roughly ten years from the time a new antibiotic is discovered until it is ready for sale. Finding new antibacterial drugs is therefore urgently needed to combat harmful germs [2].

The Schiff bases are synthesized from an aldehyde or ketone were the (C=O) group has changed with an imine or azomethine group. Schiff first created the Schiff base in 1864. Researchers are interested in the bioactivity of Schiff bases derivatives and their metal ionic complexes. The antifungal, antibacterial, anticancer, and anti-inflammatory activities of Schiff bases and their complexes with metal ions considered the subject of numerous investigations [3, 4].

Oral lomefloxacins antibacterial effectiveness has been studied for a variety of illnesses, including respiratory tract infections, obstetric, joint, skin, oral, ear, nose, and throat infections, as well as uncomplicated and complex urinary tract infections. Additionally, it has been used as an ophthalmic solution to treat eye infections and as an otic solution for otitis media patients. Its effectiveness is comparable to other quinolones in clinical trials, and it is at least as effective as other antibacterial medications typically used to treat these illnesses. In comparison to other quinolone antibacterial medications, lomefloxacin has the benefit of being simply delivered once daily, and patients using this bronchodilator concurrently do not appear to require theophylline dosage change. Thus, lomefloxacin taken orally should prove to be an effective broad range antibacterial medication for a variety of clinical infections [5, 6]. Furthermore, the negative effects of lomefloxacin (such as its strong odor, high volatility, and insolubility in water) were greatly reduced by cinnamaldehyde-amino acid with Schiff base combinations. Recently we found a number of fluoroquinolone Schiff base analogs with effective biological activities. Quantitative structure-activity relationship (QSAR) can be used to evaluate factors and descriptors for regression analysis. QSAR gives significant methods for correlation the biological activities of the synthesized compounds with their structure properties [7]. The activity of lomefloxacin was estimated in a variety of complicated and uncomplicated infectious cases. lomefloxacin possess a wide range of advantages when compared to another flouro-quinolones as it administered orally as single daily does, and its does not required adjustment for patients taking theophylline bronchodilators [8-10]. In comparison

with other antibacterial compounds, lomefloxacin is more potent than penicillin, with comparable potency to some cephalosporin [11, 12]. QSAR helps to describe a wide range of chemicals according to their biological characters as an in-silico study and, this will reduce the time, efforts, and cost required to test these compounds in vivo [13].

AIM

The aim of this research is to discover quantitative structure-activity relationship (QSAR) of Lomefloxacin as a characteristic of its antibacterial activity.

MATERIALS AND METHODS

The model of linear regression and neural networks of Bayesian were used for QSAR study. Random forest (RF) is the algorithm that used which characterized as simple and predictable. It is a type of numerous procedures depend on multiple decision trees that can forbidden the over fitting from of a single tree decision. It became the gold standard in these studies [14, 15]. The Merck Kaggle competition (2012) sparks people's interest in neural networks. The team consistently outperformed RF in their results. Even though a multi-task neural network provided excellent performance, the team finally employed an ensemble that integrated many techniques. RF and Kaggle competition strategy were used ensemble learning, a method that creates a collection of learning models and integrates many models to give final estimation [16-17]. Many hybrid methods for QSAR have been used for prediction of different chemicals, as carcinogenicity, they are used specifically for certain chemical molecule [18].

Six synthesized analogs of lomefloxacin with IUPAC name and its biological activity as concentration MIC [19] (Table 1).

COMPUTATIONAL METHODS

For the present study, a number of lomefloxacin derivatives were selected and tested for their activity, and Windows Chem SW was adopted for molecular modeling studies. The molecules were generated and energy minimization was carried out by using Molecular Modeling Program, and all calculations are carried out by General Atomic and Molecular Electronic Structure System (GAMESS) software [20, 21].

CALCULATION

Description of physicochemical properties using theoretical programs [22-23] with pMIC and its regression (R^2) calculated and reported in table II. After minimized energy, physicochemical properties were calculated for all studied molecules (table II) and show the 2D and 3D structure in fig. 1. The description of physicochemical and structural properties using theoretical programs with pMIC and its regression (R^2) [24-25] calculated and reported in Table 2.

RESULTS

There were used six descriptions (N quinoline more -ve charge, kinetic energy, potential energy, log p, log S and F6 charge) and results have highly compatible of physicochemical properties with Lomefloxacin derivatives (A, B, C, D, E, F) activity (Fig. 2). It can be used to predict of their activity depending on QSAR equation of lomefloxacin analogs.

According to Free-Wilson Analysis [26]

$pMIC = \Sigma a_i D_i$

Where

pMIC = predicted activity,

ai= contribution per description of property

MIC (µg/mL) or (mg/L), MIC of reference = 0·25 mg/L [27]

D= description

$pMIC= \mu +a_1D_1+a_2D_2+a_3D_3+a_4D_4+a_5D_5+a_6D_6$

μ = pMIC of Lomefloxacin= 0.602

D_1: N quinoline more - ve charge,

D_2: kinetic energy (kcal/Mol),

D_3: potential Energy (kcal/mol),

D_4: log P,

D_5: log S,

D_6: F6 charge.

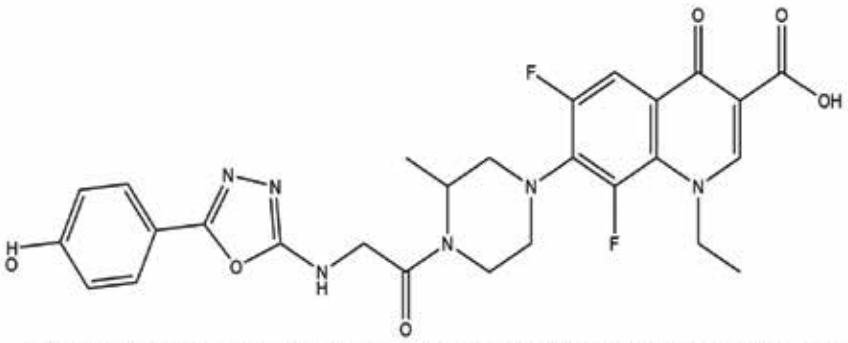
Solution of above equation as a matrix (6X6) in Microsoft office Excel gave a_i values

a_1= 0.279,

a_2= 2.25E-9,

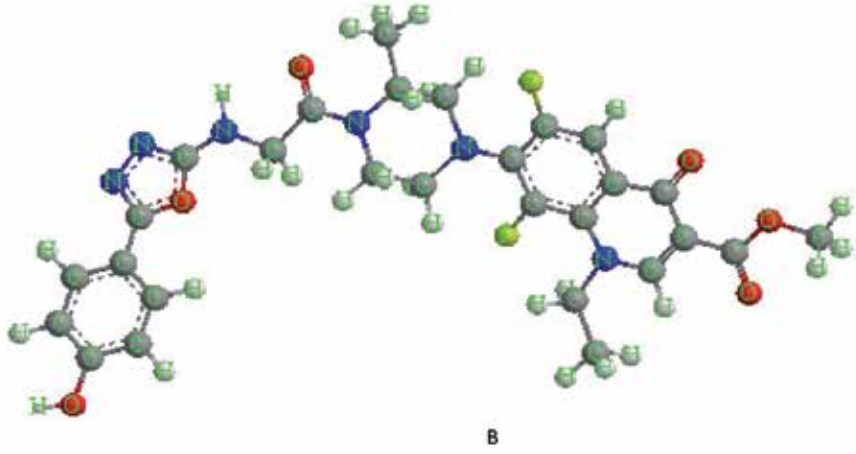
Table 1. Selected lomefloxacin derivatives

Compound	IUPAC name	MIC(µg/ml)
A	MethyI1-ethyl-6,8-difluoro-7-(4-((5-(4-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)glycyl) -3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate	16.6
B	Methyl 1-ethyl-6,8-difluoro-7-(3-methyl-4-((5-(4-nitrophenyl) -1,3,4-oxadiazol-2-yl)glycyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate	17.4
C	Methyl 7-(4-((5-(4-(dimethylamino)phenyl)-1,3,4-oxadiazol-2-yl)glycyl) -3-methylpiperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate	17.35
D	MethyI1-ethyl-6,8-difluoro-7-(4-((5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)glycyl) -3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate	17.0
E	Methyl 1-ethyl-6,8-difluoro-7-(3-methyl-4-((5-(p-tolyl)-1,3,4-oxadiazol-2-yl)glycyl)piperazin-1-yl) -4-oxo-1,4-dihydroquinoline-3-carboxylate	16.5
F	Methyl 7-(4-((5-(4-bromophenyl)-1,3,4-oxadiazol-2-yl)glycyl)-3-methylpiperazin-1-yl) -1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate	18.4

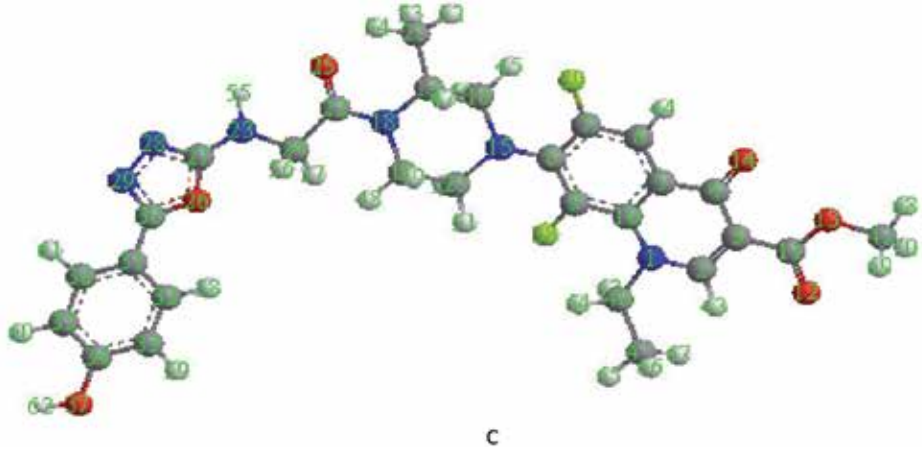


Methyl1-ethyl-6,8-difluoro-7-(4-((5-(4-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)glycyl)-3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate

A



B



C

Fig. 1. Molecular structure 2D (A) and 3D (B, C) of Lomefloxacin.

a_3 =1.25E-7,
a_4= -0.0235,
a_5= -0.024,
a_6= 3.235.

Application of a_i and D_i values according to Free-Wilson Analysis, theoretical pMIC calculated using the above equation and reported in Table 3 (Fig. 3).

DISCUSSION

The reliability of any quantitative structure–activity relationship (QSAR) model depends on multiple aspects such as the accuracy of the input dataset, selection of significant descriptors, the appropriate splitting process of the dataset, statistical tools used, and most notably on the measures of validation. Validation, the most crucial step in QSAR model development, confirms the reliability of the developed QSAR models and the acceptability of each step in the model development [28]. There are six descriptions, all of which produced a high regression correlation (R2) of over 0.9, indicating the best relationship between activity and description. Based on the activity property slopes, an increase in (N-quinoline with more negative atomic charge, potential energy, solubility) increased the activity of lomefloxacin, whereas a decrease in (potential energy,

Table 2. Regression and slope related to description of properties with pMIC

Compound/ \properties	E	A	D	C	B	F	R2	Slope
More positive charge atom in the molecule	0.893177	0.956363	1.006825	1.043771	0.997662	0.928013	0.743	-4.699
N quinoline more negative atom charge in the molecule	-0.97117	-0.97813	-0.98519	-0.99778	-1.01745	-1.05813	0.953	2.108
Kinetic energy (kcal/Mol)	1229456.103	1251979.008	1275712.136	1331673.417	1332124.976	134612.015	0.968	-4000000
Potential energy (kcal/Mol)	-2462558.215	-2506830.578	-2555705.42	-2667856.227	-2667256.343	-2691621.747	0.969	9000000
Log p	4.33759	4.53554	5.26291	5.25476	5.25476	6.15589	0.93	-33.46
Log S	-7.28323	-7.57054	-7.98463	-8.30458	-8.30458	-8.73299	0.932	25.16
F 6 charge	-0.398352	-0.397217	-0.395228	-0.394554	-0.393347	-0.391721	0.936	-0.171
C quinone charge	0.667438	0.673736	0.674344	0.687177	0.667438	0.687912	0.756	-0.411

Table 3. Practical and theoretical pMIC values

	A	B	C	D	E	F
Practical pMIC	-1.2175	-1.2201	-1.2304	-1.239	-1.2405	-1.2648
Theoretical pMIC	-1.2189	-1.2197	-1.2288	-1.2388	-1.2396	-1.2696

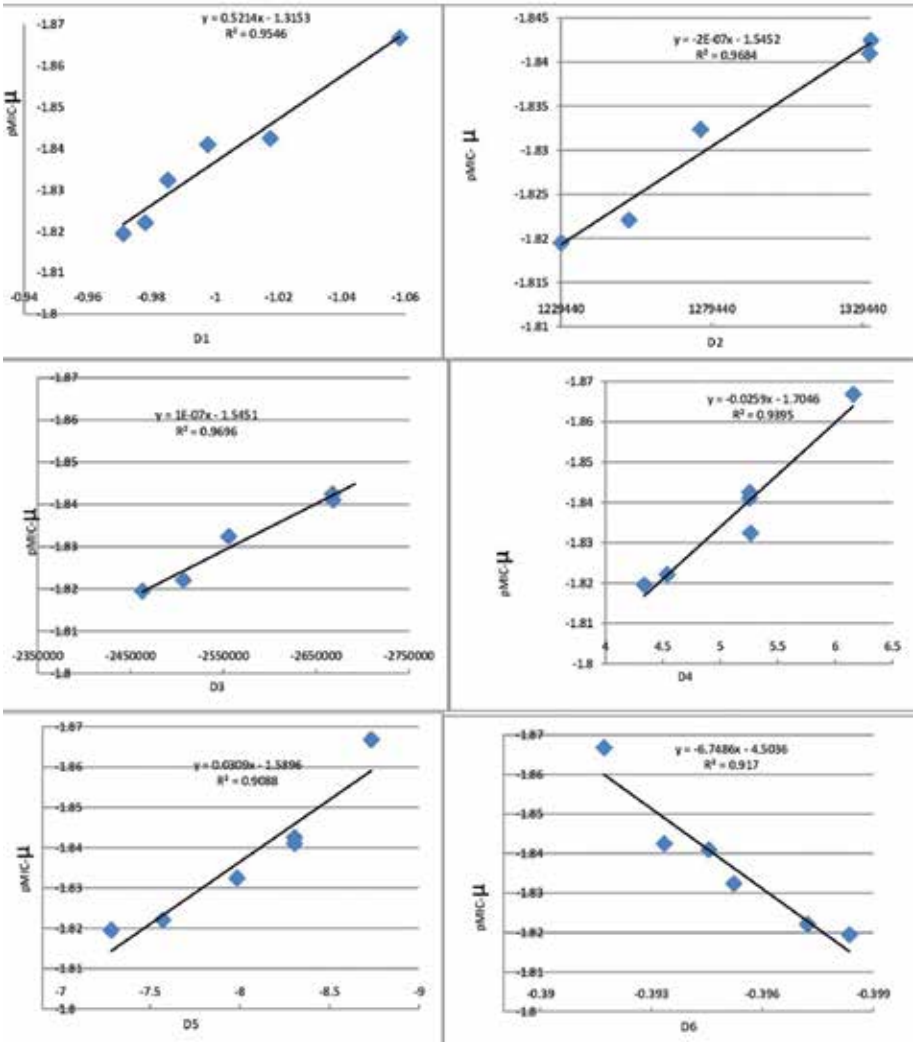


Fig. 2. Relationship of physicochemical properties (D1-D6) of Lomefloxacin derivatives A, B, C, D, E and F with its activities.

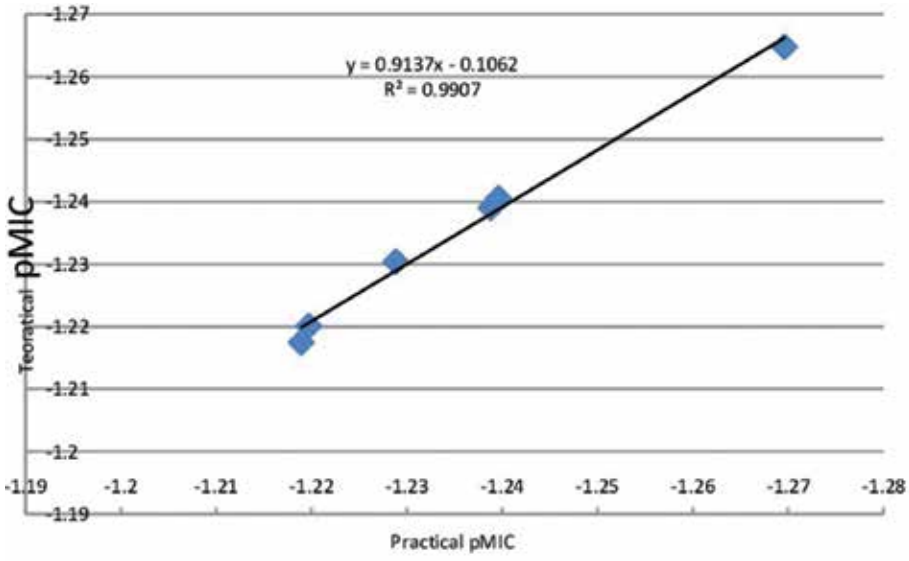


Fig. 3. Practical and theoretical pMIC values chart.

partition coefficient, Florien atomic charge at six positions) increased the activity. These description of properties will be blocks to build a considered arithmetic equation describe relationship of properties with activity [29], so QSAR equation will be:

$PMIC=0.602+0.279\ D_1+2.25E\text{-}9\ D_2+1.25E\text{-}7\ D_3-0.0235\ D_4-0.02\ D_5+3.235\ D_6$.

CONCLUSIONS

The calculated parameters, based on quantum chemical studies, could be successfully employed in deriving from computational study of properties can predict activity values of series of lomefloxacin as antibacterial. The study signified that modeling of the predicted anti-malaria for compounds under study could be done based on multiple linear regression (MLR). The best equation was derived based on twenty-nine properties calculated selected only 6 descriptions have highly compatible for 6 derivatives. The equation model was found to rely on. The best produced model is the MLR model that has a good statistical fit as clear from regression coefficient was more than 0.9.

REFERENCES

1. Wu-Wu JWF, Guadamuz-Mayorga C, Oviedo-Cerdas D et al. Antibiotic resistance and food safety: perspectives on new technologies and molecules for microbial control in the food industry. Antibiotics (Basel). 2023;12(3):550. doi:10.3390/antibiotics12030550.
2. Hafedh SA, Hussein SA, Naser NH. Antibacterial activity of Nonsteroidal anti-inflammatory agents and their derivatives. Eur. Chem. Bull. 2022;11(3):8-13. doi:10.31838/ecb/2022.11.03.002.
3. Ajjah NT, Naser NH, Abo Alard AA et al. Design, synthesis, docking study and preliminary pharmacological assessment of new norfloxacin analogues having thiazole nucleus. J Biochem Tech. 2020;11(3):58-67.
4. Adabiardakani A, Hakimi M, Kargar H. Cinnamaldehyde schiff base derivatives: a short review. World Applied Programming. 2012;2(11):472-476.
5. Gomes GC, Salgado HRN. Validation of UV Spectrophotometric Method for Determination of Lomefloxacin Pharmaceutical Dosage Form. Acta Farm. Bonaerense. 2005;24(3):406-408.
6. Mohamed GG, Abd El-Halim HF, El-Dessouky MMI et al. Synthesis and characterization of mixed ligand complexes of Lomefloxacin drug and glycine with transition metals. Antibacterial, antifungal and cytotoxicity studies. Journal of Molecular Structure. 2011;999(1-3):29-38. doi:10.1016/j.molstruc.2011.05.018.
7. Putaya HAN, Ndahi NP, Bello HS et al. Synthesis, characterization and antimicrobial analysis of Schiff bases of o-phenylenediamine and 2-aminopyridine-3-carboxylic acid with ofloxacin and their metal (II) complexes. Int. J. Biol. Chem. Sci. 2020;14(1):263-278. doi: 10.4314/ijbcs.v14i1.22.
8. Leroy A, Fillastre JP, Humbert G. Lomefloxacin pharmacokinetics in subjects with normal and impaired renal function. Antimicrob Agents Chemother. 1990;34(1):17-20. doi: 10.1128/AAC.34.1.17.
9. Freeman CD, Nicolau DP, Belliveau PP et al. Lomefloxacin clinical pharmacokinetics. Clin Pharmacokinet. 1993;25(1):6-19. doi: 10.2165/00003088-199325010-00002.
10. Counterman ED, Lawley SD. What should patients do if they miss a dose of medication? A theoretical approach.J PharmacokinetPharmacodyn. 2021;48(6):873-892. doi:10.1007/s10928-021-09777-6.
11. Majeed A, Javeedi AD, Omer MO, Mushtaq et al. Evaluation of immunomodulatory effects of Lomefloxacin in mice. Braz. J. Pharm. Sci. 2020;56:e18669. doi:10.1590/s2175-97902019000418669.

12. Wang J, Gagne JJ, Kattinakere-Sreedhara S et al. Association between initiation of fluoroquinolones and hospital admission or emergency department visit for suicidality: population based cohort study. BMJ. 2022;379:e069931. doi:10.1136/bmj-2021-069931.
13. Daneman N, Chateau D, Dahl M et al. Fluoroquinolone use for uncomplicated urinary tract infections in women: a retrospective cohort study. Clin Microbiol Infect. 2020;26(5):613-618. doi:10.1016/j.cmi.2019.10.016.
14. Jain R, Jain V, Jain N et al. Eco friendly spectrophotometric method for quantitative estimation of Lomefloxacin using hydrotropic approach. Journal of Applied Pharmaceutical Science. 2012;02(04):111-114. doi: 10.7324/JAPS.2012.2425.
15. Muro S. Challenges in design and characterization of ligand-targeted drug delivery systems. J Control Release. 2012;164(2):125-137. doi:10.1016/j.jconrel.2012.05.052.
16. Kwon S, Bae H, Jo J et al. Comprehensive ensemble in QSAR prediction for drug discovery. BMC Bioinformatics. 2019;20(1):521. doi:10.1186/s12859-019-3135-4.
17. Vardakas KZ, Siempos II, Grammatikos A et al. Respiratory fluoroquinolones for the treatment of community-acquired pneumonia: a meta-analysis of randomized controlled trials. CMAJ. 2008;179(12):1269-1277. doi:10.1503/cmaj.080358.
18. Reiter E, Ahn S, Shukla AK et al. Molecular mechanism of β-arrestin-biased agonism at seven-transmembrane receptors. Annual review of pharmacology and toxicology. 2012;52:179-197. doi:10.1146/annurev.pharmtox.010909.105800.
19. Saeed AR, Naser NH, Abo Alard AA. Design, synthesis and pharmacological evaluation of new lomefloxacin derivatives having oxadiazole nucleus. J Pharm Sci & Res. 2019;11(4):1516-1526.
20. Gether U. Uncovering molecular mechanisms involved in activation of G protein-coupled receptors. Endocr Rev. 2000;21(1):90-113. doi:10.1210/edrv.21.1.0390.
21. Hatem OA, Suhail FSA, Juda AM. Determination of physicochemical and geometrical properties of some carvedilol derivatives. Asian J Pharm And Clinical Res. 2016;9(4): 330-33.
22. Raazi A, Suhail FSA, Al-Hakeim HK. Investigation of anticancer drug activity 5-fluorouracil and some analog derivatives by QSAR: theoretical study. Journal of Physics: Conference Series. 2020;1660(1):012032. doi: 10.1088/1742-6596/1660/1/012032.
23. Adnanhatem O, Suhail FSA, Juda AM. Optimized programs and methods required for the computational study of beta blockers. Journal of Physics: Conference Series. 2020;1664(1):012088. doi: 10.1088/1742-6596/1664/1/012088.
24. Hatem OA, Suhail FSA, Juda AM. Computational and polarographic study on drug-receptor interaction for atenolol. International Journal of Pharmaceutical Sciences Review and Research. 2016;38(1):263-270.
25. Suhail FSA. Computational chemistry calculation on stavudine (d4t) and its derivatives activity to predict a new compound with best drug potency. Journal of Computational Methods in Molecular Design. 2016;6(3):1-7.
26. Kubinyi H. Free Wilson Analysis. Theory, Applications and its Relationship to Hansch Analysis, molecular information.1988;7(3):121-133. doi:10.1002/qsar.19880070303.
27. Stahlmann R, Lode HM. Risks associated with the therapeutic use of fluoroquinolones. Expert Opin Drug Saf. 2013;12(4):497-505. doi:10.1517/14740338.2013.796362.
28. Mathews B, Thalody AA, Miraj SS et al. Adverse effects of fluoroquinolones: a retrospective cohort study in a South Indian tertiary healthcare facility. Antibiotics (Basel). 2019;8(3):104. doi:10.3390/antibiotics8030104.
29. Naser NH, Hussein SA, Suhail FSA. Computational investigation of Norfloxacin derivatives properties related to their biological activity. Eur. Chem. Bull. 2022;11(3):1-7. doi: 10.31838/ecb/2022.11.03.001.

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Noor H. Naser
Al-Zahraa University for women
M357+JF6, Karbala, Iraq
e-mail: sgahmed1331962@outlook.com

ORCID AND CONTRIBUTIONSHIP

Noor H. Naser: 0000-0001-6148-3040 (A, F)
Falah S.A. Suhail: 0000-0003-4944-2259 (B - D)
Sahar A. Hussein: 0000-0002-0985-9570 (D, E)
Shakir Shinawa Salih: 0009-0000-6420-8503 (E, F)

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

RECEIVED: 16.06.2023
ACCEPTED: 08.02.2024

ORIGINAL ARTICLE

Optimization of the clinical diagnostic examination algorithm of patients with a cross bite complicated by cranio-mandibular dysfunction and postural disorder

Lyubov Smaglyuk, Anna Karasiunok, Nelia Kulish, Anastasiia Liakhovska, Hanna Voronkova, Alevtyna Bilous, Valeriy Smaglyuk

POLTAVA STATE MEDICAL UNIVERSITY, POLTAVA, UKRAINE

ABSTRACT

Aim: Optimization of the clinical and diagnostic examination algorithm of patients with cross bite, aggravated by cranio-mandibular dysfunction and postural disorders.

Materials and Methods: 22 patients aged 13-16 years with cross bite with displacement of the lower jaw were examined. The first group consisted of 15 people with a right-sided displacement of the lower jaw, the second – 7 patients with a left-sided one. The condition of the musculoskeletal system was assessed by the position of the head, shoulders, shoulder blades, back (curvature of the spine), legs, chest shape, and abdomen. To determine the state of stability of the body in space, posturological and kinesiological tests were performed. The location of TMJ elements was evaluated on orthopantomograms. Statistical processing of the material was carried out with the help of the "Excel" license package.

Results: 63.64% of patients with a cross bite have disorders of the musculoskeletal system: scoliotic posture – 40.91% and scoliosis – 22.73%. TMJ dysfunction was detected in all examined patients. It was established that the anatomical and topographic features of the joint elements depend on the side of the lower jaw displacement.

Conclusions: The functional imbalance of all structural elements of the musculoskeletal system and the cranio-mandibular complex determined during the research proved the need to optimize the clinical-diagnostic algorithm: consultation of a traumatologist-orthopedic doctor, X-ray examination of TMJ, conducting posturological tests.

KEY WORDS: cross bite, displacement of the lower jaw, posture, cranio-mandibular dysfunction, TMJ dysfunction

Pol Merkur Lek,2024;53(1):203-207. doi: 10.36740/Merkur202402109

INTRODUCTION

According to scientific research, there is a close relationship between general somatic health and dental health [1-5]. Temporo-mandibular joint (TMJ) dysfunction is among the most common dental diseases [6]. According to the World Health Organization, more than 40% of people aged 20 to 50 years suffer from dysfunction, children and teenageri – 14% to 20%. According to the American Academy of Orofacial Pain, TMJ dysfunction (or cranio-mandibular dysfunction) is a disease that combines pathological conditions in the masticatory muscles, anatomic-topographic relationships of TMJ elements and associated structures [7], such as musculoskeletal system.

The concept of the mutual influence of posture and bite was first proposed by Pierre Robin (1902) at the beginning of the 20th century, who noted that children with glossoptosis have X-shaped legs, crooked posture, and typical signs of distal bite. Over the next period of time, data was accumulated, confirming the relationship between anomalies of the dental and jaw system (DJS) and postural disorders. It has been theorized that occlusion disorders can lead to a functional imbalance between the craniofacial and cervical components of the musculoskeletal system. Disturbance of the muscle forces balance on the corresponding bone structures contributes to disharmonious growth and development of all cranio-mandibular components.

In the case of malocclusion, there is a forward-tilted position of the head in relation to the vertical axis, change in the location of the lower jaw and, accordingly, the ratio of TMJ elements. An increase in the load on the neck muscles and upper shoulder girdle over time leads to disturbances in the structure of other parts of the musculoskeletal system (sinking of the chest, reduction of its anterior-posterior size, change in the angle of inclination of the ribs, protrusion of the shoulder blades, abdomen, curvature of the lower legs, flat feet) [8, 9].

Particularly difficult, from the point of view of changes in all elements of the cranio-mandibular complex, is a cross bite with displacement of the lower jaw relative to the mid-sagittal plane [10, 11]. Therefore, this pathology is the most vulnerable for TMJ function.

Today there are not enough studies that confirm the relationship, conditioning between all structures of the musculoskeletal system and the cranio-mandibular complex

in patients with a cross bite, and there is no clear algorithm for their examination. Therefore, the conducted research is one of the steps towards achieving this goal.

AIM

The purpose of the study was optimization of the algorithm of clinical and diagnostic examination of patients with cross bite, aggravated by cranio-mandibular dysfunction and postural disorders.

MATERIALS AND METHODS

To achieve the goal, 22 patients aged 13-16 years (the period of permanent bite formation) with a cross bite with a displacement of the lower jaw were examined.

Data on the general somatic condition were obtained from patients' medical card. The correspondence of the biological, dental and passport age was noted, taking into account the height and body weight of the examinees. The condition of the musculoskeletal system was assessed by the position of the head, shoulders, shoulder blades, back (curvature of the spine), legs, chest shape, and abdomen. Posture was characterized as normal, scoliotic, scoliosis without chest deformation, and scoliosis with chest deformation (according to the classification of the G. I. Turner Children's Orthopedic Institute, 1986). The diagnosis of spinal curvature and scoliosis was distinguished by degrees of severity according to Chaklin (1995).

The clinical examination was carried out according to the standard algorithm of an orthodontic patient in accordance with the requirements of the medical card approved by the Ministry of Health of Ukraine. All patients were diagnosed with buccal cross bite with displacement of the lower jaw (according to the classification of Uzhumetskene, 1970). Patients are divided into two groups depending on the direction of displacement of the lower jaw: group 1-15 people with right-sided and group 2-7 patients with left-sided buccal cross bite.

The method of Ilyina-Markosyan (1959) was used to assess the nature of lateral lower jaw displacements. The patient was asked to close the mouth slowly, studying the direction of the lower jaw movements and observing the coincidence or divergence of the centers of the upper and lower dental arches. If the jaw is centered, and at the moment of occlusal contact it shifts sharply to the side, then there is an occlusive type of functional displacement of the lower jaw. If it smoothly shifts to the side even before occlusal contact, then we are talking about a muscle imbalance and muscular type of functional displacement of the lower jaw. If the shift with intermittent movements, like a step, then you can think about the articular variety of the functional displacement of the lower jaw to the side.

The location of TMJ elements was evaluated according to the method of Rabukhina (1966) (Fig. 1) on orthopantomograms (OPTG) performed in a state of physiological rest.

The width of the joint gap in the mesial and distal sections, the shape of the slope of the articular tubercle on both sides, the shape of the articular heads and their location in relation to the slope of the tubercle were studied.

To determine the state of stability of the body in space, posturological and kinesiological tests were performed in a state of physiological rest and in a state of habitual occlusion (patent No. 100624).

Statistical processing of the material was carried out with the help of the "Excel" license package.

RESULTS

From 22 of examined patients, 9 (40.91%) had a scoliotic posture, 5 (22.73%) had scoliosis, 1 (4.54%) had Schlatter disease, and 2 (9.09%) had flat feet. 5 (22.73%) people from the group did not have musculoskeletal disorders. The results of the musculoskeletal system state examination are presented in Table 1.

The results of the examination proved that 63.64% of patients with a cross bite have disorders of the musculoskeletal system in the transverse plane, namely: scoliotic posture – 40.91% and scoliosis – 22.73%. Clinically, the scoliotic posture in all patients was manifested by a lateral curvature of the spine in the lower thoracic or lumbar region, an

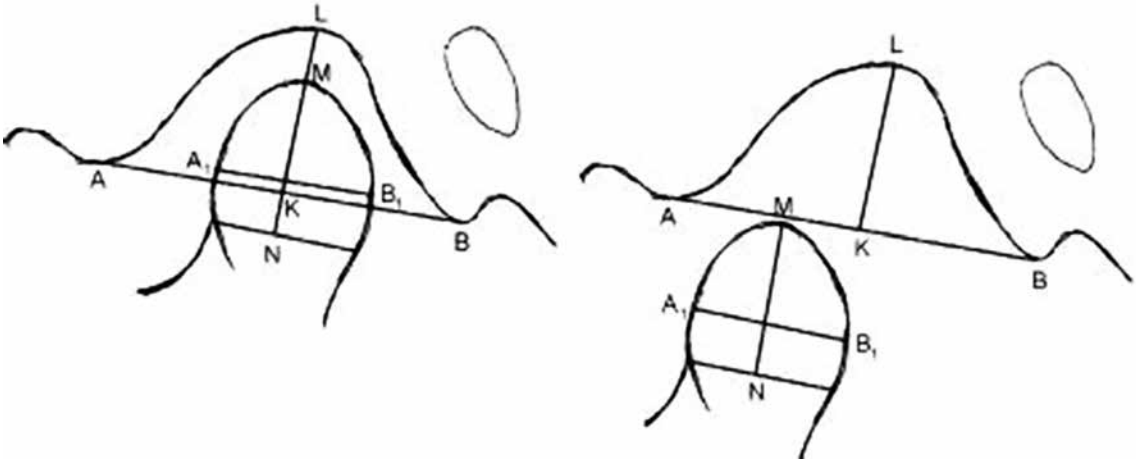


Fig. 1. Scheme of analysis of the TMJ elements location (in a state of physiological rest according to Rabukhina). AB – the width of the articular gap, A1B1 – the width of the condyle, LK – the depth of the articular fossa, MN – the height of the condyle.

Table 1. The results of musculoskeletal system state examination

Characteristics of the musculoskeletal system	Number of patients	
	abs.	%
Without posture disorder	5	22,73
Scoliotic posture	9	40,91
Scoliosis	5	22,73
Schlatter disease	1	4,54
Flat feet	2	9,09
Total	22	100%

Table 2. The results of TMJ elements examination

Research group	Condile width		Condile hight		Mesial width of articular gap		Distal width of articular gap		Height of the articular fossa		Height of the articular tubercle	
	L	R	L	R	L	R	L	R	L	R	L	R
1	11,12 ±0,33	11,85 ±0,23	9,43 ±0,31	10,04 ±0,11	2,23 ±0,18	1,77 ±0,18	5,15 ±0,92	4,50 ±0,42	11,88 ±0,53	13,58 ±0,42	11,50 ±0,74	12,19 ±0,62
2	12,64 ±0,28	10,21 ±0,10	12,93 ±0,12	10,71 ±0,63	2,60 ±0,21	1,64 ±0,14	3,71 ±0,24	3,36 ±0,83	14,14 ±0,99	13,07 ±0,01	14,21 ±1,14	13,64 ±0,95

asymmetric placement of the shoulders, the shoulder was lowered on the concave side, and raised on the convex side. The blades are placed asymmetrically. Their lower corners are not at the same level, the waist triangle on the concave side is larger than on the convex side. The pelvis is not tilted. During the examination from the front, asymmetry of the shoulders and uneven placement of the nipples are determined. The scoliotic posture was corrected in the "slender" position, its symptoms disappeared.

During clinical examination of the TMJ, 12 (54.5%) patients had complaints of tinnitus (one or both sides); 50% of the examined had a pain symptom in the TMJ area; clicking when simulating chewing movements – 10 (45.4%) patients; limited opening of the mouth was determined in 7 (31.8%) patients. 3 (13.6%) patients had joint injury in history. Only 3 (13.6%) patients aged 13 years had no complaints and symptoms that would indicate changes in the TMJ.

Examination of the mandible lateral displacement revealed a smooth movement to the side even before occlusal contact, which indicates muscle imbalance. Thus, all 22 (100%) patients had the muscular type of functional lower jaw displacement.

In the examined group of patients, a direct connection with the functional activity of the hands was established. In "right-handed" people, a cross bite with a right-sided displacement of the lower jaw is determined, in "left-handed" – a left-sided one.

The results of the assessment of TMJ elements location are shown in Table 2. The analysis of indicators of group 1 with a right-sided buccal cross bite with displacement of the lower jaw shows the following: the width of the articular head on the right is 0.73 mm wider than on the left ($p \leq 0.05$); the height of the articular head on the right is greater by 0.61 mm; the mesial width of the joint gap on the right is smaller by 0.46 mm; the distal width of the joint gap on the right side is smaller by 0.65 mm; the height of the joint fossa on the right is greater by 1.7 mm ($p \leq 0.05$); the height of the articular tubercle on the right is greater by 0.69 mm.

Analysis of indicators of the 2 groups with left-sided buccal cross bite with displacement of the lower jaw indicate similar mirror changes. The width of the articular head on the left is 2.43 mm wider than on the right; the height of the articular head on the left is greater by 2.22 mm; the mesial width of the joint gap on the left is greater by 0.96 mm; the distal width of the joint gap on the left is greater by 0.35 mm; the height of the articular fossa on the left is greater by 1.07 mm; the height of the articular tubercle on the left is greater by 0.57 mm ($p \leq 0.05$).

Analyzing clinical situations, it can be noted that the anatomical and topographic features of the joint elements depend on the side of the lower jaw displacement. So, on the side from which the jaw has shifted, the articular fossa is flatter, the articular tubercle is flattened, the shape of the articular head is flattened and elongated, its neck is elongated. The proportionality between the dimensions of the articular fossa height and the height of the articular head is preserved. On the displacement side, a different picture is observed: a deeper and wider articular fossa, the articular tubercle has a conical shape, a wider neck of the articular head, which has a spherical shape.

DISCUSSION

Determined changes in the musculoskeletal system of patients with cross bite and displacement of the lower jaw indicate the presence of interrelationship between all elements of the cranio-mandibular complex (bite, TMJ, spine). So, to ensure a stable vertical position of the body for a long time, it is important that the person's face is perpendicular to the vertical plane – this ensures anatomical and functional balance. The weight of the head is also balanced in line with the spine to minimize muscle strain. Thus, the vertical and horizontal axes are the result of the adaptation of anatomical and physiological functions in order to maintain a person's vertical position [5, 7, 9, 11].

Similar results were obtained by V. Vovk and V. Nespryadko [2], whose research revealed a positive, moderately strong correlation between the muscles of the facial skeleton and the cervical spine.

The results of our survey proved that 63.64% of patients with a cross bite have disorders of the musculoskeletal system in the transverse plane. According to L. Smaglyuk and K. O. Solovei [8], occlusal disorders lead to changes in the functional state of the human postural system. These results emphasize the need to determine the state of stability of the body in space with the help of posturological tests during the clinical examination of orthodontic patients.

V. Makeev [6] with authors. prove in their study that in the case of TMJ dysfunctions, the development of symptoms usually occurs against the background of impaired functional occlusion, which leads to impaired excursion of the lower jaw. There is an overstrain of the masticatory muscles with the development of myofascial pain syndrome involving the neck muscles. Similar results were obtained in our study: all patients had the muscular type of functional shift of the lower jaw.

Therefore, the results of the study are the basis for optimizing the algorithm of clinical and diagnostic examination and planning of orthodontic treatment of patients with cross bite, aggravated by cranio-mandibular dysfunction and postural disorders.

Thus, given the above, we recommend compulsory consultation of a traumatologist-orthopedic doctor for the correction of posture disorders with further treatment; use of posturological samples during orthodontic treatment planning (Patent No. 100624).

CONCLUSIONS

1. The assessment of the morpho-functional state of the dento-maxillary area and musculoskeletal system in patients with a cross bite with a displacement of the lower jaw enriched the data about relationship between the elements of the cranio-mandibular system and human posture.
2. TMJ dysfunction was detected in 100% of patients with a cross bite with displacement of the lower jaw and postural disturbances (crunching and clicking in the TMJ, painful mouth opening, deviation of the lower jaw, tinnitus).
3. The functional imbalance of all structural elements of the musculoskeletal system and the cranio-mandibular complex determined during the research proved the need to optimize the clinical-diagnostic algorithm (consultation of a traumatologist-orthopedic doctor, X-ray examination of TMJ, conducting posturological tests).

Prospects for further research: for further research, it is necessary to conduct an analysis of the orthodontic treatment of patients with a cross bite, for whom the construction of the appliance was carried out taking into account the results of posturological tests.

REFERENCES

1. Smaglyuk LV, Voronkova HV, Karasiunok AY et al. Interdisciplinary approach to diagnostics of malocclusions (review). Wiad Lek. 2019;72(5):918-922. doi: 10.36740/WLek.918-22.
2. Vovk VV, Nespriadko VP. Identification of correlation of temporomandibular dysfunction and cervical spine disorders. Medical science of Ukraine. 2019;15(1-2):59-64. doi: 10.32345/2664-4738.1-2.2019.08.
3. Holovanova IA, Lyakhova NA, Sheshukova OV et al. Studying the skills attitudes on factors affecting dental health of children. Wiad Lek. 2018;71(3):640-647. doi: 10.36740/WLek.640-47.
4. Lyakhova NA, Filatova VL. Sheshukova OV et al. Studying and analyze the factors that affect compliance dentist recommendations from parents of child patients. Wiad Lek. 2020;73(8):1730-1734. doi: 10.36740/WLek202008127.
5. Resende CM, Alves AC, Coelho LT et al. Quality of life and general health in patients with temporomandibular disorders. Braz Oral Res. 2013;27(2):116-21. doi: 10.1590/s1806-83242013005000006.
6. Makeev VF, Telishevska UD, Telishevska OD, Mykhailevych MY. The role and significance of costen's syndrome in dysfunctional conditions of the temporomandibular joints. Ukrainian Dental Almanach. 2020;3:34-38. doi: 10.31718/2409-0255.3.2020.06.
7. Calixtre LB, Groninger BL, Haik MN et al. Effects of cervical mobilization and exercise on pain, movement and function in subjects with temporomandibular disorders: a single group pre-post test. J Appl Oral Sci. 2016:24(3):188-97. doi: 10.1590/1678-775720150240.
8. Smaglyuk LV, Solovei KO. Otsinka Vzayem'yazku Mizh Patolohiyeyu Oklyuziyi [Assessment of the relationship between occlusion pathology and human body stability in space]. J Bulletin of problems biology and medicine. 2014;2(108):148-51.
9. Walczycska-Dragon K, Baron S, Nitecka-Buchta A, Tkacz E. Correlation between TMD and Cervical Spine Pain and Mobility: Is the Whole Body Balance J Related? Biomed Res Int. 2014;582414. doi: 10.1155/2014/582414.

10. Ries LG, Berzin F. Analysis of the postural stability in individuals with or without signs and symptoms of temporomandibular disorder. Braz Oral Res. 2008;22(4):378-83. doi: 10.1590/s1806-83242008000400016.
11. Smaglyuk LV, Kulish NV, Bilous AM, Luchko OY. X-RAY analisis of the mandible in patients with crossbite and mandibular displacement. Wiad. Lek. 2019;72(5):993-99. doi: 10.36740/WLek.993-99.

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Alevtyna Bilous
Poltava State Medical University
24 Shevchenko st. 36000 Poltava, Ukraine
e-mail: alisa6sh8@gmail.com

ORCID AND CONTRIBUTIONSHIP
Lyubov Smaglyuk: 0000-0002-7030-8313 A, E, F
Anna Karasiunok: 0000-0003-0108-6347 B, D
Nelia Kulish: 0000-0002-9375-0083 A, D
Anastasiia Liakhovska: 0000-0002-7268-9551 D
Hanna Voronkova: 0000-0002-0436-1706 C
Alevtyna Bilous: 0000-0002-7165-0516 B
Valeriy Smaglyuk: 0009-0003-6896-8974 B

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

RECEIVED: 19.04.2023
ACCEPTED: 05.03.2024

ORIGINAL ARTICLE

Evaluation of normal range of serum 25 hydroxyvitamin D in Iraqi healthy adults: demographic and socioeconomic effects

Basil E. Al-Hadithy[1], Basil Oied Mohammed Saleh[2], Zainab Nazar Hasan Anber[3], Nazar S Al-Hadad[4]

[1]AL-ANBAR HEALTH DIRECTORATE, AL-ANBAR, IRAQ
[2]COLLEGE OF MEDICINE, UNIVERSITY OF BAGHDAD, BAGHDAD, IRAQ
[3]BAGHDAD COLLEGE OF MEDICAL SCIENCES, BAGHDAD, IRAQ
[4]COLLEGE OF MEDICINE-UNIVERSITY OF BASRAH, BASRAH, IRAQ

ABSTRACT

Aim: To determine the normal range of serum levels of total 25(OH)VD in Iraqi healthy adult subjects and to relate its level with demographic profile and socioeconomic status.

Materials and Methods: This cross sectional study was carried out at Iraq and the samples were collected during the period from August 2019 to January 2020. It included 649 adult subjects apparently healthy, from three governorates (Baghdad, Al-Anbar and Al-Basrah), Investigations included serum measurement of total 25(OH)D in all included individuals by using ELISA technique. Age, gender, marital state, blood pressure, smoking, sunshine exposure (%), hours of exposure/day, percentage of body surface area exposed, body mass index (BMI) subgroups, waist circumference (WC) subgroups, diet type, sport type and time, geographic factor (governorate) were measured and / or calculated and the levels of 25(OH)D were studied according to each of these factors.

Results: The mean ± SD level of 25(OH)D in total (n=649) studied Iraqi subjects was (16.29 ± 8.22 ng/ml), with women were significantly deficient than men (15.76 ± 6.89 ng/ml, 17.14 ± 6.85 ng/ml; $p < 0.01$ respectively). However, there was no significant differences in mean values of serum 25(OH)D levels among studied governorates, subgroups of BMI, WC, marital status, smokers and nonsmoker and subgroups of age. The mean value of serum 25(OH)D was found to be decreased in January, October and November as well as according to diet and sport types. There was significant positive correlation between total 25(OH)D and sun exposed surface area, sun exposure duration and with sun exposure area.

Conclusions: The mean (±SD) value of serum total 25(OH)D in Iraqi healthy subjects was 16.29 ng/ml (±8.22) reflecting the actual body status of this vitamin with lower concentration in women than in men. Type and time of sport and diet type were the major vitamin D dependent factors.

KEY WORDS: vitamin D, 25 (OH) D, Iraqi healthy subjects, BMI, sports

Pol Merkur Lek,2024;53(1):208-215. doi: 10.36740/Merkur202402110

INTRODUCTION

Vitamin D is not precisely a vitamin since its major source is the skin [1]. The majority of D3 from sunlight and of animal origin and vitamin D2 of plant origin [2]**.** Under normal conditions, the human body can produce enough vitamin D when exposed to ultraviolet (UV)-B irradiation. 1,25-dihydrox vitamin D is the hormonally active form of vitamin D, and it is created through the interaction of several cell types and organs [3]. Steroid hormone**,** thyroid hormone, retinoic acid and 1,25(OH)2D receptors are members of a "superfamily" of structurally related gene regulators that work in a similar way [4]. Adjusting calcium absorption and homeostasis is the fundamental role of vitamin D [1]. Also it is involved in insulin secretion, synthesis and secretion of parathyroid and thyroid hormones, inhibition of production of interleukin and have additional biological actions in the adaptive and innate immune system [5]. Vitamin D is also have multifactorial heart functioning and blood pressure regulation, brain and fetal development [6]. About the source, sunlight exposure remains the major source of vitamin D for most children and adults [7]. The flesh fatty fish (such as trout, salmon, tuna, and mackerel) and fish liver oils [8], beef liver, cheese, and egg yolks have small amounts, sun-dried mushroom products and in plant-based products cow's milk with dairy products and plant-derived milk substitutes [9]. Vitamin D insufficiency is defined as levels <30 ng/ml, whereas levels below 20 ng/ml and 10 ng/ml represent deficiency and severe deficiency, respectively [10]. There is no agreement on why calciferiol levels are lower in obese people. The first (and most widely held) viewpoint is that vitamin D is fat soluble, meaning it may be stored in body fat tissues, and that extra body fat can lower T25OHVD levels in the body, particularly in obese individuals [11].

Lower educational level is associated with low food involvement and poor diet quality [12]. In the last decade, researchers have studied T25OHVD levels in a variety of athletes, including runners, basketball players, jockeys, gymnasts, and even dancers, and found that these levels are equivalent to those in the general population [13].

Recent articles, however, demonstrate that these levels will vary significantly depending on geographical region

and the sort of sport, such as whether it is indoor or outdoor, and so on. Athletes who participate in indoor sports are at danger of developing a vitamin D deficiency [14]. There has been numerous research on the link between smoking and vitamin D deficiency, with smoking being one of the predictors of vitamin D deficiency [15].

AIM

The aim of the present work was to study the total serum T25OHVD in Iraqi healthy adults and to correlate it with several demographic and socioeconomic factors.

MATERIALS AND METHODS

This cross-sectional control study was carried out at the Department of Biochemistry, College of Medicine, University of Baghdad, Iraq. The samples were collected from three cities in Iraq (Baghdad, Al- Anbar and Al-Basra), during the period from August 2019 to January 2020. It involved 649 apparently healthy volunteer subjects, 258 from Baghdad governorate, 239 were from Al-Anbar governorate and 152 volunteers were from Al-Basrah governorate.

Baghdad city (latitude: 33.312805) is located in the center of Iraq. Al-Anbar city (latitude: 33.669460) is situated in the western region of Iraq, and Al-Basrah city (latitude: 30.802088) is situated in the southern region of Iraq. The included 649 apparently healthy adults were of both gender, aged 18 years and older. Demographic profile and socioeconomic status including age, sex, marital status, smoking, education level (illiterate, primary, intermediate, secondary-school, institute, graduate and post graduate), occupation, blood pressure, seasonal variation (monthly blood sampling), physical activity (running-swimming-cycling-football) in minutes/day, duration of sun exposure, body surface area exposed to sunlight, dietary habit- intake of fatty fish, beef, liver, egg, milk or dairy product and mushroom. Furthermore, when walking outside, the body surface exposed to sunlight was classified as totally covered (only the hands, feet and face were exposed), and short sleeves were worn (with forearms exposed). Also, whether the participant had learned anything about vitamin D's physiological value through television or the internet.

Exclusion criteria included individuals with chronic diseases (coronary artery disease, kidney diseases, arthritis or bone diseases, diabetes mellitus, cancers, gastrointestinal tract diseases, and liver diseases), acute illness, metabolic or chronic inflammatory disorders, those receiving a long-term therapy for any chronic condition, individuals who taking vitamin D supplement and pregnant women.

Blood samples of 5 milliliter size was taken from each subject, left to clot for 30 minutes and subsequently centrifuged 10 minutes on 2500 rpm to get the serum that was kept near – 20° C.

The involved subjects were sub grouped according to age as: less than 20 years, 20-29 year, 30-39 year, 40-49 year, 50-59 year and more or equal to 60 years. They were also sub grouped according to results of measured blood pressure at the time of blood collection as: normotension (systolic: less than 120 mmHg and diastolic: less than 80 mmHg) and elevated blood pressure (systolic:120 – 129 mmHg and diastolic less than 80 mmHg).

Body mass index (BMI) was calculated according to following equation:

$$BMI\ (Kg/m^2) = Weight / Height^2$$

Results of BMI calculation divide according to scale: ess than 18.5 determined as underweight, from 18.5 - 24.9 – as normal, from 25 - 29.9 – as overweight and from 30 – 39.9 – as obese.

Sunshine exposure percentage was calculated according to equation:

Sunshine exposure percentage = number of hours of exposure per day × the percentage of body surface area exposed.

Percentage of body surface area exposed was estimated by used Wallace rule of nines**,** the enrolled individuals were subdivided according the hours of exposure into two subgroups: A: > 30 mint and B: ≤ 30 minute.

In addition, individuals were subgroup according to month where blood samples were collected; August, September, October, November, December and January as well as to sport type; A: one type of sport like (running, swimming, football, cycling, etc.), B: two type of sports like (running and swimming, running and football, swimming and cycling, etc.), C: three type sports like (football, swimming and running, etc.) and D: not practice each type of sports. Moreover, individuals were sub classified according to their sport time into three groups: A: Less than 30 minute, B: 30 minute and C: more than 30 minute. The enrolled individuals were also categorized into subgroups according to their dietary content of vitamin D (daily habit take it) into three groups: A: meat+ egg+ milk or dairy product; B: meat+ egg and C: others.

Total T25OHVD was measured by ELISA solid phase enzyme-linked immunosorbent assay performed on micro titer plates for professional use. Statistical Package for Social Sciences (SPSS) version 20 (SPSS Inc., Chicago, IL., USA), and Minitab analysis programs were used for all statistical studies. ANOVA and Student's t-tests were used to test for statistical significance. Linear regression was utilized to test for correlation between different studied parameters, and the significance of the R-value was assessed by related t-test. P-values of less than 0.05 were considered significant.

RESULTS

The mean (±SD) value of serum T25OHVD levels of entire included subjects was 16.29 ± 8.22 ng/ml. Those of age (35.34 ± 11.83 year), BMI (26.77 ± 4.25 Kg/m^2), and WC (98.19 ± 12.43 cm).

Table 1 shows that the mean value of serum T25OHVD levels of women (15.76 ± 6.89 ng/ml) was significantly lower than in men (17.14± 6.85 ng/ml), $p<0.01$. However, there was no significant differences in mean values of serum T25OHVD levels among governorates; Baghdad (15.95 ± 6.38 ng/ml), Al-Anbar (17.04 ± 7.27 ng/ml) and Al-Basrah (15.85 ± 7.1 ng/ml). There were also non-significant differences in mean values of serum T25OHVD levels according to subgroups of BMI: normal weight – 15.78 ± 6.28 ng/ml, over weight

Table 1. Mean ± SD values of serum T25OHVD in relation to gender, government, BMI and WC in studied group

Parameter			
	Gender*		
T25OHVD (ng/ml)	Men (n= 269)		Women (n= 380)
	17.14 ± 6.85		15.76 ± 6.89
	Governorate NS		
T25OHVD (ng/ml)	Baghdad (n= 258)	Anbar (n= 239)	Basrah (n=152)
	15.95± 6.38	17.04± 7.27	15.85± 7.1
	BMI NS		
T25OHVD (ng/ml)	Normal weighed (n= 244)	Overweight (n= 281)	Obese (n= 124)
	15.78± 6.28	16.98±7.12	16.09± 7.53
	WC NS		
T25OHVD (ng/ml)	High (n= 394)		Normal (n= 255)
	16.28± 7.34		16.40± 6.18

**ANOVA and t-test revealed significant decline in serum T25OHVD levels in women than men ($p < 0.01$), NS: non- significant difference among subgroups.*

Table 2. Mean ± SD values of serum T25OHVD in relation to blood pressure, marital status, and smoking in studied group

Parameter		
	Blood pressure*	
T25OHVD (ng/ml)	Hypertensive (n= 155)	Normotension (n= 494)
	14.86± 5.78	16.79± 7.16
	Marital status NS	
T25OHVD (ng/ml)	Married (n= 419)	Single (n= 230)
	16.45± 7.01	16.11± 6.70
	Smoking NS	
T25OHVD (ng/ml)	Smoker (n= 164)	Nonsmoker (n= 485)
	17.10± 7.45	16.07 ± 6.69

**t-test revealed significant decrease in serum T25OHVD levels in hypertensive group than normotensive ($p < 0.001$), NS; non-significant difference among groups*

Table 3. Mean ± SD values of serum total 25 hydroxyvitamin D [T25OHVD] in relation to age of study's subjects

	Age NS					
	< 20 years (n= 36)	20-29 years (n= 272)	30 – 39 years (n= 149)	40 – 49 years (n= 94)	50 – 59 years (n= 72)	≥ 60 years (n= 26)
T25OHVD (ng/ml)	16.86±8.21	16.12±6.98	17.76±7.49	15.59±5.54	15.41±6.65	14.73±4.54

**ANOVA study revealed non- significant difference among each groups*

– 16.98 ± 7.12 ng/ml and obese – 16.09 ± 7.53 ng/ml; and WC: high – 16.28 ± 7.34 ng/ml, normal – 16.40 ± 6.18 ng/ml (Table 1).

Table 2 shows the mean value of serum T25OHVD levels of elevated blood pressure individuals (14.86 ± 5.78 ng/ml) was significantly decreased in comparison with that of normotensive ones (16.79 ± 7.16 ng/ml), $p<0.001$. In addition, no significant deference in mean T25OHVD level according to marital status: married – 16.45 ± 7.01 ng/ml, single – 16.11 ± 6.70 ng/ml; and also about smoking: smoker – 17.10 ± 7.45 ng/ml, nonsmoker – 16.07 ± 6.69 ng/ml (Table 2).

Table 3 shows that the mean value of serum T25OHVD levels did not differ significantly among subgroups of age of study's individuals.

Table 4 depicts the mean values of T25OHVD of monthly blood samples aspiration. These results found that mean value of T25OHVD levels of January (14.68 ± 7.58 ng/ml,

p<0.001), October (15.46 ± 6.78 ng/ml, p=0.01), and November (15.57 ± 6.36 ng/ml, p<0.001) were significantly lower than that of August (18.36 ± 7.60 ng/ml). In addition, the mean value of serum T25OHVD levels of January (14.68 ± 7.58 ng/ml, p =0.01) and November (15.57 ± 6.36 ng/ml, p=0.01) were significantly lower than that of December (16.92 ± 5.50 ng/ml), as well as the mean value of serum T25OHVD levels of January and November were significantly lower than that of September (17.7 ± 7.57 ng/ml, p=0.02).

The same table also reveals the mean values of T25OHVD of levels of education. These results found that mean value of T25OHVD levels of illiterate (14.18 ± 5.13 ng/ml, p=0.01), primary (15.64 ± 8.0 ng/ml, p=0.02), intermediate (14.4 ± 5.34ng/ml, p=0.01), secondary (17.66 ± 7.31 ng/ml, p=0.04) and graduate (16.37 ± 6.53 ng/ml, p=0.01) were significantly lower than that of institute (25.49 ± 13.32 ng/ml). In addition, the mean value of serum T25OHVD levels of intermediate (14.4 ± 5.34 ng/ml) was significantly lower than that of secondary (17.66 ± 7.31 ng/ml, p=0.03) (Table 4).

Table 5 depicts the mean±SD values of T25OHVD of diet types, sport types, and sport duration. These results found that mean value of T25OHVD levels in diet type of group B (15.59 ± 6.54 ng/ml, p=0.001) and group C (15.05 ± 6.41 ng/ml, p<0.001) were significantly less than group A (17.76 ± 7.22 ng/ml), in addition in sport type of group A (17.32 ± 7.03 ng/ml, p<0.001), C (15.12 ±4.88 ng/ml, p<0.001) and D (15.90 ± 6.71 ng/ml, p<0.001) were significant lower than group B (24.13 ± 6.35 ng/ml), as well as the mean level of T25OHVD in sport duration/day of group A (14.68 ± 6.60 ng/ml, p=0.001) and C (17.03 ± 7.03 ng/ml, p<0.001) were significantly less than group B (17.25 ± 6.48 ng/ml) (Table 5).

Table 6 depicts that there was significant positive correlation between T25OHVD and sun exposed surface area (r= 0.14, P=0.001), sun exposure duration (r= 0.12, P= 0.001) and with sun exposure area % (r= 0.14, P= 0.001), without any other significant correlations (Table 6).

DISCUSSION

The mean level of T25OHVD in total studied Iraqi adult healthy subjects was 16.29 ± 8.22 ng/ml, the present study excluding those who taking vitamin D supplements and pregnant women, there were significant different between men and women in mean level of T25OHVD concentration, the women were more deficient than men (Table I.), this finding agree with study done in Jordan by AL-Horani et al. (2016) [16], with study done in Iraq Issa and Ibrahem (2007) [17], and study in Turkey Durmus and Cetinkaya (2017) [18]. However, Buzkurt et al. (2014) in Turkey [19] also did not found significant difference in vitamin D with gender. With respect to governorate there were no significant difference in total T25OHVD among studied three governorates (Table 1) probably because latitude not so different among them. In study done in Saudi Arabia, amount of pre-vitamin D3 production in Arar (latitude 300N) more than in Abha (latitude 180N). The high latitude has effect in T25OHVD concentration this mean that sun's rays strike the ground more directly at higher latitudes [20].

The results of present study did not found significant differences in T25OHVD levels among sub classification of BMI and WC (Table I) which agree with Mohammed (2018) study done in Iraq [21], while Al-Timimi and Salih (2016) found that the level of T25OHVD was decreased with an increased level of BMI [22]. Drincic et al. (2013) in cross-sectional study to 686 subjects found that BMI and

Table 4. Mean ± SD values of serum T25OHVD in relation to months, and education levels in study group

	Parameter							ANOVA P-value
	Month*							
T25OHVD (ng/ml)	August (n=93)	September (n=58)	October (n=100)	November (n=92)	December (n=163)	January (n=143)		0.00078
	18.36 ±7.6	17.7 ±7.57	15.46 ±6.78	15.57 ±6.36	16.92 ±5.50	14.86 ±7.58		
	Education Level*							
	Illiterate (n=18)	Primary (n=90)	Intermediate (n=64)	Secondary (n=140)	Institute (n=4)	Graduate (n=327)	PostGraduate (n=6)	0.002
	14.18 ±5.13	15.64 ± 8.0	14.4 ±5.34	17.66 ±7.31	25.49 ±13.32	16.37 ±6.53	14.15 ±1.84	

**ANOVA and t-test revealed significant decline in serum T25OHVD levels in January, October and November than August, also significant decrease in January and November than December and September. In addition, significant decrease in serum T25OHVD levels in illiterate, primary, intermediate, secondary and graduate than in institute, also significant decrease in intermediate than secondary group.*

Table 5. Mean ± SD values of serum total 25 hydroxyvitamin D [T25OHVD] in relation to diet types, sport types and sport duration in study group

	Parameter				ANOVA P-value
T25OHVD (ng/ml)	**Diet type***				
	Type A (n=277)	Type B (n=145)	Type C (n=227)		< 0.0001
	17.76±7.22	15.59±6.54	15.05±6.41		
	Sport type*				
	Type A (n=70)	Type B (n=23)	Type C (n=11)	Type D (n=545)	< 0.0001
	17.32±7.03	24.13±6.35	15.12±4.88	15.90±6.71	
	Sport duration*				
	Type A (n=202)	Type B (n=95)	Type C (n=352)		0.0001
	14.68±6.60	17.25±6.48	17.03±7.03		

** ANOVA and t-test revealed significant decline in serum T25OHVD levels in diet type of group B and C than A, in sport type the level of T25OHVD in group A, C and D are significantly less than B and about sport duration / day the level of T25OHVD of group A and C are significantly less than group B.*

Table 6. Correlation between T25OHVD and Sun exposed surface area, Sun exposure duration, Sun Exposure area %, BMI, WC and Age for studied group

		T25OHVD
Sun exposed surface area*	r	0.14
	P	0.001
Sun exposure duration*	r	0.12
	P	0.001
Sun exposure area (%)*	r	0.14
	P	0.001
BMI	r	0.05
	P	0.24
WC	r	0.001
	P	0.096
Age	r	-0.054
	P	0.168

**Positive significant correlation.*

T25OHVD plasma levels are conversely associated due to volumetric dilution [23]. Similar results were observed by Hamza and Hasan (2020) [24]. In a study done in Iran found that participants with vitamin D deficiency had higher WC [25]. This discrepancy may be attributed to degree of obesity of enrolled subjects which is mild in the present study compared to others.

The present study found subjects with elevated blood pressure had serum T25OHVD levels lower than normal blood pressure (Table 2). There was a significant negative correlation between serum vitamin D levels and systolic blood pressure in several studies [25, 26]. Vitamin D's significant endocrine suppressor effect on renin production to regulate the renin-angiotensin system could be the underlying mechanism [27]. Interestingly, vitamin D seems to reduce production of free radicals locally, with benefits on vascular health [28].

Marital state results of this study observed that there was no significant difference in serum T25OHVD levels between single and married which is in agreement with Durmus and Cetinkaya (2017) [18], but disagree with another one that found vitamin D level of married are higher than single, which may be attributed to social habit [29].

There was no significant different in serum concentrations of T25OHVD between smokers and nonsmokers (Table 2), this finding disagree with other studies in which smokers were found to have a higher risk of hypovitaminosis D than non-smokers [30].

The possible mechanisms that may be more likely disturbed and induce low serum levels of T25OHVD include:

(a) reduced intestinal absorption of vitamin D, (b) decreased cutaneous production of cholecalciferol, (c) decreased local production of calcitriol in target tissues, and (d) the regulation of genes implicated in vitamin D homeostasis, particularly CYPs [15].

About age subgroups (Table 3), there was no significant difference in serum T25OHVD levels among age subgroups, this finding disagree with Abdulrahman and Abdul Rahman (2018) [31] and Gallagher et al. (2013) [32] he claimed that as people age, their calcitriol form of vitamin D decreases by half due to a decrease in renal function and calcium absorption [32].

The present results found that blood sample collected in January, October and November was more deficient in T25OHVD level than that of August which is in agreement with other studies due to sun exposure in summer with more uncovered area in the body [19]. Also this agree with Abdullah et al. (2018) in study done in north of Iraq [33]. Al-Daghri et al. (2012) revealed that, while summer sun exposure in the Middle East and Gulf States is low, an increase could occur throughout the winter when the weather is cooler, raising T25OHVD to modest levels. As well as there were significant difference between January and November with December in mean of vitamin D level with unexplained cause [34].

Considering the different educational levels in this study, significant differences in serum T25OHVD level is recorded and the highest level was documented in institute (Table 4.). Al-Assadi et al. (2018) found that in study done in Al-Basrah no effect of educational level in T25OHVD concentration [35], while in Iran found that vitamin D deficiency is present in younger less educated, women, rural residence, higher BMI, WC, and were less physical activity [25].

The present study found differences in serum T25OHVD levels among diet type subgroups (Table 5) with T25OHVD in group B&C was more deficient than group A that pointed to fact that milk and dairy product play a role in the supplement of vitamin D [36]. Mahdi and Abbas (2019) suggested that lack of animal sources of vitamin D was one of the most common causes of its deficiency [37].

The findings of present study noted that sport type and sport time affect the T25OHVD concentration (Table 5) with type B which doing two type of sport for equal to 30 minutes was better than other types. It has been stated that subjects who undertook regular physical activity had higher serum T25OHVD than those who were inactive and that more vitamin D deficiency in participants who less physical activity [25]. Regarding correlation between total T25OHVD concentration and sun exposed surface area, sun exposure duration and sun exposure area % there were a significant positive correlation (Table 6). The role of sunlight exposure in vitamin D synthesis has been extensively known, and the effect of sunlight on vitamin D status has been well documented [38]. Area of skin exposed and duration of sunlight exposure significantly correlated with T25OHVD levels [39]. The amount of time spent under the sun is limited. In Asians, there is a tendency by most to minimize sun exposure to avoid tanning and discomfort due to heat and sweating. Tanned skin is considered less attractive by most Asians [40].

CONCLUSIONS

The mean ± SD value of serum total T25OHVD in Iraqi healthy subjects was 16.29 ± 8.22 ng/ml reflecting the actual body status of this vitamin with lower concentration in women (15.76 ± 6.89 ng/ml) than in men (17.14 ± 6.85 ng/ml). Type and time of sport considering two kinds of sport with half an hour daily is the best one as well as the type of diet which is rich in dairy products is preferable in providing adequate amount of vitamin D. The age, BMI, WC, governorate, smoking had no significant effect on serum T25OHVD level.

REFERENCES

1. Murray RK, Granner DK, Mayes PA, Rodwell VW. Harper's Biochemistry. 25th Edition, McGraw-Hill, Health Profession Division. New York. 2000, p.225.
2. Jones G. The discovery and synthesis of the nutritional factor vitamin D. Int J Paleopathol. 2018;23:96-99. doi:10.1016/j.ijpp.2018.01.002.
3. Szymczak-Pajor I, Śliwińska A. Analysis of Association between vitamin D deficiency and insulin resistance. Nutrients. 2019;11(4):794. doi:10.3390/nu11040794.
4. Bhattarai HK, Shrestha S, Rokka K et al. Vitamin D, calcium, parathyroid hormone, and sex steroids in bone health and effects of aging. J Osteoporos. 2020;2020:9324505. doi:10.1155/2020/9324505.
5. Harrison SR, Li D, Jeffery LE et al. Vitamin D, autoimmune disease and rheumatoid arthritis. Calcif Tissue Int. 2020;106(1):58-75. doi:10.1007/s00223-019-00577-2.
6. Kheiri B, Abdalla A, Osman M et al. Vitamin D deficiency and risk of cardiovascular diseases: a narrative review. Clin Hypertens. 2018;24:9. doi:10.1186/s40885-018-0094-4.
7. Gasmi A, Noor S, Tippairote T et al. Individual risk management strategy and potential therapeutic options for the COVID-19 pandemic. Clin Immunol. 2020;215:108409. doi:10.1016/j.clim.2020.108409.
8. Roseland JM, Phillips KM, Patterson KY et al. Vitamin D in foods: An evolution of knowledge. Health, Disease and Therapeutics. 2018;2:41-78. doi:10.1016/B978-0-12-809963-6.00060-2.
9. Cannell JJ, Zasloff M, Garland CF et al. On the epidemiology of influenza. Virol J. 2008;5:29. doi:10.1186/1743-422X-5-29.
10. Safar HA, Chehadeh SEH, Abdel-Wareth L et al. Vitamin D receptor gene polymorphisms among Emirati patients with type 2 diabetes mellitus. J Steroid Biochem Mol Biol. 2018;175:119-124. doi:10.1016/j.jsbmb.2017.03.012.
11. Jang H, Lee Y, Park K. Obesity and Vitamin D insufficiency among adolescent girls and young adult women from Korea. Nutrients. 2019;11(12):3049. doi:10.3390/nu11123049.

12. Jarman M, Lawrence W, Ntani G et al. Low levels of food involvement and negative affect reduce the quality of diet in women of lower educational attainment. J Hum Nutr Diet. 2012;25(5):444-452. doi:10.1111/j.1365-277X.2012.01250.x.
13. de la Puente Yagüe M, Collado Yurrita L, Ciudad Cabañas MJ et al. Role of Vitamin D in athletes and their performance: current concepts and new trends. Nutrients. 2020;12(2):579. doi:10.3390/nu12020579.
14. Sekel NM, Gallo S, Fields J et al. The effects of cholecalciferol supplementation on Vitamin D status among a diverse population of collegiate basketball athletes: a quasi-experimental trial. Nutrients. 2020;12(2):370. doi:10.3390/nu12020370.
15. Mousavi SE, Amini H, Heydarpour P et al. Air pollution, environmental chemicals, and smoking may trigger Vitamin D deficiency: Evidence and potential mechanisms. Environ Int. 2019;122:67-90. doi:10.1016/j.envint.2018.11.052.
16. Al-Horani H, Abu Dayyih W, Mallah E et al. Nationality, gender, age, and body mass index influences on Vitamin D concentration among elderly patients and young Iraqi and Jordanian in Jordan. Biochem Res Int. 2016;2016:8920503. doi:10.1155/2016/8920503.
17. Issa AM, Ibraheem SA. Alterations of Vitamin "D" level in Sera of Iraqi population. Journal of Karbala University. 2007;5(1):58-64.
18. Durmuş H, Çetinkaya F. Vitamın D status of adults in Kayseri, Turkey: summer time population based cross-sectional study. Journal of Clinical and Analytical Medicine. 2017;8(4):325-9. doi:10.4328/JCAM.5040.
19. Bozkurt S, Alkana BM, Yildiz F et al. Age, sex, and seasonal variations in the serum Vitamin D3 level in a local Turkish Population. Arch Rheumatol 2014;29(1):14-19. doi:10.5606/tjr.2014.3968.
20. Alamri F, Alshahrani F, Al-Saleh Y et al. Optimum sun exposure times for Vitamin D status correction in Saudi Arabia. European Journal of Preventive Medicine. 2015;3(5):147-154. doi:10.11648/j.ejpm.20150305.14.
21. Mohammed SJ. Association between Vitamin D and body weight in Iraqi population: case-control study. J Obes Weight Loss Ther. 2018;8:377. doi:10.4172/2165-7904.1000377.
22. Al-Timimi DJ, Salih SF. Vitamin D status among apparently healthy population in Duhok, Kurdistan region, Iraq. Duhok Med J. 2016;7:49-56.
23. Drincic AT, Armas LA, Van Diest EE et al. Volumetric dilution, rather than sequestration best explains the low Vitamin D status of obesity. Obesity (Silver Spring). 2012;20(7):1444-1448. doi:10.1038/oby.2011.404.
24. Hamza PJ, Hasan MK. The association between Vitamin D level and body mass index in a sample of childbearing age women in Erbil. Med J Babylon 2020;17:165-171. doi:10.4103/MJBL.MJBL_11_20.
25. Joukar F, Naghipour M, Hassanipour S et al. Association of serum levels of vitamin D with blood pressure status in Northern Iranian population: The PERSIAN Guilan Cohort Study (PGCS). Int J Gen Med. 2020;13:99-104. doi:10.2147/IJGM.S244472.
26. Zaleski A, Taylor B, Armstrong B et al. Associations of 25-Hydroxyvitamin D With the blood pressure response to maximal exercise among healthy adults. Int J Sport Nutr Exerc Metab. 2019;29(3):303-308. doi:10.1123/ijsnem.2017-0424.
27. McMullan CJ, Borgi L, Curhan GC et al. The effect of vitamin D on renin-angiotensin system activation and blood pressure: a randomized control trial. J Hypertens. 2017;35(4):822-829. doi:10.1097/HJH.0000000000001220.
28. Del Pinto R, Ferri C. Inflammation-accelerated senescence and the cardiovascular system: mechanisms and perspectives. Int J Mol Sci. 2018;19(12):3701. doi:10.3390/ijms19123701.
29. Travis RC, Crowe FL, Allen NE et al. Serum vitamin D and risk of prostate cancer in a case-control analysis nested within the European Prospective Investigation into Cancer and Nutrition (EPIC). Am J Epidemiol. 2009;169(10):1223-1232. doi:10.1093/aje/kwp022.
30. Cutillas-Marco E, Fuertes-Prosper A, Grant WB et al. Vitamin D deficiency in South Europe: effect of smoking and aging. Photodermatol Photoimmunol Photomed. 2012;28(3):159-161. doi:10.1111/j.1600-0781.2012.00649.x.
31. Abdulrahman RM, Rahman BMA. Prevalence of Vitamin D level in the serum of patients living in Erbil city, Iraq, referred to private clinical laboratory and effect of age and sex on it. J Biol Res Bollet Della Soc Italiana Biol Sperimentale. 2018;91(1):8-11. doi:10.4081/jbr.2018.6916.
32. Gallagher JC, Yalamanchili V, Smith LM. The effect of vitamin D supplementation on serum 25(OH)D in thin and obese women. J Steroid Biochem Mol Biol. 2013;136:195-200. doi:10.1016/j.jsbmb.2012.12.003.
33. Mohammed IH, KakeyES, Farimani MM. In Vitro Evaluation of Antioxidant Activates for Parts of Rhubarb (Rheum ribes) and Syrian Mesquite (Prosopis farcta). ICPAS. 2018. doi:10.14500/icpas2018.
34. Al-Daghri NM, Al-Attas OS, Alokail MS et al. Increased vitamin D supplementation recommended during summer season in the gulf region: a counterintuitive seasonal effect in vitamin D levels in adult, overweight and obese Middle Eastern residents. Clin Endocrinol (Oxf). 2012;76(3):346-350. doi:10.1111/j.1365-2265.2011.04219.x.
35. Al-Assadi AF, Al-Haroon DhS, Al-Rubaye AH et al. Serum Vitamin D level among infertile women at Basra city. J Women's Health Care. 2018;7(6):452.
36. Saiel AA, Rashied RM, AL-Rawi KF. The Association of Serum Irisin with Vitamin D and Body Mass Index in Healthy Adults. Medico Legal Update. 2021;21(2):58-62. doi:10.37506/mlu.v21i2.2645.
37. Dominguez LJ, Farruggia M, Veronese N, Barbagallo M. Vitamin D Sources, Metabolism, and Deficiency: Available Compounds and Guidelines for Its Treatment. Metabolites. 2021;11(4):255. doi:10.3390/metabo11040255.
38. Nair R, Maseeh A. Vitamin D: The "sunshine" vitamin. J Pharmacol Pharmacother. 2012;3(2):118-126. doi:10.4103/0976-500X.95506.
39. Hanwell HE, Vieth R, Cole DE et al. Sun exposure questionnaire predicts circulating 25-hydroxyvitamin D concentrations in Caucasian hospital workers in southern Italy. J Steroid Biochem Mol Biol. 2010;121(1-2):334-337. doi:10.1016/j.jsbmb.2010.03.023.
40. Chin WL, Jamil A, Nor NM. Sun Exposure among healthy adults in a health facility. Mal. J Med Health Sci. 2018;114(3):24-26.

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Zainab Nazar Hasan Anber
Baghdad College of Medical Sciences
M/120-G3-Building 5, Baghdad, Iraq
e-mail: zainab.anber@yahoo.com

ORCID AND CONTRIBUTIONSHIP
Basil E. Al-Hadithy: 0009-0001-2404-5591 B
Basil Oied Mohammed Saleh: 0009-0003-9407-9169 C
Zainab Nazar Hasan Anber: 0000-0002-5501-777X E, F
Nazar S Al-Hadad: 0009-0005-8927-4861 A, D

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

RECEIVED: 14.06.2023
ACCEPTED: 17.02.2024

ORIGINAL ARTICLE

Comparative study of oral microbiota in the experimental long-term opioid exposure, after its withdrawal and the use of complex drug correction

Volodymyr B. Fik[1], Roman P. Krynytskyi[1], Olha V. Dudok[1], Mariia V. Podoliyk[1], Myroslava A. Kosiuta[2], Larysa Ya. Fedoniuk[3]

[1]DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE
[2]PRIVATE HIGHER EDUCATION INSTITUTION "LVIV MEDICAL UNIVERSITY", LVIV, UKRAINE
[3]I. HORBACHEVSKY TERNOPIL NATIONAL MEDICAL UNIVERSITY, TERNOPIL, UKRAINE

ABSTRACT

Aim: To study changes of dental biofilm microbiota composition during experimental opioid exposure, after its withdrawal and when using of complex drug correction.

Materials and Methods: Microbiological studies (48 rats) included microscopic and bacteriological methods, as well as determination of antibiotic susceptibility of microbial isolates. Ceftriaxone and pentoxifylline were used to correction the changes.

Results: The action of opioid for 10 weeks caused considerable changes in the microbiocenosis, which was illustrated by a significant increasing of the opportunistic pathogens quantitative indicators and the emergence of pathogenic microbiota. Changes in the microbiocenosis at 6 weeks of opioid exposure and after its withdrawal for 4 weeks were expressed in the appearance of pathogenic microbiota and the absence of significant differences in quantitative indicators of saprophytic and opportunistic microflora compared to similar indicators in animals with 10 weeks opioid exposure. This indicated a slow progression of dysbiotic changes and the inflammatory process in the oral cavity of rats.

Conclusions: After 10 weeks of experiment with opioid administration for 6 weeks and the use of ceftriaxone and pentoxifylline on the background of 4-week opioid withdrawal, a significant reduction of quantitative indicators of opportunistic bacteria and elimination of pathogenic species of microorganisms was determined. The use of complex drug correction on the background of 10 weeks of opioid exposure led to a significant reduction in the quantitative indicators of opportunistic pathogens and contributed to the elimination of most pathogenic species of microbiota under the action of ceftriaxone.

KEY WORDS: oral cavity, rats, opioid exposure, microbiota, drug correction

Pol Merkur Lek,2024;53(1):216-225. doi: 10.36740/Merkur202402111

INTRODUCTION

Natural and synthetic opioids are potent analgesics, which are usually used in medical practice for the treatment of acute and chronic pain according to the relevant clinical indications [1, 2]. However, using of opioids in long termed period causes dependence on the effect of the drug action and forms a persistent tendency to the development and inflammatory processes chronization, in particular, in the oral cavity cells, tissues, organs [3, 4]. Among people who abuse drugs, dental caries and chronic periodontitis are significantly more common than in healthy people [5], due to the altered microbiome profile, namely the increased density of colonization of the oral mucosa opportunistic and pathogenic microbiota [6-8]. It should be noted that opportunistic and pathogenic bacteria of the dental biofilm are a determining factor in the coadaptation of the oral cavity microbiocenosis and the appearance of a new econiche – periodontal pocket [9-11]. In this case, the increase in the mass of the biofilm causes the reproduction of parodontopathogensics [10, 11] that destroys the epithelium of the gums with the release of local mediators of inflammation from the periodontal pocket into the systemic circulation, which promotes the recruitment of immune cells in the body involving neutrophils and macrophages in the affected tissue [12].

The microbial etiology of purulent-inflammatory processes determines the need of antibacterial therapy, which creates the conditions for pathogenetically oriented treatment of inflammatory diseases in the oral cavity paradental tissues [13, 14]. Since, in inflammatory diseases of the oral cavity there is an increase in the resistance of opportunistic pathogens to various antibacterial agents, to select an effective etiotropic treatment it is essential to test the degree of sensitivity of regional isolates to antibiotics – antibioticogram [15]. Conducted studies in vitro, in vivo, as well as studies in patients on opioid exposure, have shown that another side effect is their ability to suppress immune reactions – immunosuppression, which leads to increased susceptibility to infections [2, 16]. In addition, the hypoxic microenvironment caused by oral pathogens is an

important factor in disrupting of the dynamic hemostasis between the immune system and oral microbiome, resulting in periodontal infection intensifies the inflammatory reaction and causes vascular disorders [17]. Accordingly, in order to achieve a stable positive result of therapy in drug-addicted patients with periodontal disease, it is advisable to use drugs of etiopathogenetic treatment. Of particular note is the antispasmodic pentoxifylline, which also has antihypoxant, antioxidant, anti-inflammatory and immunomodulatory effects, which has been confirmed in experimental and clinical research [18-20]. However, in the available professional literature, we did not find data on the development of adequate treatment of pathological conditions and infectious foci in the oral cavity under long-term action of opioid agents, which allows to test such a scheme in the experiment. All of the above determined the direction of this investigation.

AIM

The aim of the scientific research was to identify changes in the microbiota composition of the rats dental biofilm during long-term experimental opioid exposure, after its withdrawal and when using of complex drug correction.

MATERIALS AND METHODS

48 white outbred adult male rats, aged 4.5–7.0 months, weighing 160–255 grams were taking for the experiment. The animals were administered opioid analgesic nalbuphine with the active substance nalbuphine hydrochloride intramuscularly daily, once, with a gradual increase in dose every 2 weeks, as well as after its withdrawal and the use of drug correction. In the experiment, the animals were divided into five groups. I control group included rats, which during the experiment were intramuscularly injected by saline. Group II (experimental) included rats, which were administered by *nalbuphine hydrochloride* during 10 weeks, with the increasing of dose every 2 weeks: 1-2 weeks – 0.212 mg/kg, 3-4 weeks – 0.225 mg/kg, 5-6 weeks – 0.252 mg/kg, 7-8 weeks – 0.260 mg/kg, 9-10 weeks – 0.283 mg/kg. The proposed scheme of gradually increasing dosage of the drug allowed to simulate chronic opioid exposure [21]. In Group III intramuscular injections of the opioid analgesic was performed daily for 6 weeks (1-2 weeks – 0.212 mg/kg, 3-4 weeks – 0.225 mg/kg, 5-6 weeks – 0.252 mg/kg) with subsequent 4-week withdrawal of the drug. In Group IV – animals received 6 weeks of nalbuphine (0.212 – 0.252 mg/kg) with subsequent 4-week its withdrawal and a complex drug correction, which included an 11-day course of antibiotic therapy with ceftriaxone at the end of the experiment (9-10 weeks) and intramuscular injections of pentoxifylline (7-10 weeks). In Group V the animals were injected daily (10 weeks) *nalbuphine hydrochloride* with a gradual increase doses every 2 weeks – from 0,212 to 0,283 mg/kg, and they received intramuscular injections of ceftriaxone (11 days) and pentoxifylline (during 7-10 weeks).

The animals were in general conditions of the vivarium. The Bioethics Commission of Danylo Halytsky Lviv National Medical University have made a conclussion that this scientific research uses ethical requirements due to the European Convention "for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [Strasbourg, 1985], to the order of the Ministry of Health of Ukraine № 231 of 01.11.2000 (Protocol № 5 of 24.05.2021). Microbiological studies included microscopic and bacteriological methods, as well as determination of antibiotic susceptibility of microbial isolates by disco-diffusion method. Collection of material for the study of microbiota was performed on the dental biofilm (the tooth surface in the gingival margin) of the upper and lower jaws of rats. The dental biofilm microbiota reflects the general state of the oral microbiome and is very stable. Microscopic examination of smears, and Gram stained, help to count the number of cellular elements in the field of view. Number and volume of prokaryotic gram-negative and gram-positive microflora were illustrated by microscopic method and evaluation was performed by interposition of cells. It was indicated the the presence of extracellular structures, different morphological bacterial types as elements of the biofilm. Bacteriological studies were used for the species and quantitative characteristics of the microbial composition, with culture of the material on standard nutrient media, including nutrient media for anaerobic bacteria. As a result of summarizing the general number of sprouted colonies, a quantitative index of colony-forming units was counted (CFU/ml). In order to select an antibacterial drug in the complex drug correction, the antibiotic susceptibility of strains of opportunistic and pathogenic bacteria isolated at the end of 10 weeks of opioid exposure was determined. The sensitivity of the isolated bacterial isolates to beta-lactam antibiotics of the cephalosporin series – cefazolin and ceftriaxone, as well as ciprofloxacin, from the group of fluoroquinolones was determined by the disk diffusion method. Based on the analysis of antibiotic sensitivity, it was found that the isolates of the studied microorganisms are the most sensitive to ceftriaxone. The antibacterial agent ceftriaxone was administered intramuscularly for 11 days (9-10 weeks), 1 g per day, respectively, a single daily dose for one animal with an average body weight of 200 g was 2.86 mg. Pentoxifylline (a group of peripheral vasodilators, purine derivatives) was also used as part of the complex drug correction, which was administered intramuscularly at a dose of 2.86 mg during 4 weeks at the end of the experiment (7-10 weeks) in IV and V experimental groups.

For further statistical analysis, the results were verified for normality by calculating the asymmetry and excess coefficients and by the results of the Shapiro-Wilk test ($p < 0.05$). Results are presented as $M \pm SD$, where M is the mean and SD is the standard deviation. For normally distributed results, bilateralt-test was used to identify the significance of differences between two groups of animals; to determine the significance of differences between three or more animals' groups, ANOVA with Tukey's posthoc test was used. The next pairwise comparison was made. If the distribution of the results differed from the normal distribution, nonparametric criteria were used to establish

the reliability of the differences between groups-theMann-Whitney U test for comparisons between two groups, the Kruskel-Wallis H test for comparisons between three or more independent groups, and further paired comparisons with the Dunn test Apost-hoc analysis was used for pair wise comparisons. All statistical computations were performed using RStudio v. 1.1.442 [22, 23] and R Commander v.2.4-4 [24].Figures and tables were prepared with the help of Microsoft Office Excel.

RESULTS

Microscopic examination of smears from the tooth surface in the gingival margin of the I (control) group of rats showed the one-species microbiota. In general, the smears mainly showed Gram-positive microorganisms – long filamentous Gram-positive bacteria *Leptothrix*, identified by morphological features of *Lactobacilli* of the genus *Lactobacillus*, located singly, in pairs, or in short chains. *Coccal* gram-positive microflora was located in separate clusters. Cellular elements were 3-5 leukocytes and 2-3 epitheliocytes in the field of view. Bacteriological analysis revealed the presence of microorganisms, which according to biochemical and morphotinctorial properties were divided into 3 subgroups. Gram-positive species saprophytic and opportunistic microbiota, namely, non-hemolytic *Streptococci* (43.00±6.56 CFU/ml), α-hemolytic *Streptococci* (64.33±5.13 CFU/ml), coagulase-negative *Staphylococci* (9.67±2.89 CFU/ml) and *Enterococci* (16.33±1.15 CFU/ml). were assigned to subgroup 1. On dense nutrient media, the growth of different colonies was observed, around them areas of complete or incomplete (α-) hemolysis or signs of no hemolysis were visualized. 2 subgroups were bacilli: gram-positive non-spore-forming rods (10.33±1.15 CFU/ml) and gram-positive spore-forming rods (9.67±0.58 CFU/ml). Subgroup 3 consisted of gram-negative microorganisms – lactose-positive enterobacteria – *Escherichia coli* (6.00±0.00 CFU/ml).

At the end of the 10 week of opioid exposure (Group II) in order to select an antibacterial drug in the complex drug correction, we conducted a study of antibiotic susceptibility of bacterial isolates – potential pathogens of the inflammatory process which were isolated from the dental biofilm of the oral cavity of rats. As a result of the analysis of the sensitivity of microorganisms to antibiotics, it was found that the isolated strains of microorganisms had the highest sensitivity to ceftriaxone. 89,3 % *Staphylococcus aureus* and 88,2 % *Staphylococcus intermedius* (microbial association of coagulase-positive *Staphylococci*), 87,5 % *Streptococcus pyogenes* (bacterial grouping of β-hemolytic *Streptococci*), 86,7 % non-hemolytic *Streptococci*, 80,8 % *Escherichia coli* and 76,0 % hemolytic *Escherichia coli* were highly sensitive and sensitive to this antibiotic (Tabl. 1). In addition to specified bacterial species, *Klebsiella* and *Pseudomonas aeruginosa* isolates, which were sensitive to ceftriaxone, were also studied.

In group II animals, after 10 weeks of experimental opioid exposure, microscopic studies of dental biofilm material showed changes in the correlation of components in bacterial morphotypes, fungal microbiota, neutrophil leukocytes, degeneratively altered and destroyed epitheliocytes up to 8-10 in the field of view. In the presence of formed periodontal pockets, capsule bacteria – *Klebsiella* and periodontopathogenics species – bacteroids were also detected in smear from subgingival biofilms. Bacteriological studies showed a significant increase in quantitative indicators of non-hemolytic *Streptococci* (60.22 ± 7.00 CFU/ml), gram-positive non-spore-forming rods (19.22 ± 4.63 CFU/ml), gram-positive spore-forming rods (15.11 ± 2.52 CFU/ml), α-hemolytic *Streptococci* (76.33 ± 3.32 CFU/ml), coagulase-negative *Staphylococci* (59.22 ± 5.24 CFU/ml), *Enterococci* (32.22 ± 5.74 CFU/ml) and *Escherichia coli* (58.33 ± 6.93 CFU/ml) – 1.4, 1.9, 1.5, 1.2, 6.1, 2.0 and 9.7 times, respectively, compared with similar indicators of the control group of animals ($p < 0.05$). Changes in the species and quantitative composition of the microbiota were caused by the appearance of bacterial associations that were absent in the control (Table 1).

Group of rats, in particular, hemolytic *Escherichia coli* (42.56±4.48 CFU/ml), β-hemolytic s reptococci (51.44±6.91 CFU/ml), coagulase-positive *Staphylococci* (39.11±6.68 CFU/ml), *Klebsiella* (22.44±4.00 CFU/ml), bacteria of group Pseudomonas a ruginosa (3.11±1.05 CFU/ml) and single colonies of bacteroids under anaerobic c nditions were sown. Fungal microflora consisted of Yeast-like fungi and filaments fungi in small quantities.

Microbiological studies in group III of animals, at the end of 10 weeks of the experiment (6 weeks of opioid exposure and after its withdrawal for 4 weeks) revealed stable changes in the microbiotope of dental biofilm of rats due to appearance of pathogenic and increasing number of opportunistic species of microorganisms. Mixed microbiota with the participation of Gram-positive cocci, gram-negative bacteria of different morphotypes, epithelial cells with adsorbed *coccal* microflora were detected in the studied smears from dental biofilm of animals. Changes in the composition of bacterial morphotypes in group III were less pronounced than in group II of animals (10 weeks of opioid administration), however, thay indicated violations of the microbiocenosis of the studied microbiotope, which persisted after opioid withdrawal. According to the results of bacteriological studies, it was found that the number of non-hemolytic *Streptococci* (51.7±6.04 CFU/ml) increases compared to the corresponding indicator of animals in group II. Gram-positive non-spore-forming rods (13.33±1.87 CFU/ml) significantly increased – 1.3 times compared to the control and significantly decreased – 1.4 times, compared with the same indicator of animals in group II ($p < 0, 05$). The quantitative composition of Gram-positive spore-forming rods (24.33±3.28 CFU/ml) significantly increased – 2.5 times compared to the control and 1.6 times relative to the corresponding indicator in animals in group II ($p < 0.05$). The quantitative composition of Gram-positive spore-forming rods (24.33±3.28 CFU/ml) was significantly increased – 2.5 times, compared to the control and 1.6 times compared with the corresponding indicator in animals in group II ($p < 0.05$) (Table 2). It was noteworthy, that after opioid withdrawal, both opportunistic and pathogenic bacterial

Table 1. Antibiotic sensitivity of the bacterial isolates of dental biofilm of rats, isolated after 10 weeks of opioid exposure

Bacterial species	N	Antibacterial drug											
		Ciprofloxacin				Cefazolin				Ceftriaxone			
		Degree of sensitivity				Degree of sensitivity				Degree of sensitivity			
		h/sen	sen	m/st	st	h/sen	sen	m/st	st	h/sen	sen	m/st	st
Staphylo-coccus aureus	28	n 10	n 13	n 5	-	n 8	n 14	n 6	-	n 19	n 6	n 3	-
	%	35,7	46,4	17,9		28,6	50,0	21,4		67,9	21,4	10,7	
Staphylo-coccus intermedius	34	n 20	n 8	n 6	-	n 14	n 13	n 7	-	n 23	n 7	n 4	-
	%	58,9	23,5	17,6		41,2	38,2	20,6		67,6	20.6	11,8	
Nonhemolytic streptococci	30	n 11	n 14	n 5	-	n 7	n 16	n 7	-	n 21	n 5	n 4	-
	%	36,7	46,6	16,7		23,3	53,4	23,3		70,0	16,7	13,3	
Streptococus pyogenes	32	n 17	n 10	n 5	-	n 15	n 10	n7	-	n 21	n 7	n 4	-
	%	53,1	31,3	15,6		46,8	31,3	21,9		65,6	21,9	12,5	
Esherichia coli	26	n 12	n 8	n 6	-	n 7	n 11	n 8	-	n 13	n 8	n 5	-
	%	46,1	30,8	23,1		26,9	42,3	30,8		50,0	30,8	19,2	
Hemolytic Escherichia coli	25	n 8	n 10	n 7	-	n 6	n 11	n 5	n3	n 13	n 6	n 6	-
	%	32,0	40,0	28,0		24,0	44,0	20,0	12,0	52,0	24,0	24,0	

Notes: N –total number of bacteria, n – the number of isolates with the appropriate degree of sensitivity; h/sen – highly sensitive, sen – sensitive, m/st – moderately stable, st – stable.

Table 2. The composition of the microbiota of the dental biofilm of rats at 6 weeks of opioid exposure and after its 4-week withdrawal (CFU / ml)

№	Bacterial groups	Quantitative composition		
		Control	Group II	Group III
1.	*Nonhemolytic streptococci*	43.00±6.56	60.22±7.00*	51.78±6.04
2.	*Gram-positive non-spore-forming rods*	10.33±1.15	19.22±4.63*	13.33±1.87**,***
3.	*Gram-positive spore-forming rods*	9.67±0.58	15.11±2.52*	24.33±3.28**,***
4.	*α-hemolytic streptococci*	64.33±5.13	76.33±3.32*	57.56±9.07***
5.	*Coagulase-negative staphylococci*	9.67±2.89	59.22±5.24*	60.44±4.16**
6.	*Enterococci*	16.33±1.15	32.22±5.74*	32.22±7.41**
7.	*Escherichia coli*	6.00±0.00	58.33±6.93*	26.33±5.92**,***
8.	*Hemolytic Escherichia coli*	–	42.56±4.48	3.00±1.00***
9.	*β-hemolytic streptococci*	–	51.44±6.91	1-2
10.	*Coagulase-positive staphylococci*	–	39.11±6.68	18.11±1.17***
11.	*Klebsiella*	–	22.44±4.00	1-2
12.	*Bacteroids*	–	1-2	1-2
13.	*Pseudomonas aeruginosa*	–	3.11±1.05	–
14.	*Yeast-like fungi*	–	1-2	7.00±0.71
15.	*Filamentous fungi*	–	1-2	1-2

*Notes: data are presented in the form of M ± SD, where M is the average value, SD is the standard deviation; * p <0.05 is a significant difference between the values of group II relative to the control, ** p <0.05 is a significant difference between the values of group III relative to the control, *** p <0.05 is a significant difference between the values of group III relative to the group II.*

species, such as hemolytic *Escherichia coli*, *Klebsiella*, and coagulase-negative *Staphylococci* and coagulase-positive *Staphylococci*, were seeded at the end of 10th week of the experiment. The presence of significant amounts of α-hemolytic *Streptococci* (57.56±9.07 CFU/ml) indicated a tendency to the formation of the dental biofilm, however, their number decreased significantly – 1.3 times, compared with the same indicator in animals in group II ($p < 0.05$).

Durng research it also was indicated a significant increasing of the coagulase-negative *Staphylococci* (60.44±4.16 CFU/ml) – 6.2 times compared with the corresponding indicator of control group ($p <0.05$), however, their number did not practically differ compared with the quantitative composition in animals in group II. The quantitative composition indicators of *Enterococci* (32.22±7.41 CFU/ml) also had no differences with similar indicators in animals in group II, however, significantly increased – 2.0 times compared to control ($p <0.05$). It was illustrated a prominentt difference in the contamination of *Escherichia coli* (26.33±5.92 CFU/ml), namely, a significant increase – 4.4 times compared to the control and a significant decrease – 2.2 times, compared with the similar indicator in group II ($p <0.05$).

It was noted that in experimental group of animals number III were present colonies of hemolytic *Escherichia coli* (3.00 ± 1.00 CFU/ml) and coagulase-positive *Staphylococci* (18.11±1.17 CFU/ml), in particular, *Staphylococcus aureus* and *Staphylococcus intermedius*, which were absent in the control group of animals, while their number was significantly reduced compared to the corresponding indicators in group II. Single colonies (1-2) of *Klebsiella*, β-hemolytic *Streptococci* and filamentous fungi, and bacteroids were visualized on nutrient media, indicating progression of dysbiotic changes in the rats' oral cavity. Yeast-like fungi were sown in the amount of 7.00±0.71 CFU/ml (Table 2).

The composition of the aerobic microflora in rats in group IV (10 weeks of the experiment) on the background of 6 weeks of opioid exposure, after its 4-week withdrawal and use of ceftriaxone and pentoxifylline, changed significantly and tended to reduce bacterial contamination compared with animals that were under 10 weeks of opioid exposure. During microscopic examination of smears, accumulations of *coccal* gram-positive microorganisms and adsorption of *coccal* microbiota on epitheliocytes were visualized. Bacteriological studies showed a significant decrease in non-hemolytic *Streptococci* (17.11±3.30 CFU/ml) – 2.5 times compared to control and 3.5 times compared to the corresponding indicator of animals in experimental II group ($p <0.05$) (Table 3).

Bacterial species of Grame positive non-spore-forming rods and Grame positive spore-forming rods in group IV of animals were eliminated, apparently due to the action of the antibacterial drug. A characteristic feature was also that the quantitative composition of opportunistic microbiota changed significantly, namely groups that could include species naturally resistant to ceftriaxone. In particular, antibiotic therapy with opioid withdrawal led to a significant reduction in quantitative indicators of coagulase-negative *Staphylococci* (4.00±0.71 CFU/ml) – 2.4 times compared to control and 14.8 times compared to group II ($p <0.05$). The quantitative indicator of α-hemolytic *Streptococci*

(10.33±1.22 CFU/ml) significantly decreased – 6.2 times compared to the control and 7.4 times, compared with the same indicator in animals in group II ($p < 0.05$). The quantitative composition of *Enterococci* (26.67±4.92 CFU/ml), which are naturally resistant to antibiotics, decreased slightly compared to the corresponding indicator in group II, however, significantly increased – 1.6 times compared to the control ($p < 0.05$). At the same time, the quantitative indicators of *Escherichia coli* (6.00±1.22 CFU/ml) did not differ from the control and significantly decreased – 9.7 times, compared with the same indicator of animals in group II ($p < 0.05$). Colonies of Yeast-like fungi (10.11±1.62 CFU/ml) were sown, however, pathogenic species of microorganisms were eliminated, which testified to the effectiveness of the course of antibiotic therapy under such conditions (Table 3).

The aim of the study of animals of the group V (10 weeks – opioid and concomitant drug correction) was to determine the relationship between long-term opioid exposure and the effectiveness of the release of oral cavity of rats under the influence of ceftriaxone at the end of the experiment (60-70 days). Microscopic examinations revealed a decrease in bacterial species capable of forming extracellular structures – the dental biofilm. Microscopic examinations revealed a decrease in bacterial species capable of forming extracellular structures – dental biofilm. Gram-positive microbiota prevailed in smears: rods and cocci, single gram-negative bacteria were observed. Epithelial cells with adsorbed *coccal* microbiota, leukocytes, destructively altered epitheliocytes were sometimes found in the field of view. In bacteriological studies there was a significant decrease in quantitative indicators of non-hemolytic *Streptococci* (6.00±1.50 CFU/ml) – 7.1 times, compared with controls and 10.0 times compared with the same in animals in group II ($p < 0.05$). As in the previous group, with the use of ceftriaxone and pentoxifylline, Gram-positive non-spore-forming rods and Gram-positive spore-forming rods were not detected on nutrient media. After the course of antibiotic therapy, a small number of opportunistic pathogens were isolated, namely groups that could include species that are naturally resistant to ceftriaxone. Therefore, the amount of α-hemolytic *Streptococci* (12.22±2.33 CFU/ml) significantly decreased – 5.3 times compared to the control and 6.2 times compared to the corresponding indicator in animals in group II ($p < 0.05$). Quantitative indicators of coagulase-negative *Staphylococci* (11.22±2.44 CFU/ml) slightly increased compared to control, however, they decreased significantly – 5.3 times compared to the same indicator in rats in group II ($p < 0.05$). The amount of *Enterococci* (29.33±6.67 CFU/ml) decreased slightly compared with the corresponding quantitative composition in animals in group II, however, it significantly increased – 1.8 times compared to the control ($p < 0.05$). The quantitative content of *Escherichia coli* (6.00±0.71 CFU/ml) did not differ from the same indicator of the control group, while it significantly decreased – 9.8 times compared to the corresponding indicator in group II ($p < 0.05$). Single colonies (1-2) of Gram-negative bacteria, in particular, *Klebsiella* and bacteroids, as well as filamentous fungi, were sown. The amount of Yeast-like fungi increased to 12.00±0.71 CFU/ml, indicating the presence of moderate candidiasis on the background of intensive antibacterial

Table 3. The composition of the microbiota of the dental biofilm of rats at 6 weeks of opioid exposure, after its 4-week withdrawal and drug correction (CFU / ml)

№	Bacterial groups	Quantitative composition		
		Control	Group II	Group IV
1.	*Nonhemolytic streptococci*	43.00±6.56	60.22±7.00*	17.11±3.30**,***
2.	*Gram-positive non-spore-forming rods*	10.33±1.15	19.22±4.63*	–
3.	*Gram-positive spore-forming rods*	9.67±0.58	15.11±2.52*	–
4.	*a-hemolytic streptococci*	64.33±5.13	76.33±3.32*	10.33±1.22**,***
5.	*Coagulase-negative staphylococci*	9.67±2.89	59.22±5.24*	4.00±0.71**,***
6.	*Enterococci*	16.33±1.15	32.22±5.74*	26.67±4.92**
7.	*Escherichia coli*	6.00±0.00	58.33±6.93*	6.00 ±1.22***
8.	*Hemolytic Escherichia coli*	–	42.56±4.48	–
9.	*β-hemolytic streptococci*	–	51.44±6.91	–
10.	*Coagulase-positive staphylococci*	–	39.11±6.68	–
11.	*Klebsiella*	–	22.44±4.00	–
12.	*Bacteroids*	–	1-2	–
13.	*Pseudomonas aeruginosa*	–	3.11±1.05	–
14.	*Yeast-like fungi*	–	1-2	10.11±1.62
15.	*Filamentous fungi*	–	1-2	–

*Notes: data are presented in the form of M ± SD, where M is the average value, SD is the standard deviation; * $p < 0.05$ is a significant difference between the values of group II relative to the control, ** $p < 0.05$ is a significant difference between the values of group IV relative to the control, *** $p < 0.05$ is a significant difference between the values of group IV relative to the group II.*

Table 4. The composition of the microbiota of the dental biofilm of rats at 10 weeks of opioid exposure and concomitant use of drug correction (CFU / ml)

№	Bacterial groups	Quantitative composition		
		Control	Group II	Group V
1.	*Nonhemolytic streptococci*	43.00±6.56	60.22±7.00*	6.00±1.50**,***
2.	*Gram-positive non-spore-forming rods*	10.33±1.15	19.22±4.63*	-
3.	*Gram-positive spore-forming rods*	9.67±0.58	15.11±2.52*	-
4.	*α-hemolytic streptococci*	64.33±5.13	76.33±3.32*	12.22±2.33**,***
5.	*Coagulase-negative staphylococci*	9.67±2.89	59.22±5.24*	11.22±2.44***
6.	*Enterococci*	16.33±1.15	32.22±5.74*	29.33±6.67**
7.	*Escherichia coli*	6.00±0.00	58.33±6.93*	6.00±0.71***
8.	*Hemolytic Escherichia coli*	–	42.56±4.48	–
9.	*β-hemolytic streptococci*	–	51.44±6.91	–
10.	*Coagulase-positive staphylococci*	–	39.11±6.68	–
11.	*Klebsiella*	–	22.44±4.00	1-2
12.	*Bacteroids*	–	1-2	1-2
13.	*Pseudomonas aeruginosa*	–	3.11±1.05	–
14.	*Yeast-like fungi*	–	1-2	12.00±0.71
15.	*Filamentous fungi*	–	1-2	1-2

*Notes: data are presented in the form of M ± SD, where M is the average value, SD is the standard deviation; * p <0.05 is a significant difference between the values of group II relative to the control, ** p <0.05 is a significant difference between the values of group V relative to the control, *** p <0.05 is a significant difference between the values of group V relative to the group II.*

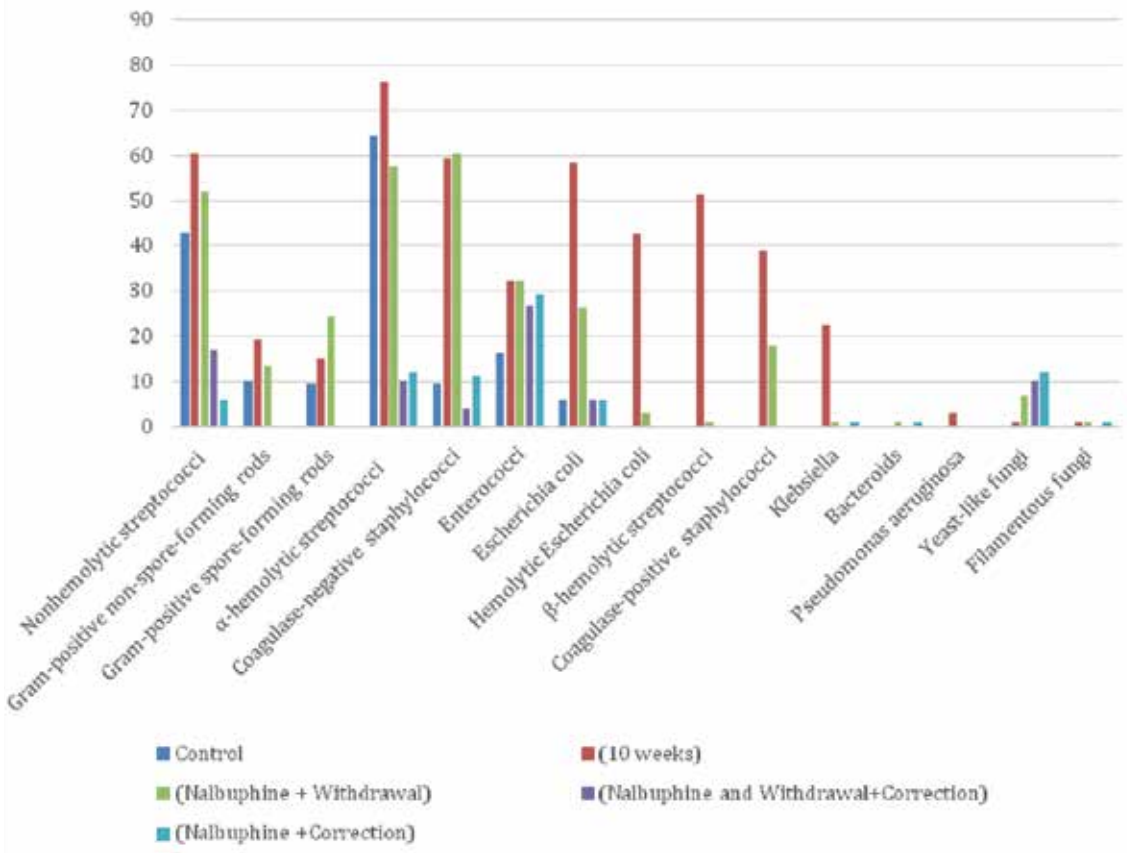


Fig.1. Changes of the quantitative and the qualitative composition of the dental biofilm microbiocenosis at the end of 10 weeks of experimental long-term opioid exposure, after its withdrawal and drug correction.

therapy. It should be noted, that after the use of ceftriaxone, on the background of chronic opioid exposure, pathogenic species of microorganisms highly sensitive to antibiotics, namely, β-hemolytic *Streptococci*, coagulase-positive *Staphylococci*, and opportunistic hemolytic *Escherichia coli* were eliminated (Table 4).

The obtained results of microbiological studies allowed to establish patterns of dynamics of changes in microbiocenosis in the studied microbiotope of the oral cavity of rats during the experiment under conditions of long-term opioid exposure and after its withdrawal. It helps to find the possibility to correct disorders with using pentoxifylline and antibacterial agent with the active substance ceftriaxone, bacterial isolates isolated at the end of 10 weeks of opioid exposure were highly sensitive to it (Fig. 1).

DISCUSSION

Accordingly, in the case of chronic opioid exposure develop he erogeneous microbiocenoses of the oral cavity, which depends on the duration of drug administration. Whereas constant and prolonged use of injecting drugs may en anger the physiological barriers of the oral mucosa and increase the risk of contamination of chronic viral and bacterial infections [6, 25-28]. It is very visible directly proportional dependence of the degree of damage of the oral cavity mucosa and periodontium on the duration of drug when using of them and concomitant infectious diseases' severity in the drug-addicted patients [8, 28-30]. Under the influence of opiates were found pathogenic microorganisms, namely, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* [30]. In addition, when the dynamic balance between autochthonous and allochthonous bacteria of the oral cavity is disturbed, periodontal pathogenic microorganisms appear and it may determine the transition of various forms of gingivitis to periodontitis [9, 31, 32]. We found similar changes in our experimental studies, in particular, at the end of 10 weeks of opioid exposure from the formed periodontal pockets were sown various microbial associations, namely periodontal pathogenic bacteroids, as well as *Klebsiella* and – *Pseudomonas aeruginosa* – microorganisms that can provoke the development of purulent foci in the oral cavity with the subsequent development of stomatogenic sepsis.

The results of our work also coincide with the data obtained in patients who inject drugs, in particular, the most common type of microorganism in such individuals was Staphylococcus aureus, it should be considered in empirical therapy, and in some cases colonization by enterobacteria was noted, in particular *Escherichia coli* is a typical causative agent of bacteremia in the general population [28]. In microbiological studies, we found that the quantitative indicators of microbiota of animals of group II (6 weeks of opioid administration and 4 weeks after its withdrawal) were slightly improved compared to the corresponding indicators of animals at 10 weeks of opioid administration. Nevertheless, the indicators of the altered microbiota of animals of group II remained consistently high and did not show significant signs of elimination of indicator pathogenic and opportunistic microbiota, compared with similar indicators of animals of the control group. It should also be noted that the microcirculatory bed of the oral cavity plays an important role in the processes to control the infection and to regulate the immune response. Bacteremia, that disrupts vascular hemostasis can cause a number of processes, including the releasing of prothrombotic mediators from endotheliocytes, which causes a vasoconstrictive reaction and it intensifies lipid peroxidation due to periodontal infection [12, 17].

Therefore, drugs, as a leading factor in the exogenous effect on the organs of the oral cavity with the progression of the inflammatory process in periodontal tissues, form the specifics of approaches to the implementation of therapeutic measures in this pathology. Analysis of the literature has shown that antibiotics as an adjunct might be used in the treatment of infective diseases the body of drug addicts in general [6, 13], and in patients with dental diseases, in order to prevent the bacterial infections development in the oral cavity [14]. In determining the antibiotic susceptibility of microorganisms isolated from the tooth surface in the gingival margin of rats exposed to 10 weeks of opioids, we found that the studied strains of bacteria – potential pathogens of purulent-inflammatory process, were highly sensitive and sensitive to ceftriaxone. In general, it can be argued that in the presence of oral dysbiosis in the area of the studied microbiotope of rats on the background of long-term opioid exposure, the therapeutic effect can be achieved with conventional doses of antibiotics against highly sensitive and sensitive bacterial isolates. Increased doses of the drug under such conditions should be applied to moderately resistant isolates of bacteria. If the pathogen is stable, the use of antibacterial drugs in this case is impractical. It is important to prove the effectiveness of complex drug correction with the use of ceftriaxone and pentoxifylline on the background of opioid effects and after its cancellation in the experiment, which forms the basis for the use of proven drugs in clinical settings.

The proven effectiveness of complex drug correction with the use of ceftriaxone and pentoxifylline on the background of opioid exposure and after its withdrawal in the experiment, is important and it forms the basis for the use of proven drugs in clinical settings. Thus, the use of pentoxifylline was pathogenetically justified due to the versatility of its pharmacodynamics. Whereas pentoxifylline is an antispasmodic and angioprotectant, the active ingredient pentoxifylline also improves antioxidant protection, tissue oxygenation and the immune response and except this the drug is effective in anti-inflammatory therapy [18, 19, 20].

Our results of microbiological studies indicate that in group IV (6 weeks – opioid, 4 weeks – withdrawal and use of pentoxifylline and ceftriaxone) and group V of animals (10 weeks – opioid and concomitant administration of pentoxifylline and ceftriaxone) that received complex drug correction allowed to achieve the most optimal stabilizing therapeutic effect, which prevented the rapid development of bacterial infections in the oral cavity on the background of long-term opioid exposure. The use of antibiotic therapy with ceftriaxone in the complex correction contributed

to the effective elimination or significant reduction of the number of indicator pathogenic and opportunistic microbiota, which testified to the effectiveness of these therapeutic measures to overcome the colonization density of bacteria of the microbiotope of the oral cavity with long-term opioid exposure.

CONCLUSIONS

The action of opioid for 10 weeks caused considerable changes in the microbiocenosis, which was manifested by a prominent increasing in the quantitative indicators of opportunistic pathogens compared with the control and the emergence of pathogenic microbiota, which led to progression of dysbiosis and development of purulent and inflammatory processes in the animals' oral cavity.

Changes in the microbiocenosis at 6 weeks of opioid exposure and after its withdrawal for 4 weeks were expressed in the appearance of pathogenic microbiota and the absence of significant differences in quantitative indicators of saprophytic and opportunistic microflora compared to similar indicators in animals with 10 weeks opioid exposure. These processes indicate a slow progression of dysbiotic changes and inflammation in the oral cavity of rats as a consequence of violations of the mechanisms of protection of the oral mucosa from infection.

Based on the analysis of antibioticograms at the end of 10 weeks of opioid exposure, it was found that the isolated bacterial isolates had the highest sensitivity to ceftriaxone. After 10 weeks of experiment with opioid administration for 6 weeks and the use of ceftriaxone and pentoxifylline on the background of 4-week opioid withdrawal, a significant reduction of quantitative indicators of opportunistic bacteria and elimination of pathogenic species of microorganisms was determined. The use of complex drug correction on the background of 10 weeks of opioid exposure led to a significant reduction in the quantitative indicators of opportunistic pathogens and contributed to the elimination of most pathogenic species of microbiota under the action of ceftriaxone, compared to animals injected for 10 weeks with opioids without any corrective action.

REFERENCES

1. Brejchova J, Holan V, Svoboda P. Expression of Opioid Receptors in Cells of the Immune System. Int J Mol Sci. 2020;22(1):315. doi: 10.3390/ijms22010315.
2. Plein LM, Rittner HL. Opioids and the immune system – friend or foe. Br. J. Pharmacol. 2018;175(14):2717-2725. doi: 10.1111/bph.13750.
3. Erim D, Obadan-Udoh E, Agaku I. Cost savings from averted prescription opioidattributable dental diseases in the United States between 2013 and 2019. Popul. Med. 2021;3:13. doi:10.18332/popmed/136488.
4. Fik VB, Fedechko YM, Paltov YeV. The composition of the microbiotes of the neck part of the teeth in the gum edge of rats at the end of the second, fourth and sixth weeks of opioid influence. World science. 2020;9(61):1-5. doi: 10.31435/rsglobal_ws/30122020/7338.
5. Hegazi F, Alhazmi H, Abdullah A et al. Prevalence of Oral Conditions Among Methamphetamine Users: NHANES 2009-2014. J. Public Health Dent. 2021;81(1):21-28. doi: 10.1111/jphd.12389.
6. Hartnett KP, Jackson KA, Felsen C et al. Bacterial and Fungal Infections in Persons Who Inject Drugs – Western New York, 2017. MMWR Morb Mortal Wkly. 2019;68(26):583-586. doi: 10.15585/mmwr.mm6826a2.
7. Kosciolek T, Victor TA, Kuplicki R et al. Individuals with substance use disorders have a distinct oral microbiome pattern. Brain Behav Immun Health. 2021;15:100271. doi:10.1016/j.bbih.2021.100271.
8. Petrushanko TO, Boichenko OM, Popovych IYu, Gryshchenko VV. The tate of Oral Cavity in the Drug-Depedent Patients. World of medicine and biology. 2021;3(77):148-151. doi: 10.26724/2079-8334-2021-3-77-148-151.
9. Dahlen G, Fejerskov O, Manji F. Current concepts and an alternative perspective on periodontal disease. BMC Oral Health. 2020;20(1):235. doi: 10.1186/s12903-020-01221-4.
10. Marcano R, Rojo MÁ, Cordoba-Diaz D, Garrosa M. Pathological and Therapeutic Approach to Endotoxin-Secreting Bacteria Involved in Periodontal Disease. Toxins (Basel). 2021;13(8):533. doi: 10.3390/toxins13080533.
11. Willis JR, Gabaldón T. The Human Oral Microbiome in Health and Disease: From Sequences to Ecosystems. Microorganisms. 2020;8(2):308. doi: 10.3390/microorganisms8020308.
12. Paul O, Arora P, Mayer M, Chatterjee S. Inflammation in Periodontal Disease: Possible Link to Vascular Disease. Front Physiol. 2021;11:609614. doi:10.3389/fphys.2020.609614.
13. Buttar R, Aleksejuniene J, Coil J. Antibiotic and opioid analgesic prescribing patterns of dentists in Vancouver and endodontic specialists in British Columbia. J Can Dent Assoc. 2017;83:h8.
14. Dana R, Azarpazhooh A, Laghapour N et al. Role of Dentists in Prescribing Opioid Analgesics and Antibiotics: An Overview. Dent Clin North Am. 2018;62(2):279-294. doi: 10.1016/j.cden.2017.11.007.
15. Bogacz M, Morawiec T, Śmieszek-Wilczewska J et al. Evaluation of Drug Susceptibility of Microorganisms in Odontogenic Inflammations and Dental Surgery Procedures Performed on an Outpatient Basis. Biomed Res Int. 2019:2010453. doi: 10.1155/2019/2010453.
16. Eisenstein TK. The Role of Opioid Receptors in Immune System Function. Front Immunol. 2019;10:2904. doi:10.3389/fimmu.2019.02904.
17. Celik D, Kantarci A. Vascular Changes and Hypoxia in Periodontal Disease as a Link to Systemic Complications. Pathogens. 2021;10(10):1280. doi: 10.3390/pathogens10101280.
18. Annamaraju P, Baradhi KM. Pentoxifylline. 2021. Treasure Island (FL): StatPearls Publishing. 2022.

19. González-Pacheco H, Amezcua-Guerra LM, Sandoval J, Arias-Mendoza A. Potential usefulness of pentoxifylline, a non-specific phosphodiesterase inhibitor with anti-inflammatory, anti-thrombotic, antioxidant, and anti-fibrogenic properties, in the treatment of SARS-CoV-2. Eur Rev Med Pharmacol Sci. 2020;24(13):7494-7496. doi: 10.26355/eurrev_202007_21921.
20. Ibrahim A, Abdel-Tawab H, Hussein T. Pentoxifylline and/or praziquantel reduce murine schistosomiasis mansoni histopathology via amelioration of liver functions. Egyptian Journal of Aquatic Biology & Fisheries. 2019;23(5):121-133. doi: 10.21608/EJABF.2019.67229.
21. Paltov EV, Fik VB, Vilkhova IV et al. Method of modeling chronic opioid influence. Danylo Halytskyi Lviv National Medical University. Ukrainian patent. UA76565. 2013 Jan 10. G09B 23/28.
22. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2020. https://www.R-project.org/ [Accessed 11 January 2024]
23. RStudio Team RStudio: Integrated Development for R. RStudio, Inc., Boston, MA. 2020. http://www.rstudio.com/ [Accessed 11 January 2024]
24. Fox J, Bouchet-Valat M. Rcmdr: R Commander. R package version 2.7-0. 2020. https://socialsciences.mcmaster.ca/jfox/Misc/Rcmdr/ [Accessed 11 January 2024]
25. Fraser AD, Zhang B, Khan H et al. Prescription Opioid Abuse and its Potential Role in Gross Dental Deca. Curr Drug Saf. 2017;12(1):22-26. doi: 10.2174/1574886311666160803111401.
26. Pang J, Tringale KR, Tapia VJ et al. Chronic Opioid Use Following Surgery for Oral Cavity Cancer. Otolaryngol.Head Neck Surg. 2017;143(12):1187-94. doi: 10.1001/jamaoto.2017.0582.
27. Schroeder AR, Dehghan M, Newman TB et al. Association of Opioid Prescriptions From Dental Clinicians for US Adolescents and Young Adults With Subsequent Opioid Use and Abuse. JAMA Intern Med. 2019;179(2):145-152. doi: 10.1001/jamainternmed.2018.5419.
28. Thønnings S, Jansåker F, Sundqvist C et al. Prevalence and recurrence of bacteraemia in hospitalised people who inject drugs – a single Centre retrospective cohort study in Denmark. BMC Infect Dis. 2020;20(1):634. doi: 10.1186/s12879-020-05357-0.
29. Yang Y, Yu X, Yang X et al. Oral Microbiota Profile of Individuals Who Abuse Methamphetamine. Front Cell Infect Microbiol. 2021;11:706961. doi: 10.3389/fcimb.2021.706961.
30. Zhan SH, French L. Sequence similarity searches for morphine biosynthesis enzymes in bacteria yield putative targets for understanding associations between infection and opiate administration. J Med Microbiol. 2019;68(6):952-956. doi: 10.1099/jmm.0.001001.
31. Lenartova M, Tesinska B, Janatova T et al. The Oral Microbiome in Periodontal Health. Front Cell Infect Microbiol. 2021;11:629723. doi: 10.3389/fcimb.2021.629723.
32. Xiao J, Fiscella KA, Gill SR. Oral microbiome: possible harbinger for childrens health. Int J of Oral Sci. 2020;12(1):12. doi:10.1038/s41368-020-0082-x.

The research highlighted in the article became a fragment of the research work of the Normal Anatomy Department of the Danylo Halytskyy Lviv National Medical University:"Morpho-functional features of organs in the pre- and postnatal periods of ontogenesis, under the influence of opioids, food supplements, reconstructive surgery and obesity", state registration number 0120U002129, deadline 2020-2024.

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Volodymyr B. Fik
Danylo Halytskyy Lviv National Medical University
69 Pekarska st, 79010 Lviv, Ukraine
e-mail: fikvolodumur@ukr.net

ORCID AND CONTRIBUTIONSHIP
Volodymyr B. Fik: 0000-0002-2284-4488 (A, B, D, F)
Roman P. Krynytskyi: 0000-0002-2764-8814 (A, B)
Olha V. Dudok: 0000-0001-9513-3460 (C, E)
Mariia V. Podoliyk: 0000-0003-3490-8976 (B, C)
Myroslava A. Kosiuta: 0009-0000-1459-5437 (B, E)
Larysa Ya. Fedoniuk: 0000-0003-4910-6888 (E, F)

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

RECEIVED: 25.10.2023
ACCEPTED: 07.02.2024

ORIGINAL ARTICLE

Causes and management of Pica disorder among central Iraqi peoples

Mohammed Rasool[1], Mahmood Jawad[2], Shathel Khalaf Noman[3], Mohammed Ejerish[4]

[1]UNIVERSITY OF KARBALA, KARBALA, IRAQ
[2]AL-ZAHRAWI UNIVERSITY COLLEGE, KARBALA, IRAQ
[3]TIKRIT UNIVERSITY, TIKRIT, IRAQ
[4]TRAINING AND DEVELOPMENT CENTER, BABYLON, IRAQ

ABSTRACT

Aim: To find the causes and factors behind the Pica disorder, which helps in early diagnosis and appropriate treatments.

Materials and Methods: A retrospective cross-section study was carried out between July 1, 2022, and April 20, 2023, enrolling 300 patients from different provinces of central and south Iraq with Pica disease whose diagnosis depended on specialized physicians according to WHO guidelines. The participants were following up for three to six months in private clinics.

Results: 92.4% of the patients were female, and 41% of patients were under 20 years old, with low ferritin, HB, and vitamin D levels (80% of cases), and these markers showed a negative correlation with the number of Pica. Chowing of ice and clay were the common types of Pica, which represent about 30% each, while 34% of cases had multiple types, which had signs and symptoms of fever, palpitation, vomiting, abdominal pain, paleness, headaches, and hair loss. Six-month flows were better than three months.

Conclusions: Pica was a disorder that could lead to behavior and emotional abnormalities that caused the patients to eat some things that were eaten by healthy people. This may be, as concluded from our results, due to reduced levels of ferritin, hemoglobin (Hb), and vitamin D that caused these psychological problems.

KEY WORDS: Pica, non-nutritive substances, clay, and ferritin

Pol Merkur Lek,2024;53(1):226-232. doi: 10.36740/Merkur202402112

INTRODUCTION

The word "Pica" is derived from the Latin word "picus" which means "magpie," possibly in reference to this bird's unusual eating habits. The magpie has a haphazard choice for both foods and nonfoods. Pica is a term used to describe the consumption of non-nutritive substances for at least one month without any connection to a dislike of eating [1]. Ice cubes (pagophagia), clay (geophagia), dried pasta (amylophagia), chalk, starch, paste, Kay exalate resin (resinphagia), tomatoes, lemons, dirt, sand, glue, beeswax, cigarette butts, hair, lead, and laundry starch are some of the most often consumed substances by individuals with Pica [2]. The behavior must be sufficiently severe to require clinical attention, be developmentally inappropriate, and not be a part of a practice that is accepted in the culture. Though it has been observed in people of all ages and genders, small children and pregnant women in particular are more susceptible to the illness, which is commonly linked to mental impairment [3]. There are numerous historical references that imply Pica can also refer to the obsessive consumption of food items, which are essentially typical foods consumed in excess, obscuring the distinction with other types of food cravings like those related to pregnancy or premenstrual syndrome [4, 5]. Although clay has been demonstrated to be an iron chelator and can exacerbate the condition, it is hypothesized that the high iron content of the clay in the southern United States is the reason for its use there. Pica and iron deficiency have been linked to celiac disease in children, according to reports [6]. There has recently been some proof that Pica is a part of the obsessive-compulsive disorder (OCD) spectrum of diseases. Patients claimed that one of the ritualistic acts they felt forced to engage in was their Pica habit, and that consuming the substance relieved stress or anxiety [4]. Five categories have been used to group the consequences of Pica [7]:

- Inherent toxicity, which includes the direct toxic effects of substances like lead or other heavy metals;
- Obstruction, as seen in in trichophagia (hair eating);
- Excessive calorie intake, typically associated with amylophagia (starch eating);
- Nutritional deprivation, as seen in the consumption of clay rather than nutrient-dense foods;
- Other, such as parasitic infections and dental injury.

It can be challenging to identify a patient's Pica behavior. The diagnosis depends on self-reporting in the absence of complications that may indicate such eating practices. Due to embarrassment or a lack of knowledge that such behavior would be important to report, patients are likely to underreport such behavior.

AIM

The aim of our research is to estimate the causes and evaluate the role of follow-up in managing patients with Pica disease.

MATERIALS AND METHODS

PATIENTS AND METHOD

A retrospective cross-sectional study was carried out between June 1, 2022 and April 2023, enrolling 300 patients from different provinces of central and south Iraq (Babylon, Karbala, Al Najaf, Al Muthanna, and Diwaniya) with Pica disease whose diagnosis depended on a specialized physician according to WHO guidelines. 75 patients were excluded due to the lack of diagnostic evidence, insufficient lab results, and inability to follow up for 6 months with them for different causes, and the study was completed on 225 patients in detail and with long-term follow-up. The participants were following up for three to six months in private clinics. The research form contained 17 fields, divided into five sections. The first section contains social information such as age, gender, and field of work. The second part consists of the diagnosis, which includes the body weight, TSH level, ferritin, haemoglobin, TGAB-IgA, and vitamin D levels. The third part includes signs and symptoms, which include fever, palpitations, vomiting, abdominal pain, paleness, headaches, and hair loss. The fourth part included the type of Pica the patient had, which was either one or multiple forms of ice, rice, clay, and cardamom. And for the final part, it included the patient follow up after 3 months and 6 months of treatment and the responsiveness of the approach. After that, we collected the obtained data in Microsoft Excel program tables and then statistically analysed 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 analyze 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 markers. Statistics were deemed significant at $P < 0.05$.

RESULTS

GENERAL CHARACTERISTICS OF STUDY SUBJECTS

There were 225 patients: 17 male (7.6%) and 208 (92.4%) females. Our results found the age range of patients that were mostly affected by the disease was less than 20 years, followed by 21–30 years old, while those more than 50 years old were rarely affected. We found that most types of Pica were: 78 (34.7%) patients had multiple types of Pica: ice 68 (30.2%) and clay 70 (31.1%), while the lowest were rice 8 (3.6%) and cardamom 1 (0.4%). Our results showed that the levels of ferritin, haemoglobin, and vitamin D were significantly lower ($p<0.05$) in patients with Pica. According to symptoms, among all 225 subjects, the patients with Pica did not differ significantly ($p>0.05$) and suffered from fever, vomiting and hair loss. On the other hand, symptoms like palpitations, abdominal pain, paleness, and headaches were significantly different ($p<0.05$) among patients (Table 1).

CORRELATION BETWEEN BOTH AGE AND GENDER WITH TYPE OF PICA

Our results were observed among those with an age less than 20 years old eating different types, but we found that ice 32 (47%), clay 27 (38.6%), cardamon 1 (100%), and multiply 32 (41%) were the most preferred as compared with other age ranges. On the other hand, we found the chowing of rice was not different between patients. According to gender, females suffered the most from disease, as mentioned above. 74 (94.9%) of them chewed multiple types; 63 (92.6%) chewed ice and clay. We found an insignificant correlation between both age and gender when compared with the type of Pica ($r = 0.005$, $r = 0.041$, $p > 0.05$, respectively) (Table 2).

CORRELATION BETWEEN SERUM FERRITIN, HB, AND VITAMIN D WITH TYPE OF PICA

According to our results, we found a negative correlation between the levels of serum ferritin ($r = -0.176$, $p = 0.008$), haemoglobin ($r = -0.252$, $p = 0.038$), vitamin D ($r = -0.332$, $p = 0.038$), and the number of Pica, as increasing the number of Pica reduced its serum levels among all types of Pica. However, lower levels of these agents were found in three types: multiply, ice, and clay, respectively. We noticed 78 (35.6%), 77 (34.7%), and 63 (35.0%) had low levels of ferritin, Hb, and vitamin D, respectively, in patients with multiple types; however, nearly 30% of patients were chewing clay and ice (Table 3-4).

CORRELATION BETWEEN FOLLOW UP AND TYPE OF PICA

We found a negative correlation between three-month follow-up ($r = -0.088$, $p = 0.186$) and the type of Pica. However, about half of patients 119(52.9%), were found to be better as compared with 106 (47.1%). We noticed patients with multiply 47 (20.89%), clay 34 (15.11%), and ice 33 (14.67%) became better. On the other hand, after six months, the number and percentage of better patients increased, as seen in Table 5. However, we found a positive correlation between six follow-ups ($r = -0.017$, $p = 0.801$) and the type of Pica. 193 (85.8%) patients became better, while 32 (14.2%) of them still suffered from Pica. Also, we found patients with multiply 47 (29.78%), clay 59 (26.22%), and ice 60 (26.67%) became better.

CORRELATION BETWEEN FOLLOW-UP TYPES ACCORDING TO TYPE OF PICA

Our results found a significant positive correlation between six-month follow-up and three-month follow-up. However, 47 (45) became better and 2 were still sick, OR = 12.69 for 6 months (better or still sick), while of the total 31 of the three months followed, 22 of them became better and 9 of them were still sick after 6 months, OR = 0.14. On the other hand, 34 (33) became better and 1 was still sick, OR = 9.205 for 6 months (better or still sick), while of the total 36 of the three months followed, 26 of them became better while 10

Table 1. Characteristics of 225 patients

		Frequency	Percent	SD	Chi-Square
Age	<20	93	41.3	1.22	0.000
	21-30	56	24.9		
	31-40	32	14.2		
	40-50	37	16.4		
	>50	7	3.1		
	Total	225	100.0		
Gender	Male	17	7.6	0.26	0.00
	Female	208	92.4		
	Total	225	100.0		
Ferritin	Normal	6	3%	0.26	0.00
	Low	219	97%		
	Total	225	100.0		
Hb	Normal	3	1.3%	0.22	0.00
	Low	222	98.7%		
	Total	225	100.0		
Vit D	Normal	45	20.0	0.40	0.00
	Low	180	80.0		
	Total	225	100.0		
Type of Pica	Ice	68	30.2%	1.62	0.00
	Rice	8	3.6%		
	Clay	70	31.1%		
	Cardamom	1	0.4%		
	Multiply	78	34.7%		
	Total	225	100.0		
Fever	Yes	110	48.9	0.50	0.739
	No	115	51.1		
	Total	225	100.0		
Palpitation	Yes	211	94%	0.50	0.00
	No	14	6%		
	Total	225	100.0		
Vomiting	Yes	100	44%	0.46	0.739
	No	125	56%		
	Total	225	100.0		
Abdominal pain	Yes	160	71%	0.48	0.00
	No	65	29%		
	Total	225	100.0		
Pale	Yes	219	97%	0.49	0.00
	No	6	3%		
	Total	225	100.0		
Headache	Yes	203	90%	0.50	0.00
	No	22	10%		
	Total	225	100.0		
Hair loss	Yes	91	40.4	0.49	0.04
	No	134	59.6		
	Total	225	100.0		

of them were still sick after 6 months, OR = 0.14 still sick. Finally, of the patients who chewed ice, all of them became better, OR = 1.29 for 6 months (better), while of the total 35 of the three months followed, 27 of them became better, while 8 of them were still sick after 6 months, (Table 6).

DISCUSSION

Pica is a term used to describe the consumption of nonnutritive substances for at least one month without any connection to a dislike of eating. The study showed that 92.4% of the patients were female, which may be due to hormonal changes and menstrual cycle (MC) blood loss, which is supported by the fact that the lowest age reported was within the minimum age for menarche and only about 5% were within the menopause range. And these findings were corroborated by the fact that women who had their blood drawn during menstruation had the lowest adjusted mean levels of haemoglobin (Hb), transferrin saturation (TS), and serum ferritin (SF), while those who had their blood drawn during the luteal or late luteal phase of the menstrual cycle had the highest adjusted mean levels [8]. 41.3% of reported female cases were below the age range of 20 years old, and the percentage gradually declined as age progressed, which may support the effect of hormonal changes and the MC blood loss effect on ferritin, Hb, and vitamin D. This may be due to lots of causes, such as low sun exposure in the young, malnutrition, child neglect, and teenage hormonal changes. The types of Pica in these age groups were the largest for ice and clay, which may be affected by environmental factors pointed out by the change in result as increased numbers for rice were shown in older groups, which may point to the different cooking habits that allow for environmental habit changes in these age groups. As for the remaining male reported cases, they showed either stressful jobs, study environments, or child neglect issues, which may point to malnutrition or loss of appetite. Stressful situations (actual or anticipated), deplorable surroundings, a lack of active engagement in activities, and insufficient amounts of human interaction are linked to the start and persistence of Pica [9]. Pica, in its various manifestations, such as acuphagia, trichophagia, hyalophagia, and coprophagia, is unquestionably an unusual, dangerous, and potentially lethal illness. Dementia,

Table 2. Correlation between age and gender with types of Pica

			Type of Pica						r	p value
			Ice	Rice	Clay	Cardamom	Multiply	Total		
Age	<20	N	32	1	27	1	32	93	0.005	0.945
		%	47.1%	12.5%	38.6%	100.0%	41.0%	41.3%		
	20-30	N	13	1	22	0	20	56		
		%	19.1%	12.5%	31.4%	0.0%	25.6%	24.9%		
	31-40	N	11	2	9	0	10	32		
		%	16.2%	25.0%	12.9%	0.0%	12.8%	14.2%		
	41-50	N	11	2	10	0	14	37		
		%	16.2%	25.0%	14.3%	0.0%	17.9%	16.4%		
	>50	N	1	2	2	0	2	7		
		%	1.5%	25.0%	2.9%	0.0%	2.6%	3.1%		
	Total	N	68	8	70	1	78	225		
		%	100%	100%	100%	100%	100%	100%		
Gender	male	N	5	1	7	0	4	17	0.041	0.538
		%	7.4%	12.5%	10.0%	0.0%	5.1%	7.6%		
	female	N	63	7	63	1	74	208		
		%	92.6%	87.5%	90.0%	100.0%	94.9%	92.4%		
	Total	N	68	8	70	1	78	225		
		%	100%	100%	100%	100%	100%	100%		

Table 3. Correlation between type of Pica with ferritin, Hb, and Vit D

Correlations		Type of Pica	Ferritin	Hb	Vit D
Type of Pica	Pearson correlation	1	-0.176**	-0.252*	-0.332*
	Sig. (2-tailed)		0.008	0.038	0.038
	N	225	225	225	225

** Correlation is significant at the 0.05 level (2-tailed).*

*** Correlation is significant at the 0.01 level (2-tailed).*

Table 4. Count and percentage of type of Pica according to ferritin, Hb, and Vit

			Type of Pica						r	p-value
			Ice	Rice	Clay	Cardamom	Multiply	Total		
Ferritin	Normal	n	5	0	1	0	0	6	0.176	.008[c]
		%	83.3%	0.0%	16.7%	0.0%	0.0%	100%		
	Low	n	63	8	69	1	78	219		
		%	28.8%	3.7%	31.5%	0.5%	35.6%	100%		
	Total	n	68	8	70	1	78	225		
		%	30.2%	3.6%	31.1%	0.4%	34.7%	100%		
Hb	Normal	n	2	0	0	0	1	3	-0.252	.038[c]
		%	66.7%	0.0%	0.0%	0.0%	33.3%	100%		
	Low	n	66	8	70	1	77	222		
		%	29.7%	3.6%	31.5%	0.5%	34.7%	100%		
	Total	n	68	8	70	1	78	225		
		%	30.2%	3.6%	31.1%	0.4%	34.7%	100%		
Vit D	Normal	n	15	2	13	0	15	45	-0.332	.032[c]
		%	33.3%	4.4%	28.9%	0.0%	33.3%	100%		
	Low	n	53	6	57	1	63	180		
		%	29.4%	3.3%	31.7%	0.6%	35.0%	100%		
	Total	n	68	8	70	1	78	225		
		%	30.2%	3.6%	31.1%	0.4%	34.7%	100%		

Table 5. Count and percentage of type of Pica according to type of follow-up

			Type of Pica						r	Total
			Ice	Rice	Clay	Cardamom	Multiply	Total		
Follow up 3 months	Better	n	33	5	34	0	47	119	-0.088	.18[6]c
		%	48.5%	62.5%	48.6%	0.0%	60.3%	52.9%		
	Still sick	n	35	3	36	1	31	106		
		%	51.5%	37.5%	51.4%	100.0%	39.7%	47.1%		
	Total	n	68	8	70	1	78	225		
		%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%		
Follow up 6 months	Better	n	60	6	59	1	67	193	0.017	.801[c]
		%	88.2%	75.0%	84.3%	100.0%	85.9%	85.8%		
	Still sick	n	8	2	11	0	11	32		
		%	11.8%	25.0%	15.7%	0.0%	14.1%	14.2%		
	Total	n	68	8	70	1	78	225		
		%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%		

schizophrenia, obsessive-compulsive disorder (OCD), and intellectual developmental abnormalities are among the biological, psychological, and developmental conditions that such indiscriminate Picas have been linked to (IDD) [10]. Among the contributing elements were low socioeconomic status, malnutrition, iron deficiency anemia (IDA), and TB of the lungs. Risperidone and iron supplementation therapy reversed Pica and partially resolved the psychosis [11]. Other chronic diseases, including sickle cell anemia, celiac disease, inflammatory bowel disease, and pediatric dialysis patients, have also been linked to Pica. Iron deficiency and the resulting anemia seem to be the most common factors in these disorders [12], which is supported by these study results: 97% of cases showed low ferritin levels, and 98.7% of cases showed low Hb levels prior to or during the symptoms of Pica, which may point to the subconscious craving of the body for metallic nutrition such as clay, which resulted in being the highest consumption of Pica, and 78% being the highest disorder for multiple consumptions, which, as pointed out earlier, consisted of clay being the highest consumption,

Table 6. Comparative between follow-up 3 months vs follow-up 6 months according to type of Pica cross tabulation

Type Of Pica	Follow-up 3 months		Follow-up 6 months					95% confidence interval		r	p-value
			better	still sick	Total	Odds Ratio follow up/ Value		Lower	Upper		
Ice	better	n	33	0	33	6 months = better	1.29	1.08	1.55	0.35	.003
		% of Total	48.5%	0.0%	48.5%						
	still sick	n	27	8	35	N of Cases	68				
		% of Total	39.7%	11.8%	51.5%						
	Total	n	60	8	68						
		% of Total	88.2%	11.8%	100%						
Rice	better	n	5	0	5	6 months = better	3.00	0.60	14.86	0.74	.034
		% of Total	62.5%	0.0%	62.5%	N of Valid	8				
	still sick	n	1	2	3						
		% of Total	12.5%	25.0%	37.5%						
	Total	n	6	2	8						
		% of Total	75%	25.0%	100%						
Clay	better	n	33	1	34	6 months better / still sick)	12.69	1.52	105.62	0.34	.004
		% of Total	47.1%	1.4%	48.6%	6 months = better	1.34	1.08	1.65		
	still sick	n	26	10	36	6 months =still sick	0.10	0.01	0.78		
		% of Total	37.1%	14.3%	51.4%	N of Valid	70				
	Total	n	59	11	70						
		% of Total	84.3%	15.7%	100.%						
Cardamom	still sick	n	1		1						
		% of Total	100.0%		100.0%						
	Total	n	1		1						
		% of Total	100%		100%						
Multiply	better	n	45	2	47	6 months (better / still sick)	9.205	1.83	46.27	0.34	.002
		% of Total	57.7%	2.6%	60.3%	6 months = better	1.34	1.06	1.70		
	still sick	n	22	9	31	6 months = still sick	0.14	0.03	0.63		
		% of Total	28.2%	11.5%	39.7%	N of Valid	78				
	Total	n	67	11	78						
		% of Total	85.9%	14.1%	100%						

may point to the psychological need for metals. Although this idea was discarded by earlier investigators, such things as ice, foam, and rubber consumed by those who practice Pica have no proven nutritional value [13]. The highest percent of low ferritin values were in multiple consumption types of Pica, followed by clay and ice, and the results were the same for Hb levels and vitamin D, which may support the cause and need for metals, especially iron, in the Pica patients. Patients showed significant improvement after 3 months of iron therapy and as high as 85.8% after 6 months of follow-up and continuous iron therapy. All reported cases showed signs and symptoms of fever, palpitation, vomiting, abdominal pain, paleness, headaches, and hair loss, which may all be a result of either iron deficiency anemia or the indigestion of foreign materials such as clay and uncooked rice. It is believed that the dopamine motive system was phylogenetically designed to control the motivations necessary for human survival. Anomalies in the system that cause dopamine depletion may cause deregulations that show up as obsessive, repetitive behaviours like addictions and maybe the stereotypical behaviours of autism spectrum disorder (ASD). Given the compulsion to consume inedible substances, it would appear that the traits of Pica are similar to those of addictions. This implies that, despite being abnormal and

aberrant, the activity is pleasurable to the person. Perhaps abnormalities in the dopaminergic system can be used to localize the neurochemical and physiological reasons. This theory is strengthened by the connection between IDA and Pica since IDA has been linked to reductions in D2 receptors in the nucleus accumbens [4].

RECOMMENDATION

Based on the findings of this investigation, it was suggested that we investigate the ferritin and Hb levels of all patients with Pica, follow up for more than six months, and build a specialized health center for the management of those patients, especially young females.

CONCLUSIONS

Pica was a disorder that could lead to behavior and emotional abnormalities that made the patients eat some things that were eaten by healthy people, which may be, as concluded from our results, due to reduced levels of ferritin, Hb and vitamin D that caused these psychological problems.

REFERENCES

1. Šálek M, Grill S, Riegert J. Nest-site selection of an avian urban exploiter, the Eurasian magpie Pica, across the urban-rural gradient. Journal of Vertebrate Biology. 2020;70(1):20086.1-11. doi:10.25225/jvb.20086.
2. Bedanie G, Tikue A, Thongtan T et al. Pica/Pagophagia-Associated Hyponatremia: Patient Presenting With Seizure. Cureus. 2020;12(7):e9330. doi:10.7759/cureus.9330.
3. Konlan KD, Abdulai JA, Konlan KD et al. Practices of Pica among pregnant women in a tertiary healthcare facility in Ghana. Nurs Open. 2020;7(3):783-792. doi:10.1002/nop2.451.
4. Schnitzler E. The Neurology and Psychopathology of Pica. Curr Neurol Neurosci Rep. 2022;22(8):531-536. doi:10.1007/s11910-022-01218-2.
5. Rodrigues N, Shih S, Cohen LL. Pica in Pediatric Sickle Cell Disease. J Clin Psychol Med Settings. 2021;28(1):6-15. doi:10.1007/s10880-019-09671-x.
6. Kaneza N. A reportorial analysis of the homoeopathic remedies indicated in Pica in pregnancy. 2022. https://openscholar.dut.ac.za/bitstream/10321/4514/3/Kaneza_N_2022_Redacted.pdf [Accessed 28 December 2023]
7. Fields VL, Soke GN, Reynolds A et al. Pica, Autism, and Other Disabilities. Pediatrics. 2021;147(2):e20200462. doi:10.1542/peds.2020-0462.
8. Chandra S, Gupta N, Patel SK. Study of iron status indicators in different phases of menstrual cycle in first year medical college females. International Journal of Research in Medical Sciences. 2017;5(1):46-49. doi:10.18203/2320-6012.ijrms20164520.
9. Rose EA, Porcerelli JH, Neale AV. Pica: common but commonly missed. J Am Board Fam Pract. 2000;13(5):353-358.
10. Goldschmidt J. Contrasting Pica behaviour for individuals with ASD and IDD against presentation in the general population Part I, II, and III. Gurze-Salucore eating disorders resource catalog E-newsletter. 2019. https://www.edcatalogue.com/ [Accessed 28 December 2023]
11. Kar SK, Kamboj A, Kumar R. Pica and psychosis - clinical attributes and correlations: a case report. J Family Med Prim Care. 2015;4(1):149-150. doi:10.4103/2249-4863.152277.
12. Katsoufis CP, Kertis M, McCullough J et al. Pica: an important and unrecognized problem in pediatric dialysis patients. J Ren Nutr. 2012;22(6):567-571. doi:10.1053/j.jrn.2011.10.038.
13. Ukaonu C, Hill DA, Christensen F. Hypokalemic myopathy in pregnancy caused by clay ingestion. Obstet Gynecol. 2003;102(5):1169-1171. doi:10.1016/s0029-7844(03)00705-1.

The authors would like to acknowledge Dr. Dhafir Fadil Hassan, Ameer Zaidon Tariq, Ameer Ahmed Jawad, and Um Al-Banin Abbas Fadil for helping with the data collection for this research.

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Mahmood Jawad
Al-Zahrawi University College
G499+JPR Karbala, Iraq
e-mail: sgahmed1331962@outlook.com

ORCID AND CONTRIBUTIONSHIP

Mahmood Jawad: 0000-0001-6542-7230 A, F
Mohammed Rasool: 0000-0003-0992-320X B, C
Shathel Khalaf Noman: 0000-0002-7065-6924 C, D
Mohammed Ejerish: 0009-0001-8078-0558 D, E

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

RECEIVED: 01.10.2023
ACCEPTED: 15.03.2024

ORIGINAL ARTICLE

Immunohistochemical study of CD74 biomarker in normal and malignant breast tissues

Hayder Neamah Hassan[1], Rouaida Kadhim A. Al-Hussein[2], Asmehan Adnan Al-Naqeeb[3]

[1]FACULTY OF MEDICINE, UNIVERSITY OF KUFA, KUFA, IRAQ
[2]NURSING COLLEGE, NATIONAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, IRAQ
[3]BASIC SCIENCE DEPARTMENT, COLLEGE OF NURSING, UNIVERSITY OF BAGHDAD, BAGHDAD, IRAQ

ABSTRACT

Aim: To detect the role of CD74 expression in breast carcinoma as a predictive marker for identifying the biological behavior of malignancy in Iraqi women.

Materials and Methods: The study used technique of immunohistochemistry for detection CD74 protein role in breast cancer, and its expression in breast cancer tissue samples. Samples were collected in Al-Najaf city in Iraq, from Al-Forat Al-Awsat Oncology Center. The study was achieved at the Laboratories of the Faculty of Science in the University of Kufa. Fifty samples of breast cancer tissue, and twenty controls benign tissue were included in the study. The study has investigated relationship between expression of biomarker with grade, age of patient and tumor size.

Results: The study showed that the cytoplasmic expression of CD74 with more clear and intensive staining in the cytoplasm, and reported that CD74 positivity rate was 52%. A significant association between CD74 expression and grade and size of tumor, so CD74 can be considered as a biomarker for prediction of breast cancer in women. No association was found between CD74 expression and each of patients' age and node metastasis.

Conclusions: The study represents an important step in our region because there are a few studies about this topic; more efforts are required to approve the function of this biomarker.

KEY WORDS: breast cancer, tumor size, tumor grade, CD74, IHC

Pol Merkur Lek,2024;53(1):233-239. doi: 10.36740/Merkur202402113

INTRODUCTION

Cancer is a noun of an uncontrolled growth of cells which tend to divide in irregular mode, and then spread (metastasize) to other different parts of the body. In all types of cancer, cells form a mass or lump named a tumor, and are called according to the region of body in which the tumor began [1]. Breast cancer (also called breast carcinoma) is a heterogeneous disorder and it has many subtypes that are variant biologically and have many molecular and clinic-pathological characteristics [2]. The causes of breast cancer can be clarified briefly by two pathways, namely, maturation and division of the epithelial cells in the breasts enhanced by hormones. BRCA1 and BRCA2 mutations are the most important and main causes of breast cancer, and PTEN and p53 mutations result in severe breast cancer syndromes [3]. If breast cancer is diagnosed and managed early, the rate of survival will be extremely elevated, however, many complex barriers, women may have it which hinders diagnosis at early stages. These barriers are found in many places around the world, including social, geographic, economic, and other similar factors, which can lower women access to right time, effective, and affordable, breast cancer health care services. Previously, Health Global Initiative (BHGI) has developed resource-stratified guidelines useful in the early detection and diagnosis of breast cancer, which can be routinely used in this field [4]. Information about the incidence and mortality of breast cancer is very critical for health measure planning [5]. Breast cancer (BC) has been found to be one of malignances that significantly spreads among the Iraqi women in the southern of Iraq, which is considered the commonest cancer in all provinces [6]. CD74, also known as the invariant chain or Li, is present in breast tumor cells and various types of immune cells [7]. Originally discovered in immune cells, such as microglia, CD74 plays a crucial role in immune surveillance. Additionally, it is found in many tumors and is involved in regulating processes like endosomal trafficking, cell migration, and cellular signaling [8]. CD74 serves a dual purpose as part of the MHC class II antigen presentation pathway and as a cytokine receptor. It also has intracellular effects that influence transcription activation. MHCII is vital for presenting peptides to CD4 T cells, impacting multiple aspects of adaptive immunity, including the activation of effector CD8 T cells [9]. The cytokine macrophage inhibitory factor (MIF) is a common factor that utilizes CD74 to enhance cell proliferation, migration, and survival pathways in both immune and epithelial cell types [10]. Hence, the expression of CD74 in tumor cells is expected to have a dual role, contributing to both pro-tumor and anti-tumor effects due to its cytokine signaling and antigen presenting functions. Previous studies, although mostly based on small groups, have indicated associations between CD74 and Estrogen receptor (ER) negative and/or triple negative subgroups [11]. CD74 acts as a chaperone, ensuring the

proper folding of MHC class II and regulating its ability to bind antigens. Additionally, it acts as a cell-surface receptor for the cytokine macrophage migration inhibitory factor (MIF). When MIF binds to CD74's extracellular domain, it triggers cellular signaling, stimulating cellular proliferation and survival programs. The MIF-CD74 axis can also play a critical role in regulating innate immune responses to cancer by creating an immunosuppressive environment that supports tumor progression [12].

AIM

To the best of the authors' knowledge, there are a few formations about the diagnosis of breast cancer in women using CD74, so the current study was designed to detect the role of CD74 expression in breast carcinoma as a possible marker for detect the biological behavior of malignancy in Iraqi women.

MATERIALS AND METHODS

COLLECTION OF SAMPLES

This project included 70 breast tissue samples divided into two groups: 50 samples (malignant group) of breast cancer tissues and 20 samples (control group) of benign breast tumor (normal breast tissue). The age range was between 19-71 years old. All samples in study were collected from Al-Forat Al-Awsat Oncology Center and a Private Laboratory in Najaf province during the period between September 2022 and March 2023. All samples kept at 10% formaldehyde and room temperature for histopathological and immunohistochemical analysis. The patient data were collected either directly from the patients as a questioner or from the histopathological reports.

HAEMATOXYLIN & EOSIN STAINING PROTOCOL

Haematoxylin & Eosin is a principal stain commonly used in histology to stain the cellular structures, like nucleus or cytoplasm of tissues under light microscope, to differentiate cellular components, also can be used to detect any abnormal changes in tissues, to diagnose some diseases, including cancer.

IMMUNOHISTOCHEMISTRY ASSAY

In this study the immunohistochemical (IHC) technique was performed on CD74 biomarker according to the methods of Alghezi for all samples. Sections of tissues were 4 µm in thick (paraffin embedded), that prepared on a slide with positive charge, then subjected to IHC technology. First step is de-parrafination, achieved by soaking samples in xylene and then dehydrated by the absolute alcohol. After that, sections were placed in water bath for 35 min at 70 C°, and soaking them with Citrate Buffer-50x (pH 6) from Path Situ, for Antigen retrieval. Then the sections were left at room temperature for 20 minutes to cool, after which they were treated with instillation of peroxidase. Then the sections were left overnight for incubation with primary antibodies (using CD74 - murine monoclonal antibodies) from the Dako Company. Next day, all the slides were subjected to washing three runs with phosphate-buffered saline (PBS) is a buffer for ten min, then dropping of goat anti-mouse or goat anti-rabbit labeled polymer-HRP conjugated secondary ABs on slides and incubated then at 25 Co, with slow, rhythmic swaying for 30 min. Then, the slides washed three times with PBS buffer for 5 min each. After that, drops of DAP chromogen were added into 1 ml of substrate buffer and gathered very heavily. After this preparation, a few drops of this solution were put on the slides and let for 5 min at room temperature. Next step, slides washed with D.W (distilled water) for 10 min. Then, drops of the counter stain (Haematoxylin) were applied onto the slides and let for incubation at 25 C° for 2 min, to stain the nucleus of cells, then, slides washed by the tap water for 2 min and distilled water for 1 min. After that, the slides dehydrated by soaking in ascending concentrations of alcohol (70, 95, and 100 %) for 1 min each one. After dehydration slides soaked in xylene 2 times for 2 min each. Then, drops of DPX applied onto slides and covered with cover slides (22 X 22 mm) and let them to dry. In the next day, the slides were ready to examine under the microscope that harbors a digital camera, and then 5 pictures of each slide were taken and scored by using scoring system to evaluate the biomarker expression [13].

STATISTICAL ANALYSIS

The data was analysed by the statistical program available from SPSS 25. Simple frequencies, percentages were used to display the data. The Pearson Chi-square test (two-test) was utilized to determine the significance of the difference between distinct % (qualitative data). Whenever the P-value was less than 0.05, statistical significance was considered. Chi square was used to test the relationship between categorial variables [14].

RESULTS

The results revealed that CD74 expression accumulated more things in the cytoplasm of malignant cell, as Fig. 1. revealed that the samples have shown the cytoplasmic expression of CD74 and it was more than nuclear staining. In the studied group, CD74 expression was reported in 26 cases out of fifty cases of breast carcinoma indicating a positivity rate of 52%, with (P-value <0.000), (Chi-square = 16.55; R= 0.39) (Table 1).

The score of CD74 expression was measured according to the stage of tumor. Strong or high expression was reported in 9 of 50 cases: 3 in T2, 4 in T3 and 2 in T4. Low expression was reported in 18 out of 50 breast carcinoma cases, 11 in T2, 5 in T3 and 2 in T4 (Table 2).

THE RELATION BETWEEN CD74 EXPRESSION AND AGE OF PATIENT IN BREAST CANCER CASES

The results of CD74 expression in relation to age distribution of breast carcinoma cases shown that CD74 expression (positive result) was 1 (1.7%) case in the age group 18-29 years, 5 (8.3%) cases in the age group 30-39 years, 9 (15%) cases in the age group 40-49 years, 8 (13.3%) cases in the age group 50-59 years and 3 (5%) cases in the age group ≥ 60 years (P-value = 0.12, R = -0.106) as it shown in table 3.

The results of CD74 expression in relation to node metastasis shown that 14 (56%) cases have positive CD74 in the positive

Table 1. Results of CD74 expression in presented breast cancer cases

Types of tissue		CD74	
		Positive	Negative
Breast Cancer	No.	26	24
	%	52.0%	48.0%
Normal tissue	No.	0	20
	%	0.0%	100.0%
Chi Square		16.55	
P-value		<0.000	

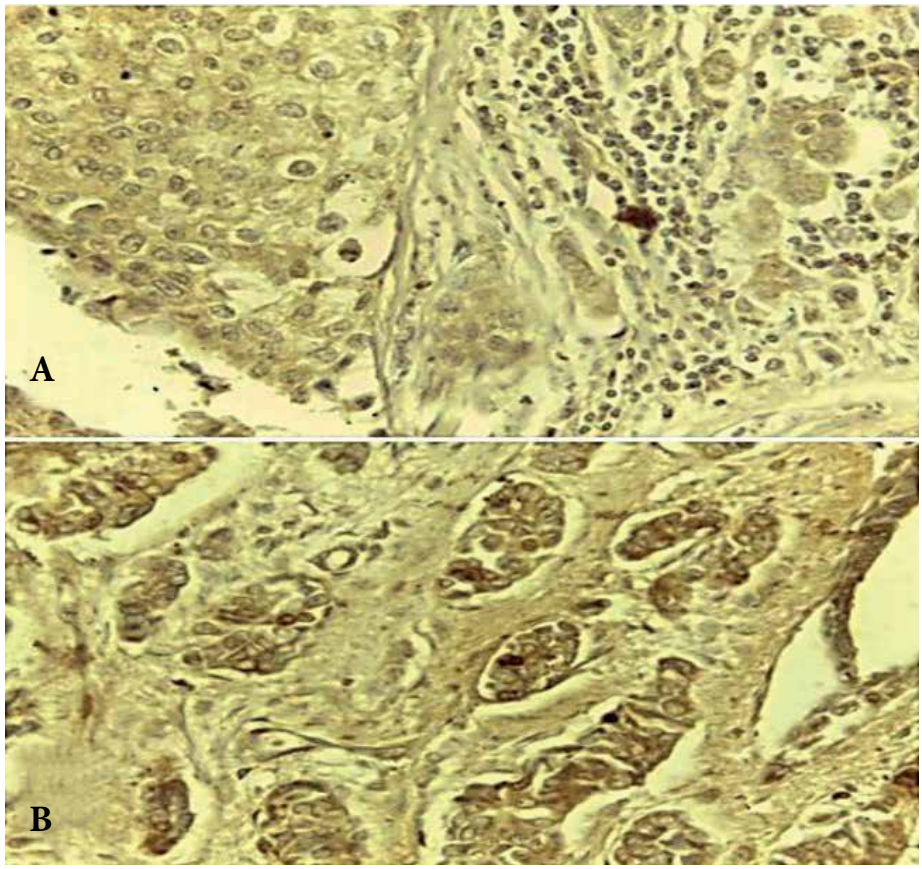


Fig. 1. A&B shows the positive result of CD74 in breast cancer. Cytoplasmic expression is more clearly visible in the more staining parts (Haematoxylin & Eosin Staining 100X).

Table 2. The positivity of CD74 score of breast carcinoma in correlation to size of tumor

Tumor stage		CD-74 Score		
		High	Low	No Score
T1	No.	0	0	1
	% within stage	0.0%	0.0%	100.0%
T2	No.	3	11	12
	% within stage	11.5%	42.3%	46.2%
T3	No.	4	5	8
	% within stage	23.5%	29.4%	47.1%
T4	No.	2	2	2
	% within stage	33.3%	33.3%	33.3%
Chi Square		2.04		
P-value		0.91		

Table 3. The relation between CD74 expression and age of patient in breast cancer cases

Age groups		CD74	
		Positive	Negative
18-29 year	No.	1	5
	%	16.7%	83.3%
30-39 year	No.	5	10
	%	33.3%	66.7%
40-49 year	No.	9	7
	%	56.3%	43.8%
50-59 year	No.	8	4
	%	66.7%	33.3%
≥ 60 years	No.	3	8
	%	27.3%	72.7%
Chi Square		7.25	
P-value		0.12	

Table 4. The relation between CD74 expression and grade of tumor

Node Metastasis		CD74	
		Positive	Negative
Positive	No.	14	11
	%	56.0%	44.0%
Negative	No.	12	9
	%	57.1%	42.9%
Chi Square		0.1	
P- value		0.93	

node metastasis, while 44% were negative CD74. The same table shows that 57.1% cases have positive CD74 in the negative node metastasis, while 42.9% were negative CD74 (P value= 0.10, Chi Square= 0.93) as it shown in table 4.

THE RELATION BETWEEN CD74 EXPRESSION AND GRADE OF TUMOR

The results of CD74 expression in relation to grade of tumor (positive result) shown that there is no cases of Grade I, 10 (20%) cases of Grade II, 16 (32%) cases of Grade III with (P-value = 0.04, Chi Square = 3.98; R = -0.28) as it shown in table 5.

THE RELATION BETWEEN CD74 IHC EXPRESSION AND SIZE OF TUMOR

The results of CD74 expression in relation to size of tumor (positive results) shown that there is no cases of T1, 11 (22%) cases of T2, 9 (18%) cases of T3, 6 (12%) cases of T4 with (P-value = 0.04, R = -0.38) as it shown in table 6.

DISCUSSION

The results revealed that the expression of CD74 accumulated in the cytoplasm of malignant cells, which is consistent with study of Wang et al. [11]. The study also showed the nuclear staining in the studied group, CD74 expression was negative in all control groups, but the CD74 positivity rate was 52%, with significant association (P-value = 0.002). This positivity is lower than that reported by Tian et al. [15], as they observed that about 468(80.69 %) of the 580 breast cases exhibited a positive expression for CD74. In another study conducted in in Antalya in Turkey Immunohistochemical staining of CD74 exhibited a very similar positivity rate of 53.7% in breast cancer patients, and they also showed that the disease-free survival (DFS) was better in CD74 negative subjects than in CD74 positive subjects, but the difference was not statistically significant [16]. CD74 plays a crucial role in effectively displaying antigens limited by MHCII. This is a critical element in promoting immunity against tumors. CD74 helps stabilize the MHCII complex within the endoplasmic reticulum, enabling the presentation of specific peptides restricted by MHCII on the cell surface. Research has demonstrated that CD74 is involved in controlling the range of antigens showcased through MHCII. This control subsequently initiates the activation of the CD4 and CD8 components of the adaptive immune response, ultimately resulting in the rejection of tumors [17, 18]. A previous study in Egypt has shown that CD74

Table 5. The relation between CD74 expression and grade of tumor

Grade		CD 74	
		Positive	Negative
Grade I	No.	0	0
	%	0%	0%
Grade II	No.	10	16
	%	38.5%	61.5%
Grade III	No.	16	8
	%	66.7%	33.3%
Chi Square		3.98	
P-value		0.04	

Table 6. The relation between CD74 expression and size of tumor in breast cancer cases

Tumor size		CD74	
		Positive	Negative
T1	No.	0	1
	%	0%	2%
T2	No.	11	16
	%	22%	32%
T3	No.	9	7
	%	18%	14%
T4	No.	6	0
	%	12%	0%
Chi Square		8.11	
P-value		0.04	

may be a good predictive factor indicating weak breast cancer patient's outcome [19]. Metodieva et al. [20] have shown that when CD74 is excessively produced in both cancerous and noncancerous epithelial cells in humans, it interacts with and disrupts the functioning of Scribble, a well-known gene product associated with suppressing tumors. Additionally, our study employs epithelial cell lines that carry CD74 with a tetracycline-inducible promoter, along with advanced mass spectrometry. Through this, we illustrate that heightened CD74 levels cause specific alterations in the phosphorylation pattern of Scribble's C-terminal portion. Consequently, this leads to the relocation of Scribble from the points of cell-to-cell contact on the cell membrane to the cytoplasm. This shift is likely to significantly boost the movement and invasive properties of cancer cells [20]. Hasby & Khalifa reported that the persistent presence of CD74 expression in triple-negative breast cancers highlights the necessity for additional research to investigate the potential of targeting it as a therapeutic approach for breast carcinoma, particularly within these specific groups. The results of CD74 expression in breast carcinoma cases in the study shown that CD74 expression has no relation with age groups (P-value = 0.41), so it is non-significant relation, this supported by assessment of CD74 in one study of Wang et al. [11]. Our study approved that CD74 has no association with Grade I, but it has a significant relationship (P value= 0.04) to both Grade II and Grade III. This result is similar to the study of Wang et al. which showed a significant association between CD74 expression and tumor grade. Results of CD74 expression in relation to size of tumor have indicated that CD74 expression is not in detectable levels at the first stage of the tumor, while it is highly expressed at the second stage because of its role in the protection against cancer prognosis, until the late stages where its expression is notably decreased. This result is supported by another study which reported similar results and found that Spearman's rho analysis (P = 0.008, r = -0.313) showed that CD74 had a negative correlation with stage of tumor [21]. The role of CD74 in the development of cancer appeared to differ depending on the specific type of cancer and the stage of the disease. Previous studies have shown that CD74 serves as a distinct prognostic element. Insufficient CD74 expression could suggest an unfavorable prognosis, whereas heightened CD74 expression was associated with a more favorable prognosis, aligning with prior studies. This marker has the potential to guide healthcare professionals in offering conceptual recommendations to enhance patient prognoses [22]. On the other hand, another study found

that the immunohistochemical presence of CD74 in invasive breast cancer was notably elevated in cases with higher tumor grades, the existence of metastasis in lymph nodes, and advanced tumor stages [19]. The current result did not find a significant association between CD74 expression and each of patients' age and node metastasis, and this result comes in agreement with some previous studies [16]; while some authors have found a significant association with node metastasis.

CONCLUSIONS

The study approved that CD74 is expressed both in the cytoplasm and in the nucleus, with more clear accumulation in cytoplasm. The study revealed an association between CD74 and grade and size of tumor, so CD74 can be used as a marker for assessment of breast cancer in women. This study represents an important step in our region because there are a few studies about this topic; more studies are needed to approve the function of this biomarker.

REFERENCES

1. Al-Janabi AAHS, Naseer ZH, Hamody TA. Epidemiological study of cancers in Iraq-Karbala from 2008 to 2015. Int J Med Res Health Sci. 2017;(61):79-86.
2. Mohammad HN, Al-Fahham AA. Maternal risk factors in women with breast cancer in Al-Najaf province. Indian Journal of Forensic Medicine & Toxicology. 2021;15(2):2458-633. doi:10.37506/ijfmt.v15i2.14741.
3. Walsh T, King MC. Ten genes for inherited breast cancer. Cancer Cell. 2007;11(2):103-105. doi:10.1016/j.ccr.2007.01.010.
4. Ginsburg O, Yip CH, Brooks A et al. Breast cancer early detection: A phased approach to implementation. Cancer. 2020;126(10):2379-2393. doi:10.1002/cncr.32887.
5. Ghoncheh M, Pournamdar Z, Salehiniya H. Incidence and mortality and epidemiology of breast cancer in the world. Asian Pac J Cancer Prev. 2016;17(S3):43-46. doi:10.7314/apjcp.2016.17.s3.43.
6. Al-Kafajy M. Breast cancer risk trends of Iraqi women. Medico Legal Update. 2020;20(1):383-386.
7. Richard V, Kindt N, Saussez S. Macrophage migration inhibitory factor involvement in breast cancer (Review). Int J Oncol. 2015;47(5):1627-1633. doi:10.3892/ijo.2015.3185.
8. Loreth D, Schuette M, Zinke J et al. CD74 and CD44 expression on CTCs in cancer patients with brain metastasis. Int J Mol Sci. 2021;22(13):6993. doi:10.3390/ijms22136993.
9. Wang RF. The role of MHC class II-restricted tumor antigens and CD4+ T cells in antitumor immunity. Trends Immunol. 2001;22(5):269-276. doi:10.1016/s1471-4906(01)01896-8.
10. Bucala R, Shachar I. The integral role of CD74 in antigen presentation, MIF signal transduction, and B cell survival and homeostasis. Mini Rev Med Chem. 2014;14(14):1132-1138. doi:10.2174/1389557515666150203144111.
11. Wang ZQ, Milne K, Webb JR et al. CD74 and intratumoral immune response in breast cancer. Oncotarget. 2017;8(8):12664-12674. doi:10.18632/oncotarget.8610.
12. Noer JB, Talman MM, Moreira JMA. HLA Class II Histocompatibility Antigen γ Chain (CD74) Expression Is Associated with Immune Cell Infiltration and Favorable Outcome in Breast Cancer. Cancers (Basel). 2021;13(24):6179. doi:10.3390/cancers13246179.
13. Alghezi DA, Whitley P, Beresford M et al. Decreased ABCG2 expression in prostate cancer and negatively associated with poorly differentiated grade and biochemical recurrence. Najaf Medical Journal. 2020;20(2):243-256.
14. Al-Fahham AA. Development of new LSD formula when unequal observations numbers of observations are. Open Journal of Statistics. 2018;8:258-263. doi:10.4236/ojs.2018.82016.
15. Tian B, Zhang Y, Li N et al. CD74: a potential novel target for triple-negative breast cancer. Tumour Biol. 2012;33(6):2273-2277. doi:10.1007/s13277-012-0489-x.
16. Yaprak M, Erdogan G, Aricic G et al. Prognostic value of CD74 and HLA-DR expressions in invasive ductal breast cancer. Advances in Breast Cancer Research. 2015;4:71-76. doi:10.4236/abcr.2015.43008.
17. Accolla RS, Lombardo L, Abdallah R et al. Boosting the MHC class II-restricted tumor antigen presentation to CD4+ T helper cells: a critical issue for triggering protective immunity and re-orienting the tumor microenvironment toward an anti-tumor state. Front Oncol. 2014;4:32. doi:10.3389/fonc.2014.00032.
18. Chornoguz O, Gapeev A, O'Neill MC et al. Major histocompatibility complex class II+ invariant chain negative breast cancer cells present unique peptides that activate tumor-specific T cells from breast cancer patients. Mol Cell Proteomics. 2012;11(11):1457-1467. doi:10.1074/mcp.M112.019232.
19. Hasby EA, Khalifa RA. Expression of CD74 in invasive breast carcinoma: its relation to Nottingham Prognostic Index, hormone receptors, and HER2 immunoprofile. Tumori. 2017;103(2):193-203. doi:10.5301/tj.5000562.
20. Metodieva G, Nogueira-de-Souza NC, Greenwood C et al. CD74-dependent deregulation of the tumor suppressor scribble in human epithelial and breast cancer cells. Neoplasia. 2013;15(6):660-668. doi:10.1593/neo.13464.
21. Liang Y, Li C, Liu Y et al. Prognostic role of CD74, CD10 and Ki-67 immunohistochemical expression in patients with diffuse malignant peritoneal mesothelioma: a retrospective study. BMC Cancer. 2023;23(1):406. doi:10.1186/s12885-023-10871-w.
22. Otterstrom C, Soltermann A, Opitz I et al. CD74: a new prognostic factor for patients with malignant pleural mesothelioma. Br J Cancer. 2014;110(8):2040-2046. doi:10.1038/bjc.2014.117.

The procedures in this study were approved by the bioethical committee of the medical college- University of Kufa (No. 232 in 2023).

The consent of participation in the present study was taken from all patients from whom the samples were taken.

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Asmehan Adnan Al-Naqeeb
University of Baghdad
Presidency of the University of Baghdad, Al-Jadriya, Iraq
e-mail: sgahmed1331962@outlook.com

ORCID AND CONTRIBUTIONSHIP
Asmehan Adnan Al-Naqeeb: 0000-0002-1821-0183 A, B
Hayder Neamah Hassan: 0000-0001-8540-9478 C, D
Rouaida Kadhim A. Al-hussein: 0009-0001-6982-5065 D-F

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

RECEIVED: 23.09.2023
ACCEPTED: 15.03.2024

REVIEW ARTICLE

Platelet-Rich Plasma - a remedy present in every human being. History, functioning, and the benefits of therapy using it

Monika Prokurat[1], Katarzyna Grudnik[1], Wojciech Niemczyk[1], Stanisław Niemczyk[1], Mateusz Migas[2], Karolina Wągrowska[3], Karolina Lau[4], Janusz Kasperczyk[4]

[1]STUDENT SCIENTIFIC CIRCLE AT THE DEPARTMENT OF ENVIRONMENTAL MEDICINE AND EPIDEMIOLOGY IN ZABRZE, FACULTY OF MEDICAL SCIENCES IN ZABRZE, SILESIAN MEDICAL UNIVERSITY IN KATOWICE, ZABRZE, POLAND
[2]ST, LUKE HOSPITAL, TARNÓW, POLAND
[3]CITY HOSPITAL NO. 4 IN GLIWICE, GLIWICE, POLAND
[4]DEPARTMENT OF ENVIRONMENTAL MEDICINE AND EPIDEMIOLOGY IN ZABRZE, FACULTY OF MEDICAL SCIENCES IN ZABRZE, SILESIAN MEDICAL UNIVERSITY IN KATOWICE, ZABRZE, POLAND

ABSTRACT

Platelet-rich plasma is an autologous product used in restorative medicine. It contains a high concentration of platelets, which are rich in growth factors and other biologically active substances known for their ability to stimulate regenerative processes in the body. Currently, research is being conducted into the use of platelet-rich plasma in many areas of medicine. This publication provides information on the nature, mechanism of action, therapeutic properties and application of autologous platelet-rich plasma in medicine. Furthermore, ongoing investigations explore its potential in wound healing, orthopedics, dermatology, and even in dentistry, showcasing its versatility and promising outcomes across various medical disciplines. Additionally, the safety and efficacy of platelet-rich plasma therapies are subjects of continual scrutiny, aiming to refine protocols and expand its clinical utility with robust scientific evidence. The growing interest in this regenerative approach underscores its potential as a valuable tool in modern medical practice. Platelet-rich plasma therapy represents a promising avenue for personalized medicine, offering tailored treatment approaches that capitalize on the body's own healing mechanisms to promote tissue repair and regeneration.

KEY WORDS: platelet-rich plasma, aesthetic medicine, orthopedics, antimicrobial activity, vascular growth factor

Pol Merkur Lek,2024;53(1):240-245. doi: 10.36740/Merkur202402114

INTRODUCTION

PRP is a blood-derived preparation that results from centrifuging a patient's own blood and extracting the platelet-rich blood fraction. PRP therapies are successfully employed in various fields of medicine [1]. An important aspect is the autologous origin of PRP, which is advantageous because it eliminates the risk of immunological reactions, cross-contamination, and the transmission of microbial diseases. Initially, PRP was successfully used in transfusion medicine. The blood platelets contained in PRP were used to improve tissue sealing but not as stimulators of tissue repair and angiogenesis in damaged tissues [1]. Nowadays, PRP concentrate is known to be used in the treatment of various disorders because PRP containsnumerous growth factors that have the potential to stimulate the repair of damaged tissues and accelerate the formation of new cells [2]. Although therapies using PRP are minimally invasive, easy to prepare, and relatively inexpensive [2], they raise uncertainties among researchers because there is a lack of standardization in the composition, acquisition methods, and application of PRP in various medical fields. The purpose of this work is to analyze and consider the consequences of actions and contemporary challenges faced by medical professionals using PRP-enriched therapy. This publication attempts to present current knowledge about PRP, explaining its mechanism of action and how historical attempts to use platelet-rich concentrate have evolved. The work draws upon materials from selected publications and scientific literature.

AIM

The aim of the work is to present current applications, mechanism of action, therapeutic properties and potential areas of use of autologous platelet-rich plasma in medicine.

REVIEW AND DISCUSSION

THE HISTORY OF PLATELET-RICH THERAPY AS A PANACEA FOR AILMENTS

The first mentions of PRP application date back to the 1970s. One of the breakthrough events during that period was the research on fibroblasts. In 1974, two researchers, Kohler and Lipton, demonstrated that blood platelets (PLT) could be treated as growth stimulators [3]. In 1972, experiments were conducted on animals using fibrinogen concentrates in the regeneration of damaged peripheral nerves. Two years after the success in animal studies,

fibrinogen concentrates were used in humans, containing stabilizing factor XIII, along with a thrombin solution, and in some cases aprotinin with antifibrinolytic properties [4]. The concept of PRP application and its initial descriptions can be traced back to hematology. Specialists in this field coined the term PRP in the 1970s to describe plasma with a higher platelet count than peripheral blood, initially used in the treatment of patients with thrombocytopenia as a transfusion product [5]. The first mentions of PRP application in surgery date back to 1987. During a cardiothoracic surgery performed by Ferrari and his collaborating physicians, a concentrate was used for the first time. It was discovered that the use of autologous platelet and red blood cell concentrates significantly reduced the need for blood transfusions during the procedure, consequently lowering the costs of surgical procedures. The first documented use of PRP in jaw reconstruction was reported in 1994 by Tayapongsak and his team [6]. The basis for PRP application in this procedure is the utilization of fibrin's potential for adhesion and homeostatic properties, while PRP, with its anti-inflammatory properties, stimulates cell proliferation. In 1997, PRP began to be employed in oral and maxillofacial surgery. Simultaneously, knowledge continued to evolve regarding the creation and use of fibrin glue [7].

PRP AS A SOURCE OF BIOLOGICALLY ACTIVE COMPONENTS

The justification for therapy using PRP lies in the fact that concentrated blood platelets at the site of damage initiate tissue repair. Concentrated blood platelets directly or indirectly affect the tissue environment, initiating a hemostatic cascade and the synthesis of new connective tissue. This occurs through the release of various biologically active factors by platelets, including growth factors, cytokines, lysosomes, and adhesive proteins [8]. PRP concentrate contains eight growth factor proteins (GFs): platelet-derived growth factor (PDGF), transforming growth factor b1 (TGF-b1), transforming growth factor b2 (TGF-b2), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), fibroblast growth factor (FGF), and insulin-like growth factor (IGF) [8].

METHODS OF PRP EXTRACTION

PRP is a natural product that requires special extraction methods, taking into account various factors such as speed, quantity, and the quality of the obtained PRP. One of the most commonly used methods for PRP extraction is the centrifugation method [9]. This process relies on the difference in blood component density, allowing the separation of PRP from other blood elements such as red blood cells and leukocytes. A blood sample is taken from the patient and placed in a specialized centrifuge. The spinning action separates the blood components into three layers: red blood cells at the bottom, PRP in the middle layer, and leukocytes at the top. PRP can then be extracted and utilized in further medical procedures. Centrifugation is relatively simple and fast, although it may not always ensure the optimal platelet concentration.

Another popular method for PRP extraction is the two-step method, which allows for obtaining more concentrated PRP [9]. The process starts with the collection of a blood sample from the patient. The blood is then subjected to the centrifugation process, similar to the previous method. After centrifugation, a portion of the PRP is separated from the rest of the blood. However, instead of collecting the entire layer, this method involves selectively collecting only the most concentrated part, which is closest to the border with platelet-poor plasma. This second phase of the process is carried out using precise tools or automated collection systems. The two-step method allows for obtaining a higher quantity of blood platelets in the obtained PRP, which can have a beneficial impact on the therapy's effectiveness.

The third method we will discuss is the whole blood collection method [9]. In this technique, the entire blood sample from the patient is collected in special tubes containing an anticoagulant substance. This allows PRP to be directly obtained from the patient's blood, without separating other blood components. After blood collection, the samples are appropriately mixed to prevent blood platelet aggregation and stimulate the release of growth factors. PRP is then separated from other blood elements through centrifugation or filtration. This method is relatively simpler to perform but may result in slightly lower platelet concentrations compared to other methods. The choice of the appropriate PRP extraction procedure is crucial for achieving optimal results and maximizing the therapeutic potential of PRP.

MOLECULAR SPECIFICS OF THE ACTION OF ACTIVE COMPONENTS IN PRP CONCENTRATE

Among all biologically active molecules present in blood platelets, growth factors play the most significant role in tissue healing and repair. GFs constitute a diverse group of proteins secreted not only by platelets but also by cells in various tissues in the body, including connective tissue cells and hematopoietic stem cells. These factors have a relatively short half-life in the body [10].

PDGF is a glycoprotein released at the site of injury through platelet degranulation. This process activates the cell receptor on the target cell to initiate specific cell activities, such as angiogenesis and macrophage activation. VEGF is produced by both platelets and macrophages and serves as an anti-proliferative factor in natural epithelial cells. TGF-β targets marrow stem cells, pre-osteoblasts, and fibroblasts. VEGF is a signaling protein secreted by cells and activates angiogenesis [11].

EGF stimulates cell growth, proliferation, and changes by binding to the EGFR receptor [12].

PLATELET-RICH PLASMA IN SPORTS MEDICINE

The first reports of PRP application in an American football player with a ruptured Achilles tendon date back to 1999 [13]. The first controlled study of PRP usage in sports medicine was published by Mishra and Pavelko in 2006 [14]. These publications initiated the use of PRP therapies in sports medicine. The use of PRP gained popularity as a biological therapy delivered through injections. PRP in

sports medicine is primarily employed for the treatment of tendon and ligament injuries. It capitalizes on the fact that PRP concentrate exhibits anabolic effects, including increased protein production, enhanced bone marrow cell chemotaxis, increased monocyte proliferation, and improved tissue histological organization, offering the potential for accelerated regeneration [15].

One common ailment in sports medicine is Achilles tendon tendinopathy, and studies assessing the use of PRP have yielded conflicting results and insufficient final guidelines. This condition occurs when the tendon is damaged or irritated, leading to pain, weakness, and reduced function. Conservative approaches are typically used in the treatment of Achilles tendon tendinopathy, involving rest, avoiding pain-inducing factors, ice application, physiotherapy, strength and stretching exercises, and proper footwear. In some cases where conservative methods fail to improve the condition, pharmacological therapy or even surgery may be considered.

Charousset and colleagues used PRP to treat 28 athletes who did not respond to other conservative treatments. During a 2-year evaluation, it was found that 75% of the patients were able to regain their pre-injury level of sports activity within three months [16]. In another study, Volpi and their research team injected a single dose of PRP into three patients and achieved a good clinical outcome in the first 3-month assessment, which was confirmed after 2 years [16].

In contrast, another study conducted in 2010 and 2011 had different findings. In 2010, De Vos and colleagues published an article, and a year later, they conducted another study involving the same patients. The research aimed to determine whether administering PRP had better therapeutic efficacy compared to injecting a saline solution. Fifty-four patients aged 18 to 70 were treated with a single injection of either 4 ml of PRP or 4 ml of saline solution. The results showed improvement in both treated groups, but there was no significant difference between the groups [17]. In the 2011 publication [18], the results after one year of observation also confirmed the lack of differences in clinical outcomes or the time to return to sports. Based on the results of this study, the authors did not provide any evidence to support the use of PRP in the treatment of Achilles tendon tendinopathy.

PLATELET-RICH PLASMA IN ORTHOPEDICS

PRP is increasingly being used in various musculoskeletal disorders due to its potential for tissue repair in cases of weak healing abilities [19]. In orthopedics, PRP is used to facilitate the regeneration of connective tissue, cartilage, tendons, and muscles. Despite advancements in spinal fusion surgical techniques, pseudoarthrosis, resulting from the failure to fuse bone implants, remains a significant problem [20]. Therefore, although autografts remain the gold standard for spinal fusion, additional materials and biologic agents, including PRP, are used to expedite fusion rates.

PRP is also utilized in the treatment of joint diseases, particularly degenerative joint diseases [21]. Degenerative joint disease is a chronic condition characterized by progressive deterioration of joint cartilage quality. As the condition progresses, the cartilage begins to wear down, leading to joint friction and damage. As the disease advances, the cartilage may become completely eroded, and the joint can lose its normal structure, resulting in pain, stiffness, swelling, and limited mobility in the affected joint. The causes of degenerative joint disease are diverse and include genetic factors, older age, obesity, joint injuries, improper joint alignment, excessive physical activity, and more. The treatment for this disease aims to alleviate symptoms, manage pain, and improve joint function. It may include the use of pain-relieving and anti-inflammatory medications, physiotherapy, muscle-strengthening exercises, occupational therapy, lifestyle changes such as maintaining a healthy weight and avoiding excessive joint strain, and, in some cases, surgical procedures such as arthroscopy or joint replacement.

Each growth factor contained in PRP has shown a positive impact on joint biology, including extracellular matrix production [22]. It has been demonstrated that the release of PRP components into human chondrocytes with degenerative joint disease increases cell proliferation and upregulates the expression of aggrecan and SOX-9 transcription factor genes (SOX-9 is a transcription factor encoded by the SOX9 gene). These characteristics are maintained for up to 14 days in in vitro culture. This solution has immense application potential because applying a patch or capsule with cells facilitates their adhesion to the defect site [22].

Before platelet-rich plasma therapies are introduced for human treatment, animal studies are conducted to assess their efficacy. Initially, platelet-rich plasma was used for spinal fusion reinforcement in animal surgical models. The experiments demonstrated that the use of this concentrate promoted bone formation and reduced the time required for spinal fusion in posterior-lateral lumbar interbody fusion (PLIF) and anterior lumbar interbody fusion (ALIF) [23].

Li and their research team came up with the idea of conducting studies on rabbit joints. The aim was to demonstrate that PRP can stimulate cartilage repair in rabbits with degenerative joint disease [24]. The results indicated the beneficial effects of PRP. Another experiment was also conducted on rabbits, but by a different research team. Wang and their colleagues used a rabbit joint inflammation model and confirmed the influence of PRP on cartilage repair and the inhibition of matrix metallopeptidase 13 [24]. Based on these and other experiments, Siclari and their research team conducted a review of 52 cases of patients following knee joint arthroscopic surgery. The study involved histological evaluation of cartilage repair tissue in the knee joint of these patients. Nine months after PRP injection in the control observation, it was found that patients undergoing histological assessment showed a homogeneous, transparent repair tissue in the cartilage. In a 4-year follow-up, it was also observed that continued PRP use resulted in cartilage repair [25].

PRP concentrate is used in the treatment of rotator cuff tears, patellar tendinopathy, elbow conditions, Achilles

tendon injuries, and to assist in the repair of the anterior cruciate ligament (ACL). Clinical studies have shown that the use of PRP reduced pain and improved function in seven out of nine studies, with earlier return to function and increased range of motion [26]. In other studies, De Almeida and their physicians conducted clinical and radiological assessments of the effects of PRP on patellar tendon healing following ACL reconstruction with patellar tendon graft. The results showed a reduction in the gap area at the patellar tendon graft site in patients treated with PRP compared to those not treated with PRP.

PRP – A METHOD FOR HEALTHY SKIN AND HAIR

PRP is a relatively new treatment method, and research indicates its usefulness in aesthetic dermatology. It is most commonly used in combination with other therapies. Numerous growth factors in PRP are believed to rejuvenate the skin, improving its smoothness. It has been reported that facial swelling following cosmetic procedures significantly decreases when PRP is used [27]. It has been shown that PRP increases skin elasticity by inducing the synthesis of new collagen by skin fibroblasts, which is necessary for rejuvenating aging skin. PRP can also be used for mesotherapy. Scientific publications have demonstrated that injecting PRP into the face and neck yields good results [28]. It has been observed that PRP induces the expression of collagen type I and matrix metalloproteinase-1and matrix metalloproteinase-2 [28]. It has also been proven that PRP has the ability to increase skin elasticity through keratinocyte and fibroblast proliferation. Therefore, PRP used for the face and neck is considered an easy technique for skin rejuvenation.

The popular "Vampire Facelift" combines PRP with dermal fillers. Another method for achieving a youthful appearance is the use of PRP in combination with laser therapy and microneedling. The idea behind this approach is to use a laser in combination with microneedling. Small openings are created in the skin, allowing for better PRP absorption and regeneration in the deeper skin layers [29]. Combining therapies using lasers and microneedling enhances wound healing and reduces post-procedure redness and shortens the recovery time.

PRP can also help alleviate hair loss in both men and women. Growth factors have been shown to promote hair regrowth by stimulating the differentiation of stem cells in hair follicles. It has been demonstrated that the use of PRP extends the anagen phase of hair follicles and promotes angiogenesis. This results in increased perifollicular vascularization and the survival of dermal papilla fibroblasts [30]. Injections of PRP can be combined with progesterone, low molecular weight heparin fragments (dalteparin), or CD34+ cells. PRP administered with progesterone naturally inhibits 5-alpha reductase, an enzyme that converts testosterone into dihydrotestosterone (DHT). DHT is responsible for hair loss as it damages hair follicles. Progesterone inhibits 5-alpha reductase, and therefore DHT synthesis, allowing for hair regrowth. Combining PRP with CD34+ cells has shown significant improvements in hair thickness and overall appearance [31]. PRP combined with dalteparin stimulates hair shaft diameter growth and the proliferation of collagen fibers and fibroblasts, as increased angiogenesis leads to thickening of the epithelium and hair follicles [32].

CONTROVERSIES RELATED TO THE USE OF PRP

The therapeutic effect of PRP is associated with various factors released by platelets after their activation. These factors play a role in tissue repair and regeneration. Activated platelets create a platelet plug that acts as an extracellular matrix, enabling cell proliferation and differentiation [33]. In addition to platelets, leukocytes also secrete VEGF, which stimulates the formation of new blood vessels. Researchers agree that the increased number of growth factors and platelets in PRP solutions accelerates the wound healing process. However, there are significant controversies regarding the presence of leukocytes in PRP solutions.

In one study, it was shown that due to the intensified inflammatory state triggered by leukocytes, wound healing is more efficient, and the recovery time is shorter [34]. In contrast, another study suggested that the presence of leukocytes in PRP could delay tissue healing. This was based on the fact that leukocytes in PRP may release matrix metalloproteinases and reactive oxygen species, potentially slowing down healing or damaging tissues [34].

Another controversy is the lack of standardized protocols for the preparation of PRP. Studies have shown that depending on the PRP preparation procedure, which includes factors like centrifugation speed, duration, and number of spins, the cellular components of PRP can differ between solutions. Thus, the lack of standardized protocols for the PRP preparation technique is another issue [35].

The use of platelet-rich plasma is contraindicated for individuals with coagulation disorders, pregnant and breastfeeding women. Local anesthetics are typically used for injections, so it's essential to inquire about the patient's allergies to anesthetics. The impact of ultraviolet (UV) radiation on the function of growth factors in PRP is not entirely understood. It is not recommended to expose areas that have been injected with PRP to UV radiation because the disturbance of signal transduction to the cell nucleus by TGF-β may predispose to the development of skin cancer [36].

PRP CONCENTRATE – EVIDENCE OF ANTIMICROBIAL ACTIVITY IN A RABBIT BLOOD EXPERIMENT

In scientific research, investigations have been conducted to determine whether blood platelets possess antimicrobial properties. An experiment was carried out in which antibacterial proteins were purified from rabbit blood platelets, and it was demonstrated that they exhibit bacteriostatic and bactericidal activity against Escherichia coli, Candida albicans, Staphylococcus aureus, and Bacillus subtilis [37].

Moreover, in vivo evidence supports the antimicrobial action of PRP. Significantly lower rates of surgical wound infections were confirmed in patients undergoing cardiothoracic surgeries when wounds were treated with PRP compared to the control group [38]. PRP therapy has exhibited antimicrobial activity against bacteria isolated from infected wounds, as indicated by negative wound cultures [39].

CONLCUSIONS

The literature review conducted leads to the conclusion that PRP represents a groundbreaking opportunity for the field of medicine. Since the historical descriptions of PRP's applications in scientific experiments and medical procedures, many years have passed. The first researchers working on developing and utilizing this innovative PRP therapy probably didn't anticipate that it would generate such significant interest and find broad applications across various branches of medicine.

The invention of PRP and the expansion of its indications have led to clinical trials targeting a wider range of medical conditions. The therapeutic potential of a high concentration of blood platelets lies in its ability to deliver a greater quantity of growth factors than is physiologically available, which is promising for new approaches to regenerative therapies. However, there remains a knowledge gap concerning the mechanism of action of PRP, which raises skepticism regarding its potential effectiveness and application.

Currently, research is ongoing in various medical fields to explore the use of PRP. It is imperative to conduct broader, independent studies and double-blind clinical trials to demonstrate the effectiveness, efficiency, and safety of PRP as an innovative approach in medicine. It seems that the most significant standardization of procedures exists in orthopedics, but there is still room for improvement. It's essential that a uniform PRP solution composition, minimal component numbers, preparation techniques, and injection doses and frequencies be established to draw conclusions about the quality of PRP's performance. The lack of standardized composition, production methods, and administration guidelines remains a significant limitation.

REFERENCE

1. Piszczorowicz Ł, Król D, Dyląg S. Autologous platelet-rich plasma therapy – a promising method for tissue repair/regeneration in many medical fields. J Transf Med, 2020;13(2):135–148.
2. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;85(6):638-646.
3. Kohler N, Lipton A. Platelets as a source of fibroblast growth-promoting activity. Exp Cell Res. 1974;87(2):297-301.
4. Evans LA, Morey AF. Current applications of fibrin sealant in urologic surgery. Int Braz J Urol. 2006 Mar-Apr;32(2):131-41.
5. Andia I, Abate M. Platelet-rich plasma: underlying biology and clinical correlates. Regen Med. 2013;8(5):645-658.
6. Tayapongsak P, O'Brien D, Monteiro C, Arceo-Diaz LY. Autologous fibrin adhesive in mandibular reconstruction with particulate cancellous bone and marrow. J Oral Maxillofac Surg. 1994;52(2):161-165.
7. Whitman DH, Berry RL, Green DM. Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. J Oral Maxillofac Surg. 1997 Nov;55(11):1294-9.
8. Gentile P, Garcovich S. Systematic review – The potential implications of dif erent platelet-rich plasma (PRP) concentrations in regenerative medicine for tissue repair. Int J Mol Sci 2020 Aug 9;21(16):570.
9. Dhurat R, Sukesh, M, Principles and methods of preparation of platelet-rich plasma: a review and author's perspective. J Cutan Aesthet Surg. 2014;7(4):189.
10. Herbst RS, Review of epidermal growth factor receptor biology. Int J Radiat Oncol Biol Phys. 2004;59(2 Suppl):21-26.
11. Senger DR, Galli SJ, Dvorak AM. Cancer cells secrete vascular permeability factor, which promotes the accumulation of ascitic fluid. Science 1983;219:983-985.
12. Uebel CO, da Silva JB, Cantarelli D, Martins P. The role of platelet plasma growth factors in male pattern baldness surgery. Plast Reconstr Surg. 2006;118(6):1458-1466.
13. Mishra A, Pavelko T. Treatment of chronic elbow tendon with platelet-rich plasma. Am J Sports Med. 2006;34(11):1774-1778.
14. Everts PA, van Erp A, DeSimone A, Cohen DS, Gardner RD. Platelet Rich Plasma in Orthopedic Surgical Medicine. Platelets 2021;32(2):163-174.
15. Schnabel LV, Mohammed HO, Jacobson MS, Santangelo KS, Fortier LA. Efects of platelet rich plasma and acellular bone marrow on gene expression patterns and DNA content of equine suspensory ligament explant cultures. Equine Vet J. 2008;40:260-265.
16. Volpi P, Quaglia A, Schoenhuber H. Growth factors in the management of sport-induced tendinopathies: results after 24 months from treatment. A pilot study. J Sports Med Phys Fitness. 2010;50(4):494-500.
17. De Vos RJ, Weir A, Van Schie HT. Platelet-rich plasma injection for chronic Achilles tendinopathy: a randomized controlled trial. JAMA, 2010;303(2):144-149.
18. De Jonge S, De Vos RJ, Weir A. One-year follow-up of platelet-rich plasma treatment in chronic Achilles tendinopathy: a double-blind randomized placebo-controlled trial. Am J Sports Med. 2011;39(8):1623-1629.
19. Martínez-Martínez A, Ruiz-Santiago F, García-Espinosa J. Platelet-rich plasma: myth or reality? Plasma rico en plaquetas: mito o realidad?, Radiology (Engl Ed). 2018;60(6):465-475.
20. Hirano K, Matsuyama Y, Sakai Y, et al. Surgical complications and management of occipitothoracic fusion for cervical destructive lesions in RA patients, J Spinal Disord Tech. 2010;23(2):121-126.
21. O'Keefe RJ, Crabb ID, Puzas JE, Rosier RN. Ef ects of transforming growth factor-beta 1 and fibroblast growth factor on DNA synthesis in growth plate chondrocytes are enhanced by insulin-like growth factor-I. J Orthop Res. 1994;12:299-310
22. Noth U, Rackwitz L, Heymer A, et al. Chondrogenic dif erentiation of human mesenchymal stem cells in collagen type I hydrogels. J Biomed Mater Res A. 2007;83:626-635.
23. Kamoda H, Yamashita M, Ishikawa T, et al. Platelet-rich plasma combined with hydroxyapatite for lumbar interbody fusion promoted bone formation and decreased an inflammatory pain neuropeptide in rats. Spine 2012;37:1727-1733.

24. Wang ZZ, Yu F, Yang B. et al. Inhibitory ef ect of platelet-rich plasma on matrix metalloproteinase-13 expression in rabbit cartilage with osteoarthritis. Journal of Capital Medical University 2020;41:923-928.
25. Siclari A, Mascaro G, Kaps C, Boux E. 5-year follow-up after knee cartilage repair with a polymer implant immersed in platelet-rich plasma. Open Orthop J. 2014;8:346-354.
26. Taylor DW, Petrera M, Hendry M, et al. A systematic review of the use of platelet-rich plasma in sports medicine as a new method of treating tendon and ligament injuries. Clin J Sport Med. 2011;21:344-352.
27. Eppley BL, Pietrzak WS, Blanton M. Platelet-rich plasma: a review of biology and applications in plastic surgery. Plast Reconstr Surg. 2006;1(18):147-159.
28. Shirvan MK, Alamdari DH, Ghoreifi A. Novel treatment of iatrogenic vesicovaginal fistula: autologous injection of platelet-rich plasma and insertion of platelet-rich fibrin glue. J Urol. 2013;189:2125-2129.
29. Sand JP, Nabili V, Kochhar A, Rawnsley J, Keller G. Platelet-rich plasma for the aesthetic surgeon. Plast Surg Face. 2017;33(4):437-443.
30. Gupta AK, Carviel J. A Mechanistic Model of Platelet-Rich Plasma Treatment for Androgenetic Alopecia, Dermatol Surg. 2016;42(12);1335-1339.
31. Kang JS, Cheng Z, Choi MJ, Lee SH, Kim DY, Cho SB. Influence injection of autologous platelet-rich plasma containing CD34+ cells for hair loss: a preliminary study. J Eur Acad Dermatol Venerol. 2014;28(1):72-79.
32. Takikawa M, Nakamura S, Ishirara M, et al. Enhanced Impact platelet-rich plasma containing a new carrier for hair growth. Dermatol Surg. 2011;37(12):1721-1729.
33. Marx RE. Platelet-rich plasma (PRP): what is PRP and what is not? Implant Dent. 2001;10(4):225-228.
34. Di Matteo B, Filardo G, Kon E, Marcacci M. Platelet-rich plasma: evidence for the treatment of patellar and Achilles tendinopathy--a systematic review. Musculoskelet Surg. 2015;99(1):1-9.
35. Jeong DU, Lee CR, Lee JH. Clinical applications of platelet-rich plasma in patellar tendinopathy. Biomed Res Int. 2014;2014:249498.
36. Ciążyńska M, Bednarski I, Lesiak A. The role of TGF-b in photodegradation and carcinogenesis. Forum Dermatolog. 2016;2(2):60-63.
37. Yeaman MR, Tang YQ, Shen AJ, Bayer AS, Selsted ME. *In vitro* purification and activity of rabbit platelet bactericidal proteins. Infect Immun. 1997;65:1023-1031.
38. Englert SJ, Estep TH, Ellis-Stoll CC. Postoperative chest and leg incision sites using plate gel: a retrospective study. J Extra Corporation Technol. 2008;40:225-228.
39. Cieślik-Bielecka A, Bielecki T, Gaździk TS. Autologous platelets and leukocytes may improve healing of infected high-energy soft tissue lesions, Transfus Apher Sci. 2009;41:9–12.

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Katarzyna Elżbieta Grudnik
Student Scientific Circle
at the Department of Environmental Medicine and Epidemiology
Silesian Medical University in Katowice, Zabrze, Poland
e-mail: katarzyna.grudnik15@gmail.com

ORCID AND CONTRIBUTIONSHIP

Monika Prokurat: 0009-0001-3924-9327 (A, B, D)
Katarzyna Elżbieta Grudnik: 0009-0006-1583-0041 (A, B, D)
Wojciech Niemczyk: 0000-0003-0172-0571 (A, B, D)
Stanisław Niemczyk: 0000-0001-5236-2298 (A, B, D)
Mateusz Migas: 0009-0001-0274-7832 (A, B, D)
Karolina Wągrowska: 0009-0009-6259-7598 (A, B, D)
Karolina Lau: 0000-0002-8654-0301 (E-F)
Janusz Kasperczyk: 0000-0002-6945-1200 (E-F)

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

RECEIVED: 10.01.2024
ACCEPTED: 21.03.2024

REVIEW ARTICLE

The right to know from the opt-out effectiveness system perspective in organ transplantation cases

Daniela Kokina, Karina Palkova
RIGA STRADINS UNIVERSITY, RIGA, LATVIA

ABSTRACT

Aim: This article examines the legal challenges associated with opt-out and opt-in systems in transplantation cases. It focuses on the low public knowledge and awareness of the national transplantation system, assessing its compliance with international prerequisites for an opt-out system. The analysis centres on the "right to know" perspective and the effectiveness of opt-out in organ transplantation.

Materials and Methods: The research methodology includes scientific principles, public surveys, relevant regulations from the Republic of Latvia and EU, and grey literature on the health system and organ donation in the EU. Scientific articles from databases such as Scopus and WOS were selected based on criteria such as language (English and Latvian) and focus on living wills and comparisons of organ donation systems. Previous EU and national studies, reports, and court judgments were used to analyse data on opt-in and opt-out transplantation systems and policy efficiency in organ transplantation.

Conclusions: Individuals' right to autonomy over their bodies extends even after death, encompassing the right to integrity. Organ donation, being a deeply personal choice reflecting one's values and beliefs, plays a crucial role in saving lives through transplantation. To enhance cooperation and donation rates, international regulations stress the significance of public awareness regarding organ and tissue transplantation. Unfortunately, inadequate compliance by authorities and low awareness pose ethical and legal dilemmas, potentially violating constitutional rights. Reports highlight limited public understanding of transplantation systems, raising concerns, particularly in opt-out systems. National governments bear the responsibility of safeguarding these rights and addressing challenges beyond legal means, thus establishing a more ethical organ donation system that upholds voluntariness, generosity, and individual autonomy.

KEY WORDS: rights to know, organ transplantation, living will, informed consent, opt-out, tissue and organ procurement

Pol Merkur Lek,2024;53(1):246-251. doi: 10.36740/Merkur202402115

INTRODUCTION

Despite remarkable advancements in medical science and technology, the European Union (EU) witnesses 10 daily deaths due to organ shortages[1]. Annual global solid organ transplants have risen by 52% since 2010, reaching approximately 150,000 procedures. However, this number covers less than 10% of the global demand, indicating a significant disparity. The COVID-19 pandemic worsened the situation, leading to an 18% decrease in global transplants[2]. For years, the international transplant community has debated the shift from default opt-in systems to opt-out policies to increase organ donor numbers and address the growing demand for life-saving transplants [3].

National organ donation and transplantation systems in the EU Member States vary concerning dying wishes [4] and strategies on the number of available organs do not always resolve shortages. Transplants in the EU must adhere to the Council of Europe's binding laws, respecting fundamental rights and human body integrity, and comply with relevant EU rules.[4] World Health Organization Guiding principles (WHO Principles) prioritize consent as the ethical foundation for medical interventions, leaving it to each State to determine consent registration methods while considering international ethical standards, organ procurement organization, and the role of consent in preventing abuse and security breaches [5].

Regarding the opt-out system, ensuring full information and unhindered expression of prohibition is crucial [6]. A study comparing opt-in and opt-out systems suggests that prioritizing education and informing the public about transplantation benefits is the preferred method for increasing organ donation rates [7]. However, effective information dissemination can be time-consuming and resource-intensive. The opt-out system considered to be possible only in a supportive society, but such support is only relevant if that society knows its rights and the consequences of refusing to enforce those rights.

In Latvia, the opt-out system is in place, but its effectiveness is hampered by inadequate implementation of the right to know one's rights, as stipulated in the Constitution of the Republic of Latvia (the Constitution)[8]. The Constitution not only grants individuals the right to be informed about their rights and obligations but also requires normative acts to be publicly accessible, clear, and understandable. Consequently, public awareness and understanding of the transplantation system play a crucial role in the effective functioning of the opt-out system.

AIM

This article examines the legal challenges associated with opt-out and opt-in systems in transplantation cases. It focuses on the low public knowledge and awareness of the national transplantation system, assessing its compliance with international prerequisites for an opt-out system. The analysis centres on the "right to know" perspective and the effectiveness of opt-out in organ transplantation.

MATERIALS AND METHODS

The research methodology includes scientific principles, public surveys, relevant regulations from the Republic of Latvia and EU, and grey literature on the health system and organ donation in the EU. Scientific articles from databases such as Scopus and WOS were selected based on criteria such as language (English and Latvian) and focus on living wills and comparisons of organ donation systems. Previous EU and national studies, reports, and court judgments were used to analyse data on opt-in and opt-out transplantation systems and policy efficiency in organ transplantation.

REVIEW AND DISCUSSION

National regulations and healthcare principles necessitate providing individuals with essential information in advance to make informed decisions about body and organ donation for transplantation after death. This requirement aligns with the presumed consent system's underlying assumptions.

Providing individuals with necessary information allows them to express their will and decide on granting permission or registering a ban [9]. Lack of information hinders both next-of-kin and individuals from exercising their rights. Additionally, informing next-of-kin is essential to reduce situations where they are unwilling to accept the deceased's choice.

The studies finds that the effectiveness of the opt-out system in Latvia is hindered by inadequate implementation of the right to know one's rights, leading to low public awareness and confusion about the transplantation system. The results of surveys indicate that the state and the Ministry of Health of Latvia are failing in their obligations under the national law "On the Protection of the Body of Deceased Human Beings and the Use of Human Tissues and Organs in Medicine"(Organs Law). Limited public awareness, knowledge, and cultural resistance towards transplantation remain significant barriers to organ donation and improving transplantation systems on national and regional levels.

Legislation alone cannot be expected to change the culture of society and make giving the norm. There is a risk that the lack of information may be perceived by the public as features of an authoritarian regime and the introduction of the opt-out system may have the opposite effect of increasing the number of bans registered [10]. The article points out the importance of building trust in the national healthcare system to promote organ donation. The right of individuals to be fully informed about their rights and obligations is essential to ensure informed choices and adherence to legal provisions.

LIVING WILL FOR ORGAN AND BODY DONATION AFTER DEATH: A GENERAL OUTLINE AND ECHR PRACTICE

The right to determine the fate of our deceased bodies, which is linked to bodily integrity after death,[11] signifies individuals' desire to have a say in what happens to their bodies post-death. States play a role in enabling this choice.

A 2019 study comparing opt-in and opt-out systems in 35 OECD countries found no significant difference in deceased-donor rates, but noted a reduction in living-donor numbers in opt-out countries.[7][4] The WHO Principles regarding the opt-out system emphasizes the need to ensure full information and unhindered expression of prohibition in opt-out systems.[5] As regards the opt-out system, there is a problem with the very presumption of public willingness to donate organs.[12] The opt-out system considered to be possible only in a supportive society, but such support is only relevant if that society knows its rights, also the consequences of refusing to enforce those rights. Trust and positive attitudes towards organ procurement and transplantation remain vital factors.

The concept of opt-out is commonly misunderstood in society, often wrongly linked to organ removal without consent [6]. The Ombudsman of the Republic of Latvia highlights that this misconception arises due to the existence of different opt-out systems in various countries with diverse regulations and implementations[6]. Latvia exhibits characteristics of a soft opt-out system[1, 13], considering next-of-kin views but not fully established. Consequently, when the deceased's living will on organ donation is communicated to next-of-kin, they may misinterpret it as seeking their explicit consent.

In the European Court of Human Rights (ECHR) case law, two significant cases, Elberte v. Latvia [14] and Petrova v. Latvia [15] shed light on the public's limited understanding of the organ donation system in the country. These cases, although pertaining to the legal framework of Latvia until 2019, played a crucial role in shaping legal norms at the national level. The two cases before the ECHR involved similar facts, where the legality of post-death tissue and organ removal was questioned, along with the interpretation of the Organs law. Hospital staff justified the removal, citing the absence of prohibition and presuming the deceased's consent, which the relatives only learned after the operation. The applicants (relatives) filed identical complaints, citing ECHR case law, and alleging violations of private life and the prohibition of inhuman treatment (torture) due to the organ removal from their deceased relatives' bodies [16].

The ECHR ruling exposed conflicting interpretations of the Organs Law in Latvia. Some experts perceived an opt-out system, where anything not explicitly prohibited was deemed permissible. However, investigators and complainants argued that the legal framework favoured the opt-in concept. Withdrawal was only permissible if explicitly authorized, either by the donor during their lifetime or by their next-of-kin after the donor's death [14].

The ECHR emphasized the need for a clear and precise legal framework in Latvia regarding organ removal and the

protection of rights, as the existing law lacked sufficient clarity and protection against arbitrariness [14]. Legislation can always be improved, especially when it comes to ensuring fundamental rights [17]. The Constitutional Court further established that human dignity remains protected even after death, necessitating continuous evaluation and improvement of the legal framework to safeguard fundamental rights [18,19]. Efforts to enhance the legal framework were made through the Amendments to the Organs Law (16.05.2019)[20], aligned with the Convention on Action against Trafficking in Human Organs[21]. The amendments reinforced the opt-out system, presuming consent for organ use if the deceased's will be not ascertained. However, challenges persist in public awareness and understanding, as medical staff mainly handle law interpretation daily, raising doubts about the law's effectiveness for all individuals [22].

PUBLIC ACCESS TO INFORMATION AS A PREREQUISITE FOR AN EFFECTIVE SYSTEM OF PRESUMED CONSENT IN A COUNTRY

According to a 2022 report by WHO [2] the global development of transplantation is inadequate and uneven, despite efforts by member states, the Secretariat, and other stakeholders. Limited public awareness, knowledge, and cultural resistance towards transplantation are significant barriers to organ donation and the overall improvement of national and regional transplantation systems.

The EU 2009 report revealed that ~28% of Europeans are aware of the legislation governing organ donation and transplantation in their country. Conversely, 68% of respondents indicated a lack of awareness, 4% were unable to provide a response [23] The report further highlighted the influence of education, geography, and historical factors in promoting organ donations.

In opt-in, individuals are informed before deciding to become donors. In opt-out, a clear understanding of the voluntary choice and its implications is ethically necessary to avoid perceiving organ donation as non-altruistic. In 2019, a comparison of information availability in countries with distinct organ donation systems [24] showed that opt-in countries had more accessible information and research on organ transplantation. However, opt-out countries heavily relied on academic studies analysed by medical students and staff, or materials presented during students' education. It is crucial to recognize that educating students cannot replace education of entire society. The study concluded that in an opt-out system, individuals might be uncertain about expressing their wishes concerning organ use after death.

Each country has unique legal requirements for organ procurement from deceased individuals, encompassing authorization procedures for obtaining organs. The choice between "expressed" or "presumed" consent depends on the country's social, medical, and cultural traditions. While both systems comply with human rights standards, specific conditions must be met for their implementation. To ensure that presumed consent in an opt-out system aligns with the human will, certain sources propose preconditions such as: 1. be aware of the national system and of the consequences of his/ her action or inaction; 2. the person must be given a reasonable time to express his or her consent or prohibition; 3. the person must be able to express or change his or her will without hindrance[6]. Others mention also: the procedure associated to the consent must be a matter of general knowledge; information about the procedure and its consequences must be easily accessible[12]. Once these conditions are met and secured, the opt-out system can be considered a legitimate means for individuals to express their will. Increased awareness of the system could enhance willingness to donate, but such presumption should be supported by empirical evidence [6]. Reliance on statistics is only valid if the public has been educated beforehand; otherwise, public opinion polls may not reflect informed decisions. In Latvia, the current presumption that a person is willing to become a donor if no contrary entry exists in the state register is based on an abstract assumption about public preferences, lacking concrete data. The Ombudsman's survey results indicated insufficient public awareness, with 49.1% of respondents describing their knowledge of the medical use of deceased human tissue and organs as poor, and 21.5% reporting no knowledge at all. Only 34% of respondents knew they could register their will to donate tissues and organs after death with national registries [6].

In 2021 the public survey [25] with the aim of understanding public awareness and whether the Amendments to the Organs Law have created greater public clarity about the legal framework for organ transplantation at national level and the rights of the public, indicated that the situation has not changed in Latvia. The survey was carried out among 300 persons residing in Latvia between 21.04.2021 and 28.04.2021. The results of it presented and summarised without disaggregation or analysis by age group or gender.

The survey provided definitions of "transplantation" that are included in both the national[26] and international frameworks, e.g. Additional Protocol to the Convention on Human Rights and Biomedicine concerning Transplantation of Organs and Tissues of Human Origin[27] (Additional Protocol on Biomedicine), and definition proposed by the survey author itself. 51% agreed with the definition of the Additional Protocol on Biomedicine, 36% agreed with the survey author's definition. Only 11.4% agreed with the definition provided in the national legislation. 1.7% of respondents mentioned that the definition should be supplemented by mandatory consent.

On the national level, the Ministry of Health is responsible for providing free information materials and conducting regular information campaigns about the use of human body, tissues, and organs[22]. However, the survey revealed that only 18% of respondents were aware of the Organs Law, while 41.8% had no knowledge of any national laws and regulations. An overwhelming 84% of respondents felt that there is insufficient information about the organ donation system, its possibilities, and regulation in Latvia, indicating a low level of public awareness of transplantation governance. Both national surveys indicate a lack of public awareness, thus not meeting the preconditions for a successful and human rights-compatible opt-out system.

A PERSON'S RIGHT TO BE FULLY INFORMED IS CRUCIAL FOR BUILDING TRUST IN THE NATIONAL HEALTHCARE SYSTEM

The Additional Protocol on Biomedicine emphasizes the need to address the shortage of organs and tissues by increasing donation rates through public awareness and promoting European cooperation in organ and tissue transplantation[28]. In addition, countries shall take appropriate measures to promote the donation of organs and tissues.

In the context of implementing an opt-out system and considering the level of public awareness in Latvia, the authors suggest that public awareness also plays a crucial role in informing the next-of-kin about their rights and reducing instances where they may be unwilling to accept the deceased person's choice. These situations can contribute to a lack of trust in the healthcare system [29].

Without trust, large-scale unwillingness to consider donation will abide, and increases in donor numbers are unlikely. Lack of information can lead to misconceptions about organ transplantation. Perverse financial incentives, perceptions of economic gain, reports of transplant practices favouring the wealthy, manipulation of transplant systems in favour of certain patients and unethical procurement practices have directly diminished public trust in organ transplantation[3]. Maintaining the support and trust of the donating public is vitally important in the organ donation system because the public supplies organs for donation and funds the healthcare system which manages these transplants.[30]

An analysis based on survey's data about the consent systems [31], identified quality indicators for organ removal policies and showed that citizens' support for the consent policy is crucial from the perspective of good policy governance in democratic societies.[24]

At the national level the Constitution establishes the subjective public right of a person to be informed of his or her rights and obligations [32] The right of a person to know his or her rights also determines the framework within which the legislator acts. Laws and regulations must not only be publicly accessible, but also sufficiently clear and understandable [19] Article 90 of the Constitution provides that everyone has the right to know his or her rights [8]. This entails understanding the legal consequences of certain actions or omissions to make informed choices and adjust behaviour accordingly. Article 90 creates a right for a private person to know both his rights and his obligations and, more generally, the actual or potential legal consequences of any legal relationship affecting his interests [33]. The constitutional right to know one's rights is aimed at ensuring legal certainty and legal clarity. By exercising this right, a person can plan his or her actions without having to worry whether he or she has correctly understood the legal provisions in question. As the state law expert J. Pleps points out that the clarification of the content of Article 90 in the practice of the Constitutional Court and in the decisions of administrative courts shows that the most essential elements of the principle of legitimate expectations are included in this constitutional norm as a fundamental right of a person. It may be concluded from the case-law that respect for legitimate expectations is an obligation of the State under Article 90 [33]. This should be closely linked to the principle of informed consent.

The presumed consent model is only feasible in a supportive society if society is well-informed about their rights and the consequences of not exercising them. However, the public in Latvia seems to lack sufficient knowledge about the transplantation system and the legal framework, despite efforts by the Ministry of Health.

CONCLUSIONS

Individuals' right to autonomy over their bodies extends even after death, encompassing the right to integrity. Organ donation, being a deeply personal choice reflecting one's values and beliefs, plays a crucial role in saving lives through transplantation. To enhance cooperation and donation rates, international regulations stress the significance of public awareness regarding organ and tissue transplantation. Unfortunately, inadequate compliance by authorities and low awareness pose ethical and legal dilemmas, potentially violating constitutional rights. Reports highlight limited public understanding of transplantation systems, raising concerns, particularly in opt-out systems. National governments bear the responsibility of safeguarding these rights and addressing challenges beyond legal means, thus establishing a more ethical organ donation system that upholds voluntariness, generosity, and individual autonomy.

REFERENCES

1. Ašnevica-Slokenberga S (ed.). Medicīnas tiesības. [Medical law]. Riga: Tiesu namu aģentūra. 2015, p.589-607. (Latvian)
2. Human organ and tissue transplantation: Report by the Director-General. WHO. https://apps.who.int/gb/ebwha/pdf_files/WHA75/A75_41-en.pdf [Accessed 15 May 2023]
3. Etheredge HR. Assessing Global Organ Donation Policies: Opt-In vs Opt-Out. Risk Manag Healthc Policy. 2021;14:1985-1998. doi:10.2147/RMHP.S270234.
4. Scholz N. Organ donation and transplantation: Facts, figures and European Union action. European Parliamentary Research Service. 2020. https://www.europarl.europa.eu/thinktank/en/document/EPRS_BRI(2020)649363 [Accessed 15 May 2023]
5. Guiding principles on human cell, tissue and organ transplantation: Report by the Secretariat, A62/15. WHO. http://apps.who.int/gb/ebwha/pdf_files/A62/A62_15-en.pdf [Accessed 15 May 2023]
6. Tiesībsargs. Pētījums par audu un orgānu izņemšanu izmantošanai medicīnā. [Research on the removal of tissues and organs for medical use]. https://www.tiesibsargs.lv/resource/latvijas-republikas-tiesiska-regulejuma-par-mirusa-cilveka-audu-un-organu-iznemsanu-izmantosanai-medicina-atbilstiba-cilvektiesibu-standartiem/ [Accessed 15 May 2023] (Latvian)

7. Arshad A, Anderson B, Sharif A. Comparison of organ donation and transplantation rates between opt-out and opt-in systems. Kidney Int. 2019;95(6):1453-1460. doi:10.1016/j.kint.2019.01.036.
8. The Constitution of the Republic of Latvia: Latvian law. Latvijas Vēstnesis No.43, 01.07.1993. Article 90. https://likumi.lv/ta/en/en/id/57980-the-constitution-of-the-republic-of-latvia [Accessed 15 May 2023] (Latvian)
9. Olsena S. Miruša cilvēka aizsardzība un audu izmantošana medicīnā. [Preservation of a dead person and the use of tissues in medicine]. Riga, Latvia: Latvian University. 2010, p.80. https://dspace.lu.lv/dspace/bitstream/handle/7/5061/17228- Solvita_Olsena_2010.pdf?sequence=1&isAllowed=y [Accessed 15 May 2023] (Latvian)
10. Bea S. Opt-out policy and the organ shortage problem: Critical insights and practical considerations. Transplant Rev (Orlando). 2021;35(1):100589. doi:10.1016/j.trre.2020.100589.
11. den Hartogh G. In the best interests of the deceased: A possible justification for organ removal without consent? Theoretical Medicine and Bioethics volume. 2011;32(4):259-269. doi: 10.1007/s11017-011-9182-0.
12. Formoso V, Marina S, Ricou M. Presumed Consent for Organ Donation: An Incoherent Justification. actabioethica. 2021;27(1):27-35.
13. Law On the Protection of the Body of Deceased Human Beings and the Use of Human Tissues and Organs in Medicine: Latvian law as amended in 27.08.2012. Latvijas Republikas Augstākās Padomes un Valdības Ziņotājs No.1/2, 14.01.1993. https://likumi.lv/ta/id/62843-par-mirusa-cilveka-kermena-aizsardzibu-un-cilveka-audu-un-organu-izmantosanu-medicina [Accessed 15 May 2023] (Latvian)
14. Judgment of ECtHR of 13 January 2015 on the case of Elberte v. Latvia, application no. 61243/08. https://hudoc.echr.coe.int/fre?i=002-10354 [Accessed 15 May 2023]
15. Judgment of ECtHR of 24 June 2014 on the case of Petrova v. Latvia, application no. 4605/05. https://hudoc.echr.coe.int/fre?i=002-9531 [Accessed 15 May 2023]
16. Convention for the Protection of Human Rights and Fundamental Freedoms. Council of Europe Treaty Series 005. Council of Europe. 1950. https://www.refworld.org/docid/3ae6b3b04.html [Accessed 15 May 2023]
17. Judgment of Constitutional court of Republic of Latvia of 2 June 2008 on the case No. 2007-22-01. https://www.satv.tiesa.gov.lv/wp-content/uploads/2016/02/2007-22-01_Spriedums.pdf [Accessed 15 May 2023]
18. Judgment of Constitutional court of Republic of Latvia of 5 March 2019 on the case No. 2018-08-03. https://www.satv.tiesa.gov.lv/cases/?search[number]=2018-08-03 [Accessed 15 May 2023]
19. Judgment of Constitutional court of Republic of Latvia of 20 December 2006 on the case No. 2006-12-01, point 16. https://www.satv.tiesa.gov.lv/wp-content/uploads/2016/02/2006-12-01_Spriedums.pdf [Accessed 15 May 2023]
20. Amendments to the Law on the Protection of the Body of Deceased Human Beings and the Use of Human Tissues and Organs in Medicine: Latvian law. Latvijas Vēstnesis No.108, 30.05.2019. https://likumi.lv/ta/id/307176-grozijumi-likuma-par-mirusa-cilveka-kermena-aizsardzibu-un-cilveka-audu-un-organu-izmantosanu-medicina- [Accessed 15 May 2023] (Latvian)
21. Par Eiropas Padomes Konvenciju par cīņu pret cilvēku orgānu tirdzniecību: Latvian law. Latvijas Vēstnesis Nr.36, 20.02.2019. https://likumi.lv/ta/id/304975-par-eiropas-padomes-konvenciju-par-cinu-pret-cilveku-organu-tirdzniecibu [Accessed 15 May 2023] (Latvian)
22. Law On the Protection of the Body of Deceased Human Beings and the Use of Human Tissues and Organs in Medicine: Latvian law. Latvijas Republikas Augstākās Padomes un Valdības Ziņotājs No.1/2, 14.01.1993. https://likumi.lv/ta/en/en/id/62843-on-the-protection-of-the-body-of-deceased-human-beings-and-the-use-of-human-tissues-and-organs-in-medicine [Accessed 15 May 2023] (Latvian)
23. Organ donation and transplantation - Publication Reports. Eurobarometer. https://europa.eu/eurobarometer/surveys/detail/804 [Accessed 15 May 2023]
24. Molina-Pérez A, Rodríguez-Arias D, Delgado-Rodríguez J et al. Public knowledge and attitudes towards consent policies for organ donation in Europe. A systematic review. Transplant Rev (Orlando). 2019;33(1):1-8. doi:10.1016/j.trre.2018.09.001.
25. Izotenko D et al. Orgānu transplantācija: nacionālais un starptautiskais tiesiskais regulējums un tā problemātika. [Organ transplantation: national and international legal regulation and its problems]. Riga, Latvia: Rīga Stradiņš University. 2021. https://dspace.rsu.lv/jspui/handle/123456789/4847 [Accessed 15 May 2023] (Latvian)
26. The survey used definitions from the law of Republic of Latvia "On the Protection of the Body of Deceased Human Beings and the Use of Human Tissues and Organs in Medicine and 29.01.2013. Regulation No. 70 "Regulations Regarding Use of Human Organs in Medicine, as well as Use of Human Organs and Body of Deceased Human Being for Medical Studies" of the Cabinet of Republic of Latvia.
27. European Treaty Series -No. 186 Additional Protocol to the Convention on Human Rights and Biomedicine Concerning Transplantation of Organs and Tissues of Human Origin. https://rm.coe.int/1680081562 [Accessed 15 May 2023]
28. European Treaty Series -No. 186 Explanatory Report to the Additional Protocol to the Convention on Human Rights and Biomedicine Concerning Transplantation of Organs and Tissues of Human Origin. https://rm.coe.int/16800d37ac [Accessed 15 May 2023]
29. Mazure L. Principles of Fulfilment of Patient Duties in Medical Treatment. Electronic Scientific Journal of Law Socrates. 2022;3(21):24-32. doi:10.25143/socr.22.2022.1.024-032.
30. Tennankore KK, Klarenbach S, Goldberg A. Perspectives on Opt-Out Versus Opt-In Legislation for Deceased Organ Donation: An Opinion Piece. Canadian Journal of Kidney Health and Disease. 2021;8. doi:10.1177/20543581211022151.
31. Molina-Pérez A, Werner-Felmayer G, Van Assche K et al. Attitudes of European students towards family decision-making and the harmonisation of consent systems in deceased organ donation: a cross-national survey. BMC Public Health. 2022;22(1). doi: 10.1186/s12889-022-14476-z.
32. Judgment of Constitutional court of Republic of Latvia of 1 November 2012 on the case No. 2012-06-01, point 7.2. https://www.satv.tiesa.gov.lv/wp-content/uploads/2012/03/2012-06-01_Spriedums_ENG.pdf [Accessed 15 May 2023]
33. Endziņš A et al. Latvijas Republikas Satversmes komentāri. VIII nodaļa. [Comments on the Constitution of the Republic of Latvia. Chapter VIII.]. Cilvēka pamattiesības. Riga. 2011, p.65.

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Daniela Kokina
Rīga Stradiņš University
16 Dzirciema iela, LV-1007, Rīga, Latvia
e-mail: 032897@rsu.edu.lv

ORCID AND CONTRIBUTIONSHIP
Daniela Kokina: 0000-0003-3358-3452 A, B, D, F
Karina Palkova: 0000-0002-6909-571X E

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

RECEIVED: 13.08.2023
ACCEPTED: 27.02.2024

VARIA

The study of dental status through determination of the degree of preservation of paleoanthropological material

Lyudmyla Kaskova[1], Nataliia V. Yanko[1], Andrii Artemyev[1,2], Olha Andriyanova[1]

[1]POLTAVA STATE MEDICAL UNIVERSITY, POLTAVA, UKRAINE
[2]POLTAVA MUNICIPAL ESTABLISHMENT "CENTER OF PROTECTION AND RESEARCH OF ARCHAEOLOGICAL MONUMENTS", POLTAVA, UKRAINE

ABSTRACT

Aim: To introduce a simple classification system for the degree of preservation and quality of the dentoalveolar apparatus into scientific circulation to further investigate dental diseases in ancestral populations.

Materials and Methods: The sample analyzed consisted of the remains of 499 individuals from the human populations that existed in the territories of Ukraine from the Copper Age to the Bronze Age. Teeth and jaws were examined macroscopically under bright light; dental changes were evaluated by probing. Dental radiographic examinations were performed using retroalveolar and panoramic X-ray films in adult individuals.

Results: The authors divide the dentoalveolar apparatus of fossil and subfossil skeletons into ten levels for further medical investigation of dental diseases. These levels depend on the preservation degree and changes in jaws and teeth.

Conclusions: The study of bone and tooth preservation, determination of skeletal sex, and age characteristics is a separate section and stage in reconstructing the physical type of individuals and the health status of a community. Studying the taphonomic features of odontologic anthropological material should be a mandatory prerequisite preceding its analysis in paleopathology. The condition of the dentoalveolar system can be classified into ten levels for paleopathological conclusions. This classification will be helpful to doctors specializing in forensic medicine and anthropologists.

KEY WORDS: preservation, jaws, teeth, remains

Pol Merkur Lek,2024;53(1):252-256. doi: 10.36740/Merkur202402116

INTRODUCTION

Due to the growing interest in dental research in ancient populations [1-4], particular attention is given to the quality of odontologic collections and tooth preservation issues. The obtained data on the manifestations of dental diseases in our ancestors are expected to clarify the evolution of occlusion and dental diseases and preventive measures for them in the modern population.

There are a lot of factors that determine bone and teeth preservation. It is objectively challenging to refute the impact of the "postmortem selection" factor. There is a documented direct correlation between the preservation degree of skeletal tissues and the biological age at reaching sexual maturity, possibly vice versa, with older individuals' skeletons being less well-preserved. However, the preservation of juvenile skeletal remains may be similar to that of adult skeletons. Additionally, the bones' size is likely a primary factor influencing their preservation quality.

The impact of various environmental factors directly affects skeletal remains [5]. These factors include 1) moisture; 2) temperature regime; 3) different soil composition: sandy, clayey, etc.; 4) pH of the soil, acidity; 5) forested areas, open fields, etc.; 6) different climatic zones and variations in weather conditions; 7) presence of various chemical substances in burial structures (such as introduced lime or other substances present in the soil); 8) type of burial structure and the location of the skeletal remains within it; 9) proximity of the material to the contemporary surface, and, of course, the factor of time – the period during which the hard tissues of the body have been subjected to different preservation conditions. Indeed, it is crucial to consider the archaeological site's condition and the circumstances of its disturbance during the research. Factors such as looting of the monument, damage to human hard tissues during the removal of the original structure, ritual influences on the skeleton, and the actions of burrowing animals, etc. should be considered.

The classifications of the degree of preservation of the bony skeleton have been described by Mamonova et al. [6], Buikstra and Ubelaker [7], Bello et al. [8], and Kondo et al. [9]. Vodanović M. et al. [10] offered levels of jaws preservation. There is no unified methodology for describing the jaws and teeth of ancestors exists to date.

AIM

The paper aims to introduce a simple classification system for the degree of preservation and quality of the dentoalveolar apparatus into scientific circulation to further investigate dental diseases in ancestral populations.

MATERIALS AND METHODS

To study the degree of preservation of hard dental tissues, 499 skeletons from the human populations that existed in the territories of Ukraine from the Copper Age to the Medieval period have been studied [3,11-13].

These skeletal remains were obtained during archaeological excavations conducted in present-day Ukraine. The anatomical specimens are currently housed in the Laboratory of Medical and Historical Anthropology of the Municipal Establishment "Center of Protection and Research of Archaeological Monuments" of Poltava Regional Council. Archaeologists defined the chronological period.

Sex in adults was diagnosed based on cranial and pelvic morphological features [7]. Adult skeletal age at death was diagnosed based on pubic symphysis morphology, the auricular surface of the ileum, and the sternal end of the ribs [14].

Teeth and jaws were examined macroscopically under bright light; dental changes were evaluated with the help of a dental probe; only the presence of a cavity was estimated as caries.

The method of extrapolating various individual and combined features in dental tissues and anatomical materials with dense structures, such as jaws, skull, and skeletal bones, was applied. An attempt was made to combine all necessary criteria required by paleopathology for dental collections while adhering to all rules and requirements for classifying anthropological, fossil, and subfossil material. Markers of artificial and natural origin were taken into account during the analysis.

RESULTS

Age at death was diagnosed from 6 months to 70 years. Seventeen skeletons were examined which belong to the Copper Age population (the average lifespan of the selected group was 16.5 years). There were eight children, three women and six men among them. The anatomical material was well-preserved. The analysis included all sections of the skeleton and corresponding teeth based on age. However, the bones had an unusual, unnatural dark gray color.

102 skeletons were examined, which belong to the Bronze Age population (the average lifespan of the sample was 21.5 years). Among them were 30 juvenile skeletons, 20 female skeletons, and 52 male skeletons. One individual was represented solely by a single tooth with an artificial pattern/defect applied to it. Pathology and mineralized deposits on the teeth were documented.

223 skeletons (40 children, 79 women, 104 men) were evaluated from the population of the Early Iron Age (the average lifespan of this population was 25 years). Among them, ten individuals were represented solely by a single tooth (meaning only one anatomical specimen, a tooth, was available for the analysis) and exhibited pathologies and defects that developed during their lifetime.

We studied 157 skeletal remains (39 children, 39 women, 79 men) belonging to individuals from the medieval period (the average age of the population was 30 years). Our investigations indicate varying preservation of the remains. The majority (154 skeletons) were well-preserved without defects or tissue loss. However, three individuals were represented either by skulls only or by jaws with incomplete teeth (only cranial bones and teeth were preserved). This population exhibited evident pathologies, including dental caries and its complications, as well as mineralized deposits on the teeth, to a greater extent than in the previous periods.

The authors divide the dentoalveolar apparatus of fossil and subfossil skeletons into ten levels for further medical investigation of dental diseases. It should be noted that from a single historical complex, the degree of tooth preservation can vary, along with different forms of pathology:

Level 1: Complete skull (skeleton) with fully preserved (100%) teeth, including intact enamel (teeth are strong, natural in color, some bone and tooth surfaces may be damaged due to traumatic injuries or affected by pathological changes);

Level 2: Complete skull (skeleton) with 2-3 missing or uncollected teeth but well-preserved tooth sockets that allow some conclusions about the condition of teeth during the individual's life. The enamel of the remaining teeth is intact (possibly sensitive), with a natural color, and may exhibit signs of trauma or pathological changes;

Level 3: Upper and lower jaws with the loss or uncollected 7-8 teeth (approximately 2/3 of the total) but well-preserved tooth sockets that provide insights into the condition of teeth during the individual's life. The enamel of the remaining teeth is intact (sensitive), with a natural color, and may show traces of mineralized dental deposits or signs of disease;

Level 4: Upper and lower jaws with the loss or uncollected up to 7-8 teeth but well-preserved tooth sockets that allow conclusions about the condition of the masticatory organs. The enamel may have cracks (peeling) or be lost in certain areas. Color changes may be present on the surviving portions of the teeth, along with possible carious lesions, other pathologies, and mineralized deposits;

Level 5: Upper and lower jaws with the loss of approximately half of the teeth. The enamel of the remaining teeth exhibits cracks (peeling) or is lost in certain areas, with color changes on the surviving portions. Possible carious defects, other pathologies, and mineralized deposits may be observed;

Level 6: Only the upper or lower jaw (or fragments thereof) with the loss of more than half of the teeth. The enamel of the remaining teeth exhibits cracks, is lost in certain areas, and continues to peel off. The teeth may be fractured, composed of fragments, and exhibit modified color. Traumatic injuries and pathological changes are possible;

Level 7: Dentoalveolar apparatus represented by a complete set of teeth with preserved enamel of natural color. Possible carious lesions, other pathologies, and mineralized deposits may be present;

Level 8: Dentoalveolar apparatus represented by teeth where half of the masticatory organs are preserved with intact enamel (sensitive). The possible presence of pathologies and mineralized deposits.

Fig. 1. Tooth with dental calculus.

Fig. 5. Caries on the cementoenamel junction.

Fig. 2. Tooth wear.

Fig. 6. Discoloration of tooth enamel.

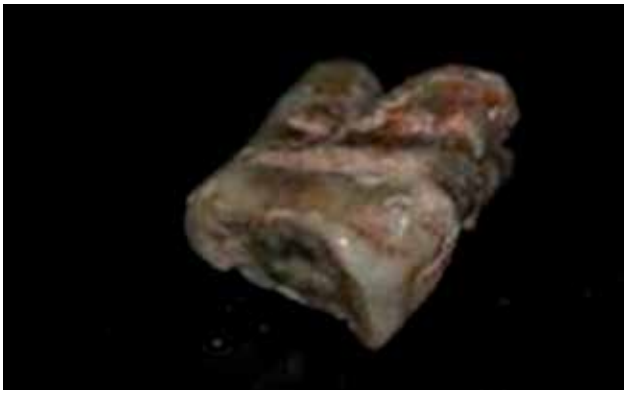


Fig. 3. Tooth with artificial intervention.

Fig. 7. Root caries.

Fig. 4. Fragmentation of tooth root.

Fig. 8. Jaw with several teeth and bone defect caused by chronic periapical inflammation.

Fig. 9. Skull with several teeth and bone defect caused by chronic periapical inflammation.

Level 9: Dentoalveolar apparatus represented by only 7-8 teeth with enamel exhibiting cracks (peeling) or loss in certain areas. There is a possible presence of fractured teeth composed of fragments, color changes, carious lesions, other pathologies, and mineralized deposits.

Level 10: Dentoalveolar apparatus represented by only 1-3 teeth with enamel exhibiting cracks (peeling) or loss in certain areas. There is a possible presence of fractured teeth composed of fragments, changed color, carious lesions, other pathologies, and mineralized deposits.

As a subclass, situations can occur where the tooth color is natural, but there is an enamel loss of various origins. Additionally, cases, where certain features are combined can be considered. For example, in a complete anatomical sample (Class 1), hard tissue preservation may be poor, corrosion, cracks, discoloration, enamel loss, etc. Therefore, we propose to pay attention to the condition of tooth enamel (Fig. 1-7) and bones (Fig. 8-9).

DISCUSSION

The consideration of methodological aspects in paleopathological analysis is an important issue often not discussed in contemporary Ukrainian paleopathology and globally. Primarily, this concerns the topic of representativeness (the case of data quality) of the odontologic series/collections that are subjected to analysis and comparative conclusions.

Unfortunately, there were no classifications for teeth preservation of our ancestors.

There are three criteria proposed for evaluating collected odontologic material [15]: 1) the material originates from entirely or nearly fully excavated historical sites, ensuring that the studied series is not a result of random selection; 2) a complete set of teeth from individuals who possessed them during their lifetime is available; 3) evidence-based sex and age determinations are made for the entire odontologic material.

The dental series that are studied are often of unsatisfactory quality due to the incomplete consideration of deciduous teeth in children (conclusions for the entire population can only be made if these teeth are included). Even a "good" sample of teeth generally lacks pronounced sexual dimorphism in children under 14 (there are no adequate forensic medical methods for obtaining such data). A similar problem exists in individuals who have reached age 40, where involutional (phenomena of reverse development) changes in the bones lead to distortions in age interpretation [16].

Consequently, the following factors should be taken into account: 1) completeness of the material as a percentage of the total number of bones/teeth (losses during life and after death); 2) preservation of different sections and surfaces of a single skeleton, bone or tooth; 3) coloration and consistency of hard tissues; 4) cracks, surface corrosion; 5) weight of anatomical preparations; 6) corrosion of tooth and bone collection specimens by metal compounds in the soil; 7) degree of mineralization of the skeletal remains (important for genetic research); 8) diseases.

Thus, the importance of the degree of tooth preservation in ancestral populations is underestimated in anthropology and forensic medicine, and developed classifications may be helpful for specialists.

CONCLUSIONS

The study of bone and tooth preservation, determination of skeletal sex, and age characteristics is a separate section and stage in reconstructing the physical type of individuals and the health status of a community. Studying the taphonomic features of odontologic anthropological material should be a mandatory prerequisite preceding its analysis in paleopathology. The condition of the dentoalveolar system can be classified into ten levels for paleopathological conclusions (with the possibility of further subclassification of anatomical specimens). The presented classification can also help identify individuals or determine the number of individuals in mass burials.

REFERENCES

1. Tomczyk J, Myszka A, Lewandowska J et al. Assessment of dental caries among children and adults inhabiting Starorypin (11th–12th c.) compared to other Early Medieval populations from Poland. Anthropological Review. 2023;86(2):39-50. doi:10.18778/1898-6773.86.2.04.
2. Kufterin VV, Sataev RM, Dubova NA. O kachestve paleoantropologicheskoy vyiborki i «posmertnom otbore» (po materialam Gonur-depe) [Taphonomic bias in preservation and representativeness of skeletal samples (a case of Gonur Depe)]. Vestnik arheologii, antropologii i etnografii. 2022;56:148-158. (Russian)
3. Yanko NV, Artemyev AV, Kaskova LF. Dental health indicators of the Chernyakhov population from Shyshaki (Ukraine). Anthropological Review. 2021;84(1):17-28. doi:10.2478/anre-2021-0002.
4. Schultz M. Zur Geschichte der Krankheiten [History of diseases]. Istorychna antropolohiia ta bioarkheolohiia Ukrainy. 2020;2:16-38 (German)
5. Buikstra JE. Ortner's Identification of Pathological Conditions in Human Skeletal Remains. 3rd Edition. Elsevier: Academic Press, 2019, p.858.

6. Mamonova NN, Romanova GP, Haritonov VM. Metodika polevyih arheologicheskih issledovaniy [Methodology of archaeological research in the field]. Leningrad. 1989, p.50-83. (Russian)
7. Buikstra JE, Ubelaker D. Standards for data collection from human skeletal remains, Arkansas. Archaeological Survey Research Series No 44. Arkansas: Fayetteville. 1994, p.224.
8. Bello SM, Thomann A, Signoli M et al. Age and sex bias in the reconstruction of past population structures. American Journal of Physical Anthropology. 2006;129(1):24-43. doi: 10.1002/ajpa.20243.
9. Kondo O, Tashiro M, Miyake Y. Human skeletal remains from Hasankeyf Höyük, a sedentary hunter-gatherer site in southeast Anatolia. Anthropological Science. 2022;130(2):121-34. doi: 10.1537/ase.220122.
10. Vodanović M, Brkić H, Slaus M, Demo Z. The frequency and distribution of caries in the mediaeval population of Bijelo Brdo in Croatia (10th–11th century). Arch Oral Biol. 2005;50:669–80. doi:10.1016/j.archoralbio.2004.11.014.
11. Kaskova LF, Chupryna LF, Artemiev AV. Evoliutsiini osoblyvosti rozvytku zakhvoriuvan na kariies zubiv u liudyny [Evolutional features of caries development in humans]. Lambert Academic Publishing: Tallinn. 2021, p.185. (Ukrainian)
12. Yanko NV, Artemyev AV, Kaskova LF. Frequency of dental caries in children in the Early Iron Age and the Medieval populations from Ukraine. Anthrop Rev. 2017;80(4):415–26. doi: 10.1515/anre-2017-0030.
13. Kas'kova LF, Artem'ev AV, Berezhnaia EE, Amosova LI. Features' dental status population of the territory of Ukraine in different historical epochs. Georgian Med News. 2014;(237):35-40. (Russian)
14. Byers SN. Introduction to forensic Anthropology: A Textbook. London: Pearson. 2005, p.468.
15. Alekseev VP. Paleodemografiya SSSR [Paleodemography of USSR]. Sovetskaya arheologiya. 1972;1:3-21. (Russian)
16. Alekseev VP. Paleodemografiya. Soderzhanie i rezultatyi. Istoricheskaya demografiya: problemyi, suzhdeniya, zadachi [Paleodemography. Content and results. Historical demography: problems, assessment, tasks]. Moskva: Nauka. 1989, pp.63-90. (Russian)

The paper is a fragment from the research and scientific project of the Department of Pediatric Therapeutic Dentistry at Poltava State Medical University, Ukraine, entitled "Improvement of Prevention and Treatment Methods for Dental Diseases in Children Taking into Account the Factors of Their Occurrence." (State registration number 0121U113868). Duration: 2022-2026.

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Nataliia V. Yanko
Poltava state medical university
23 Shevchenko st., 36000 Poltava, Ukraine
e-mail: latned@ukr.net

ORCID AND CONTRIBUTIONSHIP
Lyudmyla Kaskova: 0000-0003-0855-2865 A, C, E, F
Nataliia V. Yanko: 0000-0002-3752-4110 C-F
Andrii Artemyev: 0000-0002-8197-5533 A-F
Olha Andriyanova: 0000-0002-8332-7417 A, E, F

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

RECEIVED: 30.06.2023
ACCEPTED: 11.01.2024