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## CERVICITIS AS A CAUSE OF PRETERM BIRTH IN WOMEN

DOI: 10.36740/WLek202211201

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### ABSTRACT

**The aim:** To evaluate the prevalence of preterm birth and to determine the role of cervicitis as a cause of preterm birth in women in Ukraine.

**Materials and methods:** We conducted a retrospective multicentre cohort study from January 1st, 2019 to December 31st, 2021. This study included pregnant women aged 17–50 years admitted to the labor ward at the 13 hospitals from 10 regions of Ukraine.

**Results:** Of the 8151 participants, the prevalence of preterm birth was 2226 (27.3%, [95% CI 26.8 – 27.8]) whereas 5925 (72.7% [95% CI 72.2–73.2]) delivered at term. Preterm birth associated with cervicitis was 76.3% (4,388/2666). History of cervicitis, maternal age, previous preterm labor or premature birth, and pregnancy with twins, triplets or other multiples were identified as independent risk factors of preterm birth.

**Conclusions:** Preterm birth in Ukraine is widespread, the number of which tends to increase. Infection and inflammation of the cervix seem to play a significant role for preterm birth. Early detection and treatment of cervicitis can reduce the risk of preterm birth. Women who have a history of poor pregnancy outcomes are at greater risk of poor outcomes in following pregnancies. Health providers should be aware of this risk when treating patients with a history of poor pregnancy outcomes.

**KEY WORDS:** Pregnancy; Cervix; Cervicitis, Cervical remodeling; Cervical ripening; Spontaneous preterm birth; Ukraine

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### INTRODUCTION

Pregnancy loss and preterm birth (also known as premature) in women is an important public health care concern in worldwide. Its negative consequences are manifested in many ways in our society, including the monetary costs of its investigation, diagnosis, and treatment as well as the psychosocial stresses it imposes on women.

Each year, around 2.6 million babies are stillborn, 15 million are born preterm (<37 weeks of gestation), and 32 million are born small for gestational age (less than tenth percentile for weight, smaller than usually expected for the relevant pregnancy stage). Being born preterm or small for gestational age can increase the chance of long-term health problems [1]. Preterm labor leading to preterm delivery (<37 weeks' gestation) affects approximately 5–7% of live births in developed countries, but significantly higher in developing countries [2]. More than 60% of preterm births occur in Africa and South Asia. In the lower-income countries, on average, 12% of babies are born too early compared with 9% in higher-income countries. [3].

The term reproductive loss is often associated with miscarriage, which manifests itself as spontaneous abortion during the first trimester of pregnancy before 12 weeks (early miscarriage) or 12 to 24 weeks of gestation

(late miscarriage) in women [4]. Miscarriage can be also classified as embryonic loss (or early miscarriage) when it occurs before 10 gestational weeks and fetal loss (or fetal miscarriage) when it occurs after 10 gestational weeks [5]. According to literature, research suggests that between 10% and 20% of women with a medically confirmed pregnancy will end in miscarriage [6]. Early pregnancy loss in women is a relatively common event, occurring in 15%–25% of pregnancies, and increasing in prevalence with maternal age [7, 8]. In an Australian prospective cohort women the rate of miscarriage varied from 11.3 to 86.5 per 100 live births; overall, miscarriage occurred in 25% of the women in the study [9]. According to the American College of Obstetricians and Gynecologists, risk of miscarriage in women aged 45 years but increases to 80% [10].

Pregnancy loss and preterm birth in women is one of the most common yet under-studied adverse pregnancy outcomes [7]. Preterm birth is now recognized as a syndrome associated with multiple pathologic mechanisms, including infection, vascular disorders, uterine overdistension, breakdown in maternal-fetal tolerance, and cervical disease [11, 12]. Preterm birth is also associated with socioeconomic, lifestyle, and environmental factors [13, 14]. Previous studies have shown the association of

advanced maternal age [7, 8], smoking, drinking alcohol, illegal drug use, domestic violence, physical abuse, and exposure to environmental pollutants [13–15].

## THE AIM

The aim of this study was to evaluate the prevalence of preterm birth and to determine the role of cervicitis as a cause of preterm birth in women in Ukraine.

## MATERIALS AND METHODS

### STUDY DESIGN, SETTINGS AND PARTICIPANTS

We conducted a retrospective multicentre cohort study from January 1st, 2019 to December 31st, 2021. This study included pregnant women aged 17–50 years admitted to the labor ward at the 13 hospitals from 10 regions of Ukraine. We compiled list of the 17 women hospitals. Of these, only 13 hospitals from 10 regions (Lviv, Vinnytsia, Zhytomyr, Kyiv, Sumy, Kherson, Dnipro, Kharkiv, Zaporizhzhia, and Odesa) of Ukraine agreed to take part in our study. The inclusion criteria in this study for participants were as follows: 17–49 years old; married or cohabitational; local residents. The exclusion criteria: not a local resident of the selected regions; cancer; positive serological test for syphilis or other sexually transmitted infections.

### DEFINITION

Preterm birth occurs when a baby is born before the 37th completed week of gestation. Pregnant mothers who delivered between 28 and 36 completed weeks of gestation were classified as preterm birth and whereas pregnant mothers who delivered at 37–42 completed weeks were classified as term birth. The gestational age (GA) in the present

study was defined in weeks as the duration of pregnancy before birth based on menstrual history (date of the first day of the last menstruation), clinical examination, and ultrasonography (measuring the crown-rump length of the fetus during a first-trimester). Although preterm birth has an unknown cause, its etiologic phenotypes are broadly categorized into spontaneous preterm birth (natural onset of labor or preterm premature rupture of membranes) and provider-initiated preterm birth (induction of labor or pre-labor elective cesarean for maternal or fetal indications). Intrauterine growth restriction (IUGR) was defined as the estimated fetal weight less than the 5th centile along with abnormal Doppler velocimetry values (above 95th centile). IUGR was further examined determined by the measurement of the mother's belly from the top of the pubic bone to the top of the uterus (fundal height).

### DATA COLLECTION

Using a simple randomized sampling technique, a total of 8,151 pregnant women who are between 17 and 50 years and had reported for delivery at the labor ward from 2019 to 2021 were selected for this study. This study includes interviews, questionnaires, and examinations medical records. Full text medical records were reviewed for the all women's. A standard data collection form was created to extract demographic and clinical data, and outcome information from routine patient records. Supervision and quality control were conducted throughout the entire study. In this study adopted double entry mode of paper questionnaire data and were analyzed anonymously. Pregnant women who delivered between 28 and 36 completed weeks of gestation were classified as preterm delivery whereas those who delivered after 37–42 completed weeks were described as term. Patients' folders and the hospitals' database were used to obtain all the other information

**Table I.** Trends of preterm birth among 8,151 pregnant women in Ukraine (2019–2021)

Region of Ukraine (n, medical facilities)	Trend of preterm birth						Total (2019-2021) n (%)	95% CI <sup>a</sup>	Trend (2019-2021) <sup>b</sup>
	2019 (n=696)		2020 (n=756)		2021 (n=774)				
	n	%	n	%	n	%			
Northern region (n=2)	130	18.7	144	19.0	148	19.1	422 (19.0)	18.2 – 19.8	↑
South Region (n=3)	146	21.0	157	20.8	149	19.3	452 (20.3)	19.5 – 21.2	↓
Western region (n=2)	131	18.8	146	19.3	142	18.3	419 (18.8)	18.0 – 19.6	↓
Eastern region (n=2)	131	18.8	147	19.4	146	18.9	424 (19.0)	18.2 – 19.8	↑
Central region (n=4)	158	22.7	162	21.4	189	24.4	509 (22.9)	22.0 – 23.8	↑
Total (n=13)	696	25.3	756	27.3	774	29.4	2,226 (27.3)	26.8 – 27.8	↑

<sup>a</sup> CI, Confidence interval

<sup>b</sup> ↑ and ↓ indicate statistically significant increasing and decreasing trends, respectively;

**Table II.** Characteristics of women with preterm birth in Ukraine (2019-2021)

Variable	All pregnant women (n=8151)		Term birth (n=5925)		Preterm birth (n=2226)		P-value
	n	%	n	%	n	%	
Maternal age (years) category							
17-19	1,041	12.8	933	89.6	108	10,4	<0.001
20-22	1,557	19.1	1,308	84.0	249	16,0	
23-25	1,944	23.8	1,593	81.9	351	18.1	
26-28	1,248	15.3	951	76.2	297	23.8	
29-31	681	8.4	405	59.5	276	40.5	
32-34	549	6.7	261	47.5	288	52.5	
35-37	441	5.4	201	45.6	240	54.4	
38-41	261	3.2	117	44.8	144	55.2	
42-44	189	2.3	75	39.7	114	60.3	
45-47	129	1.6	45	34.9	84	65.1	
48-50	111	1.4	36	32.4	75	67.6	
Place of residence							
Urban	4,984	61.1	3,130	52.8	1,139	51.2	0.523
Rural	3,167	38.9	2,795	47.2	1,087	48.8	
Level of education							
Primary	2,439	29.9	784	13.2	378	17.0	0.374
High school	2,241	27.5	941	15.9	287	12.9	
Junior college degree	1,937	23.8	1,327	22.4	460	20.7	
Bachelor's degree and above	1,534	18.8	2,873	48.5	1,101	49.5	
Marital status							
Married	6,978	85.6	5,028	84.9	1,950	87.6	0.347
Unmarried	1,173	14.4	897	15.1	276	12.4	
Occupation status							
Unemployed	1,118	13.7	773	13.0	345	15.5	0.517
Head of enterprises	1,152	14.1	821	13.9	331	14.9	
Professional worker	2,985	36.6	2,229	37.6	756	34.0	
Clerk	303	3,7	245	4.1	58	2.6	
Service worker	1,114	13.7	815	13.8	299	13.4	
Agricultural and related worker	217	2.7	148	2.5	69	3.1	
Operator	107	1.3	85	1.4	22	1.0	
Other	1,155	14.2	809	13.7	346	15.5	
Smoking							
Yes	6,141	75.3	4,455	75.2	1,686	75.7	0.564
No, secondhand smoke	1,877	23.0	1,360	23.0	517	23.2	
No	133	1.6	110	1.9	23	1.0	
Drinking							
Yes	7,206	88.4	5,152	87.0	2,054	92.3	0.0582
No	945	11.6	773	13.0	172	7.7	
BMI (kg/m²)							
Thin	591	7.3	408	6.9	183	8.2	0.311

Normal	5,141	63.1	3,809	64.3	1,332	59.8	
Overweight	1,845	22.6	1,295	21.9	550	24.7	
Obese	574	70.0	413	7.0	161	7.2	
Previous preterm labor or premature birth	1,728	100.0	474	27.4	1,254	72.6	0.001
Pregnancy with twins, triplets or other multiples	945	100.0	322	34.1	623	65.9	0.009
History of Cervicitis	5,748	100.0	1,360	23.7	4,388	76.3	<0.001
Chronic conditions							
high blood pressure	641	7.9	488	8.2	153	6.9	0.537
diabetes	4,975	61.0	3,544	59.8	1,431	64.3	
autoimmune disease	1,952	23.9	1,465	24.7	487	21.9	
depression	583	7.2	428	7.2	155	7.0	
Stressful life events	711	100.0	343	48.2	368	51.8	0.311

BMI, Body Mass Index

**Table III.** Logistic multivariate regression analyses of risk factors for preterm birth among pregnant women in Ukraine (2019–2021)

Characteristics	p-value	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)
<b>Age (years)</b>				
	< 0.001		< 0.001	
17–21		Ref		Ref
22–26	0.003	9.379 (2.165–40.619)	0.011	6.862 (1.557–30.247)
27–31	0.012	6.618(1.549–28.274)	0.031	5.036 (1.163–21.83)
32–36	0.025	5.577 (1.244–25.011)	0.109	3.49 (0.758–16.071)
37–41	0.035	5.50 (1.131–26.752)	0.174	3.096 (0.607–15.797)
42–50	0.269	2.297(0.515–10.249)	0.587	1.523 (0.335–6.943)
<b>History of Cervicitis</b>				
No		Ref		Ref
Yes	< 0.001	3.611 (2.234–5.831)	< 0.001	3.063 (1.819–5.158)
<b>Previous preterm labor or premature birth</b>				
No		Ref		Ref
Yes	< 0.001	5.131 (2.662–9.878)	< 0.001	3.835 (1.908–7.712)
<b>Pregnancy with twins, triplets or other multiples</b>				
No		Ref		Ref
Yes	< 0.009	3.623 (2.231–5.841)	0.001	3.081 (1.816–5.157)
Constant			0.003	0.109

OR, Odd Ratio

needed concerning the study participants clinical and obstetric history. Pregnant women who did not give informed consent, those who were medically unstable and those with the twin pregnancies were excluded.

ETHICS

Ethics approval was given by the Committee on Human Research, Publications, and Ethics at the Shupyk National Healthcare University of Ukraine. Written permissions

were sought from the management of hospitals in which data and information were collected. This study was performed in line with the principles of the Declaration of Helsinki.

STATISTICAL ANALYSIS

The analysis of statistical data was performed using Excel (Microsoft Corp., Redmond, WA, USA). Results are expressed as median (range), mean standard deviation for



continuous variables, and number and corresponding percentage for qualitative variables. Categorical variables were analyzed and expressed as frequencies and proportions. Chi-square test/Fischer's Exact test and the binary logistic regression analysis were employed to test for associations and the strength thereof between the dependent variable (preterm birth) and independent variables. Significance level of the strength of association was determined at  $p\text{-value} < 0.05$ .

## RESULTS

### PREVALENCE OF PRETERM BIRTH

In during study period (2019-2021) we sampled 8,151 pregnant women who were 17–50 years old in 13 hospitals of 10 regions in Ukraine. Of the 8,151 participants, the prevalence of preterm birth was 2,226 (27.3%, [95% CI 26.8 – 27.8]) whereas 5,925 (72.7% [95% CI 72.2-73.2]) delivered at term. This study showed that the situation with preterm birth among pregnant women in Ukraine varies greatly by region (Table I). In general, lower preterm birth percentages were reported by Ukrainian regions in the west while higher percentages were reported in the central region, north, south and east of Ukraine.

Time trend analysis of preterm birth proportions by Ukraine was performed. The results are summarized in the Table I. Considering all hospitals that submitted data both in 2019 and 2021, the overall number of preterm birth reported was lower in 2019 than in 2021. These overall tendencies were not always observed at national level, however all but one hospital (Kyiv city, central region) reported higher numbers of preterm birth among pregnant women in 2021 than in 2019. Looking at preterm birth among pregnant women results in 2021, preterm birth was generally lowest in western part (Lviv city) of Ukraine and highest in eastern part (Kharkiv city).

### PATIENT CHARACTERISTICS AND RISK FACTORS

Table II shows association of preterm birth with socio-demographic and obstetric factors. History of Cervicitis ( $p < 0.001$ ), Maternal age ( $p < 0.001$ ), Previous preterm labor or premature birth ( $p < 0.001$ ), and Pregnancy with twins, triplets or other multiples ( $p < 0.05$ ) was significantly associated with preterm birth. However, this study did not find any significant association between pregnant women's place of residence ( $p = 0.523$ ), marital status ( $p = 0.347$ ), occupational status ( $p = 0.517$ ), smoking ( $p = 0.564$ ), Body Mass Index ( $p = 0.311$ ) and preterm birth. Univariate logistic regression indicated that participants who had drinking were at increased risk of preterm birth. However, these factors were not independent risk factors after adjusting for possible confounders on multivariate logistic regression analysis ( $p > 0.05$ ).

In Table III showed the odds ratio (OR) and 95% confidence interval (CI) for the risk factors associated with preterm birth in logistic multivariate regression analyses.

After adjusting for possible confounders in the multivariate logistic regression analysis, maternal age, history of cervicitis, previous preterm labor or premature birth, and pregnancy with twins, triplets or other multiples were identified as independent risk factors of preterm birth.

## DISCUSSION

This study presents the first estimates data on prevalence of preterm birth in pregnant women and risk factors in Ukraine. Of the 8151 participants, the prevalence of preterm birth was 27.3%, whereas 72.7% delivered at term. In the USA, the preterm delivery rate is 12–13%; in Europe and other developed countries, reported rates are generally 5–9% [16]. However, the rate of preterm birth has increased in many locations, predominantly because of increasing indicated preterm births and preterm delivery of artificially conceived multiple pregnancies. History of cervicitis, maternal age, previous preterm labor or premature birth, and pregnancy with twins, triplets or other multiples were identified as independent risk factors of preterm birth. Preterm birth associated with cervicitis was 76.3%.

Infants are born preterm at less than 37 weeks' gestational age after: (1) spontaneous labour with intact membranes, (2) preterm premature rupture of the membranes (PPROM), and (3) labour induction or caesarean delivery for maternal or fetal indications. Births that follow spontaneous preterm labour and PPROM—together called spontaneous preterm births—are regarded as a syndrome resulting from multiple causes, including infection or inflammation, vascular disease, and uterine overdistension. Risk factors for spontaneous preterm births include a previous preterm birth. A short cervical length and a raised cervical-vaginal fetal fibronectin concentration are the strongest predictors of spontaneous preterm birth [17].

Preterm deliveries are those that occur at less than 37 weeks' gestational age; however, the low-gestational age cutoff, or that used to distinguish preterm birth from spontaneous abortion, varies by location. [17]. It is estimated that 25–40% of PTBs are caused by intrauterine infections [17, 18]. Most intrauterine infections during pregnancy are caused by bacteria ascending from the vagina and the cervix [19]. How the cervix is compromised to cause the ascent of an infectious agent is still not fully understood. Infections during pregnancy may affect a developing fetus. If left untreated, these infections can lead to the death of the mother, fetus, or neonate and other adverse sequelae [19].

Infection and inflammation in the cervix appear to play a role in pregnancy and parturition. Preterm labor occurs when regular contractions result in the opening of your cervix after week 20 and before week 37 of pregnancy. Preterm labor can result in premature birth. According to literature, approximately 40% of cases of spontaneous preterm birth (sPTB) are associated with ascending intrauterine infections [12]. The cervix serves as a physical and immunological gatekeeper, preventing the ascent of microorganisms from the vagina to the amniotic cavity. The cervix undergoes remodeling during pregnancy. It remains

firm and closed from the start until the late third trimester of pregnancy and then dilates and effaces to accommodate the passage of the fetus during delivery. Remodeling proceeds appropriately and timely to maintain the pregnancy until term delivery. However, risk factors, such as acute and chronic infection and local inflammation in the cervix, may compromise cervical integrity and result in premature remodeling, predisposing to sPTB. Previous studies have established bacterial (i.e., chlamydia, gonorrhea, mycoplasma, etc.) and viral infections (i.e., herpesviruses and human papillomaviruses) as risk factors of PTB. However, the exact mechanism leading to PTB is still unknown [12].

Preterm labor can affect any pregnancy. Many factors have been associated with an increased risk of preterm labor. A viable preterm birth is defined as any delivery of a pregnancy at less than 37 completed weeks (< 259 days) and more than 23 completed weeks of gestation. It is a heterogeneous condition where 30–40% of all cases of preterm births are the result of elective delivery for a maternal or a fetal complication. The remaining 60–70% of preterm births occur spontaneously. Preterm birth complicates about 3% of pregnancies before 34 weeks' gestation and between 7 and 12% before 37 weeks' gestation [20].

If women can be identified to be at high risk in early pregnancy, they can be targeted for more intensive antenatal surveillance and prophylactic interventions. When women present with symptoms of threatened preterm labour, if the likelihood of having a spontaneous preterm birth can be determined, interventions can be deployed to prevent or delay birth and to improve subsequent neonatal mortality/morbidity. Several markers have been proposed for the identification of patients at risk for spontaneous preterm delivery, in both patients with threatened preterm labor and asymptomatic ones, with the hope that interventions could prevent preterm delivery. There is now compelling evidence that examination of the cervix with ultrasound is superior to vaginal digital examination [21] and in patients presenting with preterm labor can assist in determining the risk for preterm delivery before 34 weeks. In general, the shorter the cervix, the higher the risk for preterm delivery and vice versa [20, 22]. Transvaginal cervical sonography is a good method to assess the risk of preterm delivery in patients presenting with preterm labor, low-risk asymptomatic patients, and patients at high risk for preterm delivery. It should be also noted that endovaginal sonographic examination of the uterine cervix in women with preterm labor identifies patients at increased risk of intrauterine infections [23].

To protect the health of pregnant women and their offspring, additional research is needed to understand how these intrauterine infections adversely affect pregnancies and/or neonates in order to develop prevention strategies and treatments.

## CONCLUSIONS

Preterm birth in Ukraine is widespread, the number of which tends to increase. Infection and inflammation of the cervix seem to play a significant role for preterm birth. Early detection and treatment of cervicitis can reduce the risk of preterm

birth. Women who have a history of poor pregnancy outcomes are at greater risk of poor outcomes in following pregnancies. Health providers should be aware of this risk when treating patients with a history of poor pregnancy outcomes.

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*The Authors declare no conflict of interest*

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## ORIGINAL ARTICLE

# THE COMPARATIVE ANALYSIS OF LUMBO-PELVIC-HIP COMPLEX CAPACITY EVALUATION IN BODYBUILDERS AND FOOTBALL REFEREES

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## ABSTRACT

**The aim:** A comparative analysis of the efficiency of the lumbo-pelvic-hip complex for bodybuilders and football referees. Defining factors that are related and those that are not related to their professions that can affect their efficiency of the lumbo-pelvic-hip complex.

**Materials and methods:** 35 bodybuilders and 41 football referees were randomly selected in different associations in Łódź. The study was performed using a questionnaire and tests for the SFTR range of motion of hip joints and also a Shober test.

**Results:** It occurs that statistically the extension, flexion, abduction, external and internal rotation were significant with bodybuilders and the extension and internal rotation movement with the referees.

The mean values for the Shober test when moving forward were worse for the test group than for the control group were correct. Alongside bulking within the test group and control group the range of motion in the hip joint has decreased. The biggest statistical significance has been for the flexion and internal rotation.

**Conclusions:** In the group of bodybuilders the restriction appears to be in 5 out of 6 type of movements in the hip joint area: extension, flexion, abduction, internal and external rotation. In the second group, football referees the range of motion forward and internal rotation is restricted. The mobility of the lumbar region in both groups is within the average, but for the bodybuilders at quite low.

The efficiency of lumbo-pelvic-hip complex is determined by duration of training, body mass, type of training and also any pain ailments. Bodybuilders have less efficiency of lumbo-pelvic-hip complex.

**KEY WORDS:** bodybuilders, football referees, lumbo-pelvic-hip complex

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## INTRODUCTION

Bodybuilders and football referees are representatives of antagonistic occupational groups, even though they share passion for sports. Particularly those mentioned above should pay attention to the efficiency of the lumbo-pelvic-hip complex. This means that they must focus on the quality of exercises and the type of training, as well as overall effectiveness in the area of the hip joints, pelvic girdle and the lumbar spine [1, 2].

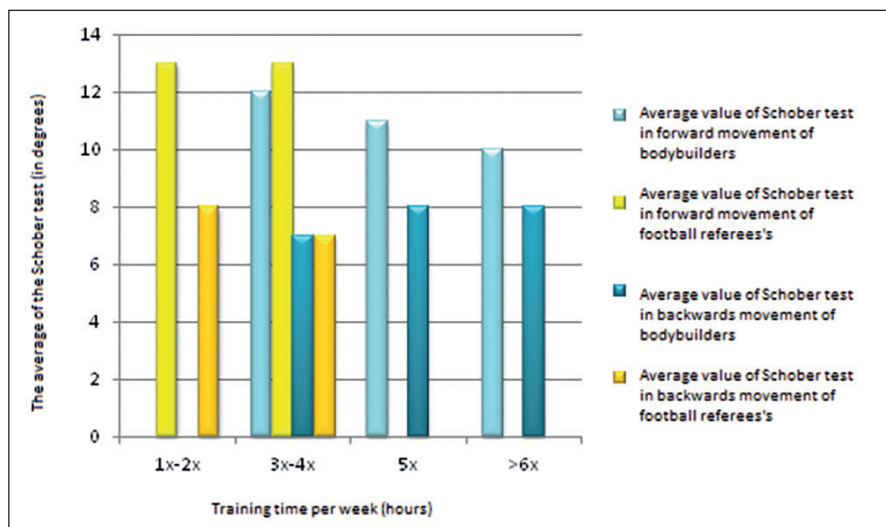
The condition of the complex is mostly determined by the state of muscle efficiency, such as: transverse abdomen, multi-sectional, internal oblique abdomen, pelvic floor, diaphragm [3]. Maintaining a proper condition in these areas is related to the correct relations between muscle length and force vectors, which are developed in more or less dynamic conditions [4, 5]. These conditions mainly apply to the profession of a football referee, where aerobic and endurance training is crucial. In the profession of a bodybuilder, strength training in less dynamic conditions dominates, which does not mean that they are not engaging in other types of training, such as: interval, balance, coordination. Unfortunately, despite an active professional life of a bodybuilder or a football referee, not everyone

knows how to properly perform some exercises to get the desired effect. Incorrect relations between tonic and phasic muscles may lead to disturbance of the arthrokinematic movement and, resulting in overload of the joint or the entire motor complex.

It is worth making an in-depth analysis of how the above professions correspond with each other, and compare their efficiency of the lumbo-pelvic-hip complex. It should also be checked which of the listed ones affect their condition, that is: body weight, type of training, time spent on training, the occurrence of pain ailments [6]. Featured topic is interesting because, among other things, bad habits, poorly chosen training and other causes can be eliminated to improve the quality of functioning of the lumbo-pelvic-hip complex.

## THE AIM

A comparative analysis of the efficiency of the lumbo-pelvic-hip complex for bodybuilders and football referees. Defining factors that are related and those that are not related to their professions that can affect their efficiency of the lumbo-pelvic-hip complex.



**Fig. 1.** The average value of the Schober test result for forward and backward movements depending on the training hours in the week.



**Fig. 2.** The average value of the range of flexion motion in the hip joints depending on the number of training hours per week.

## MATERIALS AND METHODS

The test group consisted of 35 male bodybuilders who were mainly 26-30 years old. The above-mentioned were randomly selected at gyms in Lodz while doing a strength training. The control group consisted of 41 football referees, men at the age of 26-35, who were also randomly selected from the Lodz Football Association. The average age of both groups differed because the subjects included in the study were selected randomly, but it did not affect the study itself.

All of the above mentioned subjects had to complete a questionnaire and were asked to be examined and have functional tests of the lumbar spine done.

The survey consisted of open and closed questions. The form allowed for freedom of expression. The ending was divided into: disjunctive – 1 answer out of more than 2 answers, conjunctive – more than 1 answer from the given propositions, alternative – subject can choose only „yes” or „no” answer. There were also semi-open questions, where in closed answers it was possible to choose „other”, and the respondent could enter any answer.

Measurements related to mobility in the hip joints were made with a goniometer in all aspects according to the SFTR [7].

The Schober test [7] was performed in a sitting position. On a higher level, above the post, a high level L5 process point reaching 10 cm. To help the process, the distance from the previously determined points was estimated at the forward bend at the hyperextension [7].

Statistical analysis was performed with the use of 2 programs, Statistica and Excel. In the dynamic part it will be reproduced because the superior control is turned on, that is: a comparative analysis of the lumbo-pelvic-hip efficiency. For this purpose, the ranges of layered hip supplements and the range of motion of the lumbar spine in bodybuilders and judges with access to education were examined. In the second part, the parts were related to the objectives.

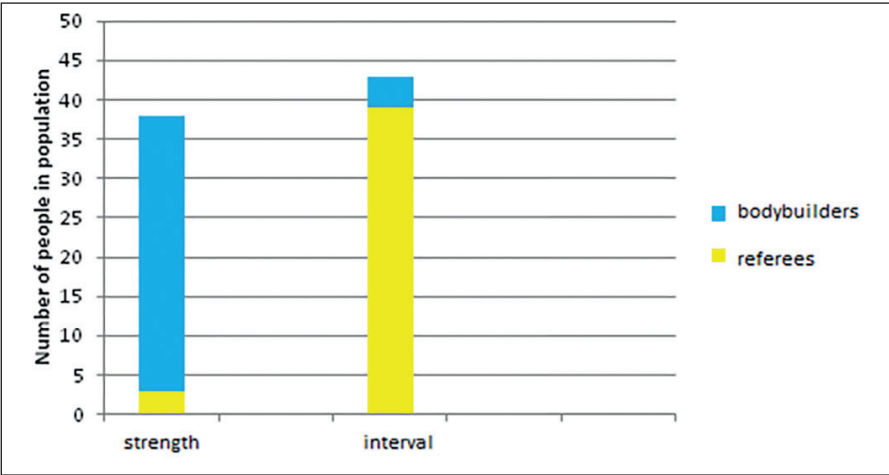
## RESULTS

The analysis of the research shows that in statistically significant movements there was a restriction of mobility in the direction of extension, flexion, abduction, external and internal rotation in bodybuilders. In football referees, the restriction of the range of motion was only visible in the direction of 2 movements – extension and internal rotation, as shown in Table I.

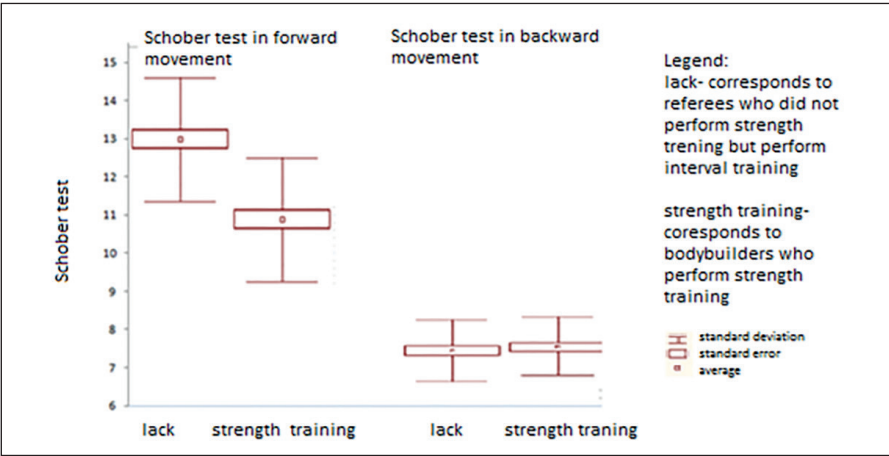
The mean values of the results (presented in Fig. 1) for both populations stayed within norms, which for the

**Table I.** Average values and t-student tests for bodybuilders and football referees compared to the norms of hip mobility.

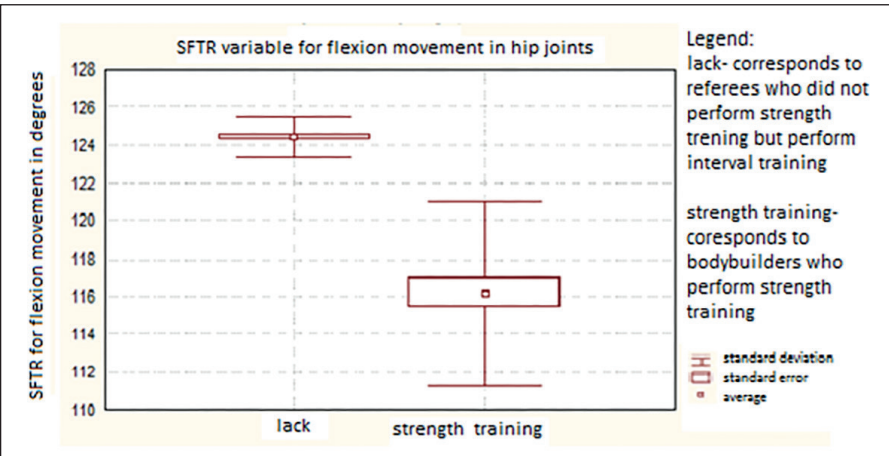
Type of movement	Average values for bodybuilders in SFTR	Average values for football referees in SFTR	SFTR norm	Bodybuilders t-student test values	Football referees t-student test values	Bodybuilders out of norm [%]	Football referees out of norm [%]
Extension	10	10	15	15,64	17,66	97,14	95,12
Flexion	115	125	125	13,03	3,23	100	24,39
Abduction	36	40	40	10,41	2,45	85,71	14,63
Adduction	30	30	30	2,055	0,0000	11,43	0
External rotation	30	35	35	9,108	2,71	80	17,07
Internal rotation	27	25	35	12,99	17,58	62,86	78,05



**Fig. 3.** Relationship between type of training - strength or interval and profession.



**Fig. 4.** Relationship between strength training and Schober test value in forward and backward movement.



**Fig. 5.** Relationship between strength training and flexion movement in hip joints.

**Table II.** Dependence of mass on the norms of ranges of mobility of hip joints.

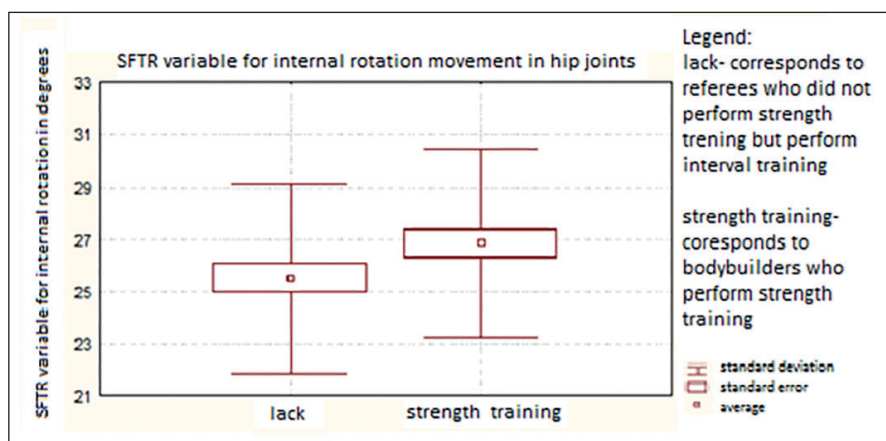
Type of movement	SFTR norm	Bodybuilders out of norm in weight 80 kg-100 kg [%]	Bodybuilders out of norm in weight >100 kg [%]	Referees out of norm in weight <80 kg [%]	Referees out of norm in weight 80kg-100 kg [%]
Extension	15	97,14	100	100	95,12
Flexion	125	100	100	33,3	5
Abduction	40	71,43	100	13	16,7
Adduction	30	8,67	16,7	0	0
External rotation	35	66,67	94,12	5,26	22,73
Internal rotation	35	95,65	100	93,75	95,83

**Table III.** Statistically significant values of ranges of movements in the hip joints and type of training in football referees and bodybuilders.

Type of movement	P level strength training	P level Interval training
Extension	0,687	0,494
Flexion	0,000	0,692
Abduction	1,000	0,954
Adduction	1,000	1,000
External rotation	1,000	0,913
Internal rotation	0,043	0,894

**Table IV.** The occurrence of pain in lumbar spine and hip joints.

	Lumbar spine		Hip joints	
	Football referees	Bodybuilders	Football referees	Bodybuilders
Occurrence of pain	11	27	5	11
No pain	30	8	36	24

**Fig. 6.** Relationship between strength training and internal rotation movement in hip joints.

Schober test for the forward movement were, somewhere between 10 cm and 15 cm but for the backward movement between 7 cm to 9 cm. It was notable that the mean values for the Schober test in forward movement were getting weaker and weaker as the number of hours for the test group increased. For football referees who train up to 4 hours a week, the values of the Schober in forward movement were higher than for bodybuilders and they reached

the value of 13 cm, while in the study group it was up to 10 cm for those who train over 6 hours a week. The average value in the Schober test for forward movement was lower in the test group than in the control group.

When analyzing the results of the Schober test for the backward movement for both groups, the increased in the number of training hours did not affect the achieved result. Based on the data in Figure 1, there was also no significant



difference in the backward Schober score between the two groups. The values achieved for bodybuilders and football referees were similar and oscillated around 7-8 cm.

The effect of the number of hours spent on training in relation to the flexion movement in the hip joints was the only statistically significant factor that was taken into account. As the number of training hours increased, the range of flexion mobility decreased, as shown in Figure 2, in relation to the study group. In bodybuilders training up to 5 times a week, the average value of forward flexion was 115 degrees, while in the group of bodybuilders exercising more than 6 times a week, they fluctuated on average around 116 degrees. Regarding the group of football referees, the values did not change depending on the number of training days, they were correct and averaged at 125 degrees. Figure 2 shows that the bodybuilders have had a reduced range of flexion motion which had an impact on the performance of the lumbo-pelvic-hip complex.

Along with the increase in weight in the test and control groups, the range of motion in the hip joints has decreased. It is very noticeable in the group of bodybuilders, who weight a lot more than the judges, as shown in Table II.

Strength training dominated in the test group, meanwhile the control group was doing an interval training. Additionally, it states that bodybuilders did not prefer interval training, and football referees rarely did strength training, as shown in Figure 3.

Strength training decreased the range of motion forward in the lumbar spine, as there was a significant difference ( $p = 0.000$ ) in the Schober test between the test and control groups. In comparison to the interval training the football referees were doing to the strength training that the bodybuilders were doing, the range of forward flexion motion reached the lower limit of normal, averaging around 10.5 cm, which means that strength training affected the forward mobility of the lumbar spine. Regarding the range of motion of the lumbar spine back, it turned out that the values of the bodybuilding referees and football referees were normal, which means that strength training did not affect the range of motion in the lumbar spine back. The values were at the lower limit of the normal range, as shown in Figure 4.

Taking into account statistically significant ranges of hip joints movements in relation to the type of training listed in Table III, the most important ones were the flexion and internal rotation movements in strength training performed by bodybuilders. Analyzing the control group in terms of the training they performed, it turned out that the type of the dominant training, which is interval training, did not affect the range of mobility in the hip joints.

Strong limitation of mobility occurred in the flexing position for bodybuilders practicing strength training, with practically normal values for the control group. Strength training influenced the range of motion of the hip joints, as shown in Figure 5.

Strength training performed by bodybuilders also restricted the range of motion towards extension. In addition, the extension movement was also limited among football

referees, but it was not related to interval training, as it was not significant (Table III) – perhaps it was due to another factor. The mobility values for internal rotation were much bigger in the group practicing strength training, as shown in Figure 6.

Not all football referees and bodybuilders felt pain in the lumbar spine and hip joints. Ailments in the lumbar region concerned 11 referees and 27 bodybuilders, which means that not all respondents declared the presence of pain in this section. In relation to pain in the hip joints, the number of people struggling with it was much smaller. The number of people in the test group was 11, while in the control group - 5, as it is shown in Table IV.

## DISCUSSION

In the literature of this subject there is not a lot comparative analysis for the profession of bodybuilder and football referee.

In the light of scientific research [8], the topics of the ranges of mobility of the hip joints, the lumbar section and the lumbar-pelvic-hip complex are very often discussed, but none of them refer to the profession of a bodybuilder or a football referee.

This study found that bodybuilders had significantly reduced performance of the lumbo-pelvic-hip complex compared to football referees. Among general bodybuilders there was a restriction in hip joints in 5 out of 6 movements: extension, flexion, abduction, external and internal rotation, while in football referees this movement was limited only in the direction of extension and internal rotation. The range of mobility of the lumbar spine in both groups was normal.

The number of hours spent training was an important factor. Bodybuilders [8] spend much more time on it [8], and often did not have enough time to rest, which is extremely important because muscles require regeneration. Lack of regeneration is also associated with a greater load within the lumbar-pelvic-hip complex.

Another important aspect influencing the efficiency of the above-mentioned complex was body weight. When weight fluctuates the function of the lumbo-pelvic-hip complex weakened. A strong increase in muscle mass in bodybuilders caused an increase in their body weight, which in turn was associated with a load on the spine and reduced mobility in the hip joints. Football referees showed less changes in body weight than the other group.

In athletes, muscle mass should prevail, so it is important to care for and strengthen certain muscle parts [9]. This applies to the local muscles stabilizing the complex, which include: transverse abdominal, multi-divided, and pelvic floor [10]. During any physical activity, the local muscles should be the first to work, causing the muscles to stiffen in the lumbo-pelvic-hip complex, and then, consequently, to support the phase muscles that are responsible for movement in the lower limbs. Some issues arise within the complex when the roles of local muscles are global and are activated first. This may lead to increased muscle tension

and pain, and, consequently, to immobilization and an antalgic posture, and in the future, to exceeding the lower limit of normal in movement [8].

The type of training significantly influenced the efficiency of the lumbo-pelvic-hip complex. For bodybuilders, the majority of training was strength training [9]. In the work of every athlete, including bodybuilder and football referee, training is an important aspect. In their work, both should approach their health condition holistically and perform various types of training, not one of them, e.g. only strength training.

The analysis of mentioned previously research shows that the most common training performed by the test group was a strength training, while among the control group, the most frequent training was interval training. It also proves that football referees rarely do strength training, and bodybuilders did not prefer interval training. The research shows that people who practiced this type of training experienced pain in the lumbar region much more often. It also turned out that the range of motion of the forward lumbar spine was at the lower limit of the norm, and with time it may be limited, which is why correctly selected exercises and the correctness of their performance were also extremely important. The advantage of strength training in bodybuilders also reduced mobility in the hip joints. In turn, football referees were dominated by interval training, which is extremely important in their profession, because motor skills are key for running in competitions [11, 12].

Any physical activity should be performed in a correct and balanced manner. Unfortunately, bodybuilders who are striving to achieve the perfect figure, often forget about stretching exercises and proper movement during training [13]. A significant factor is the dominant force character and work with increasing loads [14]. Football referees perform training in a more technical way, taking care of: quality of movement, endurance, mobility, stretch exercises [13-15]. This is due to their profession, which requires maintaining an appropriate balance, influencing the efficiency within the complex. Properly performed training, both among bodybuilders and football referees, reduces the risk of injuries and prevents muscle wasting.

All of the above-mentioned factors, including: increased number of hours of training, body weight, type of training, the occurrence of pain, affect the efficiency of the lumbo-pelvic-hip complex. It is an interesting topic, but not yet fully researched, hence it was impossible to compare the results of the work with other authors.

Bodybuilders and football referees should pay attention to ergonomics during their work and prophylaxis, because they can prevent unwanted complications in the future and reduce the generation of social costs for outpatient physiotherapy.

It is worth noticing that people performing the above-mentioned professions should in the future benefit from, among others, health resort treatment [16, 17]. Treatments performed there have a positive effect on increasing the range of mobility, eg in the lumbar spine,

and on reducing pain, and above all on obtaining adequate stabilization [17].

In addition, there are few scientific reports related to the above-mentioned professions, so further clinical trials on a larger group of patients seem to be necessary.

## CONCLUSIONS

In the group of bodybuilders the restriction appears to be in 5 out of 6 type of movements in the hip joint area: extension, flexion, abduction, internal and external rotation. In the second group, football referees the range of motion forward and internal rotation is restricted. The mobility of the lumbar region in both groups is within the average, but for the bodybuilders at quite low.

The efficiency of lumbo-pelvic-hip complex is determined by duration of training, body mass, type of training and also any pain ailments.

Bodybuilders have less efficiency of lumbo-pelvic-hip complex.

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## ORIGINAL ARTICLE

# IN VIVO ACTIVATION OF P2Y4 PURINERGIC RECEPTORS USING ATP IN RAT EPIDERMAL TISSUE

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## ABSTRACT

**The aim:** This study was carried out to examine the presence of P2Y4 receptors in rat epidermal tissue and how their in vivo activation leads to histological and genetic changes.**Materials and methods:** Thirty-six Wistar rats were separated into six groups each of six rats, the control group and five injected groups with increasing concentrations of ATP intradermally (0.1, 5.0, 10.0, 50.0, 100.0 µg/ml). The histological and genetic examination was performed from excised tissues.**Results:** Noticeable histological thickening of the epidermal layer in rats injected with high concentrations of ATP. No apparent histological damage was seen in all injected groups. The genetic expression seems to also increase following exposure to variable concentrations of ATP.**Conclusions:** Purinergic receptors activated by ATP molecules are highly involved in the development of adult tissues. Their precise location within the epidermal layer indicated their importance in cellular proliferation and differentiation of epidermal cells. Excessive exposure to ATP results in their robust genetic ectopic over expression indicative of increased cellular activity.**KEY WORD:** epidermis, ATP, P2Y4, intradermal, purinergic signaling

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## INTRODUCTION

Purinergic signaling has been linked to the development of a variety of tissues, including the retina and the ciliary marginal zone [1]. Purinergic receptors are found in a variety of embryonic mammalian organs, indicating that they play an important function in the proliferation of cells [2]. It's also been linked to the regulation and maintenance of adult stem cell populations, particularly those located in the olfactory bulb [3]. In several mammalian tissues, purinergic signaling can promote the expression of Eye field transcription factors EFTFs and enhance the proliferation of progenitor cells [2]. Purinergic receptors are found on the human epidermis, but little is known about how purinergic signaling affects epidermal cells [4]. The complex interplay of intrinsic and extrinsic pathways drives skin development [5]. Cell fate and progenitor cells determination are controlled by extrinsic signaling pathways. Extrinsic signals like neurotransmitters and neurotrophin have a major influence on progenitor cell production and fate [4]. Exocytosis and membrane leakage both release neurotransmitters like gamma-aminobutyric acid GABA and glutamate into the extracellular environment [5]. Glia and neurons in the central nervous system release purines such as ATP. Purinergic signaling, which is mediated by nucleotides like ATP and ADP, has also been demonstrated to have a role in embryogenesis [6]. The scientific focus

is shifting towards in vivo tissue activation and autologous activation of cells. This is to enhance the regenerative capacity of stem cells present in certain tissues of the human body. There is a great hope that autologous activation of stem cells or pluripotent stem cells may be used for tissue replacement therapy. One of the major obstacles facing researchers is the ability to understand the molecular signalling behind cell proliferation and differentiation. A study of canine keratinocytes showed the existence of P2 purinergic receptors. P2 receptors are stimulated by extracellular ATP, which causes a transient rise in intracellular calcium levels. In cultured human keratinocytes, a rise in intracellular calcium is an initial sign in terminal differentiation, which is critical during this process [7]. The fact that ATP activates phosphoinositide, which mobilizes [Ca<sup>2+</sup>], induces thymidine incorporation, and hence inhibits differentiation in human epidermal keratinocyte cultures, provided the first indication for the involvement of ATP in proliferation [8]. ATP generated a bipolar change in membrane potential, transitory depolarization subsequently long-lasting hyperpolarization, in whole-cell recordings from HaCaT cells (an immortalized human keratinocyte cell line) [9]. Extracellular ATP increases HaCaT cell growth by immobilizing [Ca<sup>2+</sup>] via purine receptors [10]. P2Y2 receptors were found in the basal layer of the epidermis, which is the location of cell proliferation,

and in primary cultured human keratinocytes, according to reverse transcription-PCR and in situ hybridization studies. The proliferation markers Ki-67, P2Y1, and P2Y2 receptors were used to identify a proliferating subpopulation of basal and parabasal keratinocytes in adult human skin [10]. Retinoids, which are vitamin A derivatives, are significant modulators of skin cell proliferation and differentiation and have been utilized to cure aging skin. The use of a retinoic receptor agonist increased the expression of mRNA for P2Y2 receptors in basal keratinocytes concerned with epidermal growth [11]. In human and canine keratinocytes, both extracellular ATP and UTP prompt transitory elevations in cytosolic free  $\text{Ca}^{2+}$  [7], in retrospect, it appears that P2Y2 and P2Y4 receptors were implicated. Later, utilizing whole-cell patch-clamp methods, reverse transcription-PCR, and  $\text{Ca}^{2+}$  measurements within cells, many P2X receptor subtypes in cultured human epidermal keratinocytes were identified [12]. In differentiated cells, P2Y2 receptor mRNA was reduced. Human keratinocytes have been demonstrated to respond to protein kinase by reducing cell proliferation and increasing cell differentiation [13]. Khalfa et al. [14] revealed the precise location of purinergic receptor P2Y1 in all selected anatomical sites of all age groups. The precise location is seen in the basal layer of the epidermis indicating the presence of highly mitotic cells. The presence of P2Y1 receptors in the human embryonic epidermis has not been investigated previously due to the sensitivity in using embryonic tissues [14-15]. However, several other research groups have detected their location in stem cell niches as well as highly mitotic cells in other species [16]. As suggested, most purinergic receptors drive the cellular proliferation of epidermal basal cells which agrees with other researchers who have shown that P2Y1 receptors are highly involved in cell proliferation and differentiation of vascular cell proliferation and death [17]. The effect of purinergic signaling on cellular proliferation and differentiation including stem cells is of high importance. This is reflected by the significant increase in the number of basal cells, the number of hair follicles, and the epidermal thickness of the epidermis at all stages of development. Gradual histogenesis changes with epidermal differentiation of epidermal layers can be attributed to the increased immunohistochemical expression of purinergic receptors. It is common to detect purinergic receptors in highly mitotic cells of the epidermis this is due to the increased cellular differentiation and cell turnout. Jia et al. (2009) have demonstrated the importance of purinergic receptors including P2Y1 receptors in the neural development and cell proliferation in embryonic tissues of Swiss Webster mouse olfactory epithelium [2].

## MATERIALS AND METHODS

### ATP PREPARATION

Adenosine triphosphate was purchased from Sigma Aldrich UK and the following concentrations (0.1, 5.0, 10.0, 50.0, 100.0  $\mu\text{g/ml}$ ) were made from stock solution dissolved in phosphate-buffered saline.

## EXPERIMENTAL ANIMALS

Thirty-six male Wistar albino rats weighing between 200-250 g were obtained from the University of Kufa, Iraq, between the periods of May 2021- August 2021. The animals were kept under standard laboratory conditions (13 hr light and 11 hr dark cycles). Standard food and water were available to the animals at all times. The guidelines of the animal research ethical committee were followed, which conformed to the recommendations in the health guide for laboratory animal's care. The rats were separated into six groups with each group comprising of six rats ( $n=6$ ). ATP was injected intradermally daily to the rats for 15 days. Every day, the control group was given 1 mL of phosphate-buffered saline. On the 16<sup>th</sup> day, all the rats were anesthetized and dissected; the skin tissues were excised to assess the effect of ATP on the epidermal thickness and P2Y4 gene expression.

## HISTOLOGICAL PREPARATION AND MEASUREMENT

To carry out a histological analysis, skin injection sites were excised using the surgical blade and were fixed in 4 % formaldehyde for three days. Tissues were processed and embedded in paraffin wax using TP1020 tissue processor (Leica Microsystems, UK). Five-micron sections were cut using a microtome (Leica Microsystems, UK), which were then placed on slides (Leica Microsystems, UK). The sections were deparaffanized in xylol and then rehydrated by using descending concentrations of alcohol before rehydration in distilled water. Haematoxylin and Eosin were used to stain the sections (Leica Microsystems, UK) [18]. Histological investigation of the prepared tissues was carried out by light microscopy with a magnification of 10x. Image J software (NIH, USA) was used to analyse microscopical images to measure the epidermal thickness. The epidermal thickness was measured from the base of the stratum germinativum to the surface of the keratin layer.

## QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION

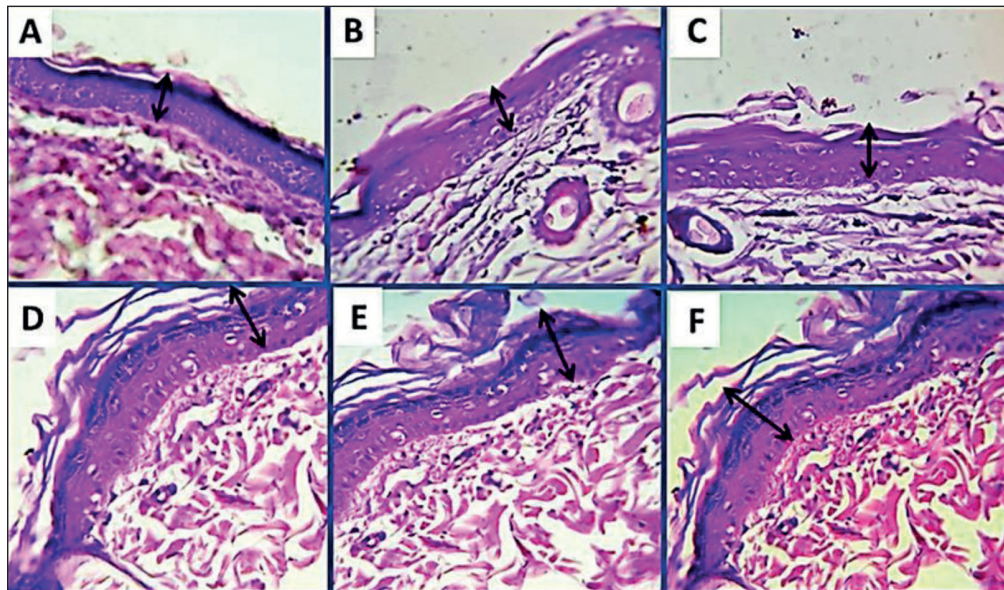
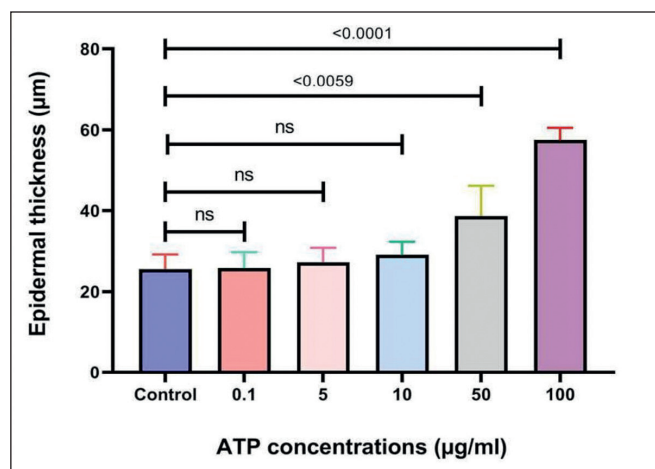
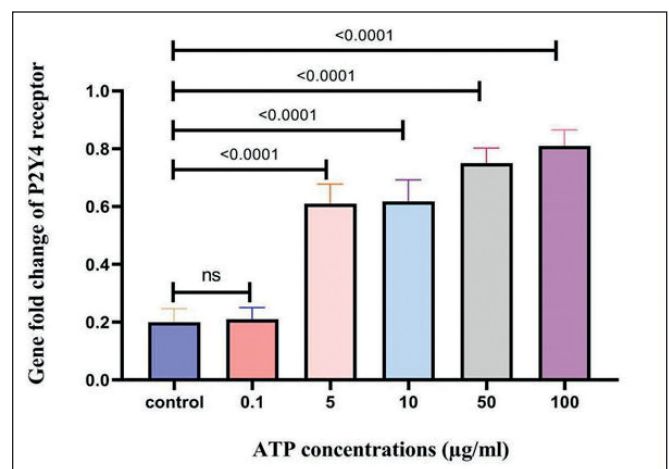
The reaction was achieved using primers for P2Y4 receptors (purchased from IDT). Total RNA Extraction was extracted from excised skin sites using a commercial purification system (abm's Excellent CT Lysis Kit) following manufacturer protocol. The qRT-PCR program used was as shown in table I. Gene fold changes were calculated using  $-2\Delta\Delta\text{CT}$ . Each reaction was repeated 3 times and mean threshold cycle value was worked out as  $-2\Delta\Delta\text{CT}$ .

## STATISTICAL ANALYSIS

The mean and standard deviation (SD) of the epidermal thickness and P2Y4 gene expression are measured. The statistical analysis of the treated groups was compared to the control group. The data were analysed using Graph Pad Prism V9.0 software by an unpaired t-test ( $P$  value  $\leq 0.05$ ).

**Table I.** Primer sequences

Primer	Left	Right	Product length (bp)
P2Y4	Gaagaagcagaacacca	caaggagtctgcactggtca	319

**Fig. 1.** Histological appearance of the skin tissues: Control group (A) is seen with no epidermal changes, intradermal administration of ATP groups show a gradual slightly increasing of the epidermal thickness in (B-D) groups and a significant increase in (E and F) groups. Corneum sloughing off is seen in all injected epidermal tissues, H&E (X10)**Fig. 2.** Histomorphometric analysis of the epidermal thickness between the control group and the groups treated with different ATP concentrations (0.1, 5.0, 10.0, 50.0, 100.0 µg/ml)**Fig. 3.** Gene expression of P2Y4 receptors in the control group and the groups treated with different ATP concentrations (0.1, 5.0, 10.0, 50.0, 100.0 µg/ml)

## RESULTS

### EPIDERMIS- HISTOLOGICAL AND HISTOMORPHOMETRIC ANALYSIS

The histological appearance of epidermal tissue shows no signs of excessive architectural damage or morphological changes in treated and control groups (Fig. 1). Histomorphometric study of the epidermis showed a significant increase in the epidermal thickness with a high concentration of ATP (50.0 and 100 µg/ml) ( $P < 0.05$ ) in injected groups in comparison to the control group (Fig. 2). At a concentration of 100 µg/ml of ATP, the epidermal thickness nearly doubles in size in comparison to the control group.

### P2Y4 GENE EXPRESSION

Gene fold change shows an increase in the genetic expression of P2Y4 receptors in all animals injected intradermally with ATP in comparison to non-injected animals (Fig. 3). Gene fold change was significantly higher in elevated ATP concentrations which indicates a stronger ectopic over expression of P2Y4 receptors in the epidermal tissue

## DISCUSSION

In vivo activation of purinergic receptors has been demonstrated by many researchers' leads to robust histological and cellular changes within many studied tissues at various de-



developmental stages. In this study, we have demonstrated the reactive response of in vivo activation of P2Y4 receptors in rat epidermal tissue. The precise location of purinergic receptor P2Y4 in the human forehead, abdomen, back, and the sole was investigated by Khalfa [11, 14-15]. The precise location is seen in the basal layer of the epidermis indicating the presence of highly mitotic cells. The presence of P2Y4 receptors alongside other purinergic receptors has been under constant observation by many researchers in the hope that they can be utilized to enhance cellular proliferation and differentiation. P2Y4 receptor is responsive to ATP which is one type of uridine nucleotides. Greig et al. (2003) have shown that in human anagen hair follicles, P2Y4 receptors are part of a signaling pathway that controls cell proliferation and differentiation in a variety of cell types. [19]. Intracellular delivery of ATP shows a vital role in wound healing by the activation of macrophages which results in an increased expression of proinflammatory cytokines including IL-1 $\beta$ , IL-6, and TNF, in addition to the secretion of MCP-1 chemokine. After that, purinergic receptor activation, platelet and platelet micro particle production, and ATP-dependent chromatin remodeling enzyme activation take place. The outcome is in vivo proliferation of macrophages, an increase in neovascularization, and, most importantly, the specific production of collagen [20-22]. P2 receptors are stimulated by extracellular ATP, which causes a transient increase in intracellular calcium levels which subsequently lead to increased cellular activity. Ectopic over expression of purinergic receptors has been demonstrated by many studies following exposure to ATP signals [23-25]. Over expression of the P2Y4 gene can be correlated to the histological finding in this study which shows the thickening of the epidermal layer in the animals following exposure to ATP concentrations. In conclusion, purinergic receptor P2Y4 is highly involved in the development of skin tissue. The precise location of this receptor within the epidermal layer indicated its importance in the cellular proliferation and differentiation of epidermal cells. Activation of P2Y4 receptors by ATP molecules could be modulated the movement of cells within the epidermal layers. Excessive exposure to ATP results in its robust genetic ectopic over expression indicative of increased cellular activity.

## CONCLUSIONS

Purinergic receptors activated by ATP molecules are highly involved in the development of adult tissues. Their precise location within the epidermal layer indicated their importance in cellular proliferation and differentiation of epidermal cells. Excessive exposure to ATP results in their robust genetic ectopic over expression indicative of increased cellular activity.

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#### Conflict of interest

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## ORIGINAL ARTICLE

# THE EFFECT OF MEDICATED INTRA-ANAL PACKING ON DECREASING OF ACUTE POST-OPERATIVE URINARY RETENTION INCIDENCE AFTER OPEN HEMORRHOIDECTOMY

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## ABSTRACT

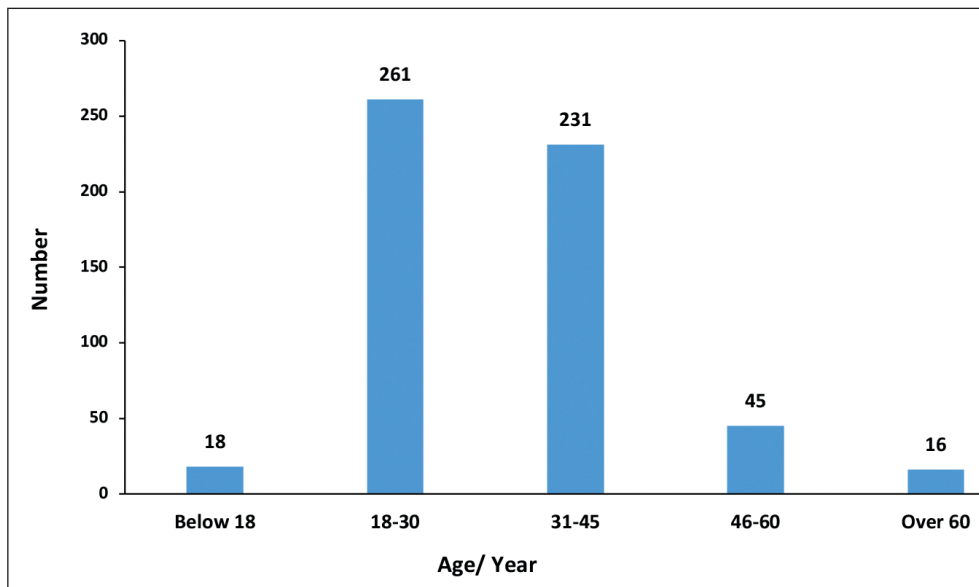
**The aim:** To evaluate the effect of decreasing post-operative anal pain and spasm on the rate of post-surgical acute urinary obstruction in patients undergoing hemorrhoidectomy.**Materials and methods:** The study was conducted from April 2013 to April 2019 on 571 patients that were undergoing conventional hemorrhoidectomy. The operation was followed by using the medicated intra-anal packing and then the patients were monitored postoperatively for the first 24 hours for the incidence of postoperative urinary retention. The hypothesis of this study was that the acute urinary retention is the most common post-operative complication of the anal surgeries and that the irritation-blockade mechanism which is induced due to anal skin injury is the main cause of that obstruction. Thus, reducing the incidence of this mechanism will reduce urinary retention and post-operative pain.**Results and conclusions:** The results found that 569 (99.64%) patients were showed no signs of urinary retention with the exception of only two (0.35%) patients were showed signs of urinary retention. Finally, it was concluded that usage of medicated anal-packing methods was of benefit in decreasing the rate of postoperative urinary retention due to its effect in decreasing postoperative anal pain and spasm.**KEY WORDS:** Hemorrhoidectomy, post-operative pain, medicated packing, post-operative urinary retention

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## INTRODUCTION

Hemorrhoid is the most common surgical affection of the anal cavity and the elective surgical operation is one of the needed treatment methods. The surgical hemorrhoidectomy could not be free from post-operative complication such as inflammation, fistula, bleeding and acute urinary retention. The latter is considered to be the most common possible post-operative complication owing to the irritation blockade mechanism as a result of the anal skin injury and spasm within the surgical field. When these patients are unable to urinate in spite of the fullness of urinary bladder post-operatively, they are considered as with post-operative urinary retention [1]. This is considered the most common post anal surgical complication and they are recorded in different studies; the incidence of this complication was ranged from 3% to 50% [1-6]. The occurrence of this complication is usually more associated with hemorrhoidectomy [34%] when compared to lateral sphincterotomy [4%] and fistulectomy [2%]. It was found that multiple factors are contributed with this condition, some of them are not modifiable such as age, sex, and the technique of surgery [4-7]. The other modifiable factors that contribute with this

condition are spinal or epidural anesthesia, using of opioids and excessive intravenous fluids, which can significantly increase the incidence of postoperative urinary retention [3-6]. Moreover, one of the modifiable factors that was associated with postoperative urinary retention is the effect of early postoperative pain through irritation blockade mechanism of pelvic nerves and reflex triggered pain [5-7]. The irritation – blockade mechanism means that irritation to sensory neurons in an area may cause blockade in the activity of motor neurons in another area if both of neuron groups share the same roots. The pudendal nerves (S2, S3, S4) carry motor nerve fibers to levator ani muscle, muscles of urogenital diaphragm, anal muscles and striated urethral sphincter muscle, as well as carries sensory neurons to the perineum, scrotum and penis. Further, through the same nerve roots (S2, S3, S4), the parasympathetic motor neurons pass to the urinary bladder, which stimulate the detrusor muscle to contract and lead to lesser degree of relaxing in bladder neck and urethra (bladder outflow region). Thus, irritation or injury to one of these nerves possibly leads to an acute urinary retention because all of these branches are originating from the same root.



**Fig. 1.** Success of technique in different group

## THE AIM

On the basis of the above-mentioned information, we aimed to use a medicated intra anal packing after conventional hemorrhoidectomy procedure in order to decrease post-operative pain and, accordingly, the post-operative acute urinary retention.

## MATERIALS AND METHODS

### PATIENTS

In this retrospective study, 571 patients of different ages and sexes were undergoing surgical hemorrhoidectomy from April 2013- April 2019 in Shahid Ahmed Ismael hospital in Sulaimani province/ Iraq. The patients were categorized into five different groups according to their age (Table I and II).

### SURGICAL TECHNIQUE

A conventional hemorrhoidectomy was performed in all the patients, using Milligan – Morgan method and followed by intra-anal packing. The anal packing component comprised a soothing ointment that composed of diclofenac suppository 75 mg plus metronidazole suppository 500 mg, mixed with 20 gm and 5 gm petroleum jelly and lidocaine gel respectively. A two finger width packs were covered with the soothing compound and placed intra-anally directly after obtaining good hemostasis at the operation site. The patients were monitored for the first 24 hours after operation for recording the incidence of acute post-operative urinary retention, and then discharged from the hospital.

## RESULTS

In this retrospective study on 571 patients, using a Milligan – Morgan technique that followed by using intra-anal medicated packing with soothing ointment, it was recorded

that all the cases were treated successfully with exception of only two patients (0.3%), who developed post-operative urinary retention. These two patients were obliged to undergo catheterization for the next 24 hours in order to release the entrapped urine and then released from hospital a day after catheterization.

Interestingly, that different ages and sexes recorded in this study were examined for the success of this technique. The higher incidence of hemorrhoids was found in the second (18-30 years) and third (31-45 years) groups. The lower incidences were found in the other groups including group one, four and five, with age below 18 years, 40-60 years and over 60 years old respectively (Figure 1). This finding indicates that this condition occurred in different age but the incidence could vary for each category. As well as, this confirms that there is no age specificity in using intra-anal packing after hemorrhoidectomy. This technique was successfully performed in different ages without post-operative complications, particularly acute post-surgical urine retention.

## DISCUSSION

Post-operative urinary retention is considered the most common implicated post-operative complication of hemorrhoidectomy, although, there is variation in the incidence of post-operative acute urinary retention in different types of surgeries. It was recorded that this incidence was 3.8% in general surgical population [8], while the incidence of post-operative urinary retention following anorectal surgery ranged between 1% to 52% with an average of 18% [6,9,10]. In a study, it was found that intravenous administration of more than 750 ml fluid during the perioperative period in patients, who underwent hernia or anorectal surgeries, increased the risk of post-operative urinary retention two-three times compared to other surgeries [11], because the excessive infusion of these fluids leading to bladder overdistension, which inhibits detrusor muscle contraction and the normal micturition reflex [12].

**Table I.** Numbers, ages and percentages of the patients who underwent hemorrhoidectomy.

Age group/ Year	Number/ Patient	Percent
Below 18	18	3.15%
18-30	261	45.7%
31-45	231	40.45%
46-60	45	7.88%
Over 60	16	2.8%
Total	571	100%

**Table II.** Number and the percentage of both sexes who underwent hemorrhoidectomy

Gender	Number	Percent
Female	327	57.3%
Male	244	42.7%
Total	571	100%

Moreover, general anesthetic agents can cause bladder atony by interfering with the autonomic nervous system [1]. As well as, anxiety was thought of as a risk factor but the use of anxiolytic agent is not effective in the treatment of this complication [1]. However, the parasympathetic agent such as bethanechol in a dose of 10 mg subcutaneously lowers the incidence of post-operative urinary retention and should be considered as an initial management for this complication [13]. Injuries to the pelvic nerves and pain evoked reflex increase in the tone of internal anal sphincter, which subsequently increases the risk of post-operative urinary retention in patients undergoing anal surgery [2,14,15]. Various methods were used to reduce pain after conventional hemorrhoidectomy, although, different trials were also being in use, such as trials to modify the surgical technique by using diathermy [16], ultrasonic dissection [17], or ligature for the excision of hemorrhoids [18], with using different forms of analgesia and anesthesia [19,20].

Despite all of these technical efforts, the primary causes of pain particularly trauma to the pain sensitive peri-anal skin and the anal epithelium is still not possible to be avoided during excision of hemorrhoids. However, this opinion to reduce the post-operative pain is supported by a study of using stapled hemorrhoidectomy, which was reduced the incidence of post-operative urinary retention [7.8%] than the conventional hemorrhoidectomy [18%] and this is likely due to the absence of a wound in the perianal region [21]. In addition to the trauma to the perianal skin, the spasm of anal sphincter muscles may contribute to the development of post-operative pain, as it was confirmed by a study of using Botulinum Toxin (Botox) injection into the surgical field reduced the post-operative pain [22]. Generally, post-surgical urinary retention is usually treated with catheterization for five days as it is suggested by most researchers [23,10].

However, in this study only two cases suffered from a post-operative urinary retention and they were treated by catheterization only for the first 24 hours and then released from the hospital, and this could refer to the reduction

of pain as a result of using the anal packing technique. Although, catheterization could increase the incidence of urinary tract infection after anal surgery, using five days post-operative catheterization, which ranged from 42% to 60% [24]. Thus, this complication produces discomfort and can lead to prolonged hospital staying, urinary tract infection, urethral injury from catheterization, and increase of the financial cost of surgery.

In this study, we tried to decrease the effect of the irritation – blockade mechanism that originates from the anal nerve injury during the operation. A special ointment was used, which consists of diclofenac (75 mg) and metronidazole (500 mg), mixed with petroleum jelly (20 gm) and lidocaine gel (5 gm), which then inserted intra-anally in a form of pack directly over the site of surgery with the width of two fingers. The idea was that the ointment will decrease pain, inflammatory response, and infection. While the anal pack maintains the sphincter in relatively relaxed state to decrease post-operative spasm as it was stated in the literature [2,14,15]. The result was a significant reduction in the incidence of post-operative urinary retention with the exception of only two patients out of 571, who produced post-operative urine retention.

CONCLUSIONS

There is a logical connection between decreasing of post-operative anal pain and sphincter spasm with less incidence of urinary retention. The use of the medicated packing for anal surgery was associated with less incidence of post-operative urinary retention.

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*The Authors declare no conflict of interest.*

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

## ORIGINAL ARTICLE

# OPTIMIZING DISTRACTION OSTEOSYNTHESIS IN PATIENT WITH THE TIBIAL SEGMENT TISSUE LOSS AFTER FRACTURES

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## ABSTRACT

**The aim:** Improving treatment outcomes in patients with segment tibial bone tissue loss after fractures by improving the ring fixators technology.

**Materials and methods:** The study includes the data of 77 patients. The patients were distributed between two groups: the main and control one, using random numbers table. The inclusion criteria were: patients' consent, their age over 18 years, tissue inflammation episodes, fibular defects 4-5cm in size and disseminated scar lesions. RF was applied with the proposed improvements. Finally, a semi-rigid external fusion system Softcast / Scotchcast was used to prevent deformation of the regenerate and create optimal conditions for fusion of fragments.

**Results:** The following results were obtained for the Modified Functional Evaluation System by Karlstrom-Olerud: positive treatment outcomes in the main group made up 97.1%, and 92.9% in the control one. Though, the specific gravity of good and excellent outcomes in the main group was 82.8%, while in the comparison group – just 45.2%

**Conclusions:** Along with considerable positive advantages of the "Ilizarov's" apparatus, some disadvantages have been noted. Elimination of the drawbacks is stipulated for the practical needs, which requires further scientific study.

**KEY WORDS:** bone defects, tissue loss, fractures

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## INTRODUCTION

Modern surgical methods of substituting bone tissue segment loss after fractures are represented with the surgery with autogenous bone grafts (ABG) of small defects less than 5cm in size; free vascularized fibula graft (FVG); distraction osteogenesis (DO) and the Masquelet technique-induced membrane technique (IMT) [1].

The choice of the huge defect substitution method depends on clinical factors, skills and preferences of the orthopedic surgeon as well as availability of the instruments necessary for successful operation.

The DO with ring fixators(RF) has been used for treatment of the fibular tissue loss after fractures in all the world, as well as the FVG and IMT. The RF fixation is low traumatic, it provides for rigid fixation sufficient for fusion of the bone. The method is characterized by low infection risk, it is economically advantageous and technically feasible even under some financial and technical restrictions [2].

As for the bone tissue defects, the distraction substitution by the distraction osteogenesis (DO), using the Ilizarov bone transport (BT) is a leading choice method. Its positive characteristics show as an opportunity of formation of the high-quality, biologically healthy bone tissue of huge size[3]. These properties make the RF a competitive method nowadays.

The RF disadvantages show as quite common joint contractures, which are mainly caused by the pins pene-

trating muscles and ligaments. Quite often inflammation is observed where the pins penetrate the tissues. Prolonged RF application is related to the "apparatus-bone" poor system accuracy, caused by the external fixation system modifications or osteoporosis manifestations. The distraction osteosynthesis union in contact points is not always observed, which needs additional interventions. Another disadvantage is represented with incomplete osteogenesis due to technical errors, with the regenerated bone deformation after removing the RF [4].

Further studies of the RF treatment technology is a commonly accepted direction, required for improving the pathology treatment methods [5].

## THE AIM

Improving treatment outcomes in patients with segment tibular bone tissue loss after fractures by improving the ring fixators technology.

## MATERIALS AND METHODS

The study includes the data of 77 patients. The patients were distributed between two groups: the main and control one, using random numbers table. The inclusion criteria were: patients' consent, their age over 18 years, tissue

**Table I.** Distribution of patients between the main and control group

Age (years)	Groups							
	Main				Control			
	n	%	gender		n	%	gender	
			m	f			m	f
18-24	12	34.3	11	1	14	33.3	12	2
25-45	11	31.4	9	2	13	30.0	11	2
45-60	9	25.7	8	1	11	26.2	10	1
>65	3	8.6	2	1	4	9.5	4	1
Totally	35	100	30	5	42	100	36	6

**Table II.** Modified Functional Evaluation System by Karlstrom-Olerud

Category characteristics	3 points	2 points	1 point
Pain	-	Mild	Severe
Walking difficulty	-	Moderate	Hard/painful
Walking difficulty on stairs	-	Needs support	Impossible
Difficulties in sport activities	-	Some kinds of sport	Impossible
Restrictions of working activity	-	Moderate	Impossible
Skin condition	Standard	Different colour	Ulcer/fistula
Deformation	---	<7°	> 7°
Muscle atrophy	<1 cm	1-2 cm	> 2 cm
Inappropriate length of legs	<1 cm	1-2 cm	> 2 cm
Loss of motion volume in the knee joint	<10°	10-20°	>20°
Loss of the motion volume in the joint	<10°	10-20°	>20°

**Table III.** Treatment outcomes by Karlstrom scale

Category characteristic (points)	Outcomes by Karlstrom-Olerud				P
	Main group n=35		Control group n=42		
	n	%	n	%	
Unsatisfactory ( 21-23)	1	2.9	3	7.1	p>0.05
Moderate function impairment (24-26)	2	5.7	7	16.7	P<0.05
Satisfactory (27-29)	3	8.6	13	31.0	P=0.05
Good (30-32)	15	42.8	14	33.3	p<0.05
Excellent (33)	14	40.0	5	11.9	p>0.05
Totally	35	100	42	100	

inflammation episodes, fibular defects 4-5cm in size and disseminated scar lesions.

The non-inclusion criteria included any somatic or skeletal disorders which could affect reparative bone tissue regeneration; subsequent postoperative supervision for less than 6 months; malignancies in anamnesis; accompanying chronic decompensated diseases; pregnancy and breastfeeding; severe infectious processes (HIV-infection, tuberculosis, syphilis, progressing viral hepatitis B and C), as well as the patient's refusal.

During the clinical-instrumental studies of both patients' groups the following data were reported: defect presence, its size, deformation, vascular-nerve insufficiency and soft tissues condition. All patients were informed about

approximate duration of the treatment and possible complications before holding the reconstructive surgery, thus agreeing with the treatment by their informed consent. The basic characteristics of the groups are represented in table I.

So, the main group was represented by 35 patients, and the control group – 42. The main group included mainly men – 30 people (85.7%) , from whom those of working age made up 28 (80%). The average age of this group of patients was  $38.09 \pm 2.34$  years. The same characteristics in control group are quite similar: men predominating in the sample – 36 (85.7%), of whom those of working age made up 31 (73.8%); and 6 women (14.3%). The average age of patients in this group was  $37.83 \pm 2.25$  years.

The size of the fibular defect in both groups ranges between 4 and 7 cm, on average  $3.9 \pm 0.9$  cm.



**Fig. 1.** Roentgenological and functional excellent treatment outcome by Karlstrom-Olerud



**Fig. 2.** Roentgenological and functional satisfactory treatment outcome by Karlstrom-Olerud

**Table IV.** Character and incidence of local complications after termination of distraction

Complication type	Main group (n = 35)	Control group (n = 42)	Totally (n = 23)	p
Soft tissue inflammation within the pin region	4	16	20	< 0.001
Ischemic skin necrosis of the operated lesion in the region of union	-	2	2	-
Regenerate deformation	-	2	2	-
Hard mobility in the ankle joint	1	5	6	< 0.02
Post-thrombophlebitis syndrome	2	4	6	> 0.1
Totally	7	29	36	< 0.05

So, no statistically significant differences by basic parameters (age, gender, severity and size of the defect,  $p < 0.05$ ) have been found between the groups.

The control group patients were treated by the traditional method of bilocal intraosseous compression-distraction osteosynthesis after Ilizarov.

The main group patients were treated by the suggested method, with the ring fixation by pins and nail, where first the nail was introduced into the distal part of the tibia, after which the ready apparatus was mounted.

The nail fixation was strengthened by the “rigidity triangle”. The pins were pen-pointed, penetrating the recom-

mended low-functional areas of the lower leg. The electric drill rotation number did not exceed 200, and after 10-15 seconds of drilling, breaks were made. The tension of the pins in the rings was continuously controlled; upon the necessity the pins were re-tightened. Pin exit points were daily treated with antiseptics.

The system accuracy was reached as follows. The Ilizarov pins Ø 2.0 mm were introduced crossing between themselves not only in the frontal, but sagittal surfaces, which provided for the construction accuracy. The crossed pins were not introduced in the upper third part of the lower leg. In the subsystem, providing for the osteotomy



fragment re-location, the pin with additional support was introduced, in order to prevent the fragment sinking back.

In order to obtain the working distraction regenerate, semi-closed tibial osteotomy was performed, which was made from the longitudinal soft tissues incision not more than 2 cm in size, with the following osteotomy of the anterior and lateral cortical walls using the direct small chisel; the posterior cortical wall was intersected using the levator. The oblique bone intersection, absent skel-etization and large area of intersection (ideally, splinter osteotomy) provided for appropriate blood supply and obtaining working regenerate at distraction. The speed of the defect substitution was 1 mm a day. The multimodal anesthesia of perioperative period and the endothelium postoperative protection was provided by pentoxifyllin and hydroethyl-starch solution. Application of low-molecular heparin in the preventive dosage of 2500U and tranexamic acid provided for safe, without considerable perioperative blood loss, microtrombosis prevention. Prescription of Ca and D3 vitamin provided for the required amount and concentration of active Ca in the regenerate area as calcium-triol.

After X-ray examination of the defect substitution, operation was performed, aimed at optimum conditions for the joined splinters union. Using the chisel and small curette, soft tissues were removed from the transverse incision, the bone fragments edges were drilled with a pin. The mixture of hydroxyapatite with the PRF plasma was introduced into the union region using the hemostatic tube. The patients started physical exercises (kinesiotherapy) right after application of the apparatus and continued it throughout all fixation period.

In order to prevent the regenerate deformation right after demounting the apparatus and providing for the complete loading of the lower leg, the individual semi-rigid fixation by Softcast/Scotchcast systems was used. The system allowed to load the extremity and use it during walking before complete regenerate reconstruction and steady consolidation of the united splinters.

So, the nail and pin apparatuses were used, with the offered by the authors modifications, including its pointing, regimens of introduction and adjustment of the pins under various angles in various planes. Inside the ring, which provided for the osteotomised segment bringing down, the pins were adjusted, in order to prevent its overriding. Safe semi-closed osteotomy was not associated with traumas, performed in a fan arrangement with a chisel, from the transverse incision less than 2cm, producing splinter fracture. Surgical intervention in the region of splinters union with plastic elements (treating the edge parts, hydroxyl-apatite-collapan, PRP plasma), early kinesiotherapy and final application of the semi-rigid fixation system Softcast/Scotchcast helped to avoid the regenerate deformation and provide for the optimum conditions for the splinters union.

The use of the suggested method provided for the low traumatic outcomes and quite rigid dynamic fixation without foreign fixators in the regeneration region.

The statistical significance of the data obtained in various groups was confirmed by the Mann-Whitney test. In all cases the bilateral criteria with 5% significance level were used. The assessment of the difference significance of the proportional characteristics was done using the on-parametric criterion  $\chi^2$ .

## RESULTS

The treatment outcomes in both groups were compared by prospective studies with parallel control, with Modified Functional Evaluation System by Karlstrom-Olerud used (Table II).

The number of points within the scale ranges between 21 to 33, where 21-23 values correspond to unsatisfactory outcome, 24-26 – moderate function impairment, 27-29 – satisfactory, 30-32- good functional condition, and 33 points evidence about excellent functional condition.

The generalized treatment outcomes are represented in table III.

The positive treatment outcomes in the main group made up 97.1%, in the control – 92.9%. But in the main group the specific gravity of good and excellent outcomes is 82.8%, while in the control group it is just 45.2%.

The correlation coefficient (CC) equaled 0.976983738, Pearson test for the data analysis regarding the points was 0.98, F-test – 0.7242314. This function determined if two samples had various dispersions. The received P-test exceeds 5%, so the null hypothesis on the dispersion equality cannot be rejected. The real relation between the compared dispersions with 95% confidence is between the 0.19 and 2.75 range.

After terminating the distraction, 36 local complications were observed, 7 in the main group and 29 in the control one. The differences are statistically significant ( $p < 0.05$ ). The structure of local complications is provided in table IV.

The number of complications per patient in the main group was 0.2, in the control one – 0.7. According to the table, most often inflammations were observed in the pin introduction area, which was related to prolonged fixation in the apparatus. The recalled complication type was more often recorded in the control group, with statistically significant differences ( $p < 0.001$ ). Hard mobility in ankle joint was also often recorded in control group, with statistically significant difference ( $p < 0.02$ ). The post-thrombophlebitis syndrome incidence was higher in control group, though no statistically significant differences were found between the groups ( $p > 0.1$ ). No deformations of regenerate and local complications in the union regions were observed in the main group. The patients were offered complete weight loading after the apparatus fixation termination in  $49.8 \pm 13.5$  days in the main group,  $57.4 \pm 11.8$  days in the control one, without statistically significant differences between the groups ( $p > 0.1$ ).

The general treatment terms in the main group made up  $144.8 \pm 19.5$  days, in the control group –  $195.5 \pm 21.6$  days, with statistically significant differences between the groups ( $p < 0.05$ ).

Shorter duration of external fixation in main group provided for decreased local complications incidence, decrease in general treatment terms and improvement of general life quality of the patients during the distraction regenerate modification.

Clinical evidences of the treatment outcomes are represented with the following Fig. 1 and Fig. 2.

## DISCUSSION

Substitution of the bone tissue defects by bone transporting is performed using various constructions with circular or monolateral facilities and the intramedullary pins.

The Ilizarov method is performed using the external fixator, being one of the most common bone reconstruction methods, stipulated for the bone physiological growth. This method provides for appropriate bone tissue distribution within the defect region [3].

The bone regenerate which is formed by this method is considered an ideal graft type, as inside the adjacent soft tissues a vascularized bone callus is formed, which further is re-shaped into the mature bone tissue [6].

The Ilizarov method provides for satisfactory treatment outcomes of the aseptic and infected bone defects, it eliminates mispositions and inappropriate extremities length. The method is relatively safe [7], so it is widely used by the world orthopedists, being considered a golden standard of treatment [8].

The disadvantages of external apparatuses use for the bone transport represent with its prolonged term of application, which needs high emotional patience of the patients (painful sensations, physical loading, treating the construction) [9], absent fusion in the contact region, fractures and deformations of the regenerate, as well as the adjacent joints contractures [10].

A thorough systemic meta-analysis of effectiveness, complications and clinical outcomes of the Ilizarov method for treatment of the tibial defects is provided in papers of Papakostidis et al. [11] and Yin et al. [12]. In these papers, the pathology is most often observed in young people of average age (34.4 years old), with the average bone defect size being 6.01 cm (ranging 3.5–10.7 cm). According to our study, main group included predominantly men 30 (85.7%), of whom 28 people were of working age (80%). The average age of the patients was  $38.09 \pm 2.34$  years. The control group characteristics were almost the same: with men predominating: 36 people (85.7%), and those of the working age – 31 (73.8%). The average age of patients in this group was  $37.83 \pm 2.25$  years.

The tibial defect size in both groups ranges between 4 and 7 cm, on average being  $3.9 \pm 0.9$  cm.

The above-mentioned authors [11, 12] also report that the bone fusion made up 90.2% (range 77–100%); good and excellent outcomes regarding the bones were observed in 87.5% (range 45–100%); good and excellent functional outcomes made up 76% (range 23–97%); the average number of complications per patient was 1.47 (range 0.12–3.35).

According to the authors' studies, no cases of union failure were observed both in the main and control group,

with average complication number per patient 0.2 in main and 0.7 in control group.

So, positive treatment outcomes in main group made up 97.1%, and 92.9% in the control. Though, the specific gravity of good and excellent outcomes in the main group is 82.8%, while in control group it is just 45.2%. The anatomic-functional outcomes in main group with high confidence (>95%) exceeded the control group results.

General treatment terms in the main group made up  $144.8 \pm 19.5$  days on average, while in the control group this figure was  $195.5 \pm 21.6$  days, with statistically significant differences between the groups ( $p < 0.05$ ).

Final use of the Softcast/Scotchcast systems eliminated the necessity of additional operative interventions, which was an advantage of the method.

## CONCLUSIONS

Our study represents the results of treatment of 77 patients with the tibial defects, by using technically improved ring fixators for strengthening the construction contact with the bone; preventing graft deformation; providing for early functional loading by the Softcast/Scotchcast systems and correcting faulty regeneration by drugs. Along with considerable positive characteristics of the Ilizarov method, several disadvantages should be noted. Elimination of the drawbacks is stipulated for the practical needs, which requires further scientific study.

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**Conflict of interest:**

*The Authors declare no conflict of interest.*

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**D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

## ORIGINAL ARTICLE

# EVALUATION OF THE EFFECT OF TOPICALLY APPLIED METHYLSULFONYLMETHANE AND THEIR COMBINATION WITH MINOXIDIL SOLUTION FOR IMPROVEMENT OF HAIR GROWTH IN MALE MICE

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**ABSTRACT****The aim:** The purpose of this research was to find out the effect of Methylsulfonylmethane in minimizing hair loss.**Materials and methods:** Twenty adult Wister Albino mice weighing 25-35g and aged 6-7 weeks were employed. Male mice's coat hairs on the dorsal skin were carefully clipped and then colored. Mice were randomly assigned into four groups, each with five animals: (1) Control group: Treated with D.W. (2), Minoxidil (5%) treated group (3), Methylsulfonylmethane (10%) treated group (4), Methylsulfonylmethane plus Minoxidil treated group.**Results:** We found that the tissue level of 8-isoprostane in the groups receiving medication are considerably lower than in the control (D.W.). We also discovered that the serum tissue vascular endothelial growth factor levels in the groups receiving medication are considerably greater than those in the control (D.W.) groups. On the other hand, we discovered that hair growth, hair follicle expansion and hair follicle number are much higher in the groups receiving medication than in the control groups.**Conclusions:** We concluded that MSM, through its antioxidant and anti-inflammatory properties, dramatically reduces hair loss in male mice.**KEY WORDS:** Methylsulfonylmethane, hair loss, Minoxidil, 8-isoprostanes and VEGF

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**INTRODUCTION**

Hair loss, often known as baldness, is a common dermatological problem that affects both men and women, with men being more impacted. It's characterized by hair shrinking, thinning, and fallout from follicles in many places of the body, including the scalp, and it's caused by a psychological condition known as unfavorable squeal [1]. Hair loss determined by two factors: first, a lack of anagen phase due to an irregular cycle, and rapid transition from anagen to telogen through catagen. The hair follicle has both dermal and epithelial components, allowing for fast remodeling and hair length reduction. The shaft is lost, and the telogen phase is reached by a substantial proportion of hairs [2]. Second, the size of the dermal papilla or hair matrix is decreased, resulting in changes in hair diameter and aspect. Hormonal changes, inherited factors, physical disorders, drug use, stress, and concern are all potential causes of hair loss [3-4]. In general, hair loss is either apparent hairlessness, referred to as alopecia, or hair loss that occurs in greater numbers than normal hair loss, referred to as effluvium [5]. Alopecia, telogen effluvium, male pattern hair loss (MPHL), and female pattern hair loss (FPHL) are all frequent hair loss forms. It's almost as though the head is engaged. Scarring and non-scarring hair loss is two types of alopecia. It's divided into two categories: localized and generalized. Hair loss can be mild or severe, affecting a small region or the entire body.

Scarring or inflammation do not generally cause baldness [6]. Baldness isn't always caused by scarring or inflammation. Alopecia can be caused by hormonal changes or stress. MPHL is mostly caused by hormonal and genetic factors, whereas FPHL is primarily caused by androgenic causes. Telogen effluvium can be caused by either psychological or physical reasons [7]. It is one of the most common causes of hair loss, although it may be brought on by a number of different conditions. Because many instances are asymptomatic and don't require medical attention, it's impossible to quantify the real number of cases [8]. The telogen effluvium is classified into two categories: acute (less than six months) and chronic (six months or more), and it includes excessive hair shedding. Women tend to have a greater frequency of the condition, although this might be due to men under reporting it [9]. Internal and external stresses can also produce acute telogen effluvium, which happens when follicles transition suddenly from anagen to telogen phase [8]. Although the specific effects of age on acute telogen effluvium are unknown, some research has shown that older women are more vulnerable [9], despite the fact that the condition is uncommon in children [10]. Telogen effluvium has shrunk in size and has become very small. In acute telogen effluvium, hair follicle regeneration occurs. Hair density has decreased in the frontal and central scalps as a result of diffuse loss and increased shedding [11]. Non-med-



ical and medicinal treatments were used to address telogen effluvium hair loss. Non-medical therapy, including acute state, is typically unnecessary since telogen effluvium is self-limiting, and hair regrowth returns to normal three to six months after the acute phase ends. This state can also be ended by removing the trigger chemicals. In the treatment of acute telogen effluvium, patient education and understanding about hair loss is crucial. Reduced nutritional supplement or medicine consumption is examples of trigger variables. In most cases, hair regeneration takes little more than a year to reach full density and look [12]. Topical Minoxidil will become more common in chronic telogen effluvium patients. Minoxidil causes artery vasodilatation by opening potassium channels. The dilatation allows blood and nutrients to reach the hair follicle. Finally, it encourages hair growth by boosting the anagen phase and reducing hair loss (telogen phase) [13]. Minoxidil has no function in the treatment of acute conditions and is only used in chronic ones. Nutritional supplements can be used to address mineral and vitamin deficiencies in telogen effluvium. In telogen effluvium, hair transplanting surgery is not required [14-15]. The 8-isoprostane isomer of prostaglandins belongs to the F2 isoprostane family. Because it is produced in numerous bodily fluids as a result of the oxidation of cellular membrane arachidonic acid, it is a reliable and long-lasting biomarker of lipid peroxidation and oxidative stress in a variety of illnesses and disorders [16]. Membrane disruption occurs as a result of lipid peroxidation (arachidonic esters of phospholipid). It is known to be a reliable biomarker of oxidative stress and to play a role in the inflammatory process [17]. The amount of 8-isoprostane was found to be higher in basal cell carcinoma of the skin, but it was reduced after applying vitamin E topically. These findings suggest that 8-isoprostane is involved in the inflammatory and oxidative stress processes. Topical vitamin E's antioxidant properties also assisted in reducing skin oxidative damage [18]. In patients with cutaneous leishmaniasis, the level of 8-isoprostane increased, which was caused by a disruption in the oxidative and antioxidant balance. The elevated 8-isoprostane level was used as a reliable indicator of oxidative stress in this investigation [19]. The signaling protein Vascular endothelial growth factor (VEGF) is involved in both vasculogenesis (the creation of the circulatory system) and angiogenesis (the production of blood vessels) (the growth of blood vessels from pre-existing vasculature). VEGF action is primarily restricted to cells of the vascular endothelium, while it does have effects on a few other cell types (e.g., monocyte/macrophage migration stimulation). Endothelial cell mitogenesis and cell migration have been demonstrated to be stimulated by VEGF in vitro. VEGF is also known as vascular permeability factor because of its potential to increase microvascular permeability [20]. Several growth factors, including vascular endothelial growth factor (VEGF), epithelial growth factor (EGF), insulin-like growth factor (IGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF), regulate the hair growth cycle. Hair loss occurs when these growth factors are dysregulated [21-23]. The basic role of VEGF in cutaneous development

as a major regulator of healthy and pathological angiogenesis. The main functional effects of VEGF were mediated by VEGF receptor-2, which is the major receptor for VEGF [24]. Methylsulfonylmethane (MSM) is an organic sulphur-containing substance that is water soluble and extremely stable [25]. It's found in trace amounts in a wide range of fruits, vegetables, grains, meat, eggs, and fish, and it's consumed in trace amounts by those who follow a healthy diet [26]. MSM is detected in the circulation of adult males at amounts of about 0.2 mg/kg [27], likely generated from food sources, as well as endogenous and bacterial metabolism [28]. MSM has long been used as a dietary supplement to treat or prevent osteoarthritis (often in conjunction with glucosamine and chondroitin). Clinical studies have shown that MSM can help with interstitial cystitis, parasites, constipation, musculoskeletal pain, and allergies [29-30]. It has anti-inflammatory and antioxidant properties, according to research [31-32]. MSM has been found to be essentially nontoxic to humans in a number of studies [33]. The full mechanistic function of MSM may involve a variety of cell types due to its improved ability to cross membranes and permeate throughout the body, making it difficult to decipher. MSM appears to act at the transcriptional and subcellular level in the crosstalk of inflammation and oxidative stress, according to results from in vitro and in vivo studies. As anti-inflammatory action, MSM inhibits nuclear factor kappa-light-chain-enhancer of activated B-cells (NF- $\kappa$ B) transcriptional activity in vitro [34] by preventing the NF- $\kappa$ B inhibitor from being degraded while preventing its translocation into the nucleus [31]. The NF- $\kappa$ B pathway is traditionally thought to be a pro-inflammatory signaling pathway that causes the upregulation of genes that code for cytokines, chemokines, and adhesion molecules [35]. In vitro, MSM inhibits NF- $\kappa$ B, resulting in downregulation of mRNA for interleukin (IL)-1, IL-6, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) [36]. Through suppression of NF- $\kappa$ B, MSM can also reduce the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) and so reduce the generation of vasodilating substances such nitric oxide (NO) and prostanoids [31]. Because NO affects both vascular tone and mast cell activation, MSM may have an indirect anti-inflammatory effect on mast cells. Flux and recruitment of immune cells to areas of local inflammation are prevented by the decrease of cytokines and vasodilating substances [37-38].

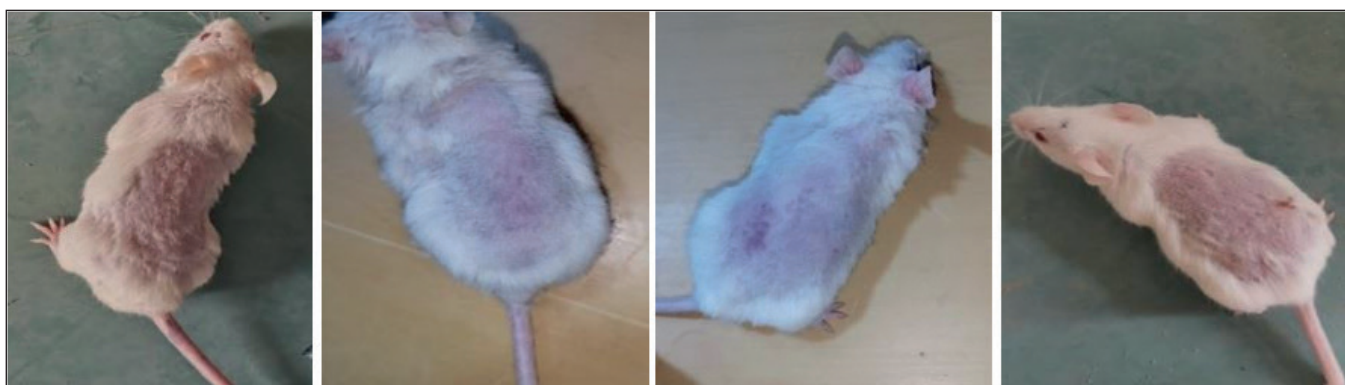
## THE AIM

The purpose of this research was to find out the effect of Methylsulfonylmethane in minimizing of hair loss.

## MATERIALS AND METHODS

### THE STUDY'S LOCATION AND ETHICAL CONSIDERATIONS

The research was carried out at the University of Kufa's Faculty of Medicine's Department of Pharmacology and Therapeutics, as well as the Middle Euphrates Unit for Can-



**Fig. 1.** Mice with dorsal coat hair clipped.



**Fig. 2.** Mice after staining of the clipped area.

cer Research. The research was approved by the University of Kufa's Bioethics Committee and its representation in the Faculty of Medicine. The Committee's recommendations were followed throughout the entire operation.

### ANIMAL GROUPING

Twenty adult Wister Albino mice, ranging in age from 6-7 weeks to 25-35 g, were utilized in this research. For 14 days prior to the treatments, the animals were kept in the animal house of the Faculty of Science/University of Kufa, where they were kept at a temperature of 20-25°C and a humidity of 60-65 percent, with a fitted 12 hour light and 12 hour dark cycle. Additionally, the mice had unrestricted access to food and water. Before the experiment, the hair mice surrounding the dorsal area were removed in one day using diethyl ether as an anesthetic (inhalation route). Hair shaving of mice in the dorsal area was avoided by employing an electrical equipment to shave the desired location softly and without causing harm. The surface layer of the skin in this area appears pink after cutting for hair with an electric shaver and removing approximately all hair. After that, each group was photographed with a digital camera after the shaving was completed (Fig. 1)

After clipping, the dorsal area of skin mice turned pink and hairless. The dorsal area of mice is stained with a commercial dye. After that, the area was washed with alcohol. The benefit of staining the dorsal part in this study is that it allows to distinguish between regrowth and denuded areas, as well as determine the ratio of white(regrowth area)

to black color(area with no hair) [39]. After staining each mouse in all groups, photographs were taken, as shown in figure 2.

The mice were subdivided into four equal groups, each with five mice, as follows:

**Group 1:** Considered as a control group, they were given 0.5 ml of D.W. vehicle solution applied to the denuded skin with a pipette once a day for three weeks. After 3 weeks of treatment, skin tissue was taken.

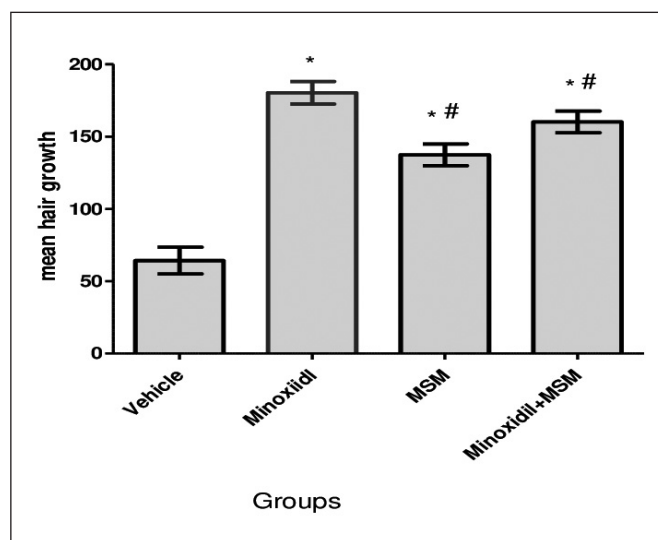
**Group 2:** Treated with Minoxidil solution 5%, 0.5 ml was applied to the denuded skin by a pipette once daily for three weeks. After 3 weeks of treatment, skin tissue was taken.

**Group 3:** Treated with MSM solution 10%, 0.5 ml was applied to the denuded skin by a pipette once a day for three weeks. After 3 weeks of treatment, skin tissue was taken.

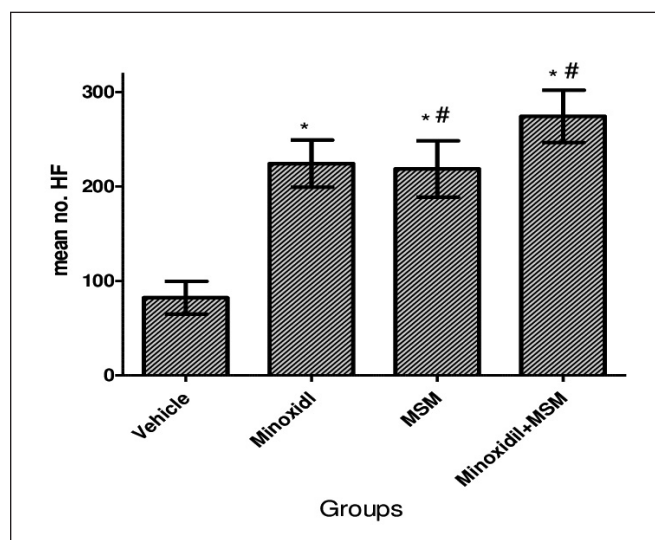
**Group 4:** Treated with equal amounts of MSM solution 10% + Minoxidil solution 5%, applied 0.5 ml of the mixed solutions once a day for three weeks. After 3 weeks of treatment, skin tissue was taken.

### ANALYZING PHOTOGRAPHIC DATA

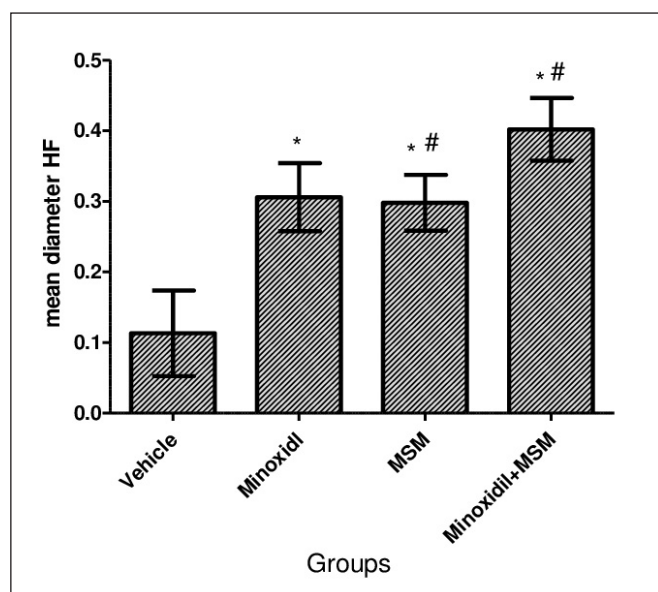
The data analysis program used in this study was Matlab 2015. This program was created specifically for the purpose of examining photographic data. In the dorsal region, Matlab software was used to calculate the ratio between the white and black sections, which represent hair growth and hair nakedness, respectively.



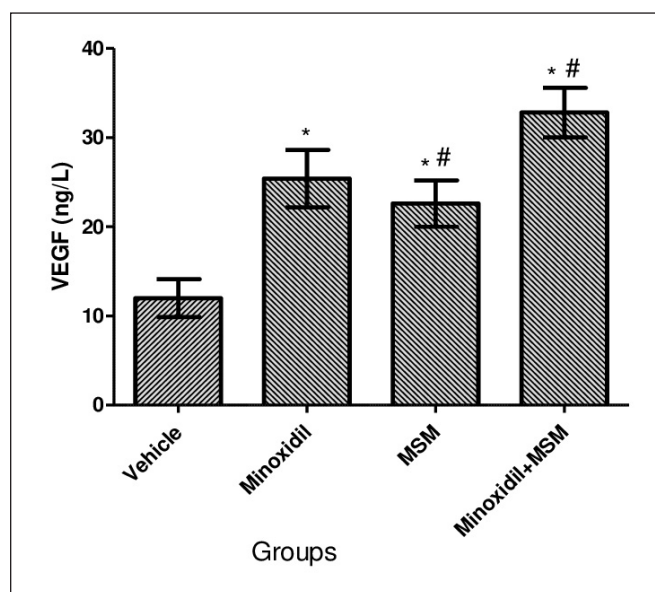
**Fig. 3.** Mean±SD, \*P-value <0.05 vs. D.W. Group, # P-value <0.05 vs. Minoxidil group.



**Fig. 4.** Mean±SD, \*P-value <0.05 vs. D.W. Group, # P-value <0.05 vs. Minoxidil group.



**Fig. 5.** Mean±SD, \*P-value <0.05 vs. control group, # P-value <0.05 vs. Minoxidil group.



**Fig. 6.** Mean±SD, \*P-value <0.05 vs. D.W. Group, # P-value <0.05 vs. Minoxidil group.

## HISTOLOGICAL SECTIONS

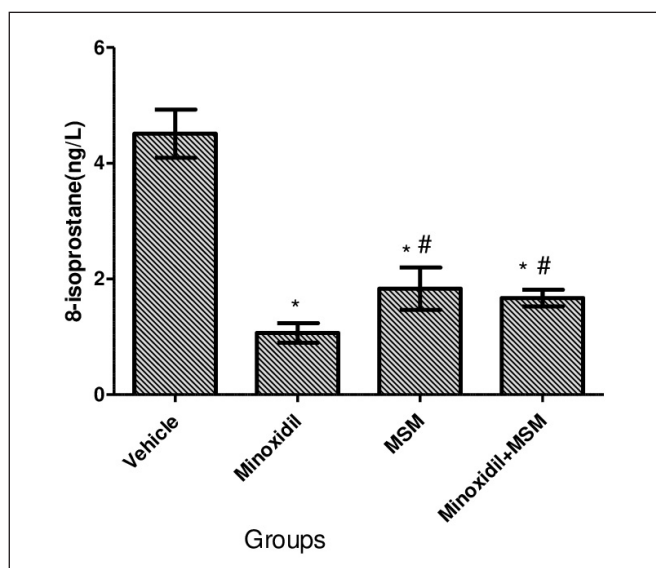
At the end of the study, histological sections were taken from samples. This treatment period lasted 21 days. To collect the samples, firstly the dorsal area of the mice had to be free of hair, which was achieved by shaving the hair. Secondly, we excised a piece of dorsal skin, flattened it, and immediately immersed it in 10% buffered formalin [40]. In both the control and treatment groups, the number and diameter of hair follicles were counted under a microscope.

## TISSUE PREPARATION FOR VEGF AND 8-ISOPROSTANE MEASUREMENTS BY ELISA KIT

After three weeks had passed since the trial ended, the skin tissues were prepared for homogenization. Skin samples were taken from the dorsal region of mice through excision,

fixation in cold phosphate buffer solution (PBS), and storage at -80°C. The samples were then taken out of the deep freeze and let to defrost. An applicator with specific processing called a high intensity ultrasonic liquid processor was used to homogenize skin tissues. The working principle was include – firstly, prepare 1 mL of standard homogenization solution (phosphate buffer, 1% triton X, and 1% protease inhibitor cocktail solution) and 100 mg of skin tissue by weighing tissue in a 1:10 (w/v) ratio. Secondly, tissue was ground in an ice bath with a mortar and pestle, and then sonicated for about 20 seconds (preferably two strokes, each stroke 10 second). Thirdly, the tissues were chilled for 30 minutes before being centrifuged for the supernatant (at 4°C for 15 minutes at 14000rpm). Finally, the supernatant was collected and the cloudy area was avoided in order to use the Elisa kit to determine VEGF and 8-isoprostane [41]





**Fig. 7.** Data are presented as Mean $\pm$ SD, \*P-value <0.05 vs. D.W. Group, # P-value <0.05 vs. Minoxidil group.

## STATISTICAL ANALYSIS

All data were presented as mean $\pm$ SD and they were tested for normal distribution using normality test (Shapiro test), one way ANOVA with post hoc test was used to test any significant difference between groups at a significance level of 0.05. Data analysis and figures were performed using Graph Pad Prism version 8.0.0 (San Diego, California USA).

## RESULTS

### EFFECT OF MSM ON HAIR GROWTH BY MATLAB PROGRAM

When compared to the control group, the groups treated with MSM, Minoxidil, and MSM + Minoxidil had significantly more hair growth, P-value < 0.05 (Fig. 3).

At the conclusion of the study, the mean hair growth ratio of four experimental groups (number of animals = five in each group) was calculated using Matlab software.

### HISTOLOGICAL EVALUATION OF THE EFFECTS OF MSM AND ITS COMBINATION WITH MINOXIDIL ON THE NUMBER AND DIAMETER OF HAIR FOLLICLES

When compared to the control group, the groups treated with MSM, Minoxidil, and MSM + Minoxidil had significantly more hair follicle numbers. P-value < 0.05 (Fig. 4).

The mean number of hair follicles was determined by histological examination of four experimental groups at the end of study (each with five animals). In addition, all treatment groups showed an increase in hair follicle diameter, P-value < 0.05 (Fig. 5).

Mean the diameter of hair follicles (in micrometer) by histological examination of four experimental groups at the end of the study (number of animals = 5 in each group).

Effect of MSM and their combination with Minoxidil of VEGF in hair follicles by ELISA

We found that the amount of VEGF in skin tissue in treatment groups (Minoxidil, MSM, MSM + Minoxidil) was considerably ( $p < 0.05$ ) greater than in control groups (Fig. 6).

Mean VEGF (in nanogram) of hair follicles measured by ELISA technique at the end of the study in four experimental groups (number of animals = 5 in each group).

### MSM REDUCED THE LEVEL OF LIPID PEROXIDATION IN SKIN TISSUE (8-ISOPRASTANE)

We found that the skin tissue level of 8-isoprostane in treatment groups (Minoxidil, MSM, MSM + Minoxidil) was considerably ( $p < 0.05$ ) lower than in control groups, figure 7.

Mean 8-isoprostane (in nanogram) of hair follicles measured by ELISA technique at the end of the research (number of animals = 5 in each group)

## DISCUSSION

Hair loss, often known as baldness, is characterized by the loss of hair in any part of the head or body, but particularly in the frontal area. Hair loss can range in severity from a few strands to the full body. Hair loss can be temporary or permanent. Some persons have hair loss as a result of psychological concerns. Hair loss is viewed as a cosmetic concern, particularly in women, and it is regarded as a difficult problem. As a result, more study on hair loss is needed in order to develop more conventional therapies to control this problem throughout the world, as present regimes have undesired side effects. MSM has both anti-inflammatory and antioxidant properties. Furthermore, MSM is rich in sulfur; this might help strengthen the connections between keratin molecules in the hair by “donating sulfur to keratin”. So, in our thesis, we tested the effect of MSM on hair growth versus control hair loss.

### EFFECT OF MSM ON PARAMETERS STUDY

#### EFFECT OF MSM ON HAIR GROWTH

After 21 days of treatment, the MSM treated group showed a significant improvement in hair growth when compared to the control group, according to the experimental study. This improvement, however, was less than that of the Minoxidil group. The findings of this study matched those of Alboreadi et al., (2021), who found that applying MSM topically in mice mixed with kombucha ferments resulted in significant increases in hair growth [42]. Shanmugam et al., (2009), found that applying MSM mixed with magnesium ascorbyl phosphate (MAP) topically in male mice for 21 days promoted hair development. MSM might be employed as a penetration enhancer that augments hair growth promotion of MAP in vivo through increased dermal accumulation of MAP as established by an in

vitro skin investigation, according to the authors. Both in vivo and in vitro, the penetration enhancing action of MSM was shown to be dose-dependent. Overall, topical MAP combined with MSM appears to be effective for the treatment of alopecia [43].

### **EFFECT OF MSM ON NUMBER AND DIAMETER OF HAIR FOLLICLE**

When compared to control groups, MSM treatment resulted in a significant increase in the number of hair follicles, but not when compared to the Minoxidil group after 21 days. This study's findings matched those of Alboreadi et al., (2021), who discovered that applying MSM topically in mice mixed with kombucha ferments resulted in significant increases in the number and diameter of hair follicles when compared to other groups [42].

### **EFFECT OF MSM ON VEGF IN HAIR GROWTH**

The current study found a significant increase in the level of VEGF in the MSM treated group when compared to the control group. The increase in VEGF level in Minoxidil, on the other hand, was thought to be more significant. Although the findings of this study differed from those of Lim et al., who found that MSM can inhibit the STAT3/VEGF pathway, reducing the formation of solid tumors in a human breast cancer cell line. STAT3 plays a role in tumor [44]. Growth by causing angiogenic factors like VEGF to be produced [45] such as Src and EGF receptor (EGFR).

### **EFFECT OF MSM ON 8-ISOPROSTANE MARKER IN HAIR FOLLICLE**

After 21 days of therapy, the level of 8-isoprostane in the MSM treated group was significantly lower than in the control group. The decrease in 8-isoprostane levels in the Minoxidil group, on the other hand, was thought to be more significant. The findings of this study corroborated those of Amirshahrokhi and Bohlooli, who found that MSM reduced PQ-induced toxicity due to its antioxidant and anti-inflammatory properties, as seen by a decrease in MDA levels. MDA is one of the most widely utilized indicators of the lipid peroxidation process in cells, and because 8-isoprostane is also a lipid peroxidation marker, MSM may be able to lower its levels [46].

### **EFFECT OF MSM PLUS MINOXIDIL ON PARAMETERS STUDY**

The current study found that combination groups of MSM and Minoxidil produce a significant increase in hair growth, enlargement of hair follicles, raising the levels of VEGF marker, and reduction of the level of 8-isoprostane after three weeks of therapy when compared to control and Minoxidil groups. However, when compared to the Minoxidil group alone, this combination produced insignificant increase in the number of hair follicles. When compared

to the distilled water group, this combination leads in a significant increase in hair count. There are no researches on combination therapy that are instructive (MSM plus Minoxidil). Our research was the first to show that this combination can promote hair growth. The pleiotropic effect of these medications on hair follicles could be one explanation for the action of the combination drugs MSM and Minoxidil.

## **CONCLUSIONS**

We concluded that MSM, through its antioxidant and anti-inflammatory properties, dramatically reduces hair loss in male mice.

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The Authors declare no conflict of interest.

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**D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

## ORIGINAL ARTICLE

# VARIATIONS OF THE STRUCTURE, TOPOGRAPHY, BLOOD SUPPLY AND INNERVATION OF THE BRACHIORADIALIS IN HUMAN FETUS

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## ABSTRACT

**The aim** of the research was to establish the features of age-related and individual anatomical variability of the brachioradialis, its blood supply, and innervation options.

**Materials and methods:** The study of the variant anatomy of the brachioradialis and its vascular and nervous structures was carried out on 25 preparations of human fetuses of 4-7 months, 81.0-270.0 mm parietal-coccygeal length (PCL) using macromicroscopic preparation, injection vessels, and morphometry.

**Results:** Spindle-like (56%) and round (24%) shapes of the brachioradialis were found in most of the studied fetuses; its elongated flat (12%) and triangular (8%) forms occur less often. In a fetus of 185.0 mm TKD, the right brachioradialis consisted of two separate parts: upper and lower, which were connected at the level of the middle of the forearm into a common short muscle belly. In another human fetus of 220.0 mm TKD, the right brachioradialis also consisted of two separate parts – upper and lower, triangular in shape, but separated by a pronounced horizontal gap.

**Conclusions:** The features of the fetal anatomy of the brachioradialis are established: its variability and bilateral asymmetry of shape and size, variability of the places of origin and attachment, etc. In individual human fetuses, the brachioradialis consists of two separate parts that have special topographical relationships. The area of the greatest concentration of both extra- and intramuscular nerves and arteries is the upper and middle third of the brachioradialis. In the area of the forearm, the course of radial vascular-nerve formations is determined by the brachioradialis.

**KEY WORDS:** brachioradialis, radial artery, radial nerve, anatomical variability, fetus

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## INTRODUCTION

One of the tasks of modern traumatology is the treatment of fractures of the shoulder and forearm bones. The use of different methods, without taking into attention the nature and severity of the damage, the topographic and anatomical features of the muscles, vessels and nerves of the shoulder and forearm, forms of anatomical variability, usually leads to unsatisfactory results. At the same time, fractures of the humerus or bones of the forearm, gunshot and stab wounds, various injuries, infections and intoxications, etc. can lead to damage of the branches of the brachial artery and long branches of the brachial plexus, and in particular, the radial nerve [1, 2, 3]. It should be noted that paresis and paralysis of the radial nerve can also be caused by such factors as abnormal position of the upper extremities during natural or narcotic sleeping, in a state of alcohol intoxication, due to forcible tying of hands in the shoulder region, in a case of the pressure of crutches, etc. Superficial location of the radial nerve in the posterior shoulder area and its close proximal position to the humerus determines quite common lesion of the radial nerve.

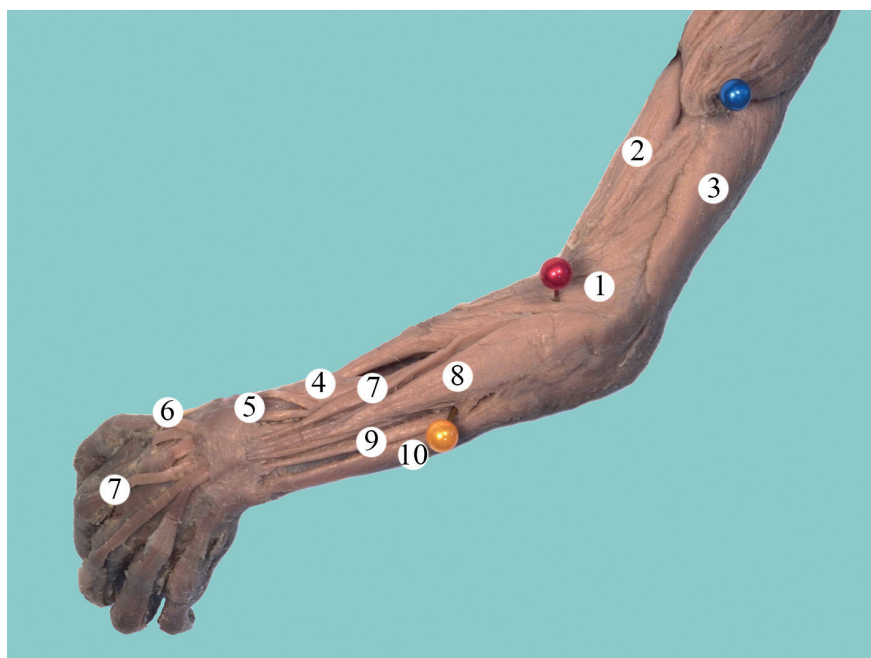
Symptopathology of the radial nerve depends on the height of the lesion. With a high lesion of the radial nerve (above the level of the branches departure to the triceps brachii), a picture of complete paralysis develops [4, 5].

There is no active extension of the forearm, supination of the forearm with an extended upper extremity is impossible, the wrist usually takes a pronation position. If, when the upper extremity hangs down, it is passively supinated, then it, like a spring, returns to the position of attraction. If the upper extremity is bent at the elbow joint, supination becomes possible due to the function of the biceps brachii.

The most typical symptom of the radial nerve paralysis is a hanging wrist ("seal's paw") [6, 7], in which, due to the dysfunction of the extensor muscles, it becomes impossible to extend the wrist and fingers.

If the radial nerve is damaged in the area of the middle third of the shoulder, i.e., below the place of branches departure to the triceps brachii, the function of the last will be preserved. In the case of damage of the radial nerve in the lower part of the shoulder or in the upper third of the forearm, the function of the brachioradialis, retractor muscle, and extensor muscles is usually preserved [8, 9].

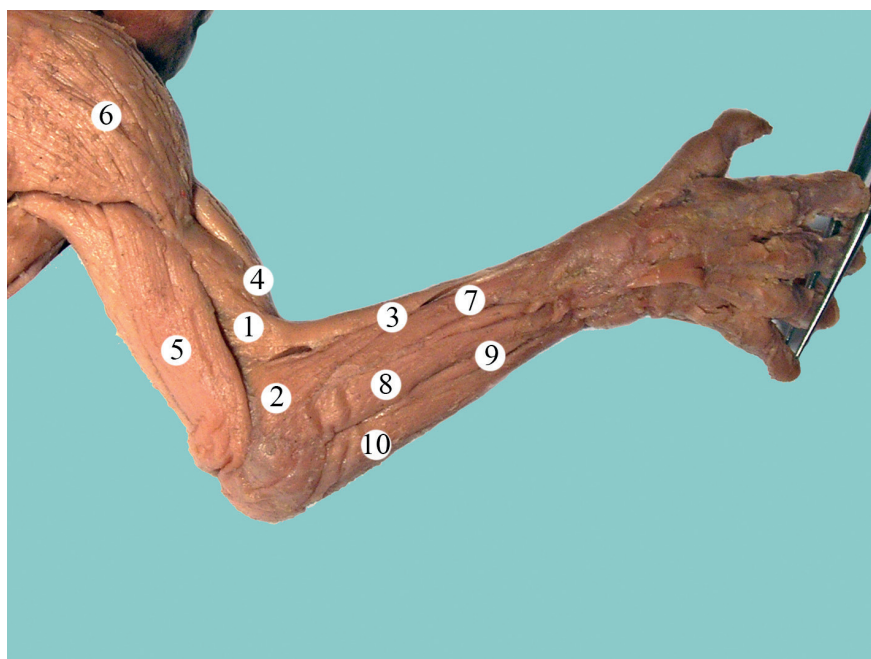
So, the clarification of the variant anatomy of the upper extremity muscles of the human fetuses, taking into attention the main and additional sources of blood supply and innervation, is actual and perspective, it has applied value for the development of anatomical standards of fetal development. Important issues of fetal surgery is information about the direction and relationship of intramuscular



**Fig. 1.** Muscles of the left upper extremity of the fetus 255.0 mm PCL.

Photo of macropreparation. X 2,1:

1 – Brachioradialis; 2 – Brachialis; 3 – Triceps brachii; 4 – Abductor pollicis longus; 5 – Extensor pollicis brevis; 6 – Extensor pollicis longus; 7 – Extensor indicis; 8 – Extensor digitorum; 9 – Extensor digiti minimi; 10 – Extensor carpi ulnaris.



**Fig. 2.** Muscles of the right upper extremity of the fetus 185.0 mm PCL.

Photo of macropreparation. X 2,4:

1 – Upper part of brachioradialis; 2 – Lower part of brachioradialis; 3 – Common muscle belly of the brachioradialis; 4 – Brachialis; 5 – Triceps brachii; 6 – Deltoid muscle; 7 – Extensor carpi radialis longus; 8 – Extensor digitorum; 9 – Extensor digiti minimi; 10 – Extensor carpi ulnaris.

arteries, nerves, and muscle bundles of the shoulder and forearm muscles in human fetuses of different age [10, 11].

However, in the scientific literature there are few information about the variant anatomy of the brachioradialis, its vessels and nerves in human fetuses [12, 13].

## THE AIM

To establish the features of age-related and individual anatomical variability of the brachioradialis, its blood supply, and innervation options in fetus.

## MATERIALS AND METHODS

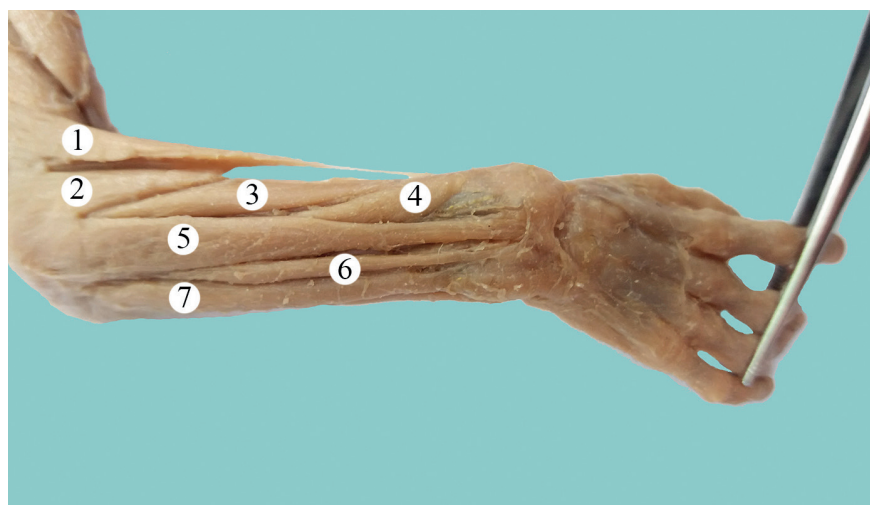
The study of the variant anatomy of the brachioradialis and its vascular and nervous structures was carried out on 25

preparations of human fetuses of 4-7 months, 81.0-270.0 mm parietal-coccygeal length (PCL) using macromicroscopic preparation, injection vessels, and morphometry.

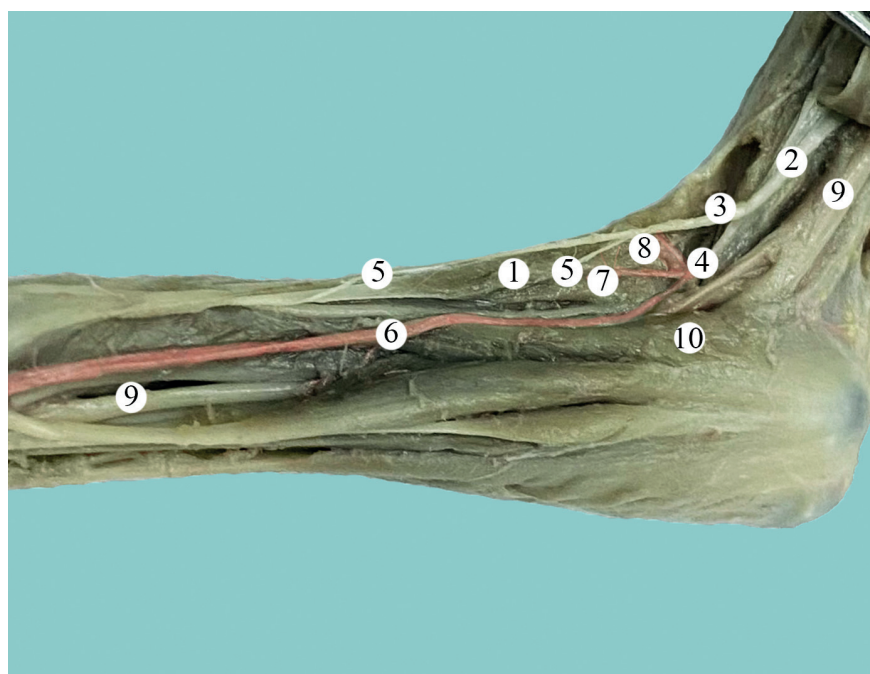
## RESULTS

In the area of the forearm, the course of radial vascular-nerve formations is determined by the brachioradialis. During the research it was established the anatomical variability and bilateral asymmetry of external shape, size and topography of the right and left brachioradialis in human fetus. Spindle-like (56%) and round (24%) shapes of the brachioradialis were found in most of the studied fetuses; its elongated flat (12%) and triangular (8%) forms occur less often. As a fact, in human fetuses, the brachioradialis is located in the lateral part of the anterior forearm, starts

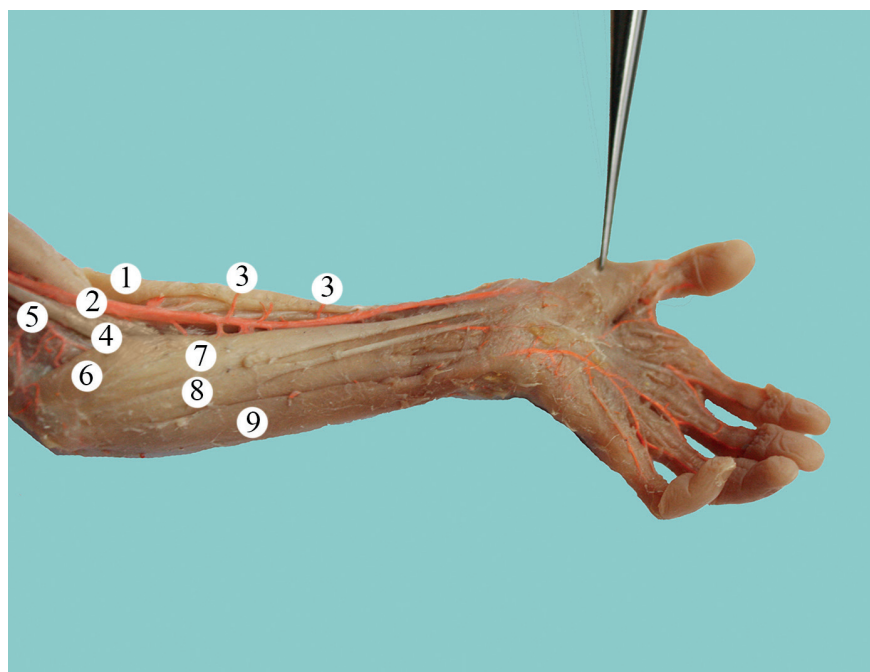




**Fig. 3.** Muscles of the right forearm of the fetus 220.0 mm PCL. Photo of macropreparation. X 2,2: 1 – Upper part of Brachioradialis; 2 – Lower part of Brachioradialis; 3 – Extensor carpi radialis longus; 4 – Abductor pollicis longus; 5 – Extensor digitorum; 6 – Extensor digiti minimi; 7 – Extensor carpi ulnaris.



**Fig. 4.** Structures of the right anterior part of the forearm of the fetus 160. 0 mm PCL. Photo of macropreparation. X 2,6: 1 – Brachioradialis; 2 – Radial nerve; 3 – Superficial branch; 4 – Deep branch; 5 – . Muscle branches of radial nerve; 6 – Radial artery; 7 – Muscle branche of radial artery; 8 – Recurrens radial artery; 9 – Median nerve; 10 – Pronator teres.



**Fig. 5.** Structures of the left forearm of the fetus 210.0 mm PCL. Photo of macropreparation. X 2,1: 1 – Brachioradialis; 2 – Radial artery; 3 – Muscular branches of the radial artery; 4 – Nervus medianus; 5 – Lower bypass ulnar artery; 6 – Pronator teres; 7 – Flexor carpi radialis; 8 – Palmaris longus; 9 – Flexor carpi ulnaris.



from the lateral epicondyle of the humerus and the lateral intermuscular septum of the brachii, and is attached to the lateral surface of the lower end of the radii near the styloid process.

In some fetuses, different variants of the beginning and attachment of the brachioradialis were observed. Thus, in a fetus with 255.0 mm PCL, the left brachioradialis began at the level of the deltoid tubercle and in the lateral edge of the middle third of the humerus, its lateral epicondyle, also in the lateral intermuscular septum of the humerus, and was located between brachialis and lateral head of the triceps brachii (Fig. 1). The left brachioradialis is characterized by a triangular shape with the apex directed to the lateral epicondyle of the humerus. The muscle fibers of the brachioradialis went obliquely in the ventrocaudal direction, outside the radius. At the border of the middle and lower thirds of the forearm, the belly of the brachioradialis turned into a wide flat tendon, which was attached together with the tendon of abductor pollicis longus to the base of the 1<sup>st</sup> metacarpal bone. It should be noted that in this fetus, the extensor digitorum was attached by three, not four, tendons to the back surface of the proximal phalanx and distal phalanx of II-IV fingers.

In a fetus of 185.0 mm TKD, the right brachioradialis consisted of two separate parts: upper and lower (Fig. 2). Upper part of the right brachioradialis began 4.0 mm below the deltoid tubercle and the middle third of the antero-lateral surface of the right humerus body. Moreover, the antero-medial edge of the upper part of the right brachioradialis was connected to the lateral edge of the right brachialis, which started from deltoid tubercle of humerus. Posterior-lateral edge of the upper part of the right brachioradialis adjoined to the antero-lateral surface of the belly of the right triceps brachii. The last one occupied the back shoulder area and only in the upper part of the triceps brachii it was the demarcation of the common belly of this muscle by shallow furrows into three heads: lateral, long and medial. The muscle bundles of the lower part of the right brachioradialis began from the lower third of the antero-lateral surface of the body and the lateral epicondyle of the right humerus. Let's indicate, that at the level of the middle of the forearm upper and lower parts of right brachioradialis were connected into a common short muscle belly, which continued into a narrow long flat tendon, which was attached to the lateral surface of the radius above its styloid process.

In another human fetus of 220.0 mm TKD, the right brachioradialis also consisted of two separate parts – upper and lower, triangular in shape, but separated by a pronounced horizontal gap (Fig. 3). At the same time, the upper part of the right brachioradialis started from the lower third of the antero-lateral surface of the body of the right humerus, and the lower part of this muscle – from the lateral epicondyle of the right humerus and the capsule of the elbow joint. At the border of the upper and middle thirds of the forearm, the lower part of the right brachioradialis was connected to the distal third of the upper part of this muscle. At the level of the middle third of

the forearm, the muscle belly of the upper part of the right brachioradialis passed into a narrow thin tendon, which went over extensor carpi radialis longus and the tendon of the abductor pollicis longus and attached to the lateral surface of the lower third of the radius.

To find out the innervation of the brachioradialis in human fetuses, the site of the exit of the radial nerve from the brachioradialis canal was dissected. To do this, the brachialis and brachioradialis were pushed apart and the trunk of the radial nerve was found deep between them. After exiting the brachialis canal, as a rule, somewhat below the lateral epicondyle of the humerus, the radial nerve was divided into superficial and deep branches. The last one deviated laterally, protruded the retractor muscle and passed to the back of the forearm. The superficial branch of the radial nerve passed under the brachioradialis, then, accompanied by the radial vessels, went into the radial furrow of the forearm. At the border of the middle and lower thirds of the forearm, the superficial branch of the radial nerve deviated laterally and passed under the brachioradialis to the back of the forearm, where it penetrated the fascia of the forearm and went to the back of the wrist. At the level of the radial carpal joint, the superficial branch of the radial nerve branched into the dorsal digital nerves, which provide innervation of the skin of the dorsal surfaces of the proximal phalanges of the I-II fingers on both sides, as well as the III finger on the radial side.

In human fetuses, one branch of the radial nerve is mainly involved in the innervation of the brachioradialis (26 observations out of 50), less often – two branches of the nerve (19 cases), three branches (4 observations) and four branches (1 case). As a rule, the trunk of the radial nerve enters the brachioradialis at the border of the upper and middle third of the belly of the muscle (40 observations out of 50), less often – within the middle third of the brachioradialis belly. At the same time, the level of entry of the radial nerve into the thickness of the brachioradialis varies from 3.0 to 9.0 mm proximal to the line connecting the epicondyle of the humerus. In the brachioradialis, which is usually spindle-shaped in fetuses, the type of branching of the radial nerve is heterogeneous: more often – magistral branching, less often – loose. In a case of the magistral type of the intramuscular branching of the radial nerve, its 1-2 branches are mostly identified, and in isolated (3) cases – 3 branches of this nerve at the level of the middle and lower thirds, as well as within the lower third of the brachioradialis belly (Fig. 4).

In some fetuses, an independent source of innervation of the upper third of the brachioradialis with a loose type of the nerve trunk branching was observed. In those cases, when the innervation of the upper third of the brachioradialis was carried out by a branch from the main trunk of the radial nerve, intramuscular distribution took place according to the magistral type. It was also found that in early fetuses, the intramuscular nerves of the brachioradialis give single trunks of the 2nd, and especially, the 3rd orders; their number, as well as the appearance of connections between them as a connecting branches, is observed

at the end of the 5th – at the beginning of the 6th months of intrauterine development.

When studying the intramuscular branching of the radial artery in the brachioradialis, first was dissected the brachial artery in the elbow fossa, which is covered by the aponeurosis of the biceps brachii and is located between the pronator teres and brachioradialis. Next, the radial artery, which in its direction is a continuation of the brachial artery, is placed superficially and is covered in the upper part only by the brachioradialis, was dissected. First, the radial artery goes between the brachioradialis and pronator teres, and below – between the brachioradialis and flexor carpi radialis.

The radial artery enters the radial furrow, where it is accompanied by the superficial branch of the radial nerve for some distance. Radial veins pass on both sides of the radial artery. In the lower half of the forearm, the radial artery lies superficially, under the fascia, and is located at the medial edge of the brachioradialis, or at a distance of  $3.0 \pm 0.5$  mm medially from the tendon of this muscle. In the lower part of the forearm, the radial artery goes in the dorso-caudal direction and goes to the back of the wrist.

As a rule, 2-5 muscular branches of the radial artery, which enter its belly in the upper and middle thirds, take part in the blood supply of the brachioradialis. In single cases, it was observed from 6 to 9 branches of the radial artery, which entered the brachioradialis along its belly length (Fig. 5).

It was established that in most of the examined fetuses, the area of greatest concentration of both extra- and intramuscular nerves and arteries is the upper and middle thirds of the brachioradialis.

The dimensions of the brachioradialis belly, as well as the type of the intramuscular distribution of the radial nerve and artery, make it possible to cut out muscle flaps from it with preserved innervation and blood supply, while the base part of the flap should be directed upwards and medially, and should be separated from the lower border entry of the main trunk of the radial nerve, not less than 10.0-12.0 mm. If necessary, it is possible to cut short and wide flaps from the upper third of the brachioradialis taking into attention its shape, while the base of the flap should be directed medially.

## DISCUSSION

Variant anatomy is an integral part of anatomical science, understanding of variant anatomy is based on thousand years of anatomical experience. Understanding variant anatomy is a basic skill not only of mere anatomists, but also of clinicians who work in fields involving both diagnostic techniques and therapeutic interventions.

Although variant anatomy is a vast topic, comprehensive sources exist. Many classical anatomical atlases contain paragraphs or pages on anatomical variants, usually concluding relevant chapters. Specific monographs on variant anatomy of the skull [14], upper limb muscles [15], arterial [16] and venous circulatory system, and peripheral nerves [17] are available.

The most variable part of the human body is the cardiovascular system, especially veins. Their arrangement, mainly in the limbs, is very heterogeneous, although they follow a certain principal axial pattern [18, 19, 20]. As for the arterial system, it has the best-described nomenclature and is recognized as part of variant anatomy. The basic and, until now, matchless publication on this topic is “Arteriensystem der Japaner” by Adachi and “Arterial Variations in Man: Classification and Frequency” [16] and the radiological “Atlas of Normal and Variant Angiographic Anatomy” [21].

The variations also are often related to the muscles, in particular different numbers of muscle heads (2–5 heads of the *musculus biceps brachii*), tendons (more tendons of the *musculus extensor carpi radialis longus et brevis*), accessory muscle slips and bands, rudimentary muscles (*musculus sternalis*, *musculus dorsoepitrochlearis*, *musculus epitrochleoanconeus* (previously mentioned in the Basiliensia Nomina Anatomica (BNA) in 1895, but then removed from the nomenclature)), tendinous arcades (*arcus axillaris* of Langer [22, 23, 24] or Struther’s canal [25]), absent *musculus palmaris longus* (missing in 4–20% of cases based on the population), absent *musculus psoas minor* (33.4–52% of cases based on the population), different numbers (3 or 4) of *intersectiones tendineae musculi recti abdominis* and their irregular arrangement, just to mention a few of the most well-known examples [26].

There exist several important literary sources, such as the classical atlas of Henle, Testut, and Le Double [27, 28], works of Macalister and Mori [29, 30], Frohse and Fränkel’s chapter concerning the muscles of the upper and lower limbs [31], Eisler’s chapter devoted to the muscles of the trunk [32], and some quite recent articles [33, 34].

As for the nervous system, the peripheral nerves are of great concern when it comes to anatomical variability, especially in the course of their trunks (e.g., the junction of the medial and lateral roots of the nervus medianus, the course of the upper nerves of the lumbar plexus (nervus iliohypogastricus, nervus ilioinguinalis, nervus cutaneus femoris lateralis, and, especially, nervus genitofemoralis), or the ansa cervicalis superficialis). A special chapter describes the rami communicantes (obsoletely called “anastomoses”), which appear between the cranial nerves [35] and spinal nerves. Clinically, the most interesting variabilities are connections between the nervus medianus and nervus ulnaris (e.g., “Martin-Gruber anastomosis”) for which terminology has not been unified [22].

In our opinion, the type of branching of the radial nerve in the brachioradialis can be explained as follows: 1) when the direction of the nerves coincides with the direction of the muscle bundles of the brachioradialis, a loose form of the branching of the radial nerve is observed, when they diverge – magisrta type; 2) if the sharper the angle of entry of the branches of the radial nerve into the brachioradialis, the direction of the intramuscular nerves coincides the more with the longitudinal axis of this muscle; 3) the regularities of the entry of the branches of the radial nerve into the thickness of the brachioradialis are associated with

the formation of the relationship between neurotome and myotome during the early period of human ontogenesis. However, in isolated cases, in the thickness of the same brachioradialis, we observed areas of overlap and divergence of the direction of intramuscular nerves and muscle bundles.

Variant anatomy includes not only life-threatening variants, but also other clinically important ones. The above-mentioned Rodríguez-Niedenführ's analyses of the upper limb arterial variations [19, 20] clarifies some potential for failure of catheterization due to a patient's anatomical variation when performed via the radial artery. Thus, knowledge of these anatomical variations is crucial for interventional cardiologists. Group of other scientists [36] present also example that is important for plastic and reconstructive surgeons, concerning the blood supply of the thumb. Based on their review, the principal arterial source for the thumb, the arteria princeps pollicis branching from the arteria radialis and corresponding to the arteria metacarpalis palmaris prima, is the dominant source of blood supply for the thumb in only 64.8% of cases. In 85.8% of cases, one artery supplying the thumb has a distinctly larger diameter than others. The current terminology is not specific enough, and, therefore, authors suggest avoiding the term "arteria princeps pollicis", which evokes exceptionality and dominance in the thumb's blood supply, and refer to all the arteries by their systemic anatomy [37].

Our understanding of variant anatomy is developing and changing, not only thanks to new diagnostic methods (CT, MRI, ultrasonography), but also by means of old and well-established methods (dissection, injection, corrosion casting).

The study of the variant anatomy of the muscles of the upper limb in human fetuses, taking into account the main and additional sources of blood supply and innervation, is relevant and promising and has applied significance to the development of anatomical standards of fetal development.

The study confirms the need for further clarification of the age-related and individual anatomical variability of the brachioradialis, its vessels and nerves in human fetuses.

## CONCLUSIONS

The features of the fetal anatomy of the brachioradialis are established: its variability and bilateral asymmetry of shape and size, variability of the places of origin and attachment.

In individual human fetuses, the brachioradialis consists of two separate parts that have special topographical relationships, and the connection of the brachioradialis with the brachii was also revealed.

The area of the greatest concentration of both extra- and intramuscular nerves and arteries is the upper and middle third of the brachioradialis.

In the area of the forearm, the course of radial vascular-nerve formations is determined by the brachioradialis: in the upper half of the forearm, the muscle covers them, and in the lower half, the radial artery is located at the medial edge of the brachioradialis, or to the inside, at a distance of  $3.0 \pm 0.5$  mm, from the tendon of this muscle.

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## ORIGINAL ARTICLE

# EXPRESSION OF TOLL-LIKE RECEPTORS 4 ON CD14 + MONOCYTES IN JUVENILE IDIOPATHIC ARTHRITIS

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**ABSTRACT**

**The aim:** The work is aimed at determining the relationship between TLR4 expression on CD14+monocytes in whole heparinized blood and B1a lymphocyte synthesis in various subtypes of JIA.

**Materials and methods:** 64 children aged 3 to 17 years were examined, including 42 children with different subtypes of JIA and 22 healthy children. The intensity of TLR4 expression on CD14+monocytes was determined in whole heparinized blood incubated with a CD14-FITC/TLR4-PE monoclonal antibody cocktail (Biolegend, USA) using flow cytometry. Monoclonal antibodies (BD Bioscience) were used to determine the main subpopulations of lymphocytes.

**Results:** A statistically significant increase in TLR4 expression has been determined in JIA compared to the control group. The most prominent TLR4 expression was detected in children with oligoarthritis, while in systemic arthritis, there was no statistical difference compared to healthy children. High TLR4 expression on peripheral CD14+monocytes inversely depends on the activity of the autoimmune process, which may have a protective effect against the aseptic inflammation. Increased TLR4 expression involves a statistically significant increase in the percentage and quantity of B1a lymphocytes ( $p \leq 0.05$ ).

**Conclusions:** A statistically significant increase in TLR4 expression on CD14+monocytes in whole heparinized blood was detected in patients with JIA compared to healthy children. Children with oligoarthritis had the highest rates, which indicates possible differences in the development of pathogenetic processes in different subtypes of arthritis. Determining the degree of TLR4 activation on CD14+monocytes is reasonable for predicting JIA activity.

**KEY WORDS:** juvenile idiopathic arthritis, adaptive, innate immunity, lymphocyte subpopulations, toll-like receptor

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**INTRODUCTION**

According to the definition of the American College of Rheumatology, juvenile idiopathic arthritis is considered a complex of non-infective chronic autoimmune processes, involving joint inflammation for more than 6 weeks with onset before age 16 [1]. Approximately one child in 1000 has a diagnosis of JIA after excluding other known causes of arthritis; 50% of these children have the oligoarticular JIA (damage to 4 or fewer joints); 40% have the polyarticular type (damage to 5 or more joints), and 10% of patients are diagnosed with systemic arthritis [1].

It has been proved that the pathologic mechanisms in juvenile arthritis develop due to dysregulation of both adaptive and innate immunity, the key ligands of which are the Toll-like receptor (TLR) family. This family includes 13 structurally unique members that are expressed in intracellular signaling pathways and play an essential role in recognizing inflammatory processes caused by the invasion of microorganisms or other pathogenic components [2].

The innate immune system relies on a limited number of germline-encoded receptors, known as pattern recognition receptors (PRRs), which have evolved for recognizing molecular pathways [3]. Toll-like receptors are just one of several PRR classes, which include NOD-

like receptors (NLRs), C-type lectin receptors (CLRs), AIM2-like receptors (ALRs), RIG-I-like receptors (RLRs), intracellular DNA sensors, etc., which recognize various structures: flagellin, nucleic acids, saccharides (mannose, lipopolysaccharide), lipoteichoic acid, peptidoglycans and lipoproteins [4]. The adaptive immune response is initiated by recognition of these antigens mediated by the synthesis of pro-inflammatory cytokines along with stimulation of antigen-presenting cells (APC).

The TLR family recognizes bacterial, fungal, protozoan, and viral components commonly referred to as pathogen-related molecular structures (PAMPs). Toll-like receptors are mostly expressed on immune system cells, mucous membranes, and skin [5]. It is proved that TLR4 expression increases in synoviocytes, chondrocytes, osteoblasts and peripheral blood monocytes that are recruited to the site of synovial inflammation [6]. After the recognition of conservative structures of pathogens, TLRs activate signaling pathways, induce transcription of the chemokines and cytokines genes that leads to activation of inflammatory mechanisms.

The formation of specific cellular antibodies, in which TLRs are inadequately activated by their components, can lead to sterile inflammation with loss of immunological tol-



erance to their own cells [7]. Consequently, organ-specific autoimmune diseases are a flawed realization of genetic features and environmental factors of adaptive regulation of the immune response to antigens [7].

Overexpression of PRR was also found in the tissues of patients with Type 1 diabetes, Crohn's disease. Therefore, TLR is currently considered an essential component of the autoimmune process initiation [8]. TLR signal transduction depends on its localization in the cell. Most TLRs interact and participate in the functioning of the central adapter protein MyD88 [6] with the involvement of members of the IRAK family (IL1-receptor-associated kinase), which significantly enhances the signal and leads to the induction of inflammatory response genes [9].

Toll-like receptor 4 (TLR4) plays a key role in the immune response and activates adaptive responses through activating dendritic cells, macrophages, and intracellular signaling pathways (NF- $\kappa$ B, MAPK, JAK-STAT etc.) with subsequent cytokine synthesis. This is considered an important but, in many cases, disputable mechanism for the development of rheumatic diseases [10].

In the pathogenesis of autoimmune disorders, B lymphocytes play an important role as elements of the adaptive immune system associated with the production of autoantibodies [11]. B lymphocytes express several cytoplasmic and surface antigens that are unique for their subpopulation and stage of development and have different effects on the course of pathological processes (infectious, autoimmune, oncological).

There are three main subpopulations of B cells: B-1, B-2, and memory B cells. The CD5 molecule is an important part of this division since it is considered as a possible marker of B cells, which makes it possible to distinguish their subpopulations: CD19+CD5+ B cells (B1-lymphocytes) and CD19+ CD5 B cells (B2-lymphocytes).

In terms of antigenic specificity of the synthesized antibodies, B1 lymphocytes differ significantly from B2 lymphocytes. B1 lymphocyte antibodies do not have a lot of variable regions of immunoglobulin molecules, but, on the contrary, are limited in recognized antigens. Compounds of bacterial cell walls are the most common among such antigens. Therefore, B1 lymphocytes are considered as one not very specialized but certainly targeted (antibacterial) clone. B1 cells are divided into two subclasses: B1a and B1b lymphocytes [12].

The role of CD5+ B cell autoreactivity in the pathogenesis of autoimmune diseases in rheumatoid arthritis, systemic lupus erythematosus, Sjogren's syndrome, myasthenia gravis, insulin-dependent diabetes, and Hashimoto's thyroiditis is gaining more and more evidence in experimental and clinical studies [13].

Given the above, it is of great interest to determine the intensity of TLR4 expression and CD19+CD5+ lymphocyte proliferation as a specific antibacterial-oriented clone to clarify their role in the pathogenesis of idiopathic arthritis in children.

## THE AIM

The aim was to determine the relationship between TLR4 expression on CD14+ monocytes in whole heparinized

blood and B1a lymphocyte synthesis in various subtypes of juvenile idiopathic arthritis.

## MATERIALS AND METHODS

The study was conducted in 64 children aged 3 to 17 years, including 42 children with a diagnosis of JIA, examined in the Department of Pediatric Rheumatology and Auto-inflammatory Diseases, Institute of Pediatrics, Obstetrics, and Gynecology named after acad. O. M. Lukyanova of the NAMS of Ukraine" and 22 healthy children (control group). The diagnosis of JIA was made according to the criteria (ILAR, 1998). Patients with JIA were stratified by subtypes: oligoarthritis – 19, polyarthritis – 17, systemic arthritis – 6 children (Table I).

Children with suspected or confirmed infections at the time of the visit were excluded from the study.

The study was conducted in compliance with the provisions of the GCP (1996), the Convention on Human Rights and Biomedicine (dated 04.04.1997), the World Medical Association Declaration of Helsinki (1964-2002), and the Order of the Ministry of Health of Ukraine No. 281 dated 01.11.2001. The study protocol was approved by the local Ethics Committee for patients and parents who participated in the study.

JIA activity was evaluated via the Juvenile Arthritis Disease Activity score (JADAS 27), which allows determining the activity of the pathological process using the sum of indicators: 1) assessment of the general condition of the child by a doctor (1 to 10 cm); 2) assessment of the condition by parents/patient (from 1 to 10 cm); 3) assessment and counting of the number of joints with active arthritis; 4) or (CRP-10)÷10 (ESR determined via the Westergren method). High process activity in oligoarthritis is diagnosed at the value of JADAS 27 > 4.2, in polyarthritis – JADAS 27 > 8.5 [14].

The intensity of TLR4 expression on CD14+ monocytes was determined in whole heparinized blood incubated with

**Table I.** General characteristics of patients with JIA

Indicator	Indicator value, n=64
The number of children, abs. n.:	64
- JIA	42
- healthy	22
Age, years	10-18
Duration of the disease, years	(4.3±3.3)
Subtypes of JIA, abs. n. (%):	
JIA-oligoarthritis	19 (45.2)
JIA-polyarthritis	17 (40.4)
JIA-systemic	6 (14.2)
ANA (+), abs.n. (%)	27 (64.2)
ANA (-), abs.n. (%)	15 (35.7)
HLA-B27 (+), abs.n. (%)	10 (23.8)
HLA-B27 (-), abs.n. (%)	32 (76.1)

Notes: ANA-antinuclear antibodies, IgA-immunoglobulin A; HLA-B27-Human leukocyte antigen B27; ACCP- anti-citrullinated protein antibody; RF - rheumatoid factor

**Table II.** TLR4 expression on CD14 + monocytes in whole heparinized blood of children with different subtypes of juvenile idiopathic arthritis

Indicator	Indicator value, (M±m) %	t	α	p
JIA, n=42	51.51±6.18	3.01	0.05	0.0033
sJIA, n=6	38.48±10.32	2.24	0.05	0.033
pJIA, n=17	52.86±7.14	3.01	0.05	0.005
oJIA, n=19	54.43±12.10	2.12	0.05	0.05

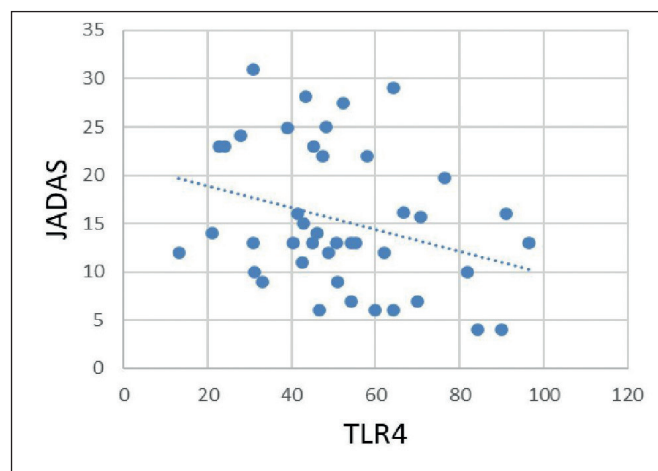
**Table III.** JIA activity at the onset of the disease on the JADAS 27 scale for different subtypes of juvenile idiopathic arthritis, n=42

JIA subtypes	JADAS indicator value, (M±m)	t	α	p
JIA (n=42)	17.14±1.86	-	-	-
sJIA (n=6)	22.98±2.45	-	-	-
pJIA (n=17)	(16.67±1.78)*	2.08	0.05	0.05
oJIA (n=19)	(11.79±1.36)**	3.99	0.05	0.0006

Notes: 1. \* – statistically significant differences between the groups of systemic arthritis and polyarthritis; 2. \*\* – statistically significant differences between the groups of systemic arthritis and oligoarthritis.

**Table IV.** Subpopulations of blood lymphocytes in JIA and healthy children

Subpopulations of lymphocytes	Indicator value, n=64		t	α	p
	Patients with JIA, n=42	Control group, n=22			
Monocytes, %	5.43±0.43	5.55±0.31	0.23	0.05	0.82
Monocytes, qty/μL	422.53±51.64	482.21±48.36	0.84	0.05	0.403
T lymphocytes (CD3+), activated (HLA-DR+), %	7.13±1.25	6.88±0.81	0.17	0.05	0.867
T lymphocytes (CD3+), activated (HLA-DR+), qty/μL	198.11±66.02	112.0±18.36	1.26	0.05	0.215
T helpers (CD3+CD4+), activated, (HLA-DR+), %	6.02±0.87	6.47±0.41	0.47	0.05	0.641
T helpers (CD3+CD4+), activated, (HLA-DR+), qty/μL	93.96±13.2	64.36±6.24	2.03	0.05	0.048
Cytotoxic T Cell (CD3+CD8+), activated, (HLA-DR+), %	15.99±2.43	16.63±2.34	0.19	0.05	0.85
Cytotoxic T Cell (CD3+CD8+), activated, (HLA-DR+), qty/μL	176.16±52.04	99.21±18.82	1.39	0.05	0.17
B lymphocytes B1a, (CD5+), %	22.87±3.11	14.72±2.57	2.02	0.05	0.049
B lymphocytes B1a, (CD5+), qty/μL	145.66±42.78	52.71±14.46	2.06	0.05	0.045



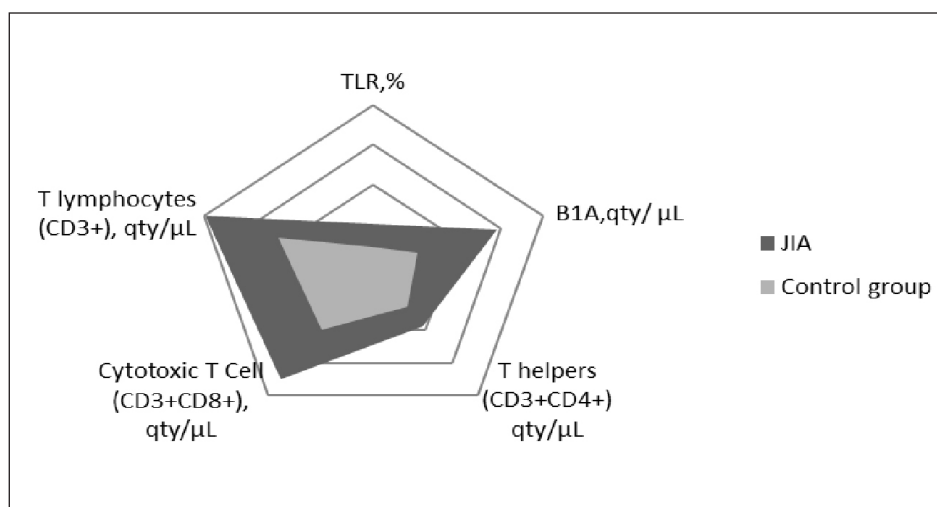
**Fig. 1.** Correlation analysis between TLR4 expression level and the JADAS scale via Pearson criterion, n=42

a CD14-FITC/TLR4-PE monoclonal antibody cocktail (Biolegend, USA) using flow cytometry for 20 minutes at room temperature. Blood for research was obtained under

standard conditions, in the morning in the fasted state. After incubation, 500 μL of lysing solution and 500 μL of distilled water were added to the samples to remove red blood cells and after, they were washed with a «Cell wash» solution. After centrifugation (5min., 1500g) and sediment drainage samples were analyzed on a FACScan instrument (Becton Dickinson, USA). Monocytes were separated based on FSC/SSC parameters; 10,000 events were included in the analysis.

Monoclonal antibodies CD19-PE, CD5-FITC, CD3-FITC, CD8-PE, CD4-PE-Cy5, HLA DR-FITC, CD56-PE, CD3-PE-Cy5 (BD Bioscience) were used to determine the main subpopulations of lymphocytes. 11 μL of monoclonal antibody cocktail was added to 75 μL of blood and incubated for 30 minutes at room temperature. The samples were then lysed with Lysing Solution (BD Bioscience) (500 μL) for 15 minutes at room temperature and washed with normal saline. After centrifugation (5min, 1500g), the samples were mixed on a Vortex and taken for analysis.

Separately, to determine the absolute number of cells, 7 μL of CD45FITC/CD14PE monoclonal antibodies (Simultest, BD Bioscience) were added to 20 μL of heparinized



**Fig. 2.** TLR4 expression on CD14 + monocytes in whole heparinized blood and separate subpopulations of lymphocytes in JIA and the control group

blood of each sample and incubated for 30 minutes at room temperature. After incubation, the samples were lysed with a Lysing Solution (BD Bioscience) (250  $\mu$ L) for 15 minutes, then 20  $\mu$ L of fluorospheres (Flow-Count Fluorospheres, Beckman Coulter) were added. Samples were mixed on a Vortex and taken for analysis. The analysis was performed on a BD FACScan device using CellQuest software.

The difference between groups in the normal distribution was assessed via the Student's t-test. Analysis of variance (ANOVA) was used to analyze the results between groups. Correlative relationships were determined by the Pearson criterion, nonparametric criteria – according to Mann-Whitney U test.

## RESULTS

Children diagnosed with JIA have shown increased TLR4 expression ( $51.51 \pm 6.18$  %), which was statistically different from the control group ( $23.47 \pm 6.68$  %), (t-test = 3.01,  $\alpha = 0.05$ ),  $p = 0.0033$  (Table II). At the same time, TLR4 expression was the highest in children with oligoarthritis and amounted to ( $54.43 \pm 12.10$  %), which significantly exceeded the indicators of healthy children (t-test = 2.24,  $\alpha = 0.05$ ),  $p = 0.033$ . The figures obtained from patients with polyarthritis also significantly differed from the control group ( $52.86 \pm 7.14$  %) (t-test = 3.01,  $\alpha = 0.05$ ),  $p = 0.005$ . However, in systemic JIA, the intensity of TLR4 expression was lower ( $38.48 \pm 10.32$  %), and there was no statistically significant difference with healthy children (t-test = 2.12,  $p > 0.05$ ). (Table II)

Thus, the level of TLR4 stimulation was the highest in children with oligoarthritis, which was 2.3 times higher than in the control group.

Evaluation of the JIA activity at its onset using the JADAS 27 scale in patients (n=42) examined for TLR4 expression have shown the overall high activity of the pathological process ( $17.14 \pm 1.86$ ). At the same time, the highest activity index was revealed in children with systemic arthritis – ( $22.98 \pm 2.45$ ), which exceeded 1.4 times the indicators in polyarthritis, where the average JADAS27 index was ( $16.67 \pm 1.78$ ) and for oligoarthritis it was ( $11.79 \pm 1.36$ ). (Table III)

The comparison has shown that the group with systemic arthritis significantly differs in the Juvenile Arthritis Disease activity score from the other two groups, and significantly differs in the TLR4 indicator from the group with polyarthritis. Correlation analysis revealed an inverse relationship between JADAS scale activity and TLR expression levels in the entire sample,  $r = -0.307$ , significance 0.048 (fig. 1).

Consequently, the activity of the autoimmune process is less with high TLR4 expression on monocytes when recognizing PAMP than in children with low TLR4 levels. That is, an increase in TLR4 may have a protective effect during the inflammatory process.

The analysis of the concentration of blood lymphocyte subpopulations in patients with JIA has revealed an increase in the absolute number of T-helper cells (CD3+CD4+) activated (HLA-DR+) to [ $93.96 \pm 13.2$ ] qty/ $\mu$ L compared to the control group [ $64.36 \pm 6.24$ ] qty/ $\mu$ L with statistical significance (t-test = 2.03,  $\alpha = 0.05$ ),  $p = 0.048$  (Table IV).

In the group of children with arthritis, there is also an increase [ $176.16 \pm 52.04$ ] qty/ $\mu$ L in the absolute number of (CD3+CD8+) lymphocytes that express (HLA-DR+), i.e. they are activated in patients with an active autoimmune process.

Children with JIA also have a relative increase in the values of B1a (CD19+CD5+) lymphocytes ( $22.87 \pm 3.11$ ) % compared to healthy children ( $14.72 \pm 2.57$  %), which is statistically significant (t-test = 2.02,  $\alpha = 0.05$ ),  $p = 0.049$ . At the same time, there is a significant difference in the quantitative value of B1a lymphocytes in JIA [ $145.66 \pm 42.78$ ] qty/ $\mu$ L compared to the control group [ $52.71 \pm 14.46$ ] qty/ $\mu$ L, (t-test = 2.06,  $\alpha = 0.05$ ),  $p = 0.045$  (fig.2).

The nonparametric Mann-Whitney U test showed a statistically significant difference in the level of B1a lymphocytes between the groups of healthy children and patients with JIA ( $p \leq 0.05$ ). The average rank in the group of children with the disease was 19.84, and in the group of healthy people, it was 13.14,  $p \leq 0.05$ .

## DISCUSSION

The paper determined the role of TLR4 as a bacterial sensor for components of the outer membrane of Gram-nega-

tive bacteria, which is the main stimulator of the innate immune system, in the pathogenesis of the autoimmune process in certain subtypes of idiopathic arthritis in children. Increased expression of TLR4, as a specific pattern recognition receptor for LPS, links infection, tissue damage, and inflammation, transmitting danger signals to intracellular signaling pathways through certain sets of adapters and transcription factors in immune and non-immune cells [15]. The first link of TLR-mediated signaling pathways is adaptive proteins, which include: MyD88 (Myeloid differentiation primary response gene 88) and TRIF (TIR domain-containing adaptor inducing interferon-beta). TLR3 is the only receptor in this family that uses only TRIF, while activation of other TLRs is associated with MyD88. TLR 4 is an exception; it activates both mechanisms: first MyD88 on the cell membrane and then TRIF localized on the endosome. Binding these adapter proteins to the TIR domain makes the next step in signal transmission possible. The second link in the activation of the TLR signaling pathway is the activation of IRAK1 and IRAK4 (interleukin-1 receptor-associated kinase;). In the third step, the activated kinase binds TRAF (tumor necrosis factor receptor-associated factor;), or more specifically, its TRAF6 domain. When the MyD88-dependent path is activated, NF- $\kappa$ B is activated and MAPK phosphorylation increases [2].

According to the data obtained, children with JIA have significantly higher TLR4 expression on CD14+monocytes in whole heparinized blood than healthy children. So, the expression of TLR4, triggered by LPS, causes an increase in the synthesis of inflammatory mediators, followed by hyperactivation of the adaptive immune response.

An increase in CD14 is required for the above LPS-activated process. Circulating peripheral blood monocytes significantly contribute to the development of inflammation; they can migrate to tissues, including synovial fluid, where they produce pro-inflammatory cytokines and chemokines, taking part in the development and maintenance of chronic aseptic inflammation. The revealed changes in TLR4 expression on peripheral blood monocytes in JIA allow assuming that the circulation of peripheral blood monocytes in children in the study groups is preactivated.

The values of TLR4 expression on monocytes were the highest in children with oligoarthritis, 1.18 times higher than in children with systemic arthritis and 1.03 higher than in polyarthritis, which indicates the possible differences in the formation of pathogenetic processes in oligoarthritis and systemic JIA.

There was an inverse relationship between the level of TLR4 expression and the activity on the JADAS scale of the inflammatory process in the entire sample,  $r = -0.307$ , significance 0.048. Therefore, with high TLR4 expression on peripheral CD14+monocytes, the activity of the autoimmune process is less than in children with low TLR4 levels, which may have a protective effect during the inflammatory process.

Along with the increase in TLR4, there was a statistically significant proliferation of B1a lymphocytes in percentage

and quantitative values ( $p \leq 0.05$ ). Antibodies of B1 lymphocytes do not have a significant variety of variable regions on immunoglobulin molecules, quite the opposite, they are limited in the repertoire of recognized antigens, and these antigens are the most common compounds of bacterial cell walls. Thus, all B1a lymphocytes are considered as one not very specialized, but certainly targeted antibacterial clone [12].

Our data show that after the activation of the autoimmune process, TLR4 increases along with this specialized lymphocytic clone. B1a cells need TLR4 expression to exhibit inhibitory effects, such as inhibition of inflammatory T-cell responses and modulation of inflammation.

Considering this, we can assume that CD19 + CD5 + lymphocytes play an important role not only in the first line of defense against PAMP and the formation of mucosal immunity but also participate in the formation of the autoimmune response, as these cells are potentially autoreactive and may cross-react with many similar antigenic determinants [16]. It is prudent to consider that endogenous ligands on CD14+ monocytes for TLR4 may be involved in promoting B1a cell proliferation during the progression of the autoimmune process.

## CONCLUSIONS

A statistically significant increase in TLR4 expression on CD14 + monocytes in whole heparinized blood was detected in patients with JIA compared to healthy children. Children with oligoarthritis had the highest rates, which indicates possible differences in the formation of pathogenetic processes in different subtypes of arthritis. Determining the degree of TLR4 activation on CD14+ monocytes is appropriate for predicting JIA activity and achieving control over the JIA. Endogenous ligands on CD14 + monocytes for TLR4 may be involved in promoting B1a cell proliferation during the progression of the autoimmune process. Identifying TLR4 transport pathways and the role of CD14+ in these processes is the key to understanding the mechanisms regulating TLR4 – induced inflammatory response in autoimmune diseases.

The TLR4 control can be a successful tool for manipulating macrophage and glial cell activation, and the development of molecules acting on TLR4 is promising disease-modifying therapeutic tactics.

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The Authors declare no conflict of interest.

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## ORIGINAL ARTICLE

# ANALYSIS OF THE PHARMACEUTICAL WORKERS STATE OF READINESS TO COUNTERACT COVID-19 IN THE HEALTH CARE SYSTEM OF UKRAINE

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**ABSTRACT****The aim:** To investigate the pharmacists level of readiness to provide assistance to pharmacy visitors on COVID-19 and ways to expand their social role in society during the pandemic.**Materials and methods:** Based on the analysis of the statistical data, sociological research and mathematical analysis, as well as generalization of the obtained results have been used in the work. The objects of the study were pharmaceutical workers of pharmacies. The survey was conducted in August 2021 via online survey of the target audience in professional groups.**Results:** The results of the survey of pharmaceutical workers indicate a high level of need (63.9% of respondents) for the continuous acquisition of relevant information on COVID-19. According to the results of the study, the growth of the social role of pharmacy specialists in combating coronavirus infection has been proved, which was supported by 86.2% of respondents in their daily activities. The possibility of introduction of 6 new functions to Ukrainian pharmacies has been considered.**Conclusions:** The practical significance of the obtained results is that together they create a scientific and methodological basis for improving the process of pharmaceutical workers participation in combating coronavirus infection.**KEY WORDS:** pharmaceutical workers, pharmaceutical care, Covid-19, continuous professional development

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**INTRODUCTION**

Considering the significant impact of COVID-19 on the economy of individual countries and the whole world, nowadays each country is developing a number of measures to combat the spread of coronavirus infection among the population, to develop approaches to adapt to the effects of this pandemic. In March 2020, the World Health Organization announced the beginning of a coronavirus pandemic. As of the beginning of September 2021, there were a total of 225331116 cases of COVID-19 infection in the world, of which 4641924 were lethal (2.06%) [1]. In Ukraine, for the same period, 2331540 confirmed cases of COVID-19 were recorded, of which 54651, unfortunately, were lethal [2].

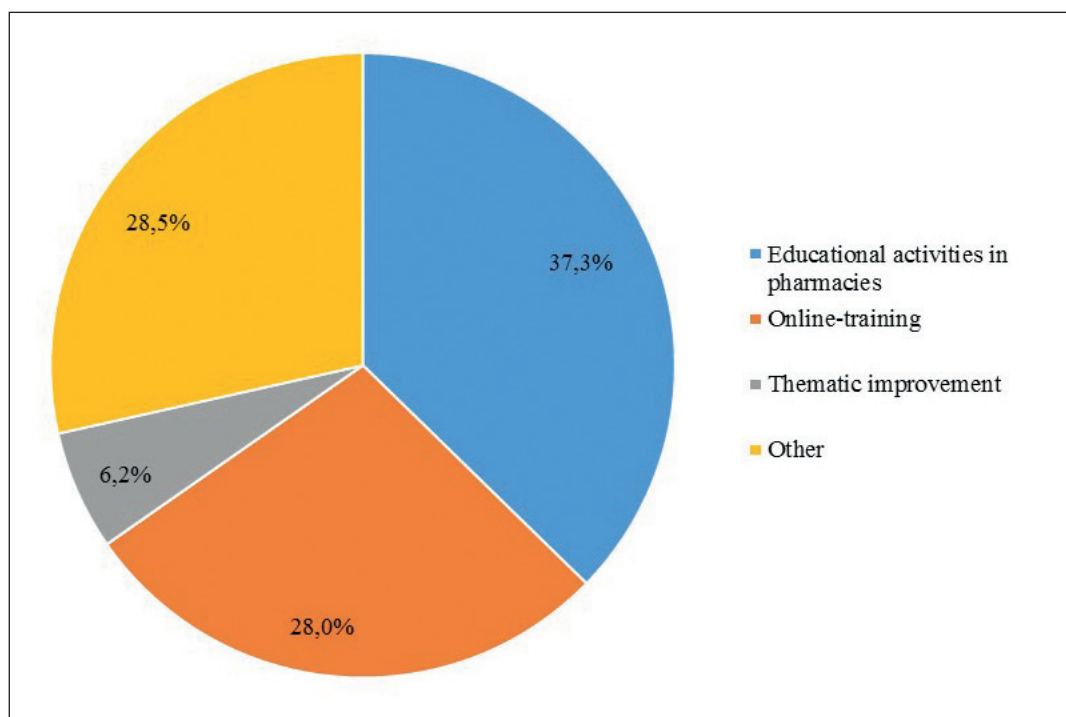
The pharmaceutical sector, as a part of the health care system, is at the forefront of the pandemic. Today, in the health care system, pharmaceutical workers are the most accessible group of professionals to provide medical and pharmaceutical care to the population. This trend continues in the context of the coronavirus infection spread around the world, as pharmacies are becoming an important point of contact for pharmacists with patients who need clear and reliable information, as well as relevant recommendations for prevention and symptomatic treatment with antipyretics. This becomes specifically important considering the general load on the health care systems.

During the fight against COVID-19, pharmacies in many countries around the world acquired new responsibilities,

which eventually led to an expansion of the range of tasks of their specialists. Research of scientific and special literature allows us to define the following pharmacists' directions of activity which have appeared in recent years:

- ensuring the safety of workers and pharmacy visitors in daily practice (observance of social distancing, possibility of making disinfectants);
- providing up-to-date information on prevention and treatment of COVID-19, conducting consultations at a distance (organization of employee training by the management of the institution, the need for continuous self-improvement on specific issues);
- organization of home delivery of drugs for patients who are on isolation;
- testing centers creation on the basis of pharmacies and participation in vaccination of the population.

The participation of pharmacists as members of the team of health professionals in the fight against Covid-19 in Ukraine was partially considered by Ukrainian scientists. Thus, in 2020 Hromovik B., Korolyov M. studied pharmacists opinion on the organizational aspects of work and ensuring their protection during quarantine due to the coronavirus pandemic [3], Zaliska O.M. et al. studied the effectiveness of quarantine measures implemented in accordance with Ukrainian legislation during the Covid-19 pandemic and the role of pharmacists in their provision in the workplace [4].



**Fig. 1.** The share of the distribution of pharmaceutical workers responses on forms of training on combating COVID-19.

## THE AIM

The aim of the study is to investigate the pharmacists level of readiness to provide assistance to pharmacy visitors on COVID-19 and ways to expand their social role in society during the pandemic.

## MATERIALS AND METHODS

For the purposes of this research, we used Ukrainian laws and policies on prevention of coronavirus disease emergence and spread, the official websites of pharmaceutical companies and pharmacy networks, statistics and other public information on the research, the results of online surveys and questionnaires of Ukrainian pharmacies.

The methods of sociological research, graphic, mathematical and statistical analysis, as well as generalization of the obtained results have been used in the work. The objects of the study were pharmaceutical workers of pharmacies. The survey was conducted in August 2021 via online survey of the target audience in professional groups at the social network "Facebook" and via direct survey of pharmacy professionals in Kyiv. The total number of respondents was 412 people, including 73.8% with higher and 26.2% with secondary pharmaceutical education. Respondents represented 18 regions of Ukraine. The gender distribution among specialists: female – 97.8%, male – 2.2%. According to professional experience, the interviewed persons were distributed as follows: 1) up to 10 years – 22.8%; 2) from 11 to 20 years – 57.3%; 3) from 21 to 30 years – 16.5%; 4) over 30 years – 3.4%.

In our study, with a total population of more than 10,000 people, i.e. the number of specialists working in pharmacies

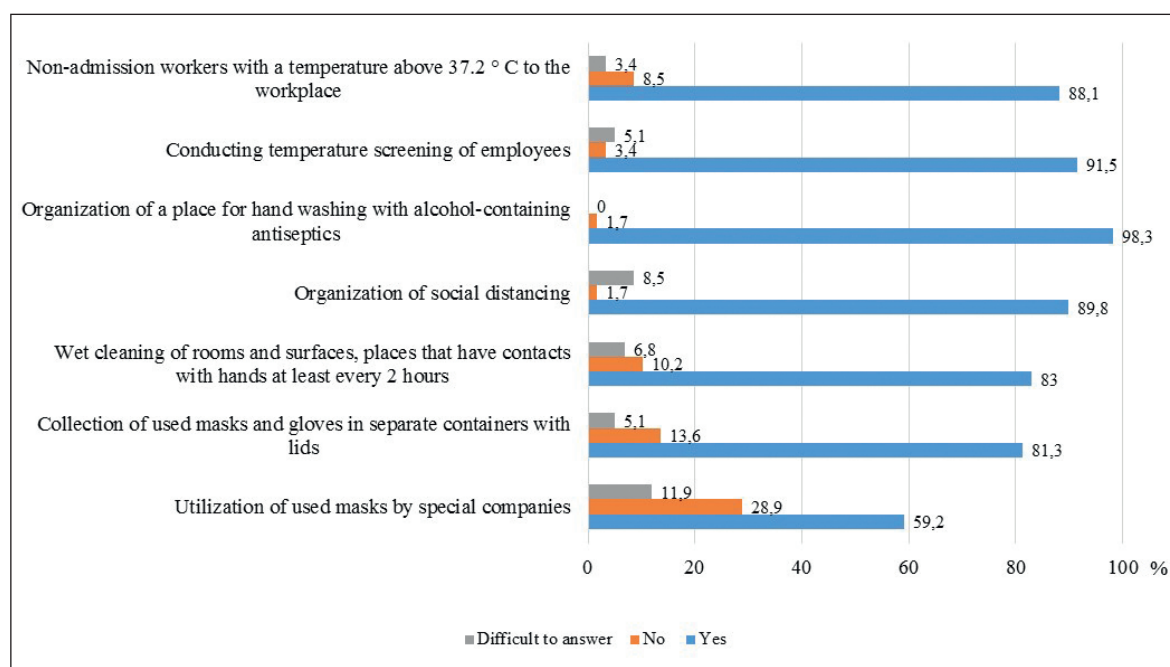
in Ukraine, the volume of a random sample is more than 385 ( $P = 0.95$ ). Thus, the obtained survey results indicate the representativeness of the sample within the allowable error limit of 5% [5].

## RESULTS

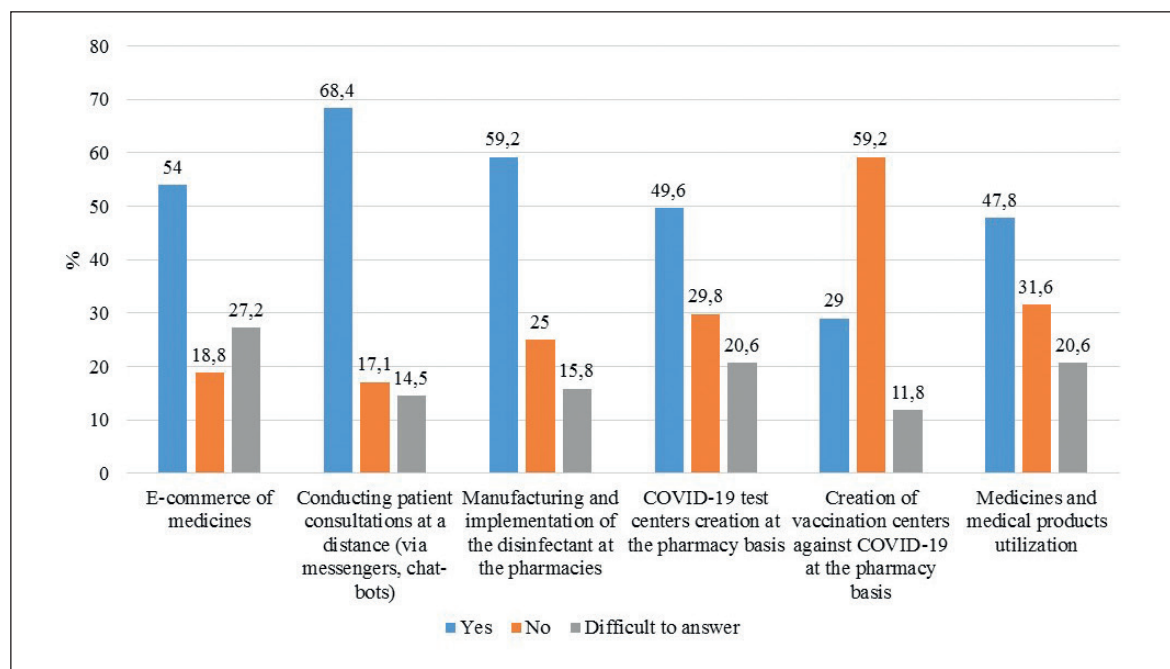
Ukrainian laws and policies on the medical and pharmaceutical care provision to the population at the time of COVID-19 pandemic have been analyzed. The results are presented in Table I [6-9].

From the pharmacists' professional activity point of view, the most important item in this list is the permission IAW the Law of Ukraine, dated 30.03.2020 № 539-IX, for use for persons suffering from a coronavirus disease unregistered medicines recommended by the official bodies of the United States of America, Member States of the European Union, the United Kingdom, the Swiss Confederation, Japan, Australia, Canada, the People's Republic of China, the State of Israel for the treatment of coronavirus disease (COVID-19) in the country concerned, and the use of registered medicinal products, provided that there is a proven efficacy in the treatment of coronavirus disease (COVID-19) and/or if such drugs are recommended by the authorities of the above countries.

Organizational aspects of safe operation of pharmacies in the period of the coronavirus infection pandemic are defined in the Standard of pharmaceutical care "Coronavirus disease (COVID-19)", approved by the Order of the Ministry of Health, dated 28.03.2020, №722 [9]. Given the importance of pharmacists' familiarization with the main points of this standard, the results of the



**Fig. 2.** The share of pharmaceutical workers' responses to the expansion of their functions, given the importance of their participation in the fight against COVID-19.



**Fig. 3.** The share of pharmaceutical workers' responses regarding the implementation of recommendations for anti-epidemic measures in pharmacies in connection with the spread of COVID-19.

survey showed that 84.5% of respondents are familiar with the content of the standard of pharmaceutical care "Coronavirus Disease (COVID-19)". However, in our opinion, a significant percentage of specialists, namely 15.5%, are not familiar with the standard and do not pay due attention to this document. An important aspect in pharmaceutical specialists' work is continuous professional development, especially during the new and unknown pandemic. Today, there is a significant variety

of forms and sources of relevant information, but only 39.1% of respondents received thematic training directly on combating COVID-19. At the same time, 63.9% of the surveyed pharmaceutical workers said that they need additional knowledge on the spread, prevention, and treatment of this disease. The most common forms of acquiring the necessary knowledge and practical skills were educational activities in pharmacies (37.3%) and participation in online training (28.0%) (Fig. 1).

**Table I.** Ukrainian laws and policies on the medical and pharmaceutical care provision at the time of COVID-19 pandemic.

Title	Official standing
Clinical guideline «Clinical management of patients with COVID-19», 2021	Recommended document based on the WHO guideline «Clinical management of COVID-19: interim guidance» (27.05.2020)
Amendments to Certain Laws of Ukraine on the Coronavirus Disease (COVID-19) Treatment Provision	The Law of Ukraine, dated 30.03.2020, № 539-IX
Clinical protocol «Medical care provision for the treatment of the Coronavirus Disease (COVID-19)»	Order of the Ministry of Health of Ukraine, dated 02.04.2020, № 762
Standard of emergency medical care «Coronavirus Disease (COVID-19)»	Order of the Ministry of Health of Ukraine, dated 28.03.2020, № 722 «Organization of medical care for patients with the Coronavirus Disease (COVID-19)»
Standards of medical care «Coronavirus Disease (COVID-19)»	
Standard of pharmaceutical care «Coronavirus Disease (COVID-19)»	

**Table II.** Classification of preventive measures for COVID-19 according to the type of prevention.

Type of prevention	Preventive measures
Non-specific	<ul style="list-style-type: none"> <li>- leading a healthy lifestyle;</li> <li>- compliance with facemasks requirements;</li> <li>- compliance with the rules of social distancing;</li> <li>- avoiding places with large crowds;</li> <li>- daily implementation of basic rules of personal hygiene (handwashing with detergent, handwashing with disinfectant, use of disposable wipes when sneezing and coughing);</li> <li>- carrying out regular cleaning and disinfection of working surfaces and personal belongings</li> </ul>
Specific	Use of vaccines against COVID-19: <ul style="list-style-type: none"> <li>- RNA vaccines (Pfizer-BioNTech, Moderna);</li> <li>- inactivated vaccines (Sinoparm, Bharat Biotech);</li> <li>- viral vector vaccine (Sputnik V, AstraZeneca, Cansino Biologics);</li> <li>- peptide vaccine (EpiVacCorona)</li> </ul>
Prophylactic medicines	<ul style="list-style-type: none"> <li>- vitamin C in daily dosage 500 mg, per os;</li> <li>- B-group vitamins;</li> <li>- vitamin D3 1000-3000 U daily;</li> <li>- Zinc compounds 30-50 mg daily;</li> <li>- Quercetin in daily dosage 250 mg;</li> <li>- Omega-3 saturated acid preparations;</li> <li>- vitamin E;</li> <li>- Magnesium compounds;</li> <li>- melatonin</li> </ul>

## DISCUSSION

According to the public information on the subject of the study, it was established that the main sources of up-to-date information on COVID-19 for medical and pharmaceutical workers, as well as for the population are the WHO recommendations for the public and the professional community, thematic pages of official websites of the Ministry of Health of Ukraine, Public Health Center of Ukraine, the official informational portal of the Ministry of Health of Ukraine "Coronavirus in Ukraine", the website "All about vaccination in Ukraine", and in particular with a version for the visually impaired users. The recommendations of the WHO and the Ministry of Health of Ukraine on the prevention of COVID-19 are based on the implementation of three main rules of anti-epidemic safety: social distance, face masks wearing and ensuring hands hygiene. In case of fever, cough, shortness of breath, it is necessary to urgently consult with a family doctor, and if you cough and sneeze, you should follow appropriate hygienic measures [9-10].

In order to eliminate the pandemic state of coronavirus infection, considerable attention should be paid to the disease prevention. Based on scientific and official information on the subject of this study, we have summarized possible preventive measures to combat COVID-19. The results are presented in the Table II. A specific method of prevention (vaccination) is now actively used by health systems of all countries [10-14].

In accordance with the principles of international standards of Good Pharmacy Practice, an important role is assigned to support and improvement of pharmacy specialists' professional activities. Pharmacists should constantly update their knowledge and be involved, where possible, in the new technologies and automatization implementation into daily practice. Due to the increasing social burden on pharmaceutical workers in society, there is a question of expanding the range of pharmaceutical services provided in the pharmacy as one of the health care facilities. Thus, according to the results of our study, 86.2% of respondents



noted the increasing importance of specialists in combating coronavirus infection. The participants of the survey in Ukraine proposed 6 new functions, which are now actively implemented in pharmaceutical practice in many countries. According to the results presented on Fig. 2, it can be noted that the vast majority of respondents agreed with the expediency of digitalizing their professional activities (e-commerce of medicines, remote consultations), but are not ready to provide vaccination in pharmacies.

Today, an important aspect in combating COVID-19 is compliance with the sanitary-epidemic regime in everyday life, and in particular by pharmacy specialists directly in the pharmacies. For the most part, pharmaceutical workers follow the recommendations for anti-epidemic measures, but more than 40% of respondents do not want to participate in the disposal of used masks with the involvement of professional companies (Fig. 3).

The current state of readiness of pharmacists to counteract COVID-19 demonstrates a significant focus of specialists on the patient, his counseling, providing evidence of drugs, as well as the feasibility and need to expand the range of pharmaceutical services that can be provided within the pharmacy. During the spread of coronavirus infection, pharmacy specialists also play a significant role in conducting health education among the population to increase medical literacy in the treatment and prevention of COVID-19, as well as the importance of vaccination to prevent serious consequences of the disease. In our opinion, the creation of vaccination centers in pharmacies and the involvement of pharmacists in this procedure will be promising.

## CONCLUSIONS

The practical significance of the obtained results is that together they create a scientific and methodological basis for improving the process of pharmaceutical workers participation in combating coronavirus infection.

1. The legal support of medical and pharmaceutical care at COVID-19 in Ukraine has been analyzed. It is established that the relevant legislative documents regulate all aspects of these processes, including organizational issues of safe operation of pharmacies in a pandemic.
2. The results of the survey of pharmaceutical workers indicate a high level of need (63.9% of respondents) for the continuous acquisition of relevant information on COVID-19, given the spread of new pathogen mutations. It was found that only 39.1% of respondents received thematic training directly on combating COVID-19, which is insufficient and requires attention from the management of pharmacies.
3. Given the importance of prevention in overcoming coronavirus infection, we grouped possible organizational measures to combat COVID-19 according to their type (non-specific, specific, and prophylactic medicines). Today, the most relevant is specific method of prevention (vaccination), which is now actively used by health systems around the world.
4. According to the results of the study, the growth of the social role of pharmacy specialists in combating coronavirus infection has been proved, which was supported by 86.2% of respondents in their daily activities. The possibility of introduction of 6 new functions to Ukrainian pharmacies has been considered, and pharmaceutical workers' concerns regarding these new functions, which are already actively realized in pharmacy practice in many countries of the world, has been investigated.
5. Based on the analysis of the implementation level of recommendations on the anti-epidemic measures in pharmacies by pharmaceutical workers, it has been established that these activities are predominantly carried out in practice. However, there is a need for a legislative solution to the issue of participation of pharmacy specialists in the process of used medical masks disposal by professional companies, given that participation in the disposal of unusable drugs and medical devices is one of the requirements of international standards of Good Pharmacy Practice.

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*The Authors declare no conflict of interest.*

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

## ORIGINAL ARTICLE

# CORRELATION BETWEEN PRIMARY TUMOR SIZES WITH PROGNOSTIC MARKERS IN BREAST CARCINOMA IN IRAQI WOMEN: IMMUNOHISTOCHEMICAL STUDY

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## ABSTRACT

**The aim:** The study aimed assessment of immunohistochemical expression of ER, PR, Ki-67 and HER2 in breast carcinoma, studied the relation between size of primary tumor and these markers and distribution of molecular subtypes between both study groups.

**Materials and methods:** The study was implemented immunohistochemistry laboratories of Al-Sadder Teaching Medical City in Al Najaf during the period from September 2020-september2021, forty four women with breast carcinoma who undergone modified radical mastectomy were involved in this study, aged between 29 -81 years, mean age being 47.3 yr. we divided study group into two categories; depending on tumor size, with cutoff point of 2 cm. Envision technique applied for evaluation of expression of ER, PR, Ki-67 and HER2.

**Results:** Among all patients, ER expressed in 70.45%, PR in 68.18%, HER2/neu in 18.18%, High ki-67 index in 52.27%.

**Conclusions:** Molecular subtype luminal A tend to occur in smaller tumor size compared to basal subtype which tend to occur in larger size of tumors. Breast carcinoma tumor size showed no significant correlation regarding histological grade, immunohistochemical expression of ER, PR, HER2, and Ki-67 labeling index.

**KEY WORDS:** breast cancer, immunohistochemistry, molecular subtypes

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## INTRODUCTION

Breast cancer ranks the first position among all cancers regarding incidence and cancer related death among Iraqi population in 2020 according to WHO international agency for research cancer. On early 2000s, breast cancer was categorized into molecular subtypes; luminal A, luminal B, HER2 positive and basal like, with increased clinical interest in these subtypes for their clinical correlation regarding response to treatment and prognosis. Breast carcinoma is the most common malignant tumor and the leading cause of carcinoma death in women [1]. Several factors are candidate to play a role in predicting possibility of distant metastasis and advanced clinical stage. Of these factors, ER, PR, Ki-67 and HER2 will be focused on in this study [2]. The choice between surgical options for breast cancer depends on a host of parameters, TNM being one of most important parameters [3].

## THE AIM

The aim of this research was in assessment of Ki-67 labeling index, HER2/neu, estrogen and progesterone over expression in breast cancer and investigate correlation of these prognostic markers with the primary tumor size.

## MATERIALS AND METHODS

We accomplished this research in the histopathology and immunohistochemistry laboratories of Al-Sadder Teaching Medical City in Al Najaf during the period from September 2014 -September 2015.

## SAMPLING OF CASES

Researchers collected formalin-fixed blocks of already confirmed cases of breast cancers from 44 ladies, who undergone modified radical mastectomy, aged between 29-81 years, mean age being 47.3yr. Total sample was classified into following groups:

- Study group: 24 cases whom tumor size was  $\leq 2$ cm.
- Comparative group: 20 cases with tumor size  $>2$  cm.

## CONTROL GROUP

We used Positive and negative control Slides for Ki-67, ER, PR and HER-2/neu, in breast cancer with each run.

## MATERIALS AND EQUIPMENT

Primary Antibody

- **Ki-67:** monoclonal mouse antihuman ki67 antigen, clone MIB-1; Dako, code-Nr.M7240.
- **HER2/neu:** Polyclonal Rabbit Anti- Human c-erbB-2 Oncoprotein; Dako, Code No. A0485.
- **Estrogen:** Monoclonal mouse anti-human estrogen receptor  $\alpha$ ; Dako, Code No. M7047.
- **Progesterone:** Monoclonal mouse antihuman progesterone receptor; Dako, Code No. M3569.

We used Envision technique in this immunohistochemical study for HER-2/neu, ER and PR.

#### Scoring System

- Herceptest system was applied for HER-2/neu (Fig. 1).
- Allred scoring guidelines used for ER and PR scoring (Fig. 2, 3).
- Ki-67 positive case diagnosis required dark brown precipitate of nucleus, then we counted the percentage of number of positive stained nuclei in a total 1000 malignant cells per 10 hpf, labeling index was calculated as demonstrated in table I and figure 4.

## STATISTICAL ANALYSIS

Statistical analyses of all results were performed by the help of SPSS software statistical package (version 15) by using Chi-Square test, P-value at level of significance  $< 0.05$ .

## RESULTS AND DISCUSSION

### IMMUNOHISTOCHEMICAL PARAMETERS

#### THE GENERAL IMMUNOHISTOCHEMICAL EXPRESSION

Our results were similar to other study from Iraq: Runnak and al. (Table II) [4].

#### THE ER EXPRESSION

There is no significant difference between the two patient groups  $P > 0.05$  (Table III, Fig.5). Our results are consistent with those of Haroon et al, Zhou et al. and Zore et al [5-7] who found no correlation between ER expression and tumor size. Our results are inconsistent with those of Azizun-Nisa et al. and Fatima et al. [8-9] who found that ER positivity decreased with increased tumor size and with Sofi G.N. et al who found that larger size tumors` tend to be ER negative [10]. These differences are likely due to preanalytic variables, thresholds for positivity, and interpretation criteria.

#### THE PR EXPRESSION

These results are consistent with those of Panahi et al and Siadati et al. both from Iran, Zore et al from Croatia, and Zhou et al., from China who didn't conclude a correlation between PR expression and tumor size [11-12]. These results are inconsistent with those of Sofi et al., Liu et al

and Thike et al. who found no correlation between tumor size and PR expression [10-14]. These differences could be due to preanalytic variables, thresholds for positivity, and interpretation criteria, or because these studies used different scoring systems and immunohistochemical techniques. Regarding Thike et al, difference in results may be due to different scoring method used as they used the H-score system while the Allred scoring system was used in the present study (Table IV, Fig. 6).

### THE HER2/NEU EXPRESSION

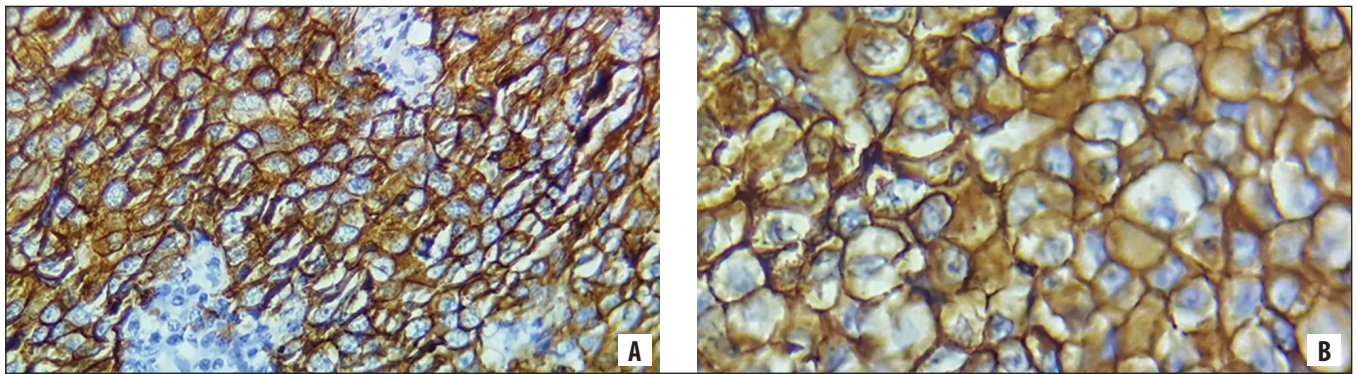
There is no significant difference between the two patient groups in HER2/neu expression ( $P > 0.05$ ). These results are consistent with those of Nag et al., Patnayak R. et al., Ning et al., Zore et al. and Ambroise et al. [15-18] who found no correlation between HER2 expression and tumor size. However, Vaidyanathan et al. and Kumar et al. reported positive correlation [19-20]. This difference could be due to different methods used in assessment of HER2 expression, in the present study we used immunohistochemistry testing alone, Vaidyanathan et al. used immunohistochemistry as a primary estimate, followed by fluorescence in situ hybridization (FISH). We agree with Ambroise et al. [21] regarding the concept of using only positive cases detected by FISH analysis will affect the results especially for cases that are equivocal (2+) in IHC HER-2 as shown in a recent article from south India (Table V, Fig. 7) [22].

### THE KI-67 LABELING INDEX

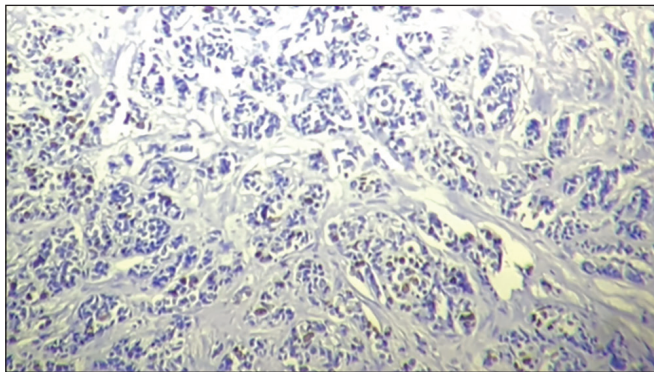
There is no significant difference between the two patient groups in Ki-67 labeling index ( $P > 0.05$ ). These results are consistent with those of Inwald et al., Bonta et al. and Zore et al. [23-24]. These results are inconsistent with those Gnozalez-Sistal A. et al., Yamamoto et al. and Rudolph et al. [25-27] who found a significant association between tumor size and immunohistochemically-augmented expression of Ki-67 and that high Ki-67 labeling index associated with increased tumor size. This difference could be due to sampling method, as Rudolph chose cases of invasive ductal carcinoma who were all without lymph node involvement, or differences may be due to different laboratory testing methods used as for Yamamoto they used real-time quantitative reverse-transcription polymerase chain reaction (RT-qPCR) assay system for detection of Ki-67 labeling index (Table VI, Fig. 8).

### MOLECULAR SUBTYPES

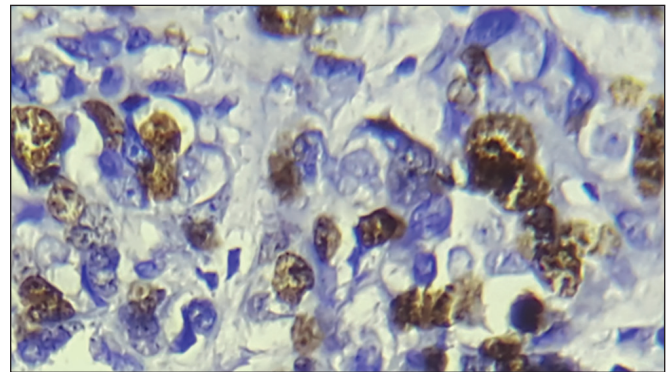
The prevalence of luminal B in this study (36.37%) was near to those of Cheang et al. (32%) [28], as compared to that of Al Tamimi et al. (16%) [29]. The reason may be that Cheang et al. have used a Ki-67 proliferation index of 13.25% as a cutoff between luminal A and Luminal B, as compared with no cutoff used by Al Tamimi et al., regarding molecular subtypes correlation to tumor size there was a significant difference between two patient



**Fig. 1.** Invasive ductal carcinoma, showing strong membranous staining (score 3+), with fishnet appearance (immunohistochemical stain for HER2/neu); (A: 20X); (B: 40X).



**Fig. 2.** Invasive ductal carcinoma, showing nuclear staining, IHC, for ER score 7 (10X).



**Fig. 3.** Invasive ductal carcinoma showing PR immunohistochemical nuclear staining score 7 (40X).

groups ( $P < 0.05$ ). Significant difference was observed between luminal A and basal subtypes regarding tumor size ( $P = 0.004$ ), which suggest that tumors of luminal A subtypes tend to be smaller size ( $T \leq 2$  cm) while tumors

of basal subtype tend to be of larger size. Our results were close to those in research by Al-Brahim et al., who found that the basal-like and HER2 subtypes were significantly associated with larger tumor size among Tunisian women with breast cancer [30]. These results are also consistent with those of Spitale et al., Garcia Fernandez A. et al. and Yang et al., who found similar correlation between tumor size and molecular subtypes [31-33]. Our results are inconsistent with those of Widodo et al., Kadivar et al. and Pracella et al., who found no correlation between

**Table I.** Ki-67 labeling index.

Ki-67 index	Labeled cells [%]
Low	<14
High	$\geq 14$

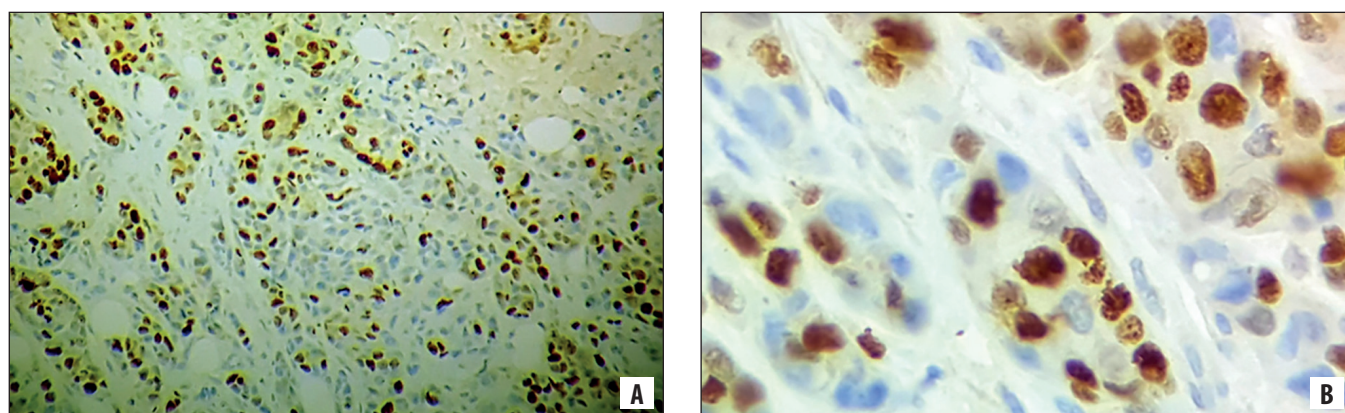
**Table II.** General Immunohistochemical Expression in the two groups of patients.

Tumor size [cm]	Number of patients	ER positive		PR + ve		HER2/neu + ve		High ki-67 index	
		No.	[%]	No.	[%]	No.	[%]	No.	[%]
$T \leq 2$	24	18	75	17	70.83	7	29.16	11	45.83
$T > 2$	20	13	65	13	65	1	5	12	60
Total	44	31	70.45	30	68.18	8	18.18	23	52.27

**Table III.** ER expression in the two groups of patients.

Tumor size [cm]	Number of patients	ER + ve		ER - ve		P value
		No.	[%]	No.	[%]	
$T \leq 2$	24	18	75	6	25	$P > 0.05$
$T > 2$	20	13	65	7	35	
Total	44	31	70.45	13	29.54	





**Fig. 4.** Invasive ductal carcinoma moderately differentiated, showing immunohistochemical nuclear staining for Ki-67 with labeling index >14%. (A: 10X), (B: 40X).

**Table IV.** PR expression in the two groups of patients.

Tumor size [cm]	Number of patients	PR +ve		PR -ve		P Value
		NO.	[%]	NO.	[%]	P>0.05
T≤2	24	17	(70.83)	7	(29.16)	
T>2	20	13	(65)	7	(35)	
Total	44	30	(68.18)	14	(31.81)	

**Table V.** HER2/neu expression in the two groups of patients.

Tumor size [cm]	Number of patients	HER2/neu +ve		HER2/neu -ve		HER2/neu Equivocal		P Value
		No.	[%]	No.	[%]	No.	[%]	
T≤2	24	7	29.16	11	45.83	6	25	P>0.05
T>2	20	1	5	10	50	9	45	
Total	44	8	18.18	21	47.72	15	34.09	

**Table VI.** Ki-67 labeling index the two groups of patients.

Tumor size [cm]	Number of patients	Low Ki-67 Labeling Index		High Ki-67 Labeling Index		P value
		No.	[%]	No.	[%]	
T≤2	24	13	54.16	11	45.83	P>0.05
T>2	20	8	40	12	60	
Total	44	21	47.732	23	52.27	

**Table VII.** Molecular subtypes in the two groups of patients.

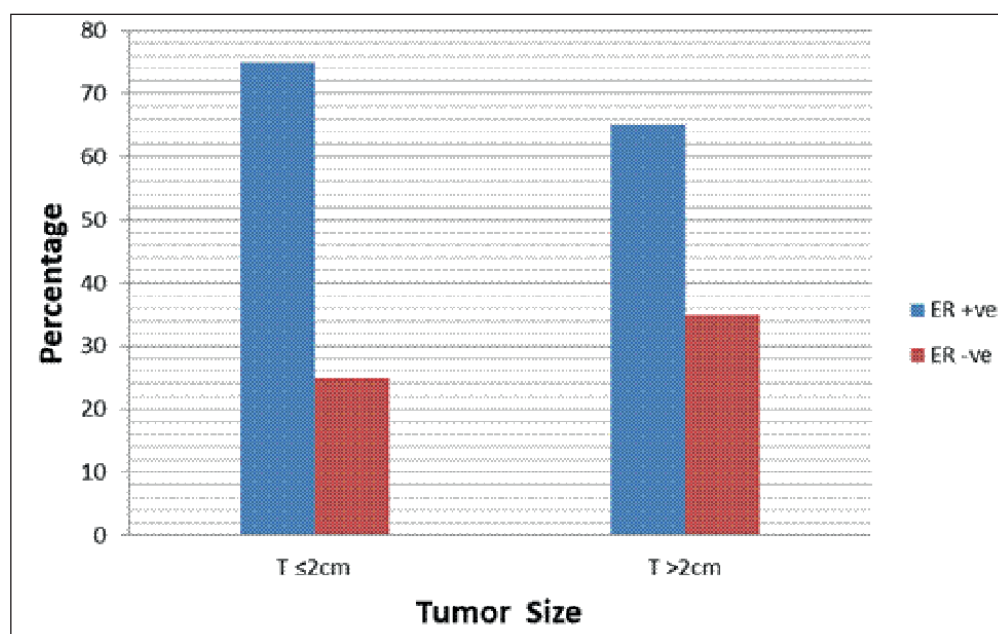
Tumor size [cm]	Number of patients	Luminal A		Luminal B		HER2/neu positive		basal like		P Value
		No.	[%]	No.	[%]	No.	[%]	No.	[%]	
T≤2	24	11	45.83	9	37.51	4	16.66	0	0	P<0.05
T>2	20	4	20	7	35	4	20	5	25	
Total	44	15	34.09	16	36.37	8	18.18	5	11.36	

tumor size and molecular subtypes [34-36]. Bhargava et al. [37] in their study concluded that these subtypes may be intrinsic and predetermined leading to weak correlation with size (Table VII, Fig. 9).

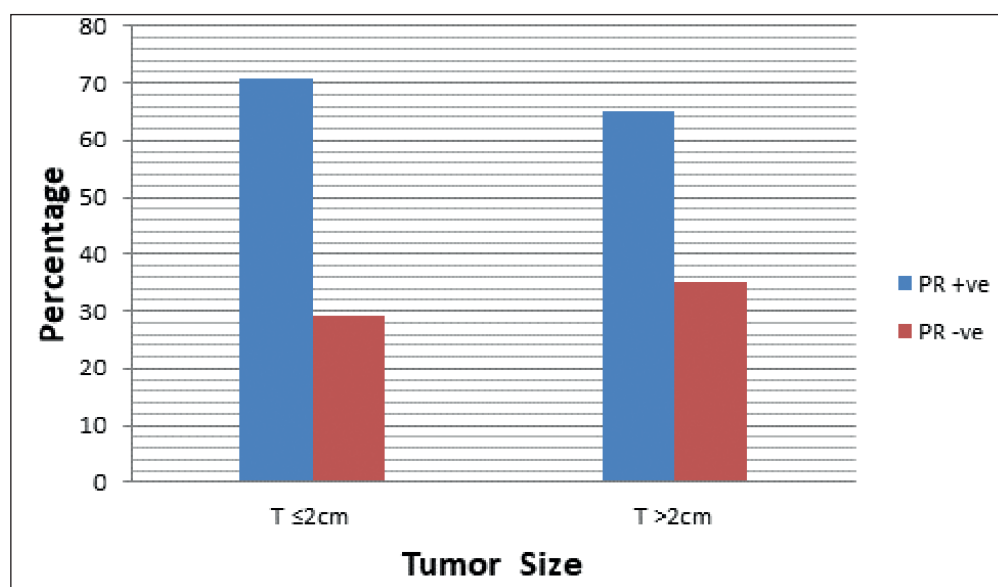
There were three main restrictions in this study:

- We used immunohistochemical technique for assessment of ER, PR and HER2 status, which gives

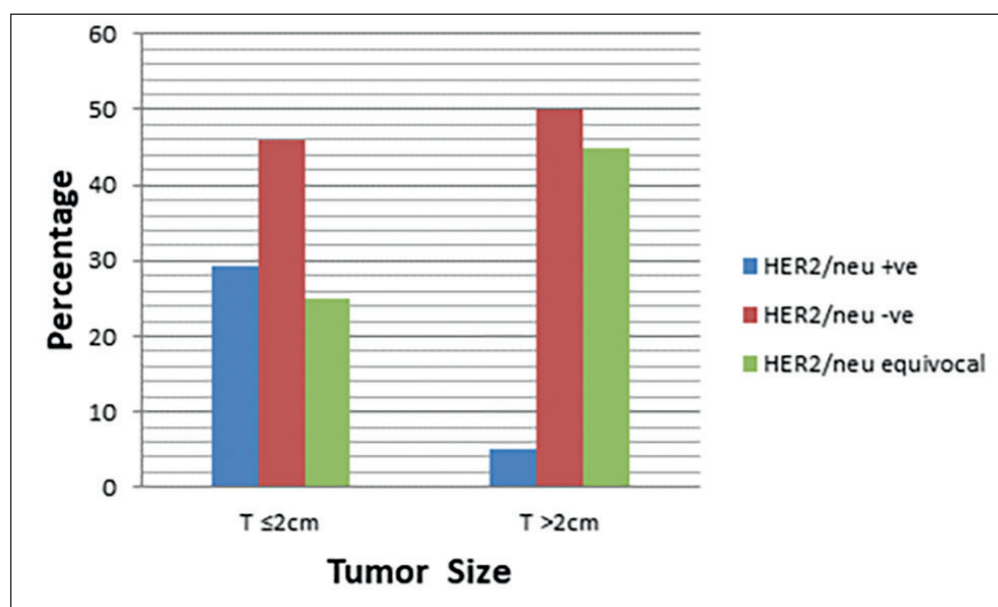
only approximate result when compared to gene expression. Nevertheless, IHC profiles used less expensive, affordable clinical receptors (Brenton et al., Carey et al., Nguyen et al., Wiechmann et al.). Compared to immunohistochemistry, Genotyping is not performed in all laboratories, needs longer time to obtain results, and has financial burden on patients Abdelkrim et al. [38-42].



**Fig. 5.** ER expression in the two groups of patients.



**Fig. 6.** PR expression in the two groups of patients.



**Fig. 7.** HER2/neu expression in the two groups of patients.

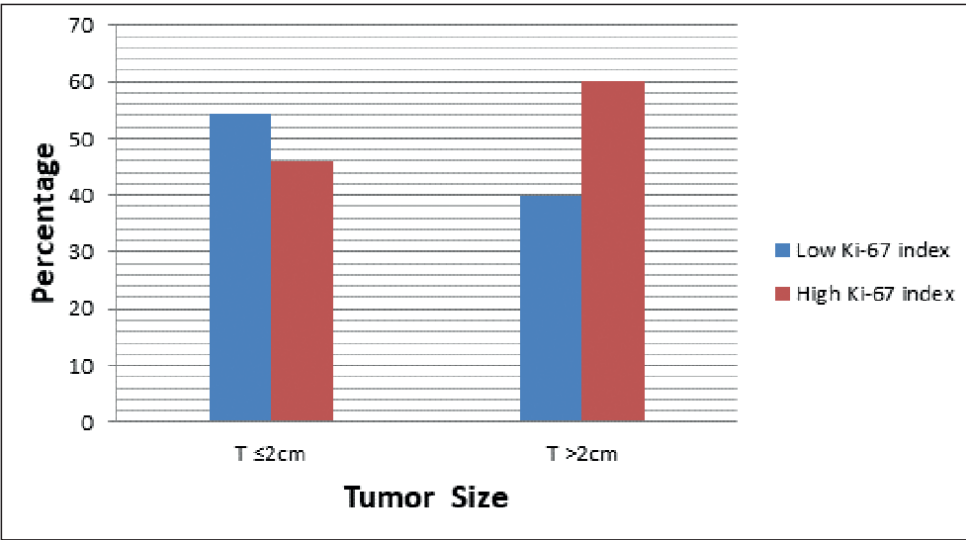


Fig. 8. Ki-67 Labeling index in the two groups of patients.

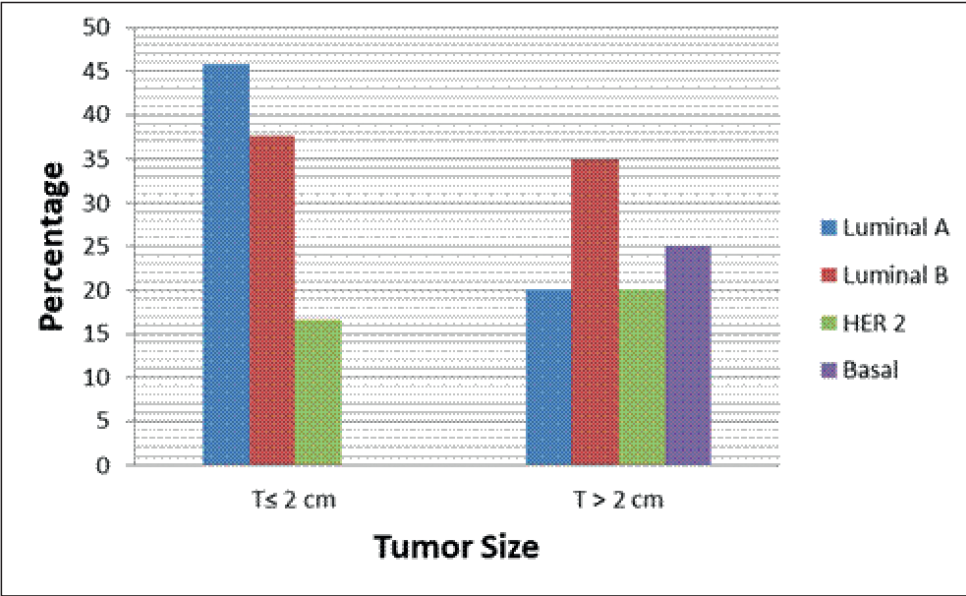


Fig. 9. Molecular subtypes in the two groups of patients.

- Cases with score 2+ for HER-2 by IHC were regarded as negative category, they needed further assessment by FISH technique.
- Carey et al. [39] defined basal-like subtype by gene expression as “ER negative, PR negative, HER-2 negative, cytokeratin 5/6 positive, and/or Her-1(EGFR) positive” which is not identical to triple negative state.

CONCLUSIONS

1. Molecular subtype luminal A tend to occur in smaller tumor size compared to basal subtype, which tend to occur in larger size of tumors.
2. Breast carcinoma tumor size showed no significant correlation regarding histological grade, immunohistochemical expression of ER, PR, HER2, and Ki-67 labeling index.

It is the biological behavior rather than clinic pathological features of breast carcinoma determine prognosis. So tumor size can't be considered as dependable parameter in determining the type of surgical operation.

RECOMMENDATIONS

Further studies focusing on differences over clinical stages of breast carcinoma using DNA/mRNA and protein levels by FISH or PCR techniques to unveil molecular aspect of mentioned biomarkers.

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## ORIGINAL ARTICLE

## EPOXID HYDROLASE SINGLE GENE POLYMORPHISM (RS1051740) AND SEVERITY OF CHRONIC OBSTRUCTIVE DISEASE

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### ABSTRACT

**The aim:** The aim of the present study was to establish a link between polymorphic variants of the microsomal epoxide hydrolase gene and the severity of COPD in patients with COPD and coronary heart disease.

**Materials and methods:** The study included 128 patients with COPD and IHD, who were divided into two groups: group 1 included 72 patients with infrequent exacerbations of COPD (0-1 per year) and group 2 included 56 patients with frequent exacerbations of COPD (exacerbation of COPD  $\geq 2$  per year). The control groups consisted of 15 smokers without COPD and IHD, 11 practically healthy non-smokers and 11 patients with IHD who do not smoke. All patients underwent DNA isolation and purification, followed by determination of the Tyr113His polymorphism of the EPHX1 microsomal epoxide hydrolase gene (rs1051740).

**Results:** There was a significant association of the carriage of the CC genotype of the EPHX1 gene in patients with COPD and IHD (RO = 21.326 [95.0% CI 4.217–107.846],  $p < 0.001$ ) with a more severe course of COPD compared with the TT genotype of the EPHX1 gene.

**Conclusions:** Patients with COPD and coronary heart disease who were carriers of a homozygous variant CC of the EPHX1 gene have a reliable association with a more severe course of COPD with frequent exacerbations (higher class according to GOLD classification and more severe symptoms of COPD according to the CAT questionnaire).

**KEY WORDS:** coronary heart disease, chronic obstructive pulmonary disease, exacerbations of chronic obstructive pulmonary disease, microsomal epoxide hydrolase, epoxide hydrolase, EPHX1

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### INTRODUCTION

Chronic obstructive pulmonary disease (COPD) due to the high prevalence and steady increase in the number of patients with this pathology, occupies one of the leading positions as a cause of mortality and disability of the world's population according to the World Health Organization (WHO). Exacerbation is considered to be the most unfavorable marker of COPD progression. Exacerbation of COPD is a long-term deterioration of the patient's condition that goes beyond normal daily changes, which has an acute onset and may require medication changes and/or hospitalization. The effect of exacerbations on lung function is cumulative and has a long-term negative impact on the patient's health [1,2].

One of the features of COPD is development over the age of 40 years, which can usually coincide with the manifesto of coronary heart disease (CHD) [3]. These diseases have a mutually aggravating effect on each other. CHD presence is associated with increased risk for hospitalization, longer length of stay and all-cause and CVD-related mortality. Patients with COPD have a higher risk of acute cardiovascular events, and about 25-30% die from cardiovascular disease [4]. Of course, coronary heart disease and COPD combine both common risk factors and common pathogenesis. Among the risk factors, the most significant is smoking [5].

Although smoking is a powerful risk factor for COPD, it should be noted that not all smokers develop the disease. According to Ward B.W. et al. COPD affects only 10-15% of smokers, which, in turn, indicates a complex interaction of genetic factors, individual behavior and environmental influences [6]. Detection of genetic markers of risk of COPD in smokers acquires both clinical and social significance. An interesting target for research, in this regard, will be polymorphic variants of genes responsible for the metabolism of toxic substances that come with tobacco smoke. Of course, the interaction of tobacco smoke with the epithelium of the bronchi has a complex character. At the same time, it has been proven that microsomal epoxide hydrolase (EPHX1) is actively involved in the detoxification of epoxy intermediates, one of the substances present in cigarette smoke and has a proven negative effect on the bronchial epithelium [7].

The human EPHX1 gene is located on chromosome 1q42.12 and consists of nine exons. Polymorphism rs1051740 in exon 3 of the EPHX1 T113C gene can reduce the activity of the enzyme approximately twice (slow allele) [8,9]. Li H. et al. presented the results of a meta-analysis that included 8,259 COPD patients and 42,883 controls. The study found an association between slow activity of the enzyme EPHX1 and an increased risk of COPD, while rapid activity of the enzymes

**Table I.** Clinical Characteristics of Patients in Comparison Groups and Control Groups (n = 165)

Indexes	1 <sup>st</sup> Group (with infrequent exacerbations of COPD) n = 72	2 <sup>nd</sup> Group (with frequent exacerbations of COPD) n = 56	3 <sup>rd</sup> group (Smokers) n = 15	4 <sup>th</sup> Group (Healthy people) n = 11	5 <sup>th</sup> group (CHD) n = 11
Age, years (Me [Lq; Uq]) <sup>c</sup>	62,0 [58,0; 70,0]	66,0 [59,0; 72,3]	62,0 [55,5; 65,0]	63,5 [61,3; 64,8]	68,0 [62,3; 78,8]
Duration of COPD years, (Me [Lq; Uq])	5,0 [3,0; 10,0]	9,0 [5,0; 11,0] <sup>b</sup>	0,0 [0,0; 0,0]	0,0 [0,0; 0,0]	0,0 [0,0; 0,0]
Duration of coronary heart disease, years, (Me [Lq; Uq])	5,0 [3,0; 10,0]	5,0 [3,0; 10,0]	0,0 [0,0; 0,0]	0,0 [0,0; 0,0]	4,5 [3,0; 8,8]
Number of COPD exacerbations per year (Me [Min; Max])	1.0 [1.0; 2.0]	2.0 [2.0; 4.0] <sup>b</sup>	0.0 [0.0; 0.0]	0.0 [0.0; 0.0]	0.0 [0.0; 0.0]
History of myocardial infarction in the anamnesis, n (%) <sup>1</sup>	21 (27.6)	7 (12.5)	0 (0.0)	0 (0.0)	4 (36.3)
Stroke, n (%) <sup>2</sup>	1 (1.3)	4 (7.1)	0 (0.0)	0 (0.0)	4 (36.3)
Diabetes mellitus, n (%) <sup>3</sup>	10 (13.2)	10 (17.5)	0 (0.0)	0 (0.0)	4 (36.3)
Hypertension, n (%) <sup>4</sup>	74 (97.4)	55 (96.5)	2 (13.3)	0 (0.0)	11 (100.0)
GOLD <sup>5</sup>	I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	II	54 (75.0)	24 (42.9)	0 (0.0)	0 (0.0)
	III	18 (25.0)	28 (50.0)	0 (0.0)	0 (0.0)
	VI	0 (0.0)	4 (7.1)	0 (0.0)	0 (0.0)
CAT, points (Me [Min; Max])	17.0 [6.0; 35.0]	21.0 [12.0; 36.0] <sup>b</sup>	0.0 [0.0; 4.0]	0.0 [0.0; 2.0]	1.0 [0.0; 2.0]
Smoking duration, years (Me [Lq; Uq])	32,0 [21,0; 42,0]	33,5 [24,5; 43,8]	31,0 [25,0; 44,0]	0,0 [0,0; 0,0]	0,0 [0,0; 0,0]

Note:

Between the main groups: <sup>1</sup> –  $\chi^2 = 4.417$  ( $p = 0.036$ ); <sup>2</sup> –  $\chi^2 = 3.004$  ( $p = 0.083$ ); <sup>3</sup> –  $\chi^2 = 0.490$  ( $p = 0.484$ ); <sup>4</sup> –  $\chi^2 = 0.086$  ( $p = 0.769$ ); <sup>5</sup> –  $\chi^2 = 15.962$  ( $p < 0.001$ ).

<sup>a</sup> – differences are significant in comparison with 1 group ( $p = 0.009$ );

<sup>b</sup> – differences are significant in comparison with 1 group ( $p < 0.001$ );

<sup>c</sup> – indicators did not differ significantly between all groups ( $H = 6.317$ ;  $p = 0.177$ ).

was not associated with a reduced risk of COPD [8]. One possible explanation for this relation is the accumulation of toxic epoxy products, prolonged exposure of the epithelium to their harmful effects while reducing the activity of the enzyme. At the same time, there is insufficient data on possible links between the presence of a mutation in the EPHX1 gene and the severity of coronary heart disease.

## THE AIM

The aim of the present study was to establish a link between polymorphic variants of the microsomal epoxide hydrolase gene and the severity of COPD in patients with COPD and coronary heart disease.

## MATERIALS AND METHODS

### SURVEYED POPULATION

Into the study conducted in the out-patient department of the State Institution “National Institute of Therapy named after L.

Malaya NAMS of Ukraine” will include 128 patients suffering from COPD with concomitant coronary heart disease, of which 29 (22.66%) women and 99 (77.34%) men. Control groups consisted of 15 smokers without COPD and coronary heart disease and 11 healthy smokers, 11 patients with coronary heart disease who do not smoke. The study included only patients who were receiving stable therapy for both COPD and coronary heart disease at the time of screening.

### DEFINITION OF COPD

The diagnosis of COPD was made according to the recommendations of Global initiative for Chronic Obstructive Lung Disease (GOLD, 2020). Patients were assigned to groups based on the assessment of FEV1 (GOLD I – FEV1  $\geq 80\%$  of the predicted; GOLD II 50-80% of the predicted; GOLD III 30-50% of the predicted; GOLD IV  $\leq 30\%$  of predicted), to assess shortness of breath and symptoms used a modified scale Medikal Research Council Dyspnea (mMRC) (mMRC 0-1 corresponds to groups A and C, and mMRC  $\geq 2$  B and D) and the CAT test (CAT  $< 10$  – group

**Table II.** Clinical characteristics of patients with COPD and coronary heart disease with different polymorphic variants of the microsomal epoxyhydrolase gene (n = 128).

Indexes		TT (n = 57)	TC (n = 52)	CC (n = 19)
Age, years (M ± SD)		65.0 [58.5; 73.0]	62.0 [57.0; 69.8]	68.0 [60.0; 73.0]
Gender <sup>1</sup>	F. abs. (%)	9 (15.8)	12 (23.1)	8 (42,1)
	M. abs. (%)	48 (84.2)	40 (76.9)	11 (57,9)
Duration of COPD years, (Me [Lq;Uq])		6.0 [4.0; 10.0]	6.0 [3.0; 10.8]	10.0 [5.0; 11.0]
Duration of coronary heart disease, years, (Me [Lq;Uq])		3.0 [2.0; 3.5]	5.0 [3.0; 10.0]	5.0 [3.0; 10.0]
Number of COPD exacerbations per year (Me [Min; Max])		1.0 [1.0; 4.0]	1.0 [1.0; 4.0]	2.0 [1.0; 3.0]
History of myocardial infarction in anamnesis, n (%) <sup>2</sup>		12 (21.4)	13 (25.0)	1 (5.3)
Stroke, n (%) <sup>3</sup>		2 (3.6)	3 (5.8)	0 (0.0)
Diabetes mellitus, n (%) <sup>4</sup>		7 (12.3)	10 (19.2)	3 (15.8)
Hypertension, n (%) <sup>5</sup>		55 (96.5)	51 (98.1)	19 (100.0)
GOLD <sup>6</sup>	I	–	–	–
	II	32 (57.1)	33 (67.3)	9 (47,4)
	III	23 (41.1)	14 (28.6)	9 (47,4)
	VI	1 (1.8)	2 (4.1)	1 (5,3)
CAT, points (Me [Min; Max])		19.0 [6.0; 35.0]	19.0 [9.0; 36.0]	21.0 [12.0; 34.0]
Smoking duration, years (Me [Lq; Uq])		32.0 [21.0; 41.5]	33.5 [22.5; 44.5]	30.0 [20.0; 40.0]

Note: Between all groups: <sup>1</sup> -  $\chi^2 = 5.640$  ( $p = 0.060$ ); <sup>2</sup> -  $\chi^2 = 3.386$  ( $p = 0.184$ ); <sup>3</sup> -  $\chi^2 = 1.260$  ( $p = 0.533$ ); <sup>4</sup> -  $\chi^2 = 0.997$  ( $p = 0.608$ ); <sup>5</sup> -  $\chi^2 = 0.834$  ( $p = 0.659$ ); <sup>6</sup> -  $\chi^2 = 3.480$  ( $p = 0.481$ ).

**Table III.** Frequency distribution of polymorphic variability of the EPHX1 gene in groups, n=165

Indexes	1 <sup>st</sup> Group n = 72	2 <sup>nd</sup> Group n = 56	3 <sup>rd</sup> group n = 15	4 <sup>th</sup> Group n = 11	5 <sup>th</sup> group n = 11
TT, n(%)	41 (56.9)	16 (28.6)	8 (53.3)	8 (72.7)	6 (54.5)
TC, n(%)	27 (37.5)	25 (44.6)	7 (46.7)	2 (18.2)	4 (36.4)
CC, n(%)	4 (5.6)	15 (26.8)	0 (0.0)	1 (9.1)	1 (9.1)
T allele, n (%)	109 (75.7)	57 (50.9)	23 (76.7)	18 (81.8)	16 (72.7)
C allele, n (%)	35 (24.3)	55 (49.1)	7 (23.3)	4 (18.2)	6 (18.8)

1 - genotype:  $\chi^2 = 22.894$  ( $p = 0.004$ )

2 - alleles:  $\chi^2 = 21.993$  ( $p < 0.001$ )

A and C, in groups B and D CAT ≥10), as well as the frequency of exacerbations or hospitalizations.

### DETERMINATION OF EXACERBATIONS OF COPD

Frequent exacerbations were understood as 2 or more episodes of COPD exacerbations during a calendar year. These exacerbations should be separated in time by a period of more than 4 weeks after the end of the treatment of the previous exacerbation or 6 weeks from the beginning of the previous exacerbation (in cases where its treatment was not carried out) (GOLD, 2020).

### DEFINITION OF CHD

Criteria for the verification of CHD were history of myocardial infarction, positive results of stress tests and the presence of atherosclerotic injury of the coronary artery on angiography.

### PATIENTS DISTRIBUTION BY GROUPS

Patients with COPD and coronary heart disease were divided into 2 groups. Group 1 consisted of patients with COPD infrequent exacerbations and group 2 consisted of patients with frequent exacerbations. Control groups were: group 3 – smokers without signs of COPD and coronary heart disease, group 4 – patients with coronary heart disease who do not smoke, group 5 – healthy people.

### LABORATORY TESTS

Blood sampling for molecular genetic studies was performed from the ulnar vein with minimal band adhesion in a VACUTEST vacutainer with K<sub>3</sub>EDTA, in the morning on an empty stomach. Determination of Tyr113His gene polymorphism was assigned to the EPHX1 (rs1051740) for visualization and purification of DNA from whole blood using an additional set of reagents “DNA-sorb-B” (Amplisens, RF) according to the instructions of the



**Table IV.** Association of clinical-anamnestic and genetic indicators with the course of COPD, accompanied by more frequent exacerbations (method of simultaneous inclusion)

Predictor	OR	95.0% CI	P
Age, years	1.043	0.968–1.124	0.266
Gender (male)	0.802	0.215–2.991	0.743
Duration of COPD, years	1.085	0.969–1.215	0.158
Duration of coronary heart disease, years	0.921	0.782–1.084	0.324
History of myocardial infarction in the anamnesis	1.056	0.274–4.074	0.937
Stroke in the anamnesis	0.075	0.005–1.054	0.055
Smoking (pack / years)	1.010	0.972–1.048	0.618
Diabetes mellitus	0.565	0.144–2.211	0.412
Hypertension	4.999	0.216–115.789	0.316
Genotype TT	Reference group		< 0.001
Genotype TC	5.909	1.904–18.323	0.002
Genotype CC	21.326	4.217–107.846	< 0.001
GOLD II	Reference group		0.063
GOLD III	3.444	1.229–9.656	0.019
GOLD VI	–	0.000–0.000	0.999
CAT, points	1.043	1.008–1.222	0.034

**Table V.** Association of clinical-anamnestic and genetic indicators with the course of COPD, accompanied by more frequent exacerbations (Wald's method of reverse exclusion)

Predictor	OR	95.0% CI	P
Stroke in the anamnesis	0.069	0.006–0.807	0.033
Genotype TT	Reference group		
Genotype TC	5.521	1.930–15.793	0.001
Genotype CC	20.627	4.693–91.718	< 0.001
GOLD II	Reference group		
GOLD III	3.681	1.378–9.833	0.009
GOLD VI	–	–	–
CAT, points	1.112	1.020–1.213	0.016

manufacturer. DNA was kept before the amplification at a temperature minus 20°C not for more than 3 months. Carrying out amplification and genotyping with the test set of reagents from the “Synthol” (RF) company is guided by the instructions of the manufacturer. Genotyping of the Tyr113His polymorphism to the EPHX1 gene was carried out with the help of software protection of the PLR Product Detection System in real time CFX96 Touch (BioRad Laboratories Pte. Ltd.) for the analysis of the threshold cycle (Quantification tab) or analyzing alleles of discrimination (tab Allelic discrimination).

## STATISTICAL DATA PROCESSING

Performed indicators evaluation determined the differences from the normal nature of the distribution, so the calculations used non-parametric statistical methods. For quantitative variables median (Me), lower quartile (Lq), upper quartile (Uq), minimum (Min) and maximum (Max)

values were determined. The probability of differences in quantitative indicators in the two unrelated groups was determined using the U-Mann-Whitney test; in 2 or more unrelated groups according to the Kruskal-Wallis H-test. Qualitative indicators were described in absolute and relative (percentage) values. Comparison of the two groups on a qualitative basis was performed using Pearson's chi-squared test.

Associations of indicators with the binomial dependent variable were determined using logistic regression analysis with the calculation of standardized coefficients  $\beta$  (Odds Ratio) and their 95% confidence intervals (CI). Methods of simultaneous inclusion (Enter) and Wald backward exclusion (Backward Wald) of variables were used.

The threshold value of the significance level in the work was taken as 0.05 ( $p = 0.05$ ). Microsoft Excel 2013 (trial version) and IBM SPSS 25.0 (trial version) for Windows were used to maintain the data bank and perform the above calculations.

## RESULTS

The median age of patients in the first group was 62.0 [58.0; 70.0] years, and in the second – 66.0 [59.0; 72.3] ( $p=0.172$ ). Table I presents the clinical characteristics of all groups that did not differ significantly in age, ratio of men and women.

In the analysis of the clinical characteristics of patients depending on the carrier of different polymorphic variants of the microsomal epoxyhydrolase gene, no statically significant differences were found in the incidence of type 2 diabetes mellitus, hypertension (table II).

It was found that the carrier of the homozygous variant of TT was statically more often observed in patients with infrequent exacerbations of COPD. The homozygous variant of the CC gene of the microsomal epoxy hydrolase gene was statistically more common in patients in the second comparison group ( $p = 0.004$ ). Also, CC genotype was not established among smokers without COPD. In the analysis of the allelic model, it was found that the carrier of the C allele was significantly more common among patients with severe COPD ( $p < 0.001$ ) (Table III).

Thus, the age and sex of patients were not reliably associated with the type of COPD. There was also no significant association with a higher frequency of exacerbations in the duration of COPD and coronary heart disease. The presence of myocardial infarction in the anamnesis did not determine a reliable association, although it should be noted that the presence of stroke identified an inverse association with more frequent exacerbations of COPD: OR = 0.075 [95.0% CI 0.005-1.054] and the indicator was on the verge of reliability ( $p = 0.055$ ). Smoking experience, concomitant diabetes mellitus, and hypertension also did not show a significant association with more severe COPD (Table IV).

At the same time, different variants of genotypes were reliably associated. Thus, in comparison with the TT genotype, the carrier of the TC genotype almost 6 times increased the chance of developing COPD with more frequent exacerbations (OR = 5.909 [95.0% CI 1.904-18.323],  $p = 0.002$ ); the carrier of the CC genotype increased the rate up to 21 times: OR = 21.326 [95.0% CI 4.217–107.846],  $p < 0.001$  (Table IV).

The upper class on the GOLD scale also significantly increased the chance of a more difficult course by almost 3.5 times. Thus, GOLD III increased this figure by 3.4 times, compared with GOLD II: OR = 3,444 [95.0% CI 1,229-0,656],  $p = 0.019$ . The increase in score on the CAT questionnaire was also associated with an increase in the chance of more severe COPD by 4.3% for each score: OR = 1,043 [95.0% CI 1,008–1,222],  $p = 0.034$  (Table IV).

As can be seen from (Table.V), reliable predictors of COPD with a higher frequency of exacerbations are the highest class according to the GOLD classification. Thus, patients with GOLD III have a 3.6 times higher chance of COPD with more frequent exacerbations (OR = 3,681 [95.0% CI 1,378–9,833],  $p < 0.009$ ). At the same time, the highest result according to the CAT questionnaire also showed a direct association with more frequent exacerbations of COPD: OR = 1,112 [95.0% CI 1,020–1,213],

$p = 0.016$ . The special attention should be given to the reliable association of genotype with the type of COPD. Thus, compared with the TT genotype, patients with the TC genotype have a 5.5 times higher chance of developing more frequent exacerbations of COPD (OR = 5.521 [95.0% CI 1.930–15.793],  $p = 0.001$ ); the presence of the CC genotype increases this indicator up to 20 times: OR = 20,627 [95.0% CI 4,593–91,718],  $p < 0.001$ . It should be noted that the existing history of stroke is significantly ( $p = 0.033$ ) associated with a milder course of stroke: OR = 0.069 [95.0% CI 0.006-0.807].

## DISCUSSION

We have not found similar studies in open literary sources of Ukraine. Contradictory data presenting clinical and genetic links in patients with COPD are presented in the world literature.

According to a study by S. Chappell, which included 1,017 white patients with COPD and 912 healthy controls, found no association of EPHX1 variation with COPD predisposition or disease severity. [10].

However, the purpose of the analysis, which included 24 studies (8,259 patients with COPD and 42,883 patients in the control group), found that the mutant homozygote EPHX1 113, a phenotype of slow activity of EPHX1, was associated with an increased risk of COPD (OR, 1.77; 95% CI, 1.23-2.55). The association between the risk of COPD and single polymorphic variants of the microsomal epoxide hydrolase gene was established only for Caucasians, and was not valid for Asians [8]. In our study, we observed patients with COPD with coronary heart disease and found that the presence of the CC genotype increased the probability of developing 20 times (OR = 21.326 [95.0% CI 4.217-107.846],  $p < 0.001$ ) more severe COPD with more pronounced symptoms of COPD according to the CAT questionnaire (OR = 1,112 [95.0% CI 1.020–1.213],  $p = 0.016$ ).

Sandford AJ and others for 5 years observed patients with COPD and evaluated FEV1% of predicted each year, in their study proved the association of homozygotes CC EPHX1 with a faster decrease in external respiration (OR = 4.9,  $p = 0.04$ ) [11]. We divided patients into GOLD groups on the basis of spirometric indicators (FEV1% of predicted each), and found a reliable probability in the presence of a larger class of GOLD school intelligence of more severe COPD, with frequent exacerbations, which in turn will contribute to faster decline functions of external lung respiration (FEV1%) [1,2].

Birsan Can Demirdöğen and others found in a stratification analysis of their study that the presence of at least one EPHX1 C allele reduced susceptibility to ischemic stroke (OR = 2.37) compared with patients with wild-type genotype (OR = 3.21). [12]. As a result of our study, we found an inverse association between the presence of stroke with more frequent exacerbations of COPD (group 2) in patients with concomitant coronary heart disease (OR = 0.075 [95.0% CI 0.005-1.054]) and the indicator was on

the verge of reliability ( $p = 0.055$ ). In the second comparison group, the occurrence of C alleles of the EPHX1 gene was significantly higher compared to the first group ( $p < 0.001$ ), which is similar to the results of the Birsan Can Demirdöğen study.

## CONCLUSIONS

The association of a higher frequency of COPD exacerbations with a higher class according to the GOLD classification and CAT score was detected. With regard to the patients with COPD and CHD, who were carriers of the homozygous CC variation of EPHX1 gene, a reliable association of this gene polymorphism variation with a more severe progression of COPD, more frequent exacerbations, a higher class according to the COPD classification and more marked COPD symptoms according to the CAT inquiry was revealed. Moreover, the CC genotype was not identified at all among smokers without COPD. It was also revealed that patients with COPD and CHD with the TC genotype of EPHX1 gene were more likely to be susceptible to development of frequent COPD exacerbations than carriers of the TT genotype of the same gene, which was found predominantly for patients with infrequent COPD exacerbations. An age, sex, smoking history, presence of associated diabetes mellitus and arterial hypertension demonstrated no reliable association with a more severe progression of COPD.

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## ORIGINAL ARTICLE

# THE MAIN ASPECTS OF COUNTERING PANDEMIC INFECTIOUS DISEASES THROUGH VACCINATION IN THE CONTEXT OF PROTECTING HEALTH AND OVERCOMING PSYCHOLOGICAL BARRIERS

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## ABSTRACT

**The aim:** Study of opinions and attitudes of citizens towards vaccination, identification of the reasons for the refusal of vaccination, the main psychological barriers.**Materials and methods:** As a result of the analysis the main reasons for the refusal of vaccination were systematized into 5 groups. For practical clarification of the main reasons for the refusal of vaccination, we conducted a survey of citizens of the family medicine outpatient clinic in one of the Ukrainian cities. A total of 30 respondents took part in the survey.**Results:** The results of the survey were systematized, according to which it was found that today there is still a negative attitude towards vaccination, although some of the respondents who have a positive attitude towards vaccination are much larger in the context of today's pandemic realities. The main reason for not vaccinating is a lack of trust and confidence in vaccine safety.**Conclusions:** To date, vaccination, including against COVID-19, is the only method of combating the pandemic. The identified reasons for the refusal of vaccination, according to the authors, are due to the lack of proper informatization of the population and the low level of trust in global and national medical institutions.**KEY WORDS:** infectious diseases, vaccination, public health

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## INTRODUCTION

In the last decade, infectious diseases have had a significant impact on the statistics of morbidity and mortality of the population and required significant expenditures from national health authorities. According to the World Health Organization (WHO), vaccines are now available for more than 30 infectious diseases. In the arsenal of immunoprophylaxis there are more than 60 effective vaccine preparations, different in composition, method of application and effectiveness. However, the improvement of immunoprophylaxis measures, the latest development methods to increase efficiency, the design of genetically engineered vaccines using virus-like particles, the creation of plant-based vaccines, as well as new adjuvants (substances that increase the immunogenicity of vaccine preparations) are an important issue in modern immunology [1].

The emergence in November 2019 of the novel coronavirus SARS-CoV-2, which caused coronavirus disease 2019 (COVID-19), is having devastating consequences around the world. Control measures such as the use of masks, maintaining social distancing, mass testing of symptomatic and asymptomatic individuals, contact tracing and isolation, and the introduction of quarantine measures

have helped to limit the transmission of the pathogen where they were severely applied; however, these actions were taken in a variety of ways and were not sufficient to prevent the spread of the novel coronavirus disease. The COVID-19 pandemic has now reached every continent, with more than 111 million laboratory-confirmed cases and more than 2.4 million deaths.

In the absence of effective therapeutic agents and preventive measures, vaccines against this disease have become the subject of increased attention. Since January 2020, when the sequencing of SARS-CoV-2 was carried out, scientists from different countries have been actively working to develop different types of vaccines against COVID-19. Vaccines are needed to reduce the morbidity and mortality associated with this disease, and many platforms have been involved in the rapid development of vaccine candidates. Usually, vaccines require years of research and testing before being used, but in 2020, scientists have begun the competition to produce safe and effective coronavirus vaccines in record time [2].

Currently, more than 170 vaccines are in preclinical development and about 60 are in clinical development. There are a number of licensed vaccines or those moving towards



phase trials. Scientists are using different approaches to create a vaccine against COVID-19. Currently, there are 7 main platforms. In particular, two categories are based on nucleic acids: RNA and DNA. These are new generation vaccines. The third platform is protein-based vaccines (subunit vaccines), for the manufacture of which various technologies for the preparation of viral immunostimulatory protein antigens are used. The next two platforms are viral vectors of the type used in gene therapy. In particular, vectors are used that both do not replicate and replicate. Two more categories are the SARS-CoV-2 viruses themselves: either inactivated or in a weakened live version. Vaccines are also being developed that do not fit into any of the above categories. These are vaccines based on virus-like particles or using killed rabies pathogen (CORAVAX) and/or modified equine pox virus (TX-1800) as viral vectors [3].

Research in the direction of obtaining vaccines by genetic engineering methods is carried out in many laboratories around the world. Significant progress has been made in the expression in bacteria and yeast of genes encoding the surface proteins of the influenza virus, hepatitis B, poliovirus, rabies, foot-and-mouth disease, etc. However, the high cost of producing new vaccines and legal issues regarding their applicability, strict regulations regarding the immunization of healthy people, and rather limited income from vaccine production are significant barriers to pharmaceutical companies from entering the vaccine business. Therefore, in recent years, the number of vaccine manufacturers has significantly decreased, which has led to a decrease in competition and interest in investing in this industry.

Today, in the practical healthcare system, vaccines are used that were developed many years ago, but improved with the development of immunology due to the need to improve their safety, tolerability and effectiveness. As a result, products with improved characteristics have appeared, but the production of which is impossible without complicating technological processes.

At the same time, some drugs developed decades ago are used (for example, the flu vaccine is still produced using outdated methods).

The goal of modern immunology is to create vaccines produced using modern technological processes, in significant volumes and at a speed that allows us to meet the existing needs in mass vaccination events.

A vaccine is a medical product intended to create in the body of vaccinated people or animals active immunity to infectious diseases. Depending on the mechanisms of formation, hereditary and acquired immunity are distinguished. The hereditary is sometimes called species, since it is inherent in all individuals of a given species and is transmitted from generation to generation. Acquired immunity is not inherited and is formed as a result of an infectious disease or as a result of immunization [4].

The problem of vaccinating the population is critically important when it comes to pandemic diseases. Today, the global medical community is faced with a new type of pandemic disease – coronavirus infection. Mass vacci-

nation against coronavirus disease today is the only way to overcome this pandemic. Given this, it is especially important to determine the current attitude of citizens to the vaccination process and to determine the main reasons for refusing to vaccinate.

## THE AIM

The main purpose of the article is to study the current state of readiness of citizens for vaccination and re-vaccination against COVID-19 in the context of today's pandemic realities. In addition, an important issue of our study is to identify the main reasons for the refusal of vaccination during the survey.

## MATERIALS AND METHODS

Recent years have been marked by the spread of the SARS-CoV-2 coronavirus around the world as a challenge to all mankind. The pandemic has affected all aspects of the public and private life of people, entire states. The words “quarantine”, “lockdown”, “state of emergency”, “epidemic”, “pandemic”, “herd immunity” and other related concepts have entered daily use. We began to use masks, disinfect, wash our hands more often and physically distance ourselves from each other. COVID-19 has affected the global economy, world politics, international relations and has changed the values and attitudes of societies, habits and everyday realities. One of the most important tools on the path to normalizing the situation after the pandemic is universal vaccination, the result of which should be the formation of herd immunity. The main barriers to vaccination are the lack of confidence in the effectiveness of vaccines, the lack of adequate information in sufficient quantities and the fear of side effects [5].

Even in the relatively recent past, the population did not think about the need for vaccination, about complications – the need for vaccinations was an axiom. The effectiveness of vaccination was evident, as evidenced by the lower number of infectious diseases compared to today.

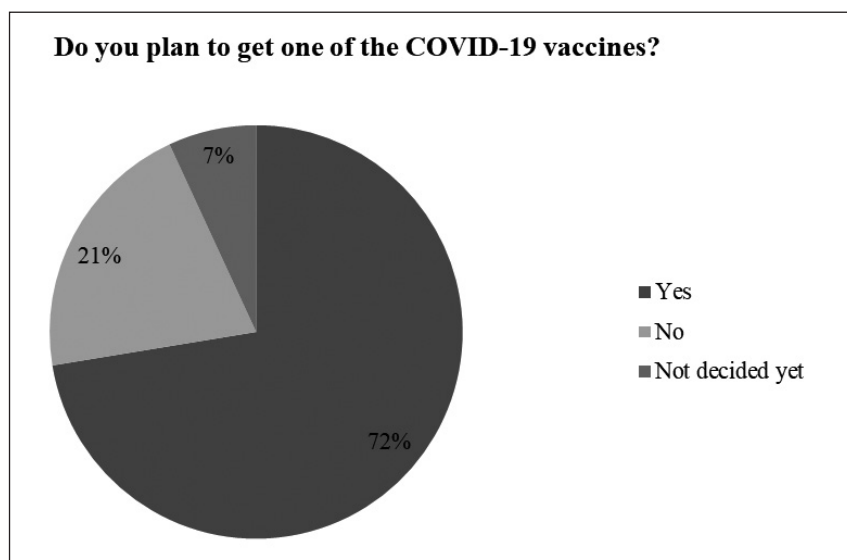
Today, attitudes towards immunization have changed dramatically. The population hears about the positive aspects of vaccination only in «dry» statistics, and any case of a post-vaccination complication, real or imaginary, is inflated, causing lively public interest. As a result, confidence in vaccination, especially against pandemic diseases, is falling sharply.

In particular, more than 1.5 million users discuss the problems of vaccination on Internet forums every month, two-thirds of which demand that mandatory vaccination be abolished altogether. Due to this, the issues of routine vaccination over the past few years have gone far beyond the competence of physicians, affecting the interests of politics, economics, ethics, philosophy and even religion [6].

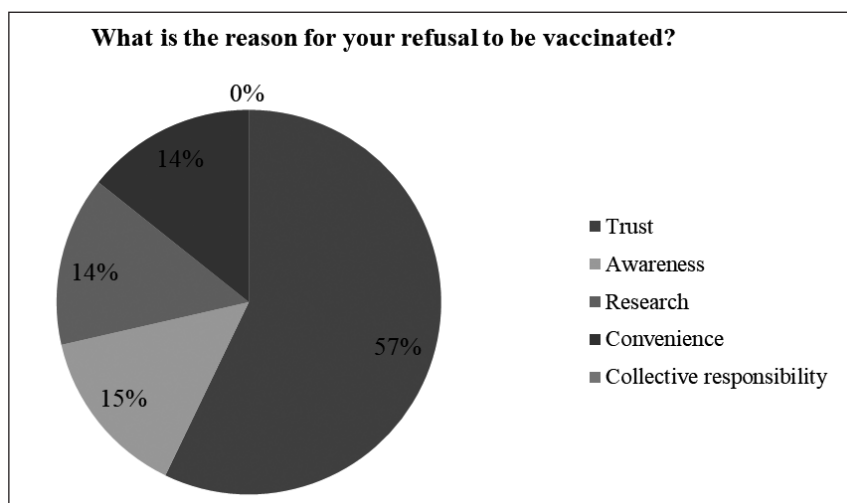
Scientists began to study doubts about vaccination long before the appearance of the coronavirus. They studied different models of human behavior and found out the most promising ones. After analyzing the relevant scientific

Trust	<ul style="list-style-type: none"> <li>the individual's confidence in the effectiveness and safety of vaccines and the medical institutions that offer them, as well as trust in the authorities conducting mass vaccination</li> </ul>
Awareness	<ul style="list-style-type: none"> <li>the disease itself does not always seem so serious to a person enough to be vaccinated against him</li> </ul>
Research	<ul style="list-style-type: none"> <li>a person's desire to seek information about vaccinations and weigh the pros and cons</li> </ul>
Convenience	<ul style="list-style-type: none"> <li>getting a vaccine is not always an easy process</li> </ul>
Collective responsibility	<ul style="list-style-type: none"> <li>low awareness of the social importance of the vaccination process as the most powerful factor in countering the pandemic</li> </ul>

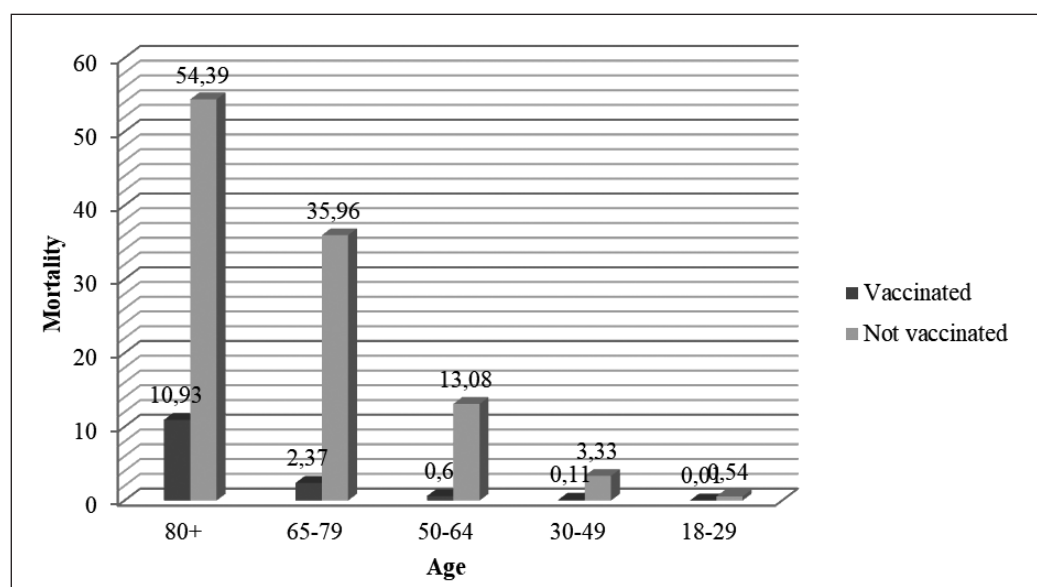
**Fig. 1.** Major psychological factors and barriers to vaccination



**Fig.2.** The results of the answer to the question «Do you plan to get one of the COVID-19 vaccines?»



**Fig.3.** The results of the answer to the question «Do you plan to get one of the COVID-19 vaccines?»



**Fig.4.** Mortality of vaccinated and unvaccinated persons during the course of illness caused by the COVID-19 virus

literature and systematizing the results, we formed five groups of vaccine refusal factors (Fig.1) [7-10].

Of course, there are other factors too. A recent Oxford University study found that fear of the needle is a major barrier for almost 10% of the population. But the above model covers the most common causes of fluctuations [11].

Vaccination is a form of medical intervention and is performed not for the purpose of diagnosing a disease or its treatment, but in order to prevent the possible occurrence of an infectious disease. Therefore, this problem, like no other, is related to issues of biomedical ethics. The ethical standard of the medical profession, expressed in the «Do No Harm» formula, requires doctors to do everything in their power to avoid the adverse consequences of any medical intervention.

Therefore, when carrying out vaccination, the unconditional professional duty of a doctor lies in an individual approach to each patient. However, in pediatric practice, it is much more difficult to do this, since the doctor mostly deals not with the patient himself, but with his representative, most often the parent. The latter can act as both a positive and a negative factor influencing the results of interaction. Thus, the social aspect of vaccination becomes a subject of discussion that goes beyond the professional competence of physicians into the field of issues requiring philosophical, legal and ethical expertise[12].

So, today, most scientists identify the following psychological barriers to a full-fledged vaccination against COVID-19 [16]:

1. Lack of awareness of the importance of vaccination in the context of the risk of disability or loss of life.
2. Fear of needles and other invasive medical procedures.
3. Feeling of helplessness and hopelessness before the epidemic, where the vaccine is considered absolutely ineffective.
4. Distrust of the components of the vaccine.
5. Feelings of mistrust towards modern technologies and policies of international organizations.

As can be seen, according to the analysis of most scientific sources, today it is customary to distinguish five main groups of reasons for refusing vaccination.

After we have conducted a theoretical analysis of the most relevant and advanced scientific literature on the main reasons for refusal of vaccination and the psychological barriers that exist, it is important to conduct a practical survey of ordinary citizens about how ready they are for vaccination and, in case of refusal of the latter reason, for which they refuse to be vaccinated.

This survey was conducted on the territory of Ukraine in the outpatient clinic of family medicine. 30 respondents took part in the survey. The reason for choosing such a limited number of respondents was that the survey was conducted in the midst of the COVID-19 epidemic. In addition, in order to comply with all ethical standards and permissions of the management of the outpatient clinic, the number of respondents was limited to 30 respondents.

Among which were different representatives by gender and age groups. The entire interview process was conducted in accordance with the ethical rules of the interview process. All data obtained during the survey was used for scientific purposes only. All personal data of the respondents, at the request of the latter, remained anonymous. It should be noted that the sample of respondents consisted exclusively of those citizens who had no absolute and relative contraindications to vaccination.

During the survey, respondents were asked the following questions:

1. Do you plan to get one of the COVID-19 vaccines?
2. If the answer is «no», indicate the reason for not vaccinating out of the five given.

After the survey, all respondents gave their informed consent to the use of their answers in the process of this research. The whole research process was agreed with the administration of the family medicine clinic.

## RESULTS

After conducting a theoretical analysis and a practical survey of respondents in a family medicine outpatient clinic regarding their attitude to vaccination of one of the vaccines against

COVID-19, and, in case of refusal of the latter, identifying the reason for refusal to vaccinate, we systematized all the answers and, for the convenience of displaying the received results are shown graphically.

As we can see from Fig.2. 72% of respondents have a positive attitude towards vaccination and are aware of the social importance of the COVID-19 vaccination process as the main measure to combat the pandemic. However, 21% of those surveyed showed a negative attitude towards vaccination, while 7% have not yet reached a final opinion on their attitude towards the COVID-19 vaccination process.

In order to find out the main reasons for the refusal of vaccination, those respondents who revealed a negative attitude towards the vaccination process were asked to choose the main reason for the refusal of vaccination among five existing groups. Fig.3. shows the answer to the question: « What is the reason for your refusal to be vaccinated?».

How can we create from Fig.3. the majority of respondents point out that the main reason for refusing to vaccinate is a lack of confidence in the vaccination process and doubts about the safety of the vaccine.

In our opinion, the reason for choosing such a factor of refusal to vaccinate is that the COVID-19 vaccines, like the disease itself, have appeared relatively recently, and, in the opinion of a large number of ordinary citizens, have not yet had time to pass all possible tests and safety and reliability studies.

But, despite the existing myths and warnings, today statistics show that vaccination was and is the only way out of the pandemic situation. Thus, according to the CDC [13], vaccination against COVID-19 is a powerful factor in reducing mortality in all age categories (Fig. 4).

Fig.4. it can be seen that in all age categories, the mortality of the vaccinated is significantly less than that of the unvaccinated, which can be affirmative evidence of the safety and reliability of the vaccine and the reason for increasing confidence in it.

Considering the results obtained, we can say that for today, vaccination, especially in the context of the current COVID-19 pandemic, is the only possible and available method to combat the pandemic. Despite significant amounts of information about the severity of the course, high mortality, both in Ukraine and around the world, a certain part of the population refuses to receive vaccinations against COVID-19, creating a significant threat of strengthening and continuing the pandemic. Given that the main reason for refusing to vaccinate is an insufficient level of trust and doubts about the safety of the vaccine, in our opinion, the main way to overcome this refusal factor is to strengthen the educational work of medical institutes, medical organizations at international and national levels in the vector of conveying information about the safety of vaccines to the population and that lack of vaccination is a factor in the increased mortality rate during illness.

## DISCUSSION

In a pandemic, it is important to protect yourself and not die from COVID-19, for which there is no cure. Therefore, it is desirable for teachers to be vaccinated as soon as possible, especially since teachers are classified as priority groups for vac-

cination in our state. In addition, your opinion is authoritative for people in your environment, and your example positively influences the decision to get vaccinated. Most likely, friends, acquaintances and parents of pets, students or students will look up to you and also want to protect themselves from COVID-19.

Each country has its own regulatory authorities that monitor the safety and efficacy of vaccines before they are widely used. Around the world, WHO coordinates a number of independent technical bodies that test the viability of vaccines before and even after they are introduced. Vaccines approved for use by the WHO have passed rigorous tests and clinical trials to prove they are safe and effective in dealing with the pandemic.

The issue of refusing to vaccinate today is becoming a priority and affects not only the medical system, but the entire system of social security of the countries of the world[14].

The issue of identifying the reasons for refusal to vaccinate against COVID-19 was investigated with the development and introduction of vaccines into medical practice. For example, scientists Ivanov and Petrov conducted a similar survey in their research and found that the main reasons for refusing to vaccinate are the following: side effects and effectiveness of the vaccine; purported benefits, including immunity, reduced fear of infection, and protection of self and the environment. In our opinion, such a list of causes is not systematized and does not make it possible to further develop mechanisms for effectively counteracting these failures[15].

Even a vaccinated person can contract COVID-19. The goal of vaccination is to effectively prevent severe disease, complications and death. Having been vaccinated, a person, even if he gets sick, will endure the disease more easily: most likely he will not need oxygen therapy and will not go to the hospital.

All vaccines approved for use by WHO are highly effective in preventing severe COVID-19 disease, hospitalization and death.

## CONCLUSIONS

Vaccines are one of the most effective public health practices in promoting health and reducing the burden of infectious diseases. They also make a significant contribution to social and economic development, not only in terms of health protection and mortality reduction, but also in terms of poverty reduction, equity, production, education and the strengthening of health systems in general. Vaccines are extremely safe. However, the safety of vaccines is receiving increased attention from the public, and rightly so.

We can talk as much as you want about the safety of the vaccine, but it will not help to eliminate such fears and ultimately affect people's behavior. It was previously thought that people were either unequivocally in favor of vaccines or against them. Most really have nothing against vaccination: according to the WHO, depending on the type of vaccine, 9 out of 10 people support immunization. There are also groups in the population who are strongly opposed to vaccination and cannot be persuaded. Usually, such people have long held alternative views on health care. Recently, however, distrust of vaccines among the population has begun to increase. People are becoming more and more reluctant to be immunized, and some are becoming relentless opponents of vaccines.



To find out the attitude and possible psychological barriers of citizens to vaccination, we conducted a theoretical analysis and a practical survey of respondents in the family medicine outpatient clinic. As a result of the study, it was found that most scientists identify five main groups of factors and barriers to refusing to be vaccinated. As a result of the survey, it was revealed that the majority of respondents who showed a negative attitude towards vaccination against COVID-19 chose the lack of trust and doubts about the safety of the vaccine as the reason for the refusal.

In our opinion, the reason for choosing this factor is that these vaccines, like the virus itself, have not been around for so long, and, according to the population, have not had time to pass all the reliability and safety tests. However, the statistics presented in the study confirm the fact that vaccination against the COVID-19 virus is currently the only way to overcome the global pandemic and significantly reduces the mortality rate during the disease.

This study has its limitations, since the survey was conducted on the territory of Ukraine and may take into account the peculiarities and dynamics of the vaccination process in this particular territory. In addition, the survey was conducted among the population, not counting the age indicator, since the goal was to identify the most common reasons for refusal to vaccinate. In future studies, the authors plan to investigate more specific factors and psychological barriers to vaccine refusal among different ages.

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## ORIGINAL ARTICLE

# NEPHROPROTECTIVE EFFECT OF OLMESARTAN ON RENAL ISCHEMIA REPERFUSION INJURY IN MALE RATS: THE ROLE OF NRF2/HO-1 SIGNALING PATHWAY

DOI: 10.36740/WLek202211213

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## ABSTRACT

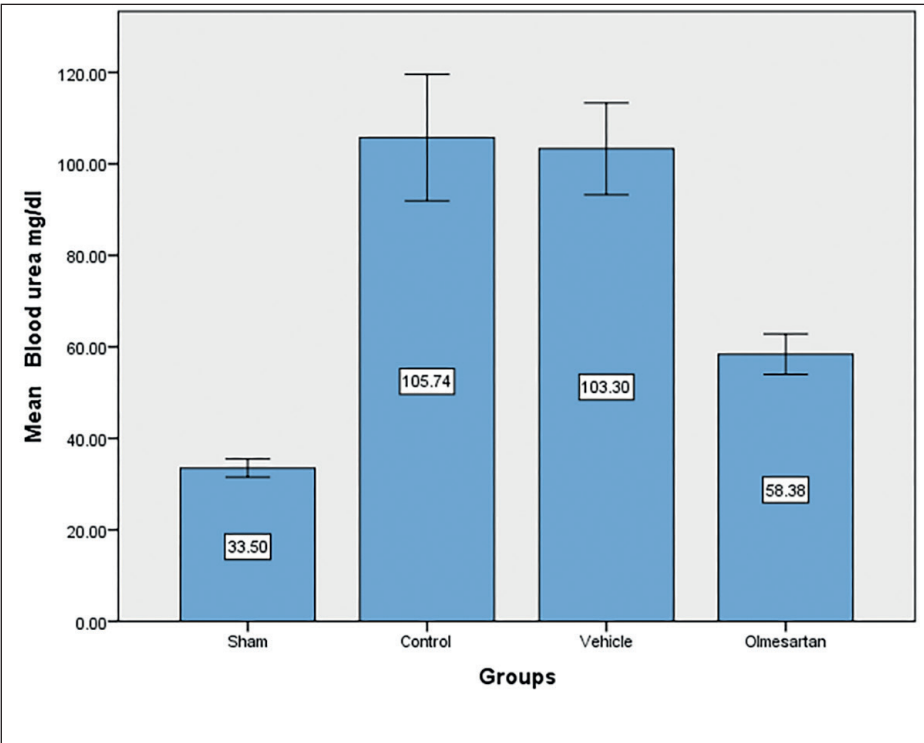
**The aim:** To investigate the Nephroprotective potential of Olmesartan in RIRI via modulation of the Nrf2/OH-1 signaling pathway.**Materials and methods:** Thirty male rats were equally divided into four groups. The sham group was exposed to surgical conditions without induction of RIRI. The control group was exposed to ischemia by clamping the renal pedicles for 30 min, followed by 2h of blood restoration. The vehicle-treated group was received dimethyl sulfoxide (DMSO) by intraperitoneal injection (IP) 30 min before clamping.**Results:** Olmesartan-treated group was pretreated with Olmesartan a dose of 10 mg/kg IP; 30 min prior to induction of ischemia. Following 30 min of ischemia, the clamps were released and allowed to the reperfusion for 2 h. Blood samples were collected to examine the levels of serum urea and creatinine. Kidney tissue was used to measure the levels of cytokines (TNF $\alpha$ , IL6, MCP, BAX, BCL2 and isoprostane F2. Immunohistochemistry was used to assess the levels of Nrf2 and HO-1. Histological analyses were used to detect the tubular damage in the kidney.**Conclusions:** The results showed that Olmesartan alleviates renal tissue damage through activating the antioxidant effect mediated by Nrf2 signaling.**KEY WORDS:** Olmesartan, renal ischemia reperfusion injury, oxidative stress, inflammation, apoptosis, Nrf2/HO-1 pathway

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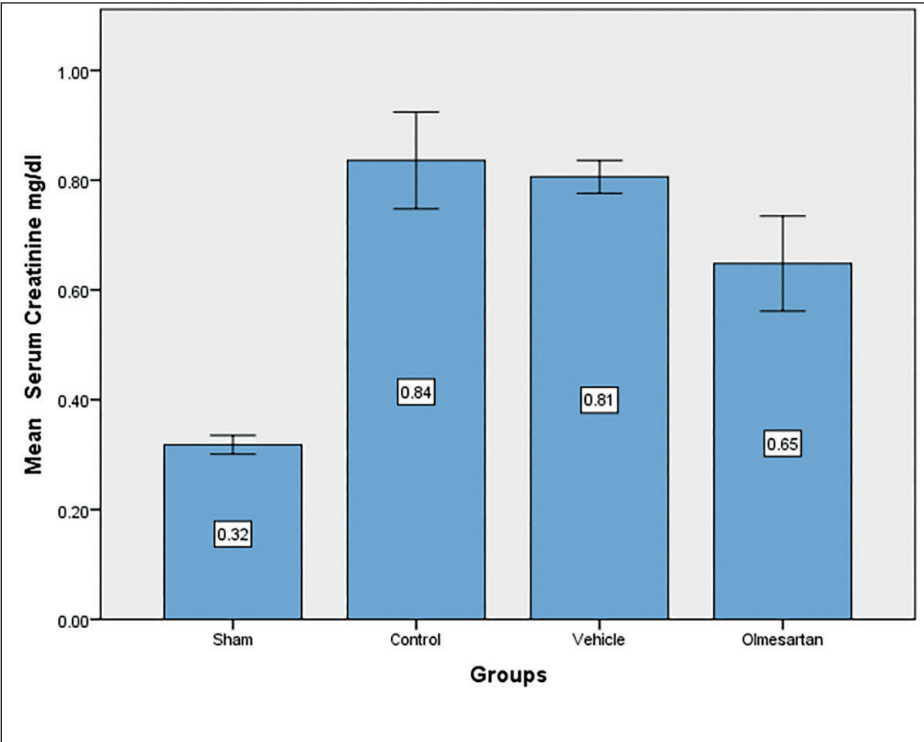
## INTRODUCTION

Acute kidney injury (AKI) is a clinical illness characterized by a rapid loss in renal function, which is associated with the buildup of fluid and metabolic waste products, as well as electrolyte imbalances. AKI has been found in 3–18% of patients, and related to in-hospital mortality. The most prevalent cause of acute kidney damage is the renal ischemia and reperfusion [1]. Ischemia reperfusion injury (IRI) is a transient decrease in blood and oxygen flow to an organ (hypoxia or anoxia), followed by reoxygenation and blood supply [2]. In the ischemia process, a lack of oxygen and nutrients will cause oxidative stress and inflammation, among other things [3]. Despite significant advances in basic research, knowledge, and care of AKI, patient mortality remains a risk [4]. A variety of the pathophysiological processes of ischemia is involved including a decrease in arterial blood flow resulting in a hypoxia and glucose deprivation. This leads to mitochondrial dysfunction. Ischemic acute kidney damage triggers a multitude of pro-inflammatory pathways, making it easier to injure other organs by triggering the host's innate and adaptive immune systems, which are supported by soluble mediators [5]. In the advanced stages of renal ischemia-reperfusion, a significant number of reactive oxygen species are synthesized, leaving the kidney in a state of acute oxidative stress and initiating a cascade of harmful cellular processes including reactive oxygen species (ROS) generation, cytokine and chemokine release, leukocyte activation,

culminating in renal damage [6]. IRI is induced by circulating the pro-inflammatory cytokines and chemokines that are released by ischemia-damaged blood cells and organs. Blood cells and cells from numerous organs generate interleukin (IL), an anti-inflammatory cytokine [7]. When the degree of the kidney damage grows, IL-6 expression rises, increasing the deleterious inflammatory reaction Monocyte chemoattractant protein-1 (MCP-1) is one of the most recently studied renal markers and found sites of injury and inflammation. MCP-1 expression rises in the kidneys when renal disease develops and an interstitial inflammatory infiltrate occurs [8]. Apoptosis is one of the processes that is involved in ischemic tissue of the kidney [9]. Apoptosis is a type of cell death that happens in reaction to hypoxia during ischemia and the creation of reactive oxygen species following reperfusion injury. After a time of ischemia, blood flow is restored, which limits infection and ROS formation, resulting in secondary damage. Extended ischemia can cause apoptosis, autophagy, necrosis, and necroptosis, among other factors-injury due to re-perfusion [10]. The B-cell lymphoma (BCL-2) is a family of pro or anti-apoptotic proteins. Typically, Bcl-2 prevents cell death by sequestering pro-apoptotic proteins via a hydrophobic groove on its surface. This mechanism of Bcl-2 can be blocked by BH3 mimetic, small molecules that bind the hydrophobic groove and prevent capturing of pro-apoptotic proteins by Bcl-2 [11], by inducing apoptosis and necrosis, Bcl2-associated X-protein (BAX) causes kidney fibrosis and



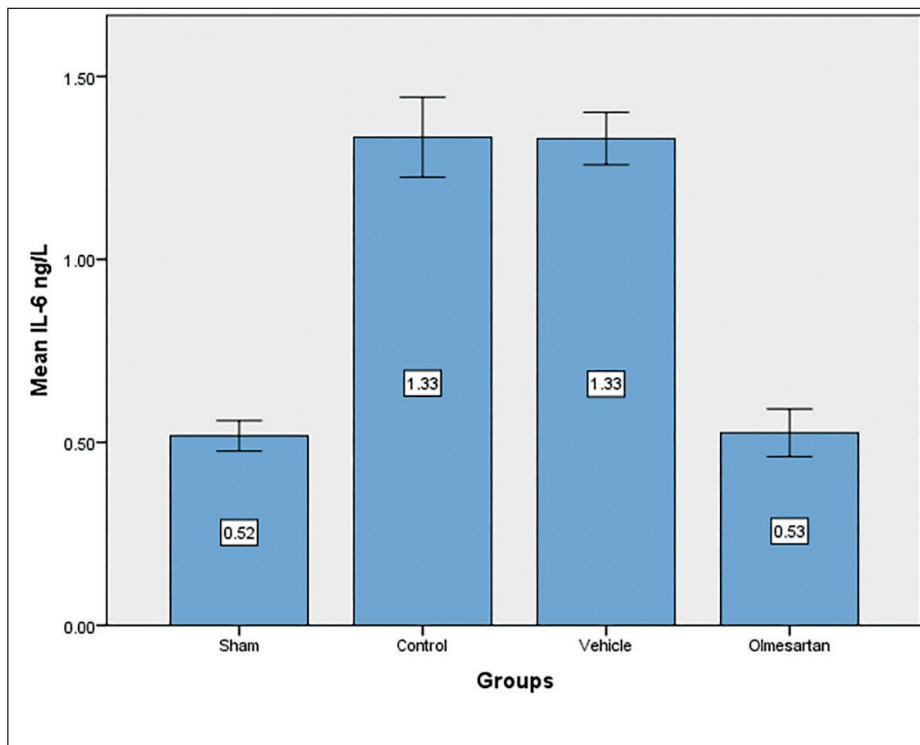
**Fig. 1.** Mean levels of urea (mg/dl) of the four experimental groups. Data are represented as mean  $\pm$  sem. Statistical analysis was performed using a one-way ANOVA followed by post hoc test,  $n=5$ , sham vs. control & vehicle groups,  $p$ -value  $< 0.01$  (significant). Olmesartan control & vehicle groups,  $p$ -value  $< 0.01$  (significant).



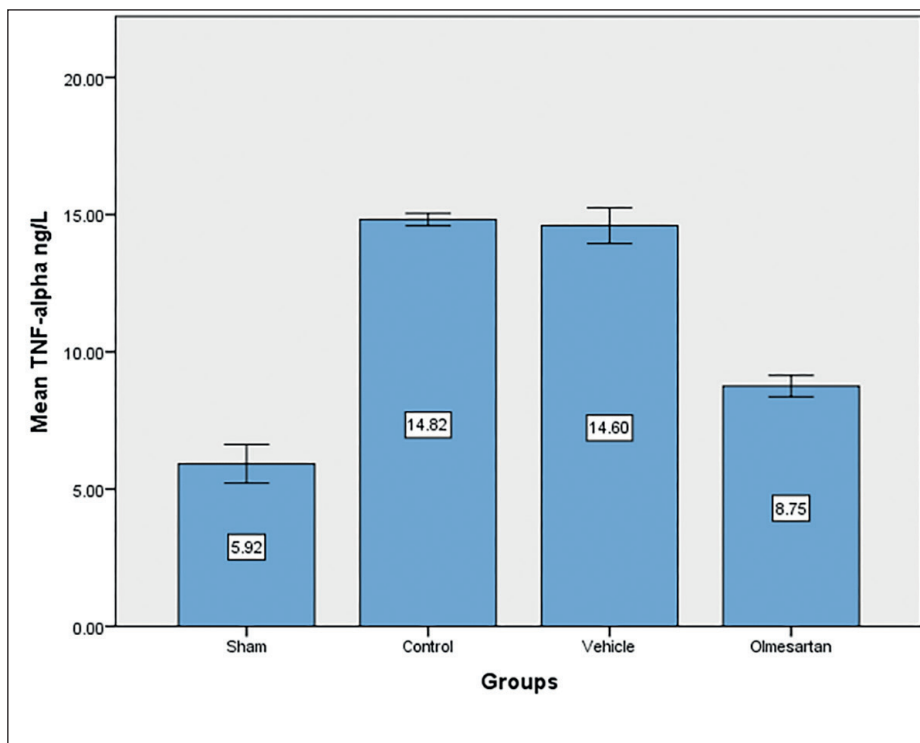
**Fig. 2.** Mean level of creatinine (mg/dl) of the four experimental groups. Data are represented as mean  $\pm$  sem. Data were analyzed using a one-way ANOVA followed by post-hoc test,  $n=5$ . Sham vs. control & vehicle groups,  $p$ -value  $< 0.01$  (significant). Olmesartan control & vehicle groups,  $p$ -value  $< 0.01$  (significant).

renal function loss, contributing to kidney fibrosis and failure [12]. BAX is an endogenous pro-apoptotic chemical that initiates apoptosis by increasing mitochondrial membrane permeability to discharge cytochrome c and then activating caspase flow to initiate apoptosis via the intrinsic pathway [13]. Nuclear Factor-Erythroid-2-related factor 2 (Nrf2) is a transcription factor that controls antioxidant genes and other cytoprotective phase II detoxifying enzymes in a number of tissues and organs, including the heart, brain, liver, kidney, and skin [14]. Nrf2 is a key regulator of the redox balance

and has been shown to provide a protection against kidney disease by limiting the formation of ROS. Nrf2 levels and transcriptional activity are decreased in many kidney diseases, with increased levels of ROS thereby making Nrf2 is a potential target in terms of IRI [15]. Olmesartan, in particular, can prevent AKI complications caused by IR injury by acting as an antioxidant to counteract the negative effects of reactive oxygen species, as an anti-inflammatory to block the pathological role of cytokines and chemokines during renal IR, and as an anti-apoptotic and anti-prolif-



**Fig. 3.** Mean level of IL-6 (ng/l) of the four experimental groups. Data are represented as mean  $\pm$  sem. Data were analyzed using a one-way ANOVA followed by post-hoc test,  $n=5$ . Sham vs. control & vehicle groups,  $p$ -value  $< 0.01$  (significant), Olmesartan control & vehicle groups,  $p$ -value  $< 0.01$  (significant).

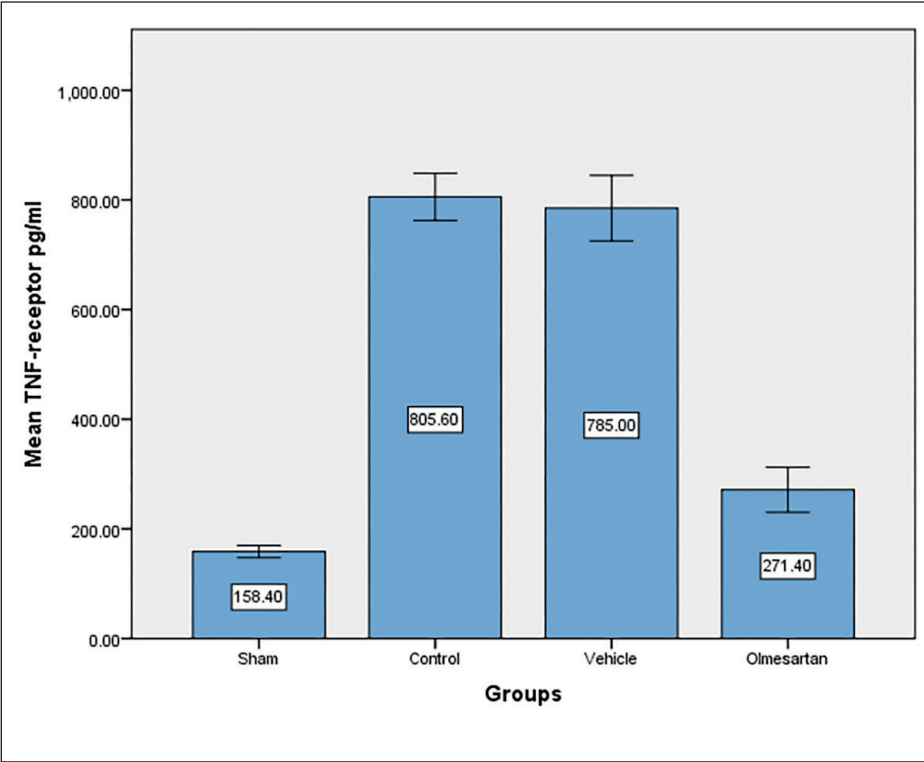


**Fig. 4.** Mean level of TNF- $\alpha$  in renal tissue (ng/l) of the four experimental groups. Data are represented as mean  $\pm$  sem. statistical analysis was performed using a one-way ANOVA followed by post-hoc test,  $n=4$ . Sham vs. control & vehicle groups,  $p$ -value  $< 0.01$  (significant), Olmesartan control & vehicle groups,  $p$ -value  $< 0.01$  (significant).

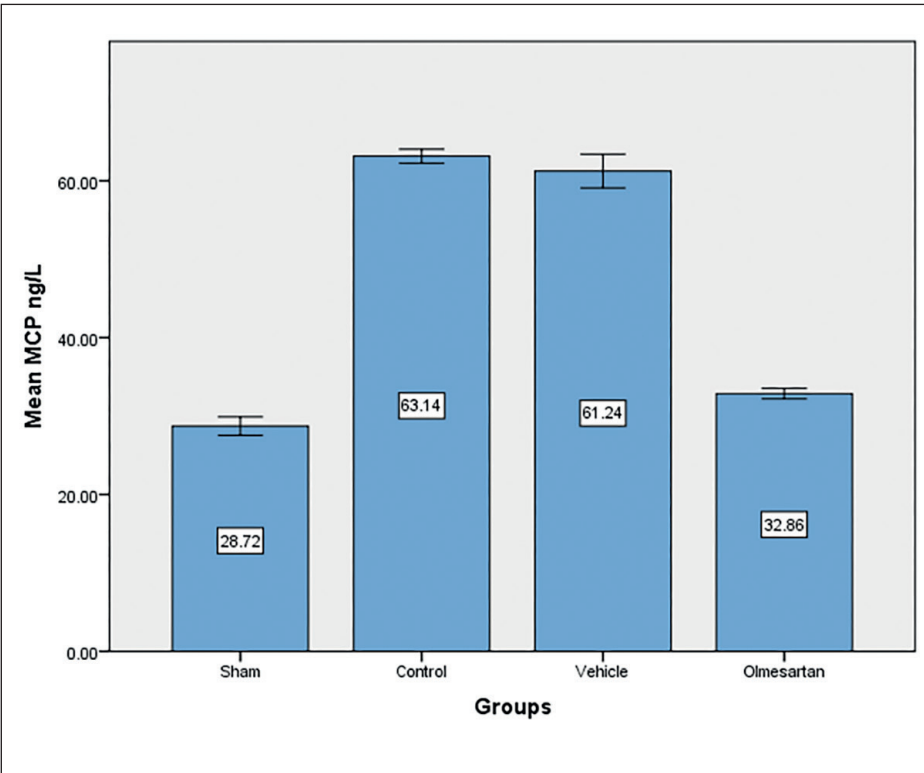
erative to prevent apoptosis and cell death during renal ischemia and hypoxia [16]. The renin-angiotensin system is important in the development of renal IRI because it plays a role in the inflammatory process. Angiotensin II (Ang II), the main peptide of the renin-angiotensin system, causes intrarenal vasoconstriction via an Ang II type 1 receptor (AT1). Non-hemodynamic consequences of Ang II include oxidative damage, inflammation, and renal tissue death. Blocking Ang II by Angiotensin II Receptor Blockers (ARBs) after renal IRI can provide a protection [18]. Olmesartan

medoxomil is a selective angiotensin II receptor antagonist used to treat hypertension. Olmesartan has no drug-drug or drug-food interactions because cytochrome p450 (CYP 450) enzymes are not involved in ester hydrolysis or first-pass metabolism (CYP 450 inhibition or induction) [19]. The kidneys excrete around 40% of the olmesartan medoxomil, and the remaining is eliminated in feces; the active metabolite is expelled without further metabolism. Adult patients with hypertension should take 10–40 mg of olmesartan medoxomil orally once a day [20].





**Fig. 5.** Mean levels of TNF- $\alpha$  receptor in renal tissue (pg/ml) of the four experimental groups. Data are represented as mean  $\pm$  sem. Data were analyzed using a one-way ANOVA followed by post-hoc test, n=5. Sham vs. control & vehicle groups, p-value < 0.01 (significant), Olmesartan control & vehicle groups, p-value < 0.01 (significant).



**Fig. 6.** Mean levels of MCP-1 (ng/L) of the four experimental groups. Data are represented as mean  $\pm$  sem. Statistical analysis was performed using a one-way ANOVA followed by post-hoc test, n=5. Sham vs. control & vehicle groups, p-value < 0.01 (significant), Olmesartan control & vehicle groups, p value < 0.01 (significant).

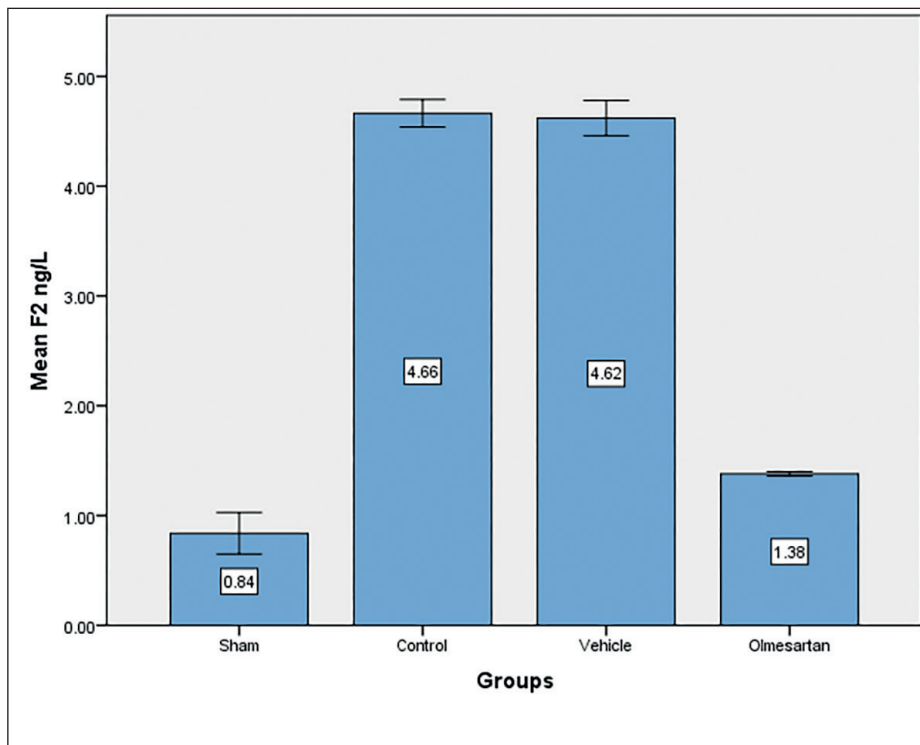
MATERIALS AND METHODS

ETHICAL STATEMENT

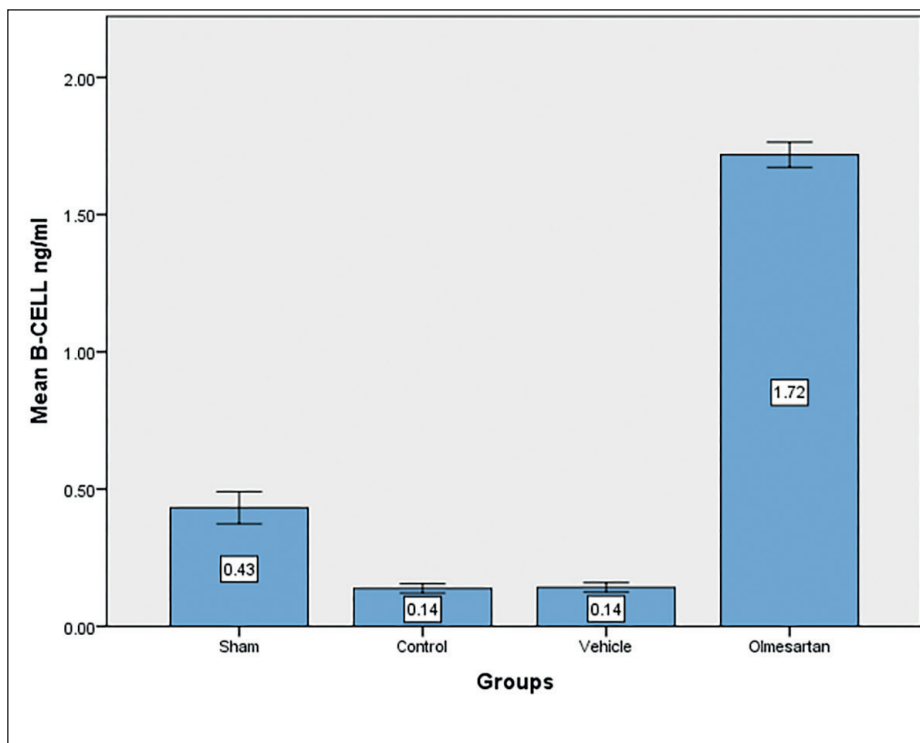
This study was followed the instruction of the Association for Laboratory Animal Science’s Guide for the Clinical and Laboratory standards, all animal were reviewed by the Animal Care Committee.

DESIGN OF THE STUDY

Male adult rats weighting 200-350 g were randomly allocated into 4 groups, each with 5 rats. Sham group n=5 was exposed to the same operation condition without Ischemia and reperfusion. Control group n=5 was exposed to 30 min ischemia and 2 h of reperfusion [21]. Vehicle croup was pretreated with dimethyl sulfoxide (DMSO) 30 min prior



**Fig. 7.** Mean levels F2 ng/L of the four experimental groups. Data are represented as mean  $\pm$  sem. Data were analyzed using a one-way ANOVA followed by post-hoc test,  $n=5$ . Sham vs. control & vehicle groups,  $p$ -value  $< 0.01$  (significant), Olmesartan control & vehicle groups,  $p$ -value  $< 0.01$  (significant).



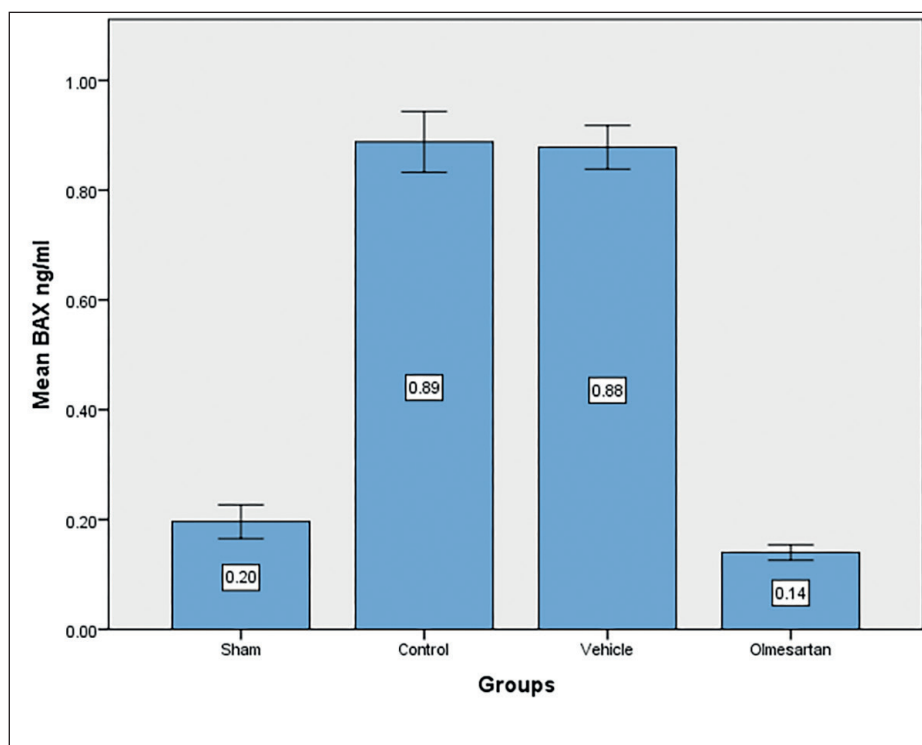
**Fig. 8.** Mean levels of Bcl2 ng/ml of the four experimental groups. Data are represented as mean  $\pm$  sem. Data were analyzed using a one-way ANOVA followed by post-hoc test,  $n=5$ . Sham vs. control & vehicle groups,  $p$ -value  $< 0.01$  (significant), Olmesartan control & vehicle groups,  $p$ -value  $< 0.01$  (significant).

to ischemia. Olmesartan-treated group was pretreated with Olmesartan 30 min before induction of ischemia [22].

#### EXPERIMENTAL PROCEDURE

After anesthesia with ketamine 100mg/kg and xylazine hydrochloride 10mg/kg, rats were placed on its back and fixed their limbs and tail with a medical plaster to assure their stability during surgery, after that hair in the abdomen had been shaved and the area disinfected. When the rats

were adequately anesthetized, the abdomen was opened midline through a laparotomy incision. Bilateral renal ischemia is made by micro vascular clamps then covers the abdomen by warm and moist gauze. After 30 minutes, the blood was returned to the kidneys by removing the clamps and the abdomen were closed prewarmed isotonic sodium chloride 0.9% solution (1 ml) was injected to avoid the dehydration. After 2 hours of reperfusion, the animals were euthanized by taking the blood from the heart by cardiac puncture and harvesting both kidneys for examination.



**Fig. 9.** Mean levels of BAX ng/ml of the four experimental groups. Data are represented as mean  $\pm$  sem. Data were analyzed using a one-way ANOVA followed by post-hoc test,  $n=5$ . Sham vs control & vehicle groups,  $p$ -value  $< 0.01$  (significant), Olmesartan control & vehicle groups,  $p$ -value  $< 0.01$  (significant).

### COLLECTION OF BLOOD SAMPLE FOR MEASUREMENT OF RENAL FUNCTION

The blood was collected from the heart and put in a tube at room temperature without anticoagulant, centrifuged for 10 minutes at 3000 rpm and supernatant was used measure the urea and creatinine [23].

### TISSUE PREPARATION FOR TNF-ALPHA, TNF-ALPHA-RECEPTOR, IL-6, F2-ISOPROSTANE, BCI2, BAX AND MCP

The kidney was cleaned with a cold isotonic sodium chloride solution and stored in a deep freezer at  $-80^{\circ}\text{C}$ . The renal segment was then homogenized in 1:10 W/V phosphate buffered saline with 1% Triton X-100 and 1% protease inhibitor cocktail using a high-intensity ultrasonic liquid processor. The homogenates were centrifuged at 3000 rpm for 20 minutes at  $4^{\circ}\text{C}$  [24], and the supernatant was used to determine tumor necrosis factor TNF- $\alpha$ , TNF- $\alpha$ -receptor, IL-6, F2-Isoprostane, BCI2, BAX and MCP using Eliza kits (Bioassay Technology Laboratory) according to the manufacturer's instructions.

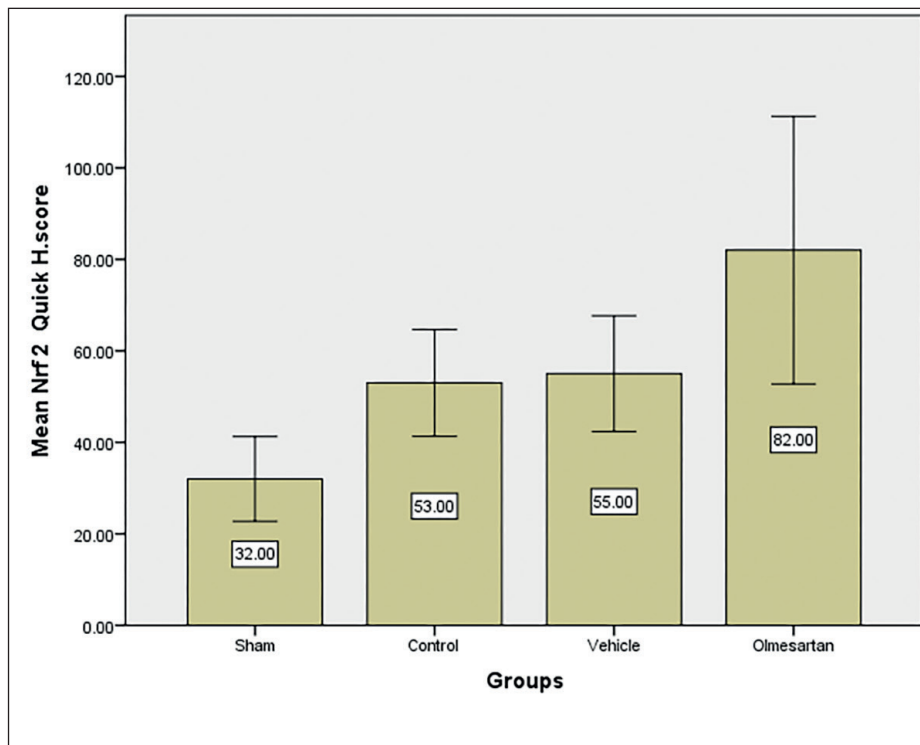
### HISTOLOGY

Renal tissues were collected and washed in a cold isotonic sodium chloride solution to remove red blood cells and clots. The renal tissues were put in 10% formalin and processed in paraffin blocks. These blocks were sliced in  $5\ \mu\text{M}$  in thickness and stained with hematoxylin and eosin dye [25]. Cellular swelling, cytoplasmic eosinophilia, tubular dilatation, loss of brush boundaries, formation of protein casts, epithelial cell desquamation into lumen, inflam-

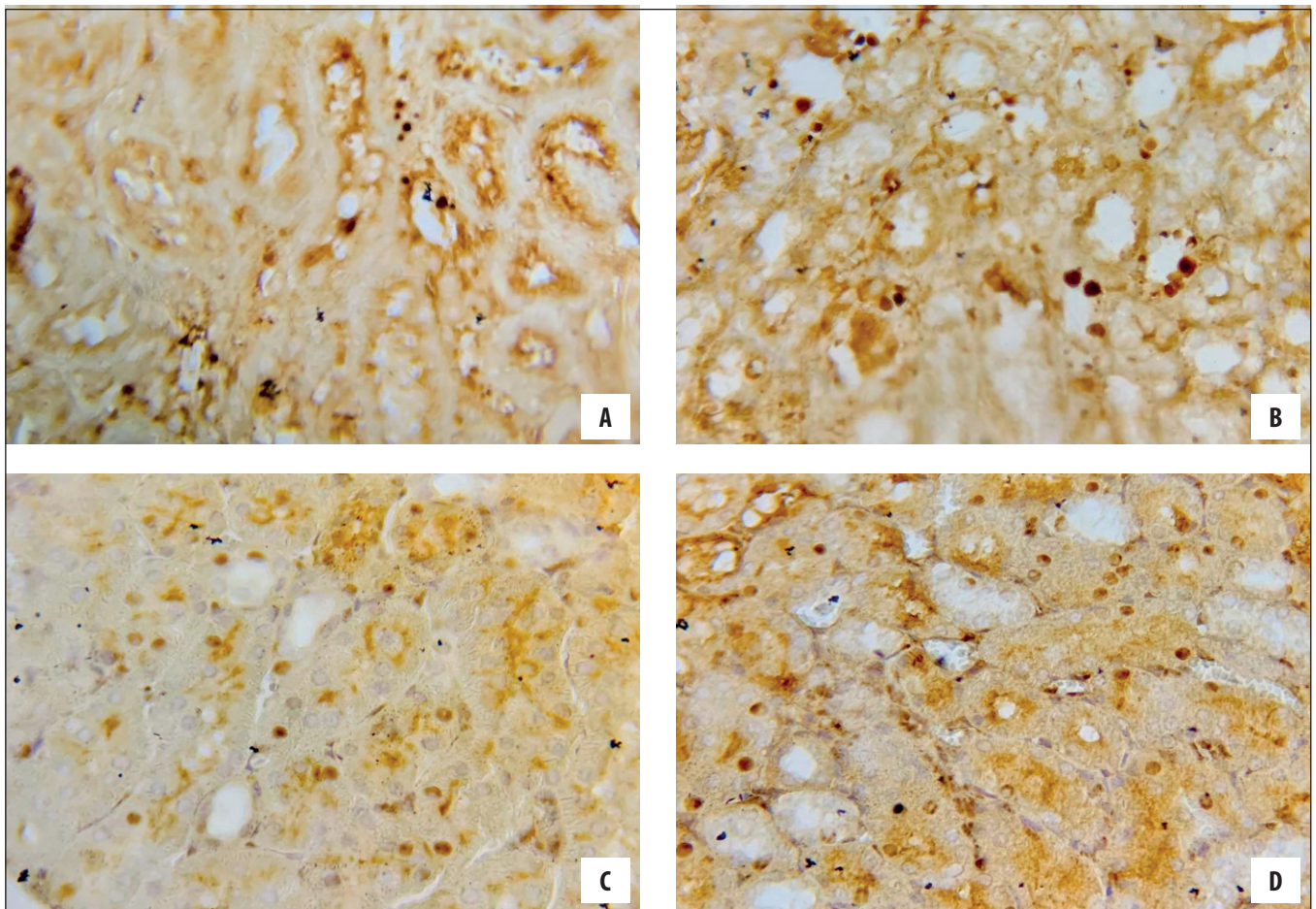
matory response, cell lysis, and necrosis were all assessed [26]. The renal tissues were investigated under bench microscope using magnification lenses of X100 to X400. A histopathologists was independently examined the tissue in a blinded manner. The tissue damage was scored from 0 to 4 based on percentage of tubular damage: 0: indicates that no damage has occurred. 1: less than 25 %, 2: 25–50% of the total, 3: 50 to 75%. 4: more than 75% [27].

### IMMUNOHISTOCHEMISTRY TECHNIQUE

In order to investigate the levels of Nrf2 and Heme Oxygenase-1 (Ho-1) in kidney tissue, immunohistochemistry was used. The immune-staining technique was used to dye  $5\ \mu\text{m}$  paraffin embedded sections. Briefly, sections were deparaffanized, rehydrated, antigen repaired using retrieval buffer, and endogenous peroxidase activity was inhibited with 3%  $\text{H}_2\text{O}_2$ . The sections were incubated with Polyclonal antibody against Nrf2 or Ho-1 (diluted 1:100 for Nrf2, Elabscience) and (1:100 for Ho-1, Elabscience). After washing, the slices were incubated for 1 hour with a conjugated secondary antibody, rinsed, and exposed for half an hour to horseradish peroxidase. After that, the slices were incubated for 8 minutes with fresh 3,3'-Diaminobenzidine. Hematoxylin stain was then used to counter stain, then, using a microscope, examine the staining. The H-score technique (ranged 0-300) was used to calculate the protein expression of Nrf2 or Ho-1, which was derived by multiplying the intensity and percent of the stained region [28]. The stain strength was graded from 0 to 3, with 0 indicating no staining, 1 indicating weak staining, 2 indicating moderate staining, and 3 indicating strong staining. From 0% to 100%, the percentage of cells stained was graded [29].

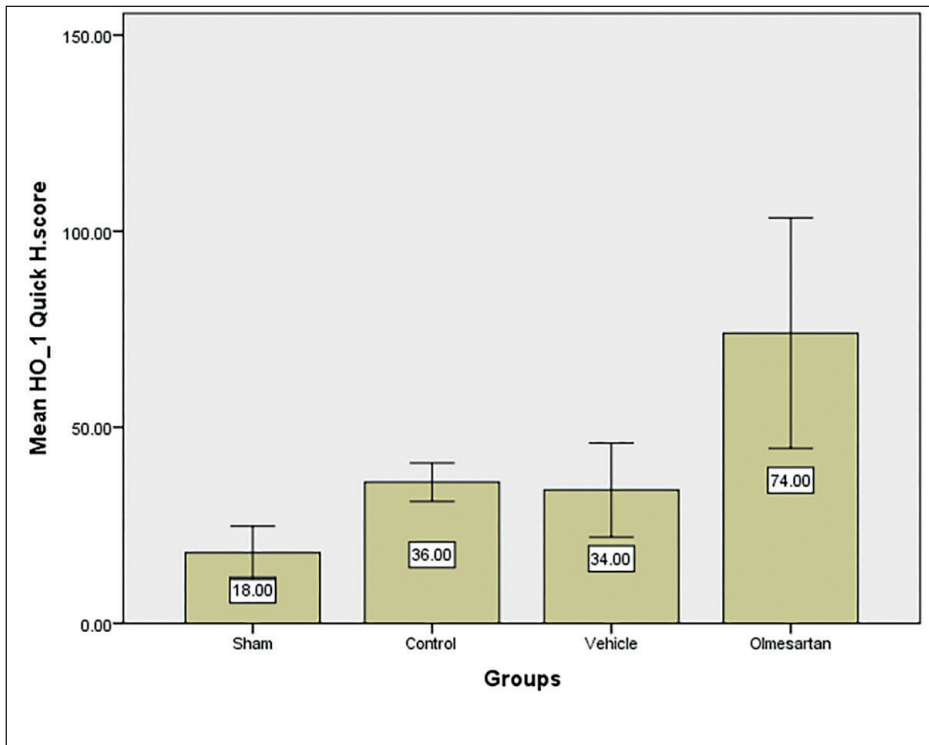


**Fig. 10.** Mean Quick H. score of cytoplasmic stain of Nrf2 renal tissue level of the four groups. Data are represented as mean  $\pm$  sem and analyzed using Kruskal-Wallis test, n=5.



**Fig. 11.** Representative images of renal tissues probed with polyclonal antibody against Nrf2: A: sham group shows strong intensity in 10% of the examined cells (arrows) IHC 10x40, B: control group shows a strong intensity in 20% of the examined cells (arrows) IHC 10x40, C: vehicle group shows a moderate intensity in 30% of the examined cells (arrows) IHC 10x40, D: Olmesartan group shows a strong labeling intensity in 40% of the examined cells (arrows) IHC 10x40.





**Fig. 12.** Mean Quick H. score of cytoplasmic stain of HO-1 renal tissue level of the four experimental groups. Data are represented as mean ± sem and analyzed using Kruskal-Wallis test, n=5.

### STATISTICAL ANALYSIS

SPSS 26.0 was used to analyze the data. The data of were firstly tested for the normality using a Kolmogorov-smirnov and shapiro to confirm that the data are normally distributed. Analysis of Variance (ANOVA) was used to compare the mean differences among the groups. Kruskal- Wallistest was used for comparing the non –parametric data.  $P \geq 0.05$  is regarded as a statistically significant.

## RESULTS

### EFFECTS OLMESARTAN ON KIDNEY FUNCTIONS (CREATININE AND UREA IN THE BLOOD)

The results showed that levels of serum urea and creatinine in the control and vehicle groups were significantly increased ( $p \text{ value} \leq 0.01$ ) compared to the sham group (figures 1 and 2). The serum levels of creatinine and urea were lower in the Olmesartan group in comparison with control and vehicle groups ( $p < \text{value } 0.01$ ) (Fig. 1-2).

### EFFECT OLMESARTAN ON INTERLEUKIN 6 (IL- 6)

In this study, the levels of IL-6 in the renal tissue were substantially higher in the control group  $p \leq 0.01$  than that of in the sham group (Fig. 3). Olmesartan pretreatment resulted in a marked decrease in the tissue levels IL-6 compared to the vehicle and control groups  $p \leq 0.01$  (Fig. 3).

### EFFECT OF OLMESARTAN ON TUMOR NECROSIS FACTOR –ALPHA (TNF-A)

The tissue levels of TNF- $\alpha$  were significantly greater than ( $p\text{-value} \leq 0.01$ ) in control and vehicle groups in compari-

son with sham group. These levels were significantly lower in Olmesartan-treated group ( $p\text{-value} \leq 0.01$ ) (Fig. 4).

### EFFECT OF OLMESARTAN ON TUMOR NECROSIS FACTOR RECEPTOR

The levels of TNF- $\alpha$  receptors in the renal tissue were considerably higher ( $p\text{-value} \leq 0.01$ ) in control and vehicle groups than that of in the sham group. Pretreatment with Olmesartan reduced the levels of TNF- $\alpha$  receptors in comparison with vehicle and control groups ( $p\text{-value} \leq 0.01$ ) (Fig. 5).

### EFFECT OLMESARTAN ON MONOCYTE CHEMOTACTIC PROTEIN-1 (MCP-1)

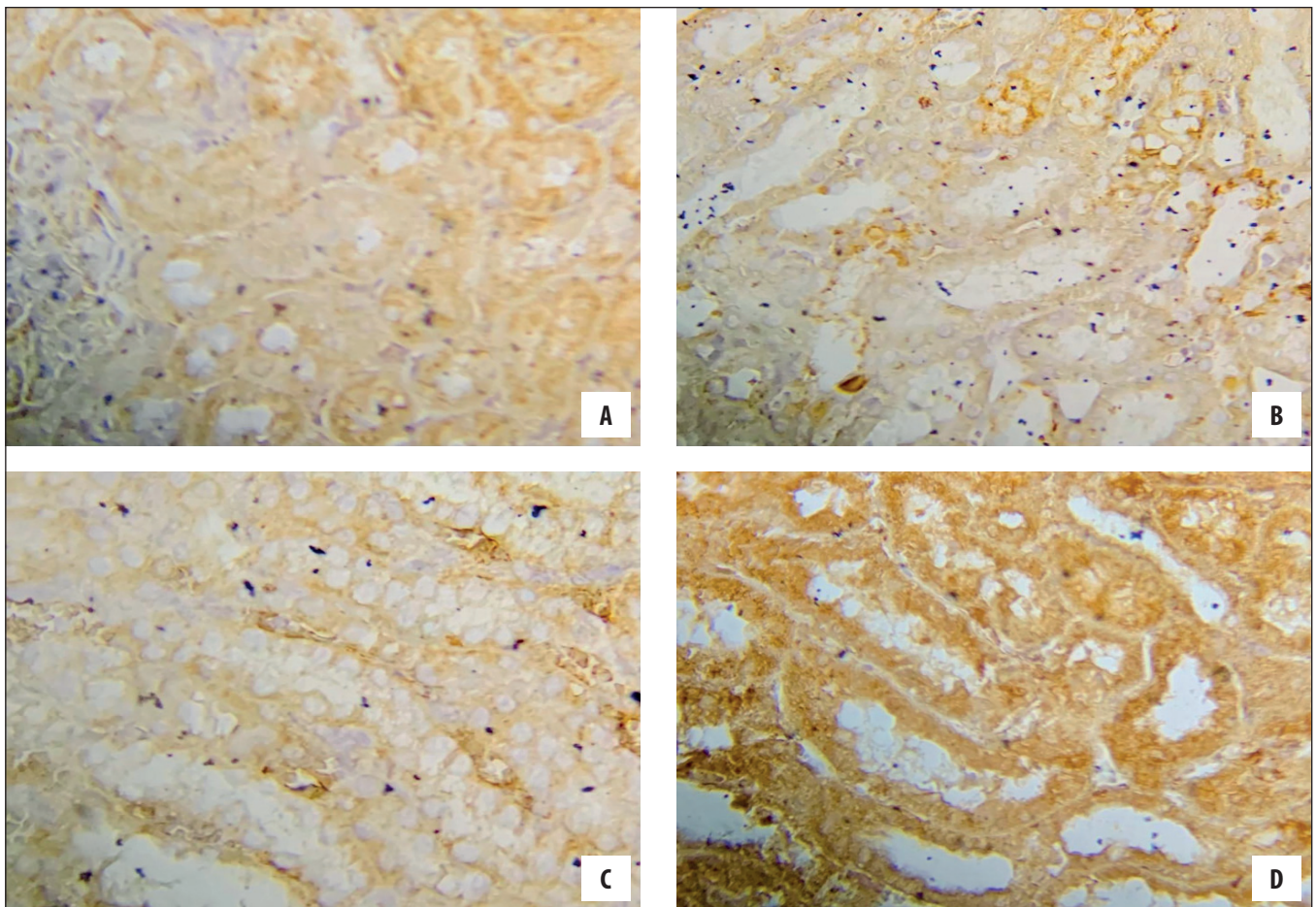
The current study showed that the renal tissue levels of MCP-1were significantly increased ( $p\text{-value} \leq 0.01$ ) in control and vehicle groups compared to the sham group. In contrast, these levels were decreased in Olmesartan-treated group groups was significantly ( $p\text{-value} \leq 0.01$ ) (Fig. 6).

### EFFECT OF OLMESARTAN ON F2 ISOPROSTANE

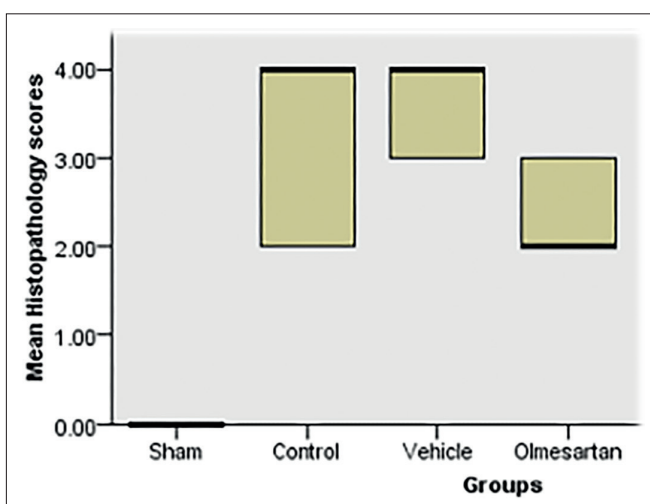
The results showed that tissue levels of F2isoprostanewere significantly elevated ( $p \text{ value} \leq 0.01$ ) in control and vehicle groups in comparison with sham group. Pretreatment with Olmesartan resulted in a marked decrease in the levels of F2 isoprostane (Fig. 7).

### EFFECT OF OLMESARTAN ON BCL-2

In this study, the renal tissue levels of Bcl-2 were significantly decreased ( $p \text{ value} \leq 0.01$ ) in control and vehicle groups compared



**Fig. 13.** Representative images of renal tissue sections showing the intensity of immunoreactivity among study groups: A: Renal section of IHC of the sham group showing a weak immunoreactivity (blue arrows) in 15% of examined renal tubules IHC (10x40); B: Renal section of IHC of the control group shows a moderate intensity staining (blue arrows) in 45% of examined renal tubules IHC 10x40; C: Renal section of IHC of the vehicle group shows a moderate intensity of the cytoplasmic staining (blue arrows) in 35% of examined renal tubules IHC10x40; D: Renal section of IHC of Olmesartan group showing a strong intensity of the cytoplasmic staining (black arrows) for HO-1 in 60% of examined renal tubules IHC 10x40.



**Fig. 14.** Mean histopathological scores of renal tissues of the four experimental groups. Data are represented as mean  $\pm$  sem and analyzed using Kruskal-Wallis test, n=5.

to the sham group. In contrast, pretreatment with Olmesartan resulted in a marked increase in the levels of Bcl2 (Fig. 8).

#### EFFECT OF OLMESARTAN ON BAX

The results showed that levels of the BAX in the renal tissues of control and vehicle groups were increased in comparison with sham group. However, these levels were decreased in Olmesartan treated group ( $p$ -value  $\leq 0.01$ ) (Fig. 9).

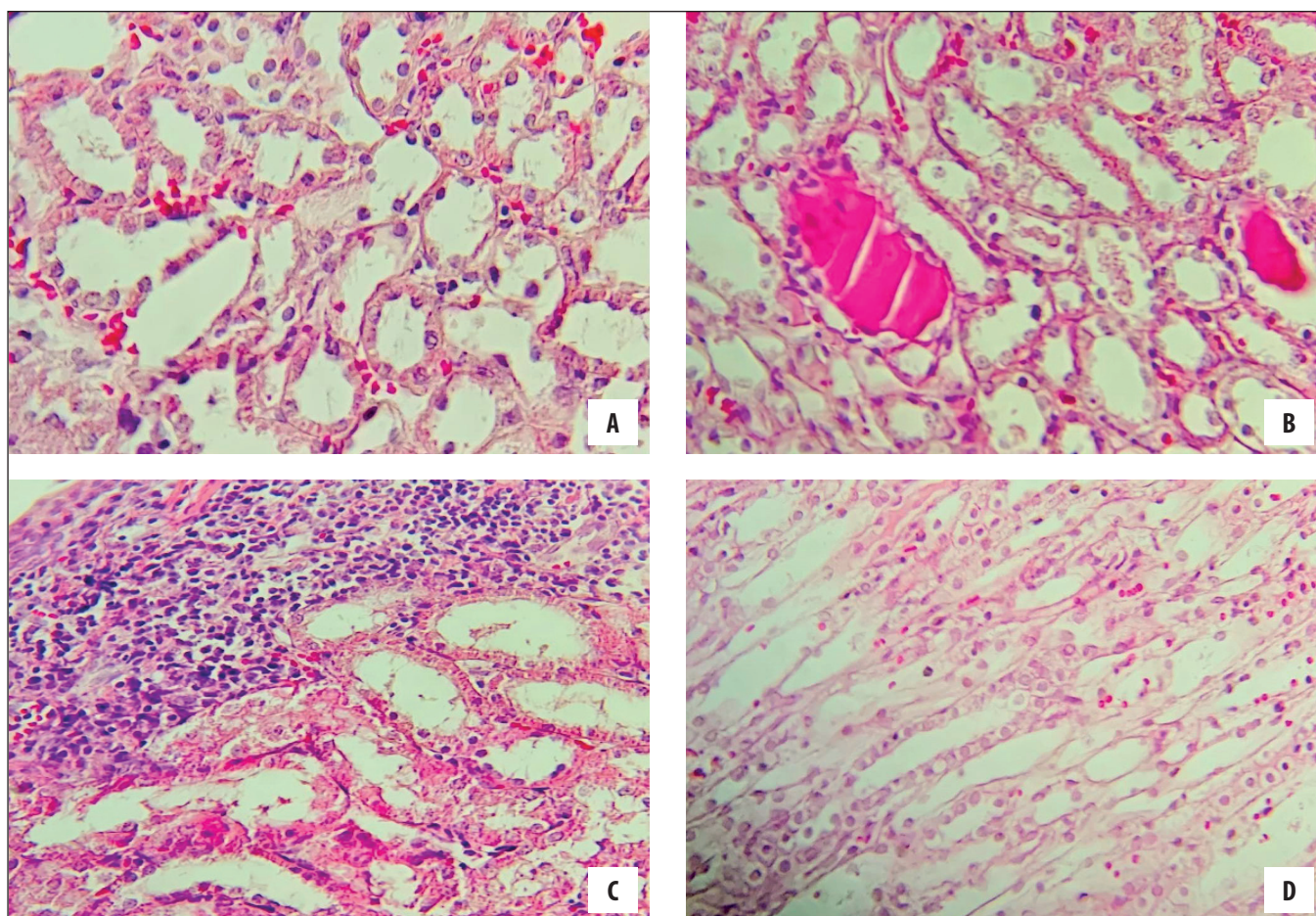
#### EFFECT OF OLMESARTAN ON NRF2/HO-1 LEVELS USING IMMUNOHISTOCHEMISTRY ANALYSIS

To investigate the levels of Nrf2 in the renal tissue, immunohistochemistry was employed by probing the Nrf2 with polyclonal antibody against Nrf2 or HO-1. Data showed a strong intensity of labeling with Nrf2 and HO-1 compared to control and vehicle groups showed in Quick H score (Fig. 10, 12 and represented in Fig. 11 and 13. A-D).

#### HISTOLOGICAL ANALYSIS

The histopathology analysis revealed a minor tubular damage in the kidney. In compared to the sham group,





**Fig. 15.** Representative images of renal tissue sections stained with hematoxylin and eosin: A: sham group shows normal histology of renal tubules sections; B: control group showing a marked damage in the renal tubules including three ischemic changes (cellular swelling, cytoplasmic eosinophilia (yellow arrows) and Eosinophilic cast (red arrows); C: vehicle group showing a marked damage in the renal tubules including inflammation (black arrows), cellular swelling and increase in cytoplasmic eosinophilia (red arrow); D: Olmesartan group shows a 40% renal tubular damage (black arrows), and normal tubules (red arrows).

there were more damages tubules and cell dilatation in the control and vehicle groups ( $p \leq \text{value } 0.001$ ) (Fig. 14 and 15A-C). In contrast, the Olmesartan pretreatment group showing improvements in the renal tissues ( $p\text{-value} \leq 0.001$ ) (Fig. 14 and 15D).

## DISCUSSION

IRI is the underlying cause of AKI thereby highlighting this condition is of the interest of researchers. It is the result of a decreased in oxygen supply and nutrition delivery to cells. The imbalance of local tissue oxygen demand, resources, and wastes are produced by cell metabolism can cause tubular epithelial damages, and if the damage is severe enough, the cells could die through necrosis and apoptosis, resulting in acute tubular necrosis (ATN), which includes organ features such as electrolyte, water homeostasis, and decreased waste excretion output of metabolism [30]. AKI caused by ischemia-reperfusion injury is associated with a high mortality and morbidity rate, as well as increased therapeutic costs in both adult and pediatric populations [31]. The results of the current study show that urea and creatinine levels were significantly in-

creased in the control and vehicle groups compared to the sham group. This result is in consistent with results of a study performed by Alawadi et al. [21] showing that levels of urea and creatinine in rats subjected to 30 min ischemia followed by 2h of reperfusion were considerably elevated. Some of these pathogenic stages or mechanisms may result in tubular and epithelial cell injury and destruction, as well as loss of kidney functions as a deficiency in GFR, which results in a rise in serum urea and creatinine, as well as a decrease in urine output [3]. Pretreatment with Olmesartan decreased the levels of urea and creatinine compared to the control and sham groups indicating that Olmesartan could improve the renal function through its effect on urea and creatinine. These findings are in line with those of previous studies [22]. This study shows that levels of IL-6 were markedly elevated in control and vehicle groups compared to the sham group. These results are in accord with recent studies indicating that levels of IL-6 were markedly increased following 30 min of ischemia and 24 h of restoration in rat model [32]. In contrast, levels of IL-6 decreased in Olmesartan pretreated group suggesting that Olmesartan may have a Nephroprotective effects on the renal tissues exposed to IRI. Several reports showing

the potential protective effect of Olmesartan by reducing the damage and inflammation in colon where tissue levels of IL-6, TNF- $\alpha$ , IL-1 and serum C-reactive protein in colon were decreased [33]. Furthermore, the current study found that TNF- $\alpha$  and TNF- $\alpha$  receptor levels in the renal tissues were increased in the control and vehicle groups in comparison with sham group. These results seem to be consistent with other research which found that levels of TNF- $\alpha$  were elevated following 30 min of ischemia 48 h of restoration [34]. Many inflammatory kidney diseases have been associated with increased TNF- $\alpha$  including glomerulonephritis, acute renal failure, and IR of the kidney. When TNF- $\alpha$  binds to its surface receptor, particularly TNFR1 the Fas-associated death domain (FADD) is activated resulting in a cell death by promoting apoptosis through release of cytochrome c from mitochondria [35]. Pretreatment with the Olmesartan reduced the levels of TNF- $\alpha$  and TNF- $\alpha$  receptor in ischemic kidney highlighting its potential role in reducing the renal deterioration [22]. Another important finding was that elevated levels of MCP-1 in renal tissues of the control and vehicle groups compared to the sham group. These results are in agreement with other reports where showed that MCP-1 promotes monocyte trafficking from the bone marrow to inflamed tissue in response to inflammatory signals [36]. In contrast, Olmesartan pretreatment resulted in a marked drop in levels of MCP-1 in renal tissues in comparison with control and vehicle groups. In accordance with present results, previous studies have demonstrated that large doses of Olmesartan reduced CD8+ T cell infiltration and M1 macrophage activation, resulting in fewer necrotic lesions [37]. Moreover, the results of this study show that tissue levels of F2-isoprostane were higher in control and vehicle groups compared to sham group. These results reflect those of Alawadi et al who also found that rats subjected to 30 min ischemia and 2 h of reperfusion caused a marked increase in levels of F2-isoprostane in the renal tissues [21]. These elevated levels were decreased in Olmesartan treated group. These results are in line with those of previous reports where they found that three days of the oral administration of Olmesartan to male rats resulted in a reduction in levels of F2-isoprostane which is a marker of the oxidative stress [38]. The current study shows that levels of Bcl2 were dropped in control and vehicle groups compared to sham group while levels of BAX were elevated. The apoptosis pathway is an important cell death process that is involved in both healthy and pathological cell states. The mitochondrial outer membrane permeabilization (MOMP) route, which results in the release of apoptogenic components such as cytochrome c, is one of the two primary apoptotic processes involved in death receptors and mitochondria. Bcl2 family proteins govern the permeabilization of the mitochondrial outer membrane during apoptosis. MOMP is caused by the pro apoptotic protein (Bax), while MOMP is inhibited by the anti-apoptotic protein (Bcl2) [39]. Pretreatment with Olmesartan resulted in an increase and decrease in levels of Bcl2 and BAX respectively. These results are in agreement with previous reports showing the Nephroprotective effect of ARBs is mediated by increase of Bcl-2 and decreased BAX and caspase-3 expression, the activity of caspase-3 and the

amount of Bax were both reduced by a novel angiotensin II receptor antagonist, whereas the level of Bcl-2 was enhanced [22]. The current study shows RIRI caused a severe damage in renal tissues as shown in histological analysis in comparison with sham group, pretreatment with Olmesartan reduced the degree of renal tissue damage. These results seem to be consistent with other research which found that Olmesartan reduced the deterioration in the renal tissues supporting the idea that Olmesartan could modulate the inflammatory responses and oxidative stress in the ischemic tissues [40]. The most interesting finding was that Nrf2 and HO-1 were elevated as indicated by immunoreactivity using immunohistochemistry technique. These results are in agreement with other reports in which they found that Nrf2 and HO-1 labeling intensities in the renal tissues were high in rats exposed to 30 min ischemia and 2 h of reperfusion [41]. Similarly, Olmesartan pretreatment resulted in a further increase levels of Nrf2 and HO-1 (represented as labeling intensity) but this increase was Significantly higher than that of those in control and vehicle groups highlighting the role of Olmesartan as potential renoprotective agent by regulating Nrf2/HO-1 signaling pathway [42].

## CONCLUSIONS

This study has identified that Olmesartan reduces the tissue levels of IL-6, TNF- $\alpha$ , MCP-1, F2-isoprostane, proapoptotic BAX and increases levels of the antiapoptotic Bcl2. In addition, Olmesartan reduces the deterioration of renal tissues following renal IRI. The most obvious finding to emerge from this study is that Olmesartan increases the expression levels of Nrf2 and HO-1 in the renal tissues following renal IRI. These findings suggest that Olmesartan may be a potential renoprotective medicine in renal IRI by its effects on the inflammatory responses, oxidative stress and modulation of the proapoptotic and antiapoptotic molecules.

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## ORIGINAL ARTICLE

# SYSTEM OF PROFESSIONAL COMMUNICATIVE LITERACY FORMATION FOR FUTURE MEDICAL WORKERS THROUGH THE PRISM OF LANGUAGE TRAINING

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## ABSTRACT

**The aim** of the study is to analyze the formation of professional communicative literacy of future medical workers, which includes a personal development component provided by independent language preparation for the use of medical terminology.

**Materials and methods:** The diagnostic stage, which was conducted in 2019-2020, included 400 students from four higher educational institutions: I. Horbachevsky Ternopil National Medical University, Danylo Halytsky Lviv National Medical University, Ivano-Frankivsk National University, Uzhhorod National University. These are students who completed the study of such disciplines as "Latin language and basics of medical terminology", "Human anatomy", "Histology", "Pharmacology" in the 1st year of studying.

**Results:** The comparative analysis shows that this determined the level of formation of the students' motivational sphere in the direction of speech preparation for the use of medical terminology in Latin language classes, and the following results were obtained. With regard to the main motivating points for Latin classes, it was established that 115 (28.75%) young people study it with great interest, strive to improve their knowledge of medical terminology further, as they are sure that they will definitely need it in their future professional activities.

**Conclusions:** Research and experimental verification of the effectiveness of pedagogical conditions and the structural-functional model of professional language training of future doctors for the use of medical terminology took place during the training of first-year-students at the higher educational establishment. Innovative methods of developing critical thinking, information and interactive technologies were used. Positive changes in the results of professional and language training of CG students (increase in average figure from 3.51 to 3.69 points) are caused by the natural influence of the traditional educational process, and changes in average figures in EG (from 3.51 to 3.92 points) show the effectiveness of implementation of defined pedagogical conditions.

**KEY WORDS:** professional communicative literacy, medical workers, language training, medical terminology, students

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## INTRODUCTION

Professional training is the process of learning at classes, independent work and the result, which is characterized by a certain level of future specialists' development, the formation of their professional knowledge, abilities and skills. At present, professional training, which originally arose in connection with the professional division of labour, depending on the level of qualification and complexity of the profession being mastered, involves obtaining higher and secondary special education [1; 2]. In a higher school professional training is carried out on the basis of a secondary comprehensive school or secondary special educational institution.

Characteristic features of the professional training of a medical specialist are: optimality (choice of appropriate educational material); structurality (distinguishing the components of professional training, their ordering and classification); integrativeness (the result of combining the

components of professional training into a single whole, which contributes to its preservation and functioning); functionality (a number of functions that regulate relationships and connections in professional training) [3-5].

Therefore, we define readiness for professional activity as a student's personal quality, which is manifested in the teacher's positive assessment of the level of future doctor's preparation for the use of medical terminology, as well as the student's self-assessment of himself as a subject of future professional speech activity [6; 7]. It helps to implement professional functions successfully, to use acquired knowledge and experience in practice correctly.

## THE AIM

The aim of the study is to analyze the formation of professional communicative literacy of future medical workers,

**Table 1.** Results of the diagnostic stage of the experimental study

Readiness components	Levels of students' readiness to use medical terminology								Averagereadiness (AR)
	High		Medium		Satisfactory		Low		
	Number of students	%	Number of students	%	Number of students	%	Number of students	%	
Personal development	110	27,50	108	27,00	121	30,25	53	13,25	3,67
Generalized results	113	28,25	111	27,75	117	29,25	59	14,75	3,70

which includes a personal development component provided by independent language preparation for the use of medical terminology.

## MATERIALS AND METHODS

The diagnostic stage, which was conducted in 2019-2020, included 400 students from four higher educational institutions: I. Horbachevsky Ternopil National Medical University, Danylo Halytsky Lviv National Medical University, Ivano-Frankivsk National University, Uzhhorod National University. These are students who completed the study of such disciplines as "Latin language and basics of medical terminology", "Human anatomy", "Histology", "Pharmacology" in the 1st year of studying.

## RESULTS

In order to establish the level of motivation to learn Latin among future medical workers, a survey was conducted, observations were made, and students were offered to work out the appropriate questionnaire (need, independence of study, difficulties that arose, areas of application of knowledge). This determined the level of formation of the students' motivational sphere in the direction of speech preparation for the use of medical terminology in Latin language classes, and the following results were obtained. With regard to the main motivating points for Latin classes, it was established that 115 (28.75%) young people study it with great interest, strive to improve their knowledge of medical terminology further, as they are sure that they will definitely need it in their future professional activities. However, 119 (29.75%) future doctors believe that classes in the Latin language are needed only for general development and they are only one of the means of obtaining new professionally significant information to some extent. The processing of the questionnaire data showed that for 105 (26.25%) students, the application of knowledge of the Latin language will take place only during studies, so they do not know whether the acquired knowledge, skills and abilities will help them to communicate in a professional environment. According to the results of the questionnaire, it was established that for 61 (15.25%) people, knowledge of the Latin language will not be used anywhere, therefore these students do not seek to learn it, they attend classes only to pass the exam, which indicates the need to work on the motivation of students.

To establish the level of medical terminology knowledge among future medical workers, a terminological dictation

was conducted. We created the content of the questions to determine the level of theoretical professional language readiness of students on the discipline "Latin language and basics of medical terminology", conducted an analysis of the success of professional knowledge of medical terms on the professional disciplines: "Human Anatomy", "Histology" and "Pharmacology" in the form of a test control, which established that a high level is characteristic of 110 (27.50%) students, an average level was assessed for 108 (27.00%) future doctors, a satisfactory level was found for 121 (30.25%) students, and a low level was revealed for 53 (13, 25%) students.

The analysis of tabular data shows that after the completion of the 1st year of study, the formation of professional and language readiness for the use of medical terminology among the students of the Medical University requires improvement in the direction of the formation of each component, as it is characterized by the following generalized indicators: a high level was found by 113 (28.25%) students; the average level is a characteristic of 111 (27.75%) future medical workers; 117 (29.25%) students have a satisfactory level; a low level was distinguished for 59 (14.75%) persons. Provided that each of the specified levels was characterized by a certain number of points (high – 5 points, average – 4, satisfactory – 3, and low – 2 points), then the average figure (AR) of the formation of the professional and language readiness of the future doctors to use medical terminology at the diagnostic stage of the study was 3.7 points.

The results of the diagnostic stage of the experimental study proved the need to improve the professional speech training of future medical workers for the use of medical terminology through the implementation of defined pedagogical conditions and the use of the developed structural-functional model.

The experimental study was divided into several stages. The ascertainment stage of the experimental study lasted during 2019-2020, during which control group (hereinafter – CG) and experimental groups (hereinafter – EG) of the students were determined. A total of 386 future doctors took part in the experiment. The basic approaches to the definition of CG and EG were compliance with the following conditions: 1) CG and EG had almost the same number of students: CG – 192 people, and EG – 194 students; 2) students in groups of both categories at the stage of entrance control had close peculiarities of the formation of each component and, in general, professional language readiness to use medical terminology.



**Table II.** Results of the ascertainment stage of the experimental study according to the data of entrance control

Components readiness	Gr.	Levels of students' readiness to use medical terminology at the stage of entrance control								Average
		High		Medium		Satisfactory		Low		
		Number of students	%	Number of students	%	Number of students	%	Number of students	%	
Personal development	CG	50	26,04	46	23,96	55	28,65	41	21,35	3,55
	EG	48	24,74	49	25,26	57	29,38	40	20,62	3,54
Generalized results	CG	48	25,00	44	22,92	57	29,69	43	22,39	3,51
	EG	47	24,23	47	24,23	58	29,89	42	21,65	3,51

In the control groups (CG), students studied according to the traditional method. In the experimental groups (EG), the professional language training of future doctors for the use of medical terminology was carried out according to the experimental method. While dividing the students into CG and EG, it was taken into account that the number of students in both categories of groups and the initial level of formation of all components of the professional language readiness of future doctors at the stage of entrance control (EC) should be close in value. The results of the ascertainment stage of the experimental study according to the entrance control data are shown in Table II.

Entrance control was carried out by means of questionnaires and testing after studying the first topic in the 1st semester of study in the 1st year, when students had already mastered a certain set of knowledge of medical terminology, since according to the curriculum, the Latin language is studied during the 1st year, and had the opportunity to demonstrate the ability to use them under time studying other disciplines "Human Anatomy", "Histology", "Pharmacology".

During the study of the first topic, CG and EG students got acquainted with the history of the Latin language and the development of medical terminology; studied the Latin alphabet and classification of sounds, pronunciation of vowels, consonants, letter combinations, diphthongs; prefixes and roots of Latinized Greek words with the letter "u"; Latin accent rules; the structure of anatomic-histological and pharmaceutical terms; grammatical categories of the noun; signs of nouns belonging to one or another declension and determining their base; anatomical, histological and pharmaceutical lexical minimum of nouns of five declensions.

Future medical workers of CG and EG learned to read and write in Latin; to use a Latin dictionary in accordance with the Latin alphabet, to write medical (anatomical, histological, and pharmaceutical terms) orthographically correctly; to determine the number of syllables in a Latin word; to emphasize trisyllabic and polysyllabic words; to determine the gender, number, and case of different types of nouns and find their base.

CG and EG students were offered the exercise of writing words in Latin letters: *karbo*, *al'oe*, *kefir*, *kardiakus*, *dispepsia*, *ekvalis*, *sapo*, *acidum*, *majus*, *keratoma*, *tetracyklinum*, *ekzakte*, *menta*, *kol'or*, *mikstura*, *kuprum*, *ljak*, *gipovitaminozus*, *formicikus* and so on; a list of Latin words

is presented in order to reveal their ability to determine the length or shortness of the penultimate syllable of each word and to put stress, since the correct stress of Latin medical terms is a key condition in their use: *purpurea*, *globulus*, *medicamentum*, *Leonurus*, *diaeta*, *toxoplasma*, *sanguis*, *vertebra*, *bifolia*, *extractum*, *reflexus*, *naturalis*, *nomen*, *lamella*, *cerebrum*, *anatomia*, *oxydatio*, *Kalanchoe*, *injectio*, *gangraena*, *ligamentum*, *infusum*, *vesica*, *stomachus*, *palpebra*, *thermometrum*, *amylum* and others. In addition, the entrance control was conducted at the classes on human anatomy, histology, and pharmacology to determine the ability of CG and EG students to transform these terms in interdisciplinary integration.

To self-monitor the knowledge of CG and EG students on the studied topic, to determine the level of mastery of the educational material, the teacher used sample test tasks:

*In which variant is the Latin consonant "c" pronounced like the Ukrainian "u" [ts]:*

- carbo*
- crystallus*
- decoctum*
- lac*
- coeruleus*

Therefore, the initial level of formation of the professional language readiness of future doctors for the use of medical terminology was almost identical. The analysis of the data of the results of the entrance control (EC) testifies to the same conditions for the entry of CG and EG students into the experimental study.

The analysis of the results of the formation of the personal development component of the professional and language readiness of future doctors to use medical terminology at the stage of entrance control in the CG and EG groups shows that before the start of the formative stage of the experimental research, the EG students had slightly lower indicators of the average score i.e. in the CG – 3.55 points, and in EG – 3.54 points, which is 0.01 points less than the students of the control groups; with a high level of formation of the personal development component of professional language readiness in CG there were 26.04%, in EG – 24.74% of students; there were 23.96% young people with an average level in CG, and in EG – 25.26% of students; with a satisfactory level – 28.65% in CG and 29.38% of students in EG; 21.35% of CG students and 20.62% of experimental students showed a low level.

The comparative analysis of the data in Table 2 shows that according to the generalized results, 25% of the students in the CG and 24.23% of the students in the EG have a high level of professional-language readiness to use medical terminology; there were 22.92% of students with an average level in CG and 24.23% of people in EG; a satisfactory level is characteristic of 29.69% of CG students and 29.89% of EG students; 22.39% of people in CG and 21.65% of EG students showed a low level; the value of the average result in CG and EG was the same – 3.51 points.

Thus, the analysis of the results of the ascertainment stage of the experimental study shows that according to the data on the formation of all components and in general the professional and language readiness of future doctors to use medical terminology, the students of CG and EG entered the experimental study with almost the same indicators. Meanwhile, the obtained figures indicate that the professional training of future doctors needs improvement and the organization of training based on the implementation of outlined pedagogical conditions, which must be verified experimentally. Therefore, the level of reliability of the main results of scientific research increases significantly if they are based on experimental data.

In order to analyze the results of the experimental study, a comparison was made of indicators of professional language readiness of future doctors to use medical terminology at the stages of entrance and final control of CG and EG students. By surveying students, assessing their knowledge of the Latin language (test control), the ability to use the acquired knowledge in practical situations (reading and understanding medical terms in Latin, writing prescriptions with the names of drugs in Latin), analyzing the results of self-education activities, digital indicators of the formation of each were established readiness component at different stages of the experiment at four levels: high, medium, satisfactory, low. The results of the formation of the motivational and value component of the professional and language readiness of future doctors who studied in CG and EG are shown in Table III.

The correlation between the indicators in Table 3 shows the effectiveness of the implementation of the first pedagogical condition in the formation of the motivational and value component of the professional and language readiness of future doctors to use medical terminology, since the dynamics of changes in the number of students in CG and EG, which are characterized by a certain level, have significant differences:

- according to figures of the average level, there was an increase of students in CG from 24.48% to 29.69%, and in EG this data shows a decrease in the number of such students from 24.74% to 10.83%, which is explained by a significant increase of EG students with a high level.

According to the data in Table 3, a comparative analysis of the formation of the personal development component in CG and EG students was carried out:

- according to high-level figures, there was an increase in the number of students in CG from 26.04% to 27.08%, and in EG – from 24.74% to 43.30%;

- according to the figures of the average level, there is an increase in the number of CG students from 23.96% to 26.56%, and in EG – a decrease from 25.26% to 9.79%, which is explained by a significant increase in EG students with a high level;

- according to figures of a satisfactory level, an increase in the number of students is noted in both categories of groups: in CG – from 28.65% to 30.21%, and in EG – from 29.38% to 37.12%;

- according to low-level figures, there was a decrease in the number of students in CG from 21.35% to 16.15%, and in EG – from 20.62% to 9.79%;

- according to the average figures, there was an increase in CG from 3.55 to 3.65 (by 0.1 points), and in EG – from 3.54 to 3.87 (by 0.33 points), which is by 0.23 points more than in CG.

In fig. 1 it is shown more positive dynamics of changes in the formation of the personal development component among EG students compared to CG students.

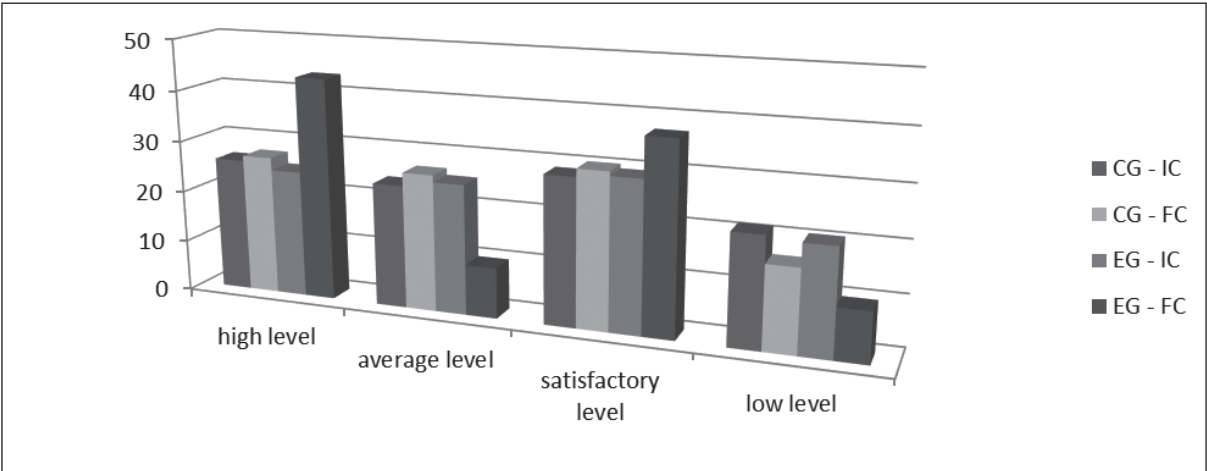
Therefore, the results obtained during the experimental testing of our proposed method of professional language training of future doctors for the use of medical terminology testify to its effectiveness and feasibility of introduction into the educational process of a higher medical school.

The analysis of the results of the ascertainment stage of the experiment proved that according to digital indicators of the formation of all components and in general the professional and language readiness of future doctors to use medical terminology, students of CG and EG entered the experimental study with the same indicators.

At the formative stage of the research, the effectiveness of the implementation of pedagogical conditions of professional speech preparation for the use of medical terminology of EG students during the study of the disciplines: "Latin language and basics of medical terminology", "Human anatomy", "Histology", "Pharmacology" was experimentally verified. The author's methodology was based on the systematic use of innovative methods. In the practical classes, a special database of thematically selected schemes was used, reflecting the educational material from the discipline "Latin language and the basics of medical terminology" taught in close connection with anatomic-histological, clinical and pharmaceutical terminology [8; 9]. Oral and written exercises were based on lexical material related to the profession of a doctor or a scientist in the field of medicine. This contributed to the formation of professional language readiness of future doctors for the use of medical terminology. The classes used technologies for the development of critical thinking, interactive forms and methods of learning ("Basket of concepts and terms", "Graphic schemes", "Fishbone", "Clusters", "Sinquain", dialogue, demonstration and solving of situational problems, etc.), means of information technologies. In order to activate the self-educational activity of students, it was organized that they maintain a "Medical and Terminological Dictionary", which contributed to the formation of students' professional language readiness to use medical terminology [10; 11].

**Table III.** Results of the formation of the personal development component of the professional language readiness of future doctors to use medical terminology

Groups and number of students	EC	Levels of the personal development component formation of the future doctors' professional language readiness for the use of medical terminology								Average
		High		Medium		Satisfactory		Low		
		Number of students	%	Number of students	%	Number of students	%	Number of students	%	
CG – 192 st.	EC	50	26,04	46	23,96	55	28,65	41	21,35	3,55
	FC	52	27,08	51	26,56	58	30,21	31	16,15	3,65
EG – 194 st.	EC	48	24,74	49	25,26	57	29,38	40	20,62	3,54
	FC	84	43,30	19	9,79	72	37,12	19	9,79	3,87



**Fig. 1.** Dynamics of changes in the formation of the personal development component of the professional language readiness of future doctors to use medical terminology

### DISCUSSION

Technologies and methods based on active interpersonal interaction, communication, and cooperation are of particular importance in the professional and language training of future medical workers for the use of medical terminology. Therefore, teachers, psychologists, and researchers use active and interactive teaching methods not only to create comfortable conditions for educational activities, to improve the perception of educational material, but also to improve the language activity of future specialists. Meanwhile, they associate the semantic meaning of the term “interactive learning” with such concepts as “activity”, “interaction”, “interactivity”, “active learning methods”, “interactive learning methods” and consider the learning process as communication, cooperation of a teacher and a student. A common feature (characteristics) of these concepts is the activity of students, which is interpreted in the pedagogic dictionary as “the quality of the personality, expressed in increased activity, in the external manifestation of views and beliefs” [12; 13].

Professionally oriented medical Latin is an integrated discipline, the study of which combines the cycles of learning phonetics, grammar, vocabulary, word formation and orthography of the classical Latin language, as well as the study of grammar, vocabulary and stylistics of the actual medical Latin language. During the study of the Latin lan-

guage and the basics of medical terminology, the teacher lays the foundations for preparing to use the professional terminology of a future doctor or a medical worker, who will later be able to use medical terminology of Greek-Latin origin in his practical and scientific activities consciously, which will indicate his professional terminological literacy, because every specialist, in order to master the profession completely and to carry out successful professional activity, needs to understand correctly and apply the appropriate special vocabulary accurately, therefore, to know the terminology of his specialty. Features of the professional and language training of medical workers for the use of medical terminology outline the need to expand students' knowledge of the history of the Latin language use, the need to understand the knowledge of the Latin language for the professional activity of a contemporary doctor, interdisciplinary integration in the study and use of professional vocabulary by students of the medical higher schools as a means of communication in professional activities, in order to read medical literature freely, understand oral messages, be able to communicate with specialists in the medical field. However, the professional language readiness of the future doctor is not limited only to the acquisition of knowledge, skills and abilities. The collective formation of mental, volitional and emotional qualities of the individual is necessary [14; 15].

The main features of professional language training for future medical workers are motivational-value, cognitive-orientation, functional-activity and personal development components. The formation of the personal development component of the professional and language readiness of future doctors to use medical terminology is largely the result of the self-educational activities of the students at the medical higher schools, who apply the acquired knowledge in the course of studying the discipline "Latin language and the basics of medical terminology" for solving modern professional tasks; focusing on personal development of students, formation of professionally important qualities of future doctors, development of their interests, awareness of modern problems of practical health care, which implies continuity of work in the direction of self-actualization, self-knowledge and self-development, aimed at revealing professional and personal potential [16].

The formation of the personal development component is determined by such qualities of the student as perseverance, determination, emotionally adequate behavior in various professional situations, the desire to solve various complex tasks using medical terminology independently, a sense of responsibility for the results of one's work. This indicates the development of personality i.e. the formation of personality as a social quality of an individual as a result of his socialization and upbringing [17]. Moreover, education is dominant in the development of the future medical worker's personality. Indicators of the formation of this component are: students' work with information sources (dictionaries, textbooks, Internet resources); development and implementation of didactic exercises aimed at professional speech self-improvement; maintenance of the "Medical and Terminological Dictionary" [18].

The formation of the personal development component affects the formation of all other components, contributing to the creation of an educational atmosphere, the desire to achieve high results in the future professional activity of a doctor. For an in-depth and purposeful study of the problem of professional language preparation of future doctors for the use of medical terminology, three stages of experimental research were determined: diagnostic, ascertaining and formative ones [19; 20].

## CONCLUSIONS

The effectiveness of the formation of the professional and language readiness of future doctors to use medical terminology depends on the purposeful implementation of the defined pedagogical conditions, the developed structural and functional model and the application of the methodology of the professional and language training of students proposed in the dissertation during the study of the disciplines: "Latin language and the basics of medical terminology", "Human anatomy", "Histology", "Pharmacology".

In order to improve the effectiveness of professional language training of future medical workers, the criteria and components of readiness to use medical terminology for their professional development have been defined and

substantiated. The indicators of the personal development component are substantiated – students' work with information sources (dictionaries, textbooks, Internet resources), development and implementation of didactic exercises aimed at professional and speech self-improvement, maintenance of the "Medical and Terminological Dictionary". Four levels (high, medium, satisfactory, low) of the components' formation of the professional language readiness of future doctors for the use of medical terminology have been specified.

Research and experimental verification of the effectiveness of pedagogical conditions and the structural-functional model of professional language training of future doctors for the use of medical terminology took place during the training of first-year-students at the higher educational establishment. Innovative methods of developing critical thinking, information and interactive technologies were used. In order to analyze the results of the formative stage of the experimental study, the figures of the results of the entry (EC) and final (FC) control of the students of the control (CG) and experimental groups (EG) were compared according to the levels of all components formation and, in general, the professional and language readiness of future doctors to use medical terminology. Positive changes in the results of professional and language training of CG students (increase in average figure from 3.51 to 3.69 points) are caused by the natural influence of the traditional educational process, and changes in average figures in EG (from 3.51 to 3.92 points) show the effectiveness of implementation of defined pedagogical conditions.

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*The Authors declare no conflict of interest.*

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## ORIGINAL ARTICLE

# THE RELEVANCE OF RS6777038 AND RS6444082 OF IGF2BP2 GENE POLYMORPHISM AND TYPE 2 DIABETES MELLITUS: A CASE CONTROL STUDY

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## ABSTRACT

**The aim:** We investigate IGF2BP2 gene polymorphisms (rs6777038 and rs6444082) association with T2DM of Iraqi sample.**Materials and methods:** The study involves 800 participants that divided to a healthy control group (400) and T2DM patients (400). Fasting blood sugar (FBS), triglycerides (Tgs), high-density lipoprotein cholesterol (HDL-Ch), total cholesterol (T-Ch), low-density lipoprotein cholesterol (LDL-Ch), and fasting insulin measured for both participant groups. IGF2BP2 gene has been genotyped for polymorphisms, rs6777038 and rs6444082 using the PCR-RFLP technique.**Results:** Logistic regression analysis testing for rs6777038 revealed that the genotype and allele frequency differ significantly ( $p=0.009$ ) between T2DM and control group. In codominant model, TT genotype carriers had higher risks for diabetes than control also in the recessive model TT genotype significantly had higher risk for diabetes than control group. The other models of rs6777038 failed to reveal significant differences. The rs6777038 genotypes as codominant model showed significant differences with phenotypic characters of BMI, insulin and HOMA-IR. As well as, this SNP as dominant model showed significant differences with fasting insulin and HOMA-IR. However, rs6444082 genotypes only as dominant model reveal significant variation with HOMA-IR.**Conclusions:** This study confirmed the variant rs6777038 of IGF2BP2 possibly associated with T2DM risks and some anthropometric parameters such as lower fasting insulin, HOMA-IR and BMI in Iraqi T2DM participants.**KEY WORDS:** rs6777038, rs6444082, IGF2BP2 gene, type 2 diabetes mellitus

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## INTRODUCTION

Diabetes is an epidemic that has been identified in all regions and populations, including rural areas in middle- and low-income countries. With changing lifestyles and rising obesity, diabetes prevalence had globally increased [1]. Type 2 diabetes mellitus (T2DM) represents the most common diabetes type, as it accounts for over 90% of the cases and affects between 10% and 20% of patients over the age of 45 in diverse developed countries [2]. It is usually linked to peripheral tissues' physiological resistance to insulin activity. Despite the fact that insulin resistance (IR) and increasing pancreatic cell dysfunction were established as two important aspects in the pathophysiology of T2DM [3]. Several gene polymorphisms have been discovered to play a role in the disease's progression. Numerous polymorphisms have also been shown to influence T2DM development [4]. Insulin-like growth factor 2 (IGF 2) is a polypeptide growth factor that regulates development and growth [5]. It belongs to insulin family of polypeptide growth factors. During development, epigenetic, transcriptional, and translational mechanisms delicately modulate its expression [6]. RNA-binding proteins (RBPs), binding to the

transcripts of IGF-2 and governing their processing, like stability, localization, and translation, also influence it post translationally [7]. The IGF2BP2 gene, which has 24 exons and is present on chromosome 3q27.2, has been involved in the embryogenesis and pancreatic development [8]. Three separate genome-wide association studies published in 2007 found a substantial link between genetic variation in the IGF2BP2 gene and human T2DM [9-11]. Previous research has found that IGF2BP2 single nucleotide polymorphisms (SNPs), specifically rs1470579 and rs4402960, are associated with occurrence of T2DM in a variety of ethnic groups, which include the majority of the European ancestry populations, Asian ancestry populations, Indian populations, and Latino/Hispanic populations [12-17]. The preceding studies of IGF2BP2 polymorphisms with different disease are directed towards intron 2 variants solely [18-20]. Population's haplotype variation of IGF2BP2 association with T2DM possibly presented upstream or downstream different from the commonly known SNPs. However, SNPs that presents as risk factors of some diseases are variable amongst the ethnic's groups [21-22]. In spite of this, limited studies about alternative variants of IGF2BP2

association with T2DM such as rs6777038 and rs6444082. This study aimed to estimate these two alternative SNPs association with T2DM risks in the Iraqi population.

## THE AIM

The aim of our research was to investigate IGF2BP2 gene polymorphisms (rs6777038 and rs6444082) association with T2DM of Iraqi sample.

## MATERIALS AND METHODS

### STUDY PARTICIPANTS

The Medical Ethics Committee at Faculty of Medicine\University of Kufa had approved the study project. Participant's agreements taken as signed written informed consent. This case-control study includes 400 T2DM patients diagnosed according to American Diabetes Associations features [2] at Al-Najaf Center for Diabetes and Endocrine in Al-Saddr Medical City. The control group includes 400 apparently healthy individuals. Individuals with heart, renal, hepatic or malignant diseases were excluded from the study. The age of participants between (35-65 y) and biochemical parameters measured for them including fasting blood glucose (FBG), triglycerides (Tgs), T-Ch, HDL-Ch, LDL-Ch, and fasting insulin.

### SAMPLE COLLECTION AND ASSAYS

Fasting participants prepared for 5 ml venous blood withdrawal, this blood divided to 2 ml for genotype analysis and 3 ml for lipids, glucose and insulin measurements. The biochemical analysis performed by measuring FBG, Tgs, T-Ch, HDL-Ch, and LDL-Ch spectrophotometrically using Randox kits as the manufacturer recommended. Insulin measured by using of Human sandwich ELISA kit of Abcam Company (ab278123) where the procedure carried out as manufacturer recommended. Insulin resistance (IR) calculated by using of equation of Homeostatic Model postulated by Matthews [23]  $HOMA-IR = [FSG \text{ (mg/dL)} * FSI \text{ (}\mu\text{U/mL)}] / 405$ , where FSG and FSI represent fasting serum glucose and insulin respectively.

### EXTRACTION OF DNA AND GENOTYPE ANALYSIS

DNA extracted from whole blood by the use of the Blood DNA Isolation Mini Kit (Cat. 46380) of NORGEN Biotek Corp Company. Extracted DNA inspected for purity and quantity using A260/A280 ratio spectrophotometrically as well as electrophoresis technology. Method of RFLP-PCR used to replicate DNA and detection of allele genotype. The primers designed by NCBI online site. Designed primers sequences of rs6777038 forward primer is GTTTCTCGTTGGCTAAGCTG and reverse primer is CCAGCCCTAATACCTGTGA. In addition, rs6444082 forward primer is ACCTGTGACTTGGCTTTCACA and the reverse primer is TCAGTGGTGTATGTGCAAGTGT. PCR

reaction mix (25 $\mu$ L) consisted from 1 $\mu$ L of the DNA sample, 1 $\mu$ L of every primer (10pm/ $\mu$ L), 15 $\mu$ L of master mix and 7  $\mu$ L of demineralized-water. Thermal cycles of PCR process are; initial denaturing step at 95°C for 5min; which is followed by 35 cycles of denaturing at 95°C time of each one is 30s; then annealing step for 30s at 53 and 54°C of rs6777038 and rs6444082 respectively; and extension step at 72°C for 35 s. The final extension step at 72°C for 5min. PCR products are 335 and 247 b of rs6777038 and rs6444082 respectively. The restriction enzyme BccI (NEB & cat. R0704S) used to cut rs6777038 (335 b) of wild genotype to 132 and 203 b segments. Whereas, BsrI (NEB Cat. R0527S) endonuclease cut wild genotype of rs6444082 segment (247b) to 78 and 169 b segments. The products approved by gel electrophoresis techniques as 2% agarose gel used in each run. Allele type discriminated by UV transilluminator for each sample and at least 25% re-genotyped for result approval.

### STATISTICAL ANALYSES

Hardy Weinberg Equilibrium tested for control group deviation by chi-square analysis, which was within Hardy principles. Biochemical features and anthropometric measurers expressed as mean and SD for each groups of participants. Statistical analysis has been performed with SPSS software V25.0 (SPSS Inc., Chicago, IL, US). Differences between groups for continues variables inspected by Student's t-test. Risk factor of T2DM with genotype alleles expressed as odd ratio (OR) with 95% CI and significances < 0.05 by logistic regression analysis. The genotype results of Odd Ratio adjusted for BMI, gender and age as cofounders.

## RESULTS

The study includes 400 T2DM (231 male/169 female) and 400 control group (226 male/174 female) as final approved results. The diabetic and anthropometric parameters differed significantly between the control group and T2DM as shown in table I. The two SNPs rs6777038 and rs6444082 tested for Hardy equilibrium of control group, using non-parametric chi-square tests, which were within Hardy equilibrium ( $p = 0.64$  &  $0.95$  respectively). Logistic regression analysis testing for rs6777038 revealed that the genotype and allele frequency differ significantly ( $p=0.009$ ) between T2DM and control group. In codominant model, carriers of TT genotype had higher risk for diabetes than control (OR = 4.25, 95% CI = 1.62-11.14 and  $p = 0.003$ ) after adjusted for age, BMI and gender. In the recessive model TT genotype significantly had higher risks for diabetes (OR = 3.84, 95% CI = 1.49-9.9 and  $p = 0.005$ ) than control group. The other models of rs6777038 failed to reveal significant differences after adjustment with age, BMI and gender. However, rs6444082 reveals insignificant difference ( $p=0.46$ ) for H (H = C, A or T) allele compared with control for all models, as shown in table II.

Analysis of variance for rs6777038 genotypes as co-dominant model showed significant differences with phenotypic characters of BMI, insulin and HOMA-IR ( $p = 0.012$ ,  $0.001$  and  $0.006$  respectively) listed in table III.

**Table I.** Diabetic and anthropometric parameters for the control and T2DM groups.

Biochemical parameters	T2DM (n=400)	Control (n=400)	p
	Mean $\pm$ SD	Mean $\pm$ SD	
Gender (M/F)	231/169	226/174	0.72
Age (yr.)	48.1 $\pm$ 7.59	46.88 $\pm$ 7.57	0.023
BMI (kg/m <sup>2</sup> )	26.45 $\pm$ 2.11	22.38 $\pm$ 1.45	<0.0001
FBG (mg/dl)	207.92 $\pm$ 23.28	80.01 $\pm$ 11.44	<0.0001
Tgs (mg/dl)	175.97 $\pm$ 44.51	76.4 $\pm$ 26.33	<0.0001
Total-C(mg/dl)	222.65 $\pm$ 43.08	144.17 $\pm$ 37.99	<0.0001
HDL-C (mg/dl)	25 $\pm$ 8.92	50.46 $\pm$ 11.95	<0.0001
VLDL-C(mg/dl)	35.19 $\pm$ 8.9	22.9 $\pm$ 10.46	<0.0001
LDL-C (mg/dl)	162.46 $\pm$ 44.86	70.82 $\pm$ 15.79	<0.0001
Insulin ( $\mu$ U/ml)	6.69 $\pm$ 1.56	1.93 $\pm$ 0.66	<0.0001
HOMA-IR	3.44 $\pm$ 0.9	0.38 $\pm$ 0.14	<0.0001

**Table II.** SNPs genotype and allele frequencies of T2DM and control groups.

IGF2BP2 polymorphisms	T2DM	Control	Unadjusted model		Adjusted model *	
			OR (95% CI)	p	OR (95% CI)	p
rs6777038	n=400	n=400				
Allele (%)						
C	586(73.25)	641(80.12)				
T	214(26.75)	159(19.88)	1.47(1.17-1.86)	0.001	1.59(1.12-2.25)	0.009
Genotype (%)						
CC	213(53.25)	254(63.5)				
CT	160(40)	133(33.25)	1.43(1.07-1.92)	0.016	1.31(0.84-2.04)	0.24
TT	27(6.75)	13(3.25)	2.48(1.25-4.92)	0.01	4.25(1.62-11.14)	0.003
Dominant Model (%)						
CC	213(53.25)	254(63.5)				
CT+TT	187(46.75)	146(36.5)	1.53(1.15-2.03)	0.003	1.51(0.99-2.31)	0.058
Recessive Model (%)						
CC+CT	373(93.25)	387(96.75)				
TT	27(6.75)	13(3.25)	1.1(4.24-0.005)	0.026	3.84(1.49-9.9)	0.005
rs6444082						
Allele (%)						
G	614(76.75)	635(79.375)				
H**	186(23.25)	165(20.625)	0.92(1.48-0.46)	0.2	1.14(0.8-1.63)	0.46
Genotype (%)						
GG	236(59)	254(63.5)				
GH	142(35.5)	127(31.75)	0.89(1.62-0.37)	0.22	1.23(0.78-1.92)	0.37
HH	22(5.5)	19(4.75)	0.66(2.36-0.37)	0.5	1.23(0.78-1.92)	0.37
Dominant Model (%)						
GG	236(59)	254(63.5)				
GH+HH	164(41)	146(36.5)	0.91(1.61-0.39)	0.19	1.21(0.79-1.86)	0.39
Recessive Model (%)						
GG+GH	378(94.5)	381(95.25)				
HH	22(5.5)	19(4.75)	0.62(2.19-0.96)	0.63	1.02(0.4-2.64)	0.96

\* Adjusted for age, sex, BMI; H\*\*Nucleotide = A, C or T



**Table III.** Analysis of variance for T2DM group of rs6777038 as codominant genotypes with anthropometric parameters.

Parameters	Mean ± SD			p
	CC (213)	CT (160)	TT (27)	
Age	48.15 ± 7.86	47.98 ± 7.24	48.33 ± 7.68	0.961
BMI	26.26 ± 2.09	26.81 ± 2.11	25.82 ± 2.01	0.012
FBG (mg/dl)	207.76 ± 23.73	207.96 ± 22.21	208.96 ± 26.63	0.968
Tgs(mg/dl)	172.11 ± 42.71	180.81 ± 46.68	177.7 ± 44.01	0.171
Total-C(mg/dl)	221.99 ± 42.97	224.58 ± 43.31	216.48 ± 43.42	0.631
HDL-C (mg/dl)	25.08 ± 8.82	24.64 ± 9.15	26.56 ± 8.39	0.58
LDL-C (mg/dl)	162.49 ± 45.26	163.78 ± 44.64	154.39 ± 43.84	0.604
Insulin (μU/ml)	6.93 ± 1.78	6.37 ± 1.41	6.08 ± 1.38	0.001
VLDL-C(mg/dl)	34.42 ± 8.54	36.16 ± 9.34	35.54 ± 8.8	0.171
HOMA-IR	3.55 ± 0.99	3.28 ± 0.85	3.14 ± 0.84	0.006

**Table IV.** Analysis of variance for T2DM group of rs6777038 as dominant genotypes with anthropometric parameters.

Parameters	Mean ± SD		p
	CC (213)	CT+TT (187)	
Age	48.15 ± 48.03	7.86 ± 7.29	0.87
BMI	26.26 ± 26.67	2.09 ± 2.12	0.053
FBG (mg/dl)	207.76 ± 208.11	23.73 ± 22.83	0.88
Tgs (mg/dl)	172.11 ± 180.36	42.71 ± 46.2	0.06
Total-C (mg/dl)	221.99 ± 223.41	42.97 ± 43.3	0.74
HDL-C (mg/dl)	25.08 ± 24.92	8.82 ± 9.05	0.86
VLDL-C (mg/dl)	34.42 ± 36.07	8.54 ± 9.24	0.06
Insulin (μU/ml)	6.93 ± 6.33	1.78 ± 1.41	0.0002
LDL-C (mg/dl)	162.49 ± 162.42	45.26 ± 44.53	0.99
HOMA-IR	3.55 ± 3.26	0.99 ± 0.84	0.0017

**Table V.** Analysis of variance for T2DM group of rs6444082 as dominant genotypes with anthropometric parameters.

Parameters	Mean ± SD		p
	GG (236)	GH*+HH* (164)	
Age	48.19 ± 47.95	7.63 ± 7.55	0.753
BMI	26.44 ± 26.47	2.16 ± 2.04	0.89
FBG (mg/dl)	207.33 ± 208.77	22.99 ± 23.74	0.543
Tgs (mg/dl)	179.53 ± 170.84	43.85 ± 45.08	0.055
Total-C (mg/dl)	222.8 ± 222.45	43.53 ± 42.55	0.936
HDL-C (mg/dl)	24.81 ± 25.29	8.96 ± 8.87	0.596
VLDL-C (mg/dl)	35.91 ± 34.17	8.77 ± 9.02	0.055
LDL-C (mg/dl)	162.09 ± 162.99	45.48 ± 44.09	0.843
Insulin (μU/ml)	6.53 ± 6.83	1.57 ± 1.73	0.07
HOMA-IR	3.34 ± 3.53	0.89 ± 0.99	0.049

H\* = C, A or T

As well as, this SNP as dominant model showed significant differences with fasting insulin and HOMA-IR (p = 0.0002 and 0.0017 respectively) shown in table IV. However, rs6444082 genotypes only as dominant model reveal significant variation with HOMA-IR (p = 0.049) as can be seen from table V.

**DISCUSSION**

This study conducted alternative variants of IGF2BP2 association with risk of T2DM, positioned at intron 2 represented by rs6777038 and rs6444082. The later has no significant risk factor on T2DM nor affect anthropometric parameters except for HOMA-IR. The former reveals strong association

of codominant and recessive TT genotype of rs6777038 with T2DM risks. This is consistence with Salem et al. [21] in Malaysian population but inconsistent with Rong et al. [24], who found no correlation of this SNP in Pima Indians. Samples of our study reveal that the risk association with T2DM of this allele (TT) related mainly with lower fasting insulin concentrations and insulin resistance as well as BMI. This was agreed with previous studies [25-26], and it has been postulate of direct influence of IGF2BP2 on T2DM. Such an association also assumed with obesity and could influence insulin resistance, which is finality resulting in T2DM [27]. In addition, lowering insulin and insulin resistance as well as BMI suggest that this alternative IGF2BP2 variant directly affect  $\beta$ -cell function, causing a decrease insulin levels. Consequently, this would be reliable with lower body insulin may result in decline of insulin anabolic effects, which is the main factor of lowering BMI. This association may be characteristic of genetic factors as well as genetic-environmental interfaces that may have influenced by differences of gen associations with disease among racial or ethnic group variabilities [28]. On the other hand, it may be attributed to ethnic variabilities and linkage disequilibrium design, complexed with contribution of non-genetic issues and daily life changes that could qualify T2DM risks [29]. The alternative SNP rs6777038 is located within an extended haplotype block spanning greatest intron 2 region and possibly intron 1 (www.hapmap.org), suggesting that this is a conserved region. Essentially, the gene HMGA2 is a regulator to IGF2BP2 that binds to pyrimidine rich motif crossing part of intron 1 [30]. Therefore, the HMGA2 gene may regulate rs6777038 alleles variably that alter its function and eventually expression. The influences of these SNPs on T2DM risks in Iraqi population might need more investigations, prompting possible epistatic interaction assumptions between those SNPs and determining entire risks of T2DM [30]. The study conducted on regional hospital and sample collecting manner non-prospective so the outcome of the study narrow its completely Iraqi population generality. Further sub-divided participants to groups according to environmental and regional factors results in small sample size in our population. Briefly, alternative variants rs6777038 of IGF2BP2 possibly associated with T2DM risks and some anthropometric parameters such as lower fasting insulin, HOMA-IR and BMI in Iraqi T2DM participants.

## CONCLUSIONS

This study confirmed the variant rs6777038 of IGF2BP2 possibly associated with T2DM risks and some anthropometric parameters such as lower fasting insulin, HOMA-IR and BMI in Iraqi T2DM participants.

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#### Conflict of interest:

*The Authors declare no conflict of interest.*

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**D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

## ORIGINAL ARTICLE

# CAUSES OF SUPERINFECTIONS: DEADLY BACTERIA OF TUBERCULOSIS

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## ABSTRACT

**The aim:** To improve early diagnosis of drug-resistant superbacteria and interrupt the ways of its formation through molecular technological and surgical methods.**Materials and methods:** The operated patients were divided into two groups: group 1 – 351 (51.25 %) patients, who were operated with the use of minimally invasive technologies, and this was the main group; group 2 – 334 (48.75 %) patients who were operated on open wide thoracotomy, which was the comparison group. Among 351 patients in the main group, in 301 – acute pleural tuberculous empyema was detected, and in 50 – chronic one. Among patients in the comparison group, acute pleural empyema was observed in 284 patients and chronic in 50 patients.**Results:** According to our data, video thoracoscopy is a highly informative method of diagnosis of pleural effusions, detection of pleural tuberculous empyema in the first, second and third stages of its development.**Conclusions:** The introduction of modern molecular-genetic and surgical technologies will allow to accurately establish the etiology process, to conduct the identification of pathogen microorganisms and to determine the phenotypic and genotypic sensitivity of bacteria to Antimycobacterial drugs. Such diagnostics will promote effective treatment of patients who are already infected with persistent strains of bacteria and viruses.**KEY WORDS:** Superinfection, Tuberculosis (TB), Mycobacterium (MBT), antimycobacterial Drugs (AMBD), absolute resistance of MBT to drugs (XXDR)

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## INTRODUCTION

The high rate of formation of resistance of bacteria now have ahead of the introduction of new antimicrobial agents, which poses a threat to an ageing planet. Due to the resistance of microorganisms, mixed specific and non-specific flora to the main drugs worldwide, there is the emergence of global disease with superinfection, which is caused by “superbacteria – nightmare bacteria – Superbug”. Due to the low level of modern molecular diagnostics this level of “superinfections” is hidden and the quantity is significantly understated. In the absence of effective global steps to reduce the rate of resistance to microorganisms, the planet’s population by 2050 may decrease by 10 million people per year, due to reactivation of atypical septical pneumonia, secondary tuberculosis (TB), expansion on the planet of total resistant tuberculosis (XXDR).

According to WHO (2018), tuberculosis (TB) is one of the top 10 causes of death in the world. In particular, in 2017, 10.4 million patients became ill with TB, 1.7 million died – 10.3 % including 0.4 million with HIV infection. In the same year, 1.0 million children became ill with tuberculosis, and 250,000 died of it – 25.0 %, including children with TB/HIV co-infection. WHO estimates (2017) that 600,000 patients with newly diagnosed tuberculosis had resistance to rifampicin, of whom 500,000 had

multidrug-resistant tuberculosis. WHO estimates (2017) that 600,000 patients with newly diagnosed tuberculosis had resistance to rifampicin, of whom 500,000 had multidrug-resistant tuberculosis. In Ukraine, despite the improvement of tuberculosis detection rates, the situation remains difficult and even prognostically unfavorable (Yu.I. Feshchenko, V.M. Melnyk et al., 2017). Regarding the dead, 25.0 % of those who died before the age of 1 year report a hidden epidemic and late detection of neglected forms of generalized tuberculosis. 33.0% of the deceased persons die at home. According to the cohort analysis for 2015 (analytical and statistical reference book 2007–2017): cured – 27.1 %; treatment completed – 48.9 %; died – 9.4 % of patients. Therefore, ineffective treatment of patients is: for the first time diagnosed with tuberculosis – 25.0 %; TB recurrence rate – 34.9 %; multiresistant TB – 54.0 %. Such low effectiveness of treatment of patients adversely affects the epidemic situation in Ukraine (I.T. Piatnochka et al., 2016), which threatens the world with the development of mycobacteria that will acquire extremely drug-resistant tuberculosis (XXDR), the so-called “supertuberculosis infection” [1, 2].

According to the head of the Center for Disease Control and Prevention USA (CDC), T. Frieden (2016), “bacteria that are resistant to all drugs are called superbacteria, and



**Table I.** Characteristics of the study groups on the severity of empyema

Indicator of tuberculosis process	Group 1		Group 2	
	n = 351		n = 334	
	Abs	(M±m)%	Abs	(M±m)%
Acute TBE	301	85,7 ± 1,3*	284	85,0 ± 1,7*
Chronic TBE	50	14,2 ± 0,3*	50	14,9 ± 0,3*
P	p < 0.05			

Note: \* - significant difference between groups (p<0.05)

because of their spread, humanity is at risk of becoming in the post-antibiotic world” [3]. In other words, if superbacteria conquer the world, then various infections will be caused, and the world community will find itself without antimicrobials, making this study highly relevant in the light of today’s COVID-19 pandemic. Superinfection is a natural stage of bacterial involution. Even O. Fleming accidentally noticed that fungi grown by him in vitro can inhibit bacterial growth. He also revealed the lysozyme enzyme, which is present in the secret of sweat glands of the skin, tears, saliva and urine of man. Prior to penicillin synthesis, 100 % of patients died of diphtheria, pneumonia, syphilis and infectious complications from postoperative surgery. Antibiotics became a powerful weapon of the mankind against bacteria, and they significantly reduced the number of deaths from infections [5]. It is well known that some bacteria become resistant from birth. According to Z. Waxman, primary resistant information was already contained in mycobacterium tuberculosis (MBT) populations, which showed streptomycin resistance in 25.0 %. This is confirmed by soil samples from places where streptomycin and other modern antibiotics have never been used. The data studied is the fact that some bacteria transmit their resistance through the DNA-plasmid loop to their offspring, which become not sensitive to the prescribed antibiotics and do not kill them but only inhibited in growth. This led to the emergence and spread of bacteria that are almost untreatable with the available antibiotics, the so-called “superbacteria – superbug”:

1) methicillin-resistant *Staphylococcus aureus* (MRSA); 2) penicillin-resistant *Streptococcus pneumoniae*; 3) vancomycin-resistant *Enterococcus faecium*; 4) carbapenem-resistant *Pseudomonas aeruginosa*; 5) clarithromycin-resistant *Helicobacter pylori*; 6) multidrug and extensively drug-resistant tuberculosis (M-XDR). This method of gene transfer of drug resistance can occur even between different types of bacteria. In this case, resistance has existed for thousands of years before the antibiotic era. However, the very process of formation of resistance was initiated with the advent of the first antibiotics, and already the drug resistance of germs has become a global problem. Based on the above analysis, there is practically no antibiotic in the world to which bacterial resistance has not yet been proven. And with the advent of resistance to macrolides and carbapenems, it can be predicted that in 17–20 years (2037–2040), humanity will become defenseless against superbacteria unless a new drug is created that will subjugate these “horrible bacteria” to non-pathogens [6].

## THE AIM

The aim of the work: to improve early diagnosis of drug-resistant superbacteria and interrupt the ways of its formation through molecular technological and surgical methods.

## MATERIALS AND METHODS

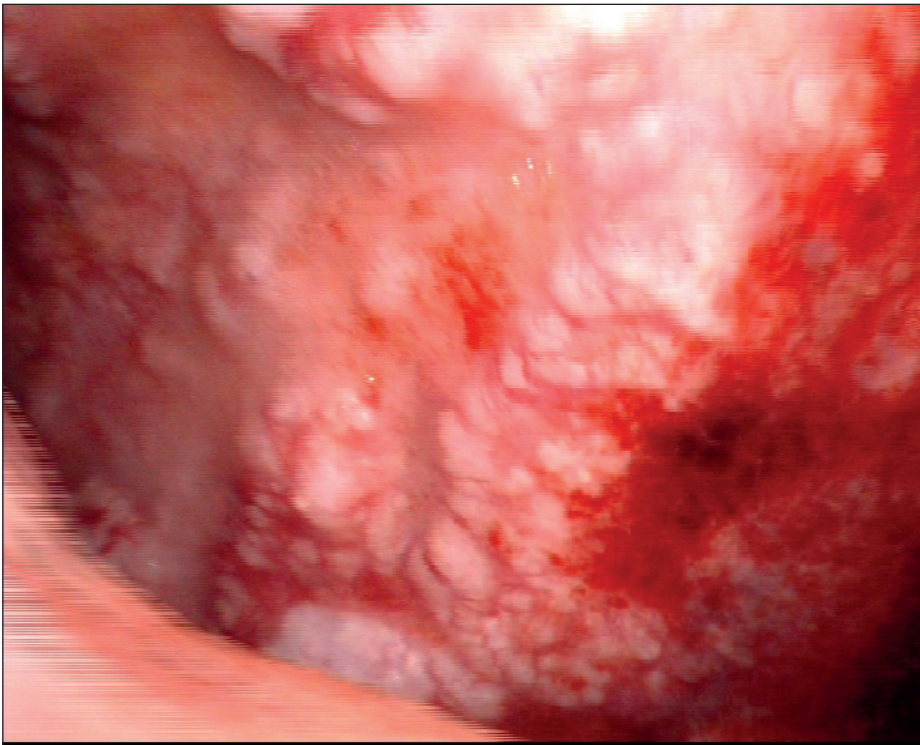
According to our data, video thoracoscopy is a highly informative method of diagnosis of pleural effusions, detection of pleural tuberculous empyema in the first, second and third stages of its development. The operated patients were divided into two groups: group 1 – 351 (51.25 %) patients, who were operated with the use of minimally invasive technologies, and this was the main group; group 2 – 334 (48.75 %) patients who were operated on open wide thoracotomy, which was the comparison group. Among 351 patients in the main group, in 301 – acute pleural tuberculous empyema was detected, and in 50 – chronic one. Among patients in the comparison group, acute pleural empyema was observed in 284 patients and chronic in 50 patients, as shown in Table I.

The above data confirm that the majority of patients are diagnosed with acute pleural TB empyema at 1–2 months of the disease. As a result of errors in treatment, this process goes into a periodically progressing chronic course.

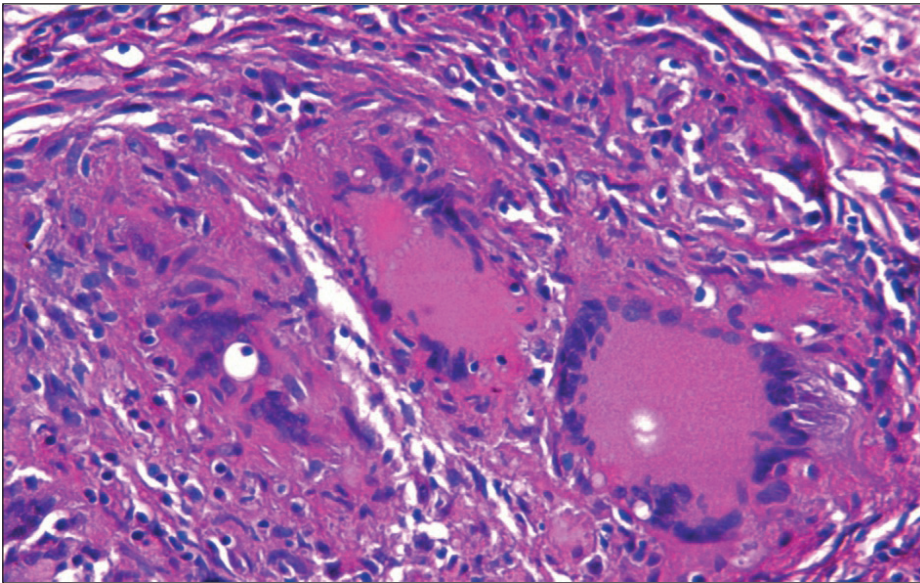
Statistical processing of the material was performed using a personal computer and a Microsoft Excel spreadsheet application using the “STATISTICA-10 for Windows®-6, 0” package. Graphs were designed using the programs “Microsoft Excel 7.0”. P < 0,05 was considered statistically significant.

## RESULTS

Ways of superbacteria forming. According to a report by K. Okamoto at the University of Chicago Rush Clinic, it was proved that the main reason for the spread of resistant and multidrug-resistant strains in outpatient and hospital institutions is 1) a form of resistance – errors by doctors and nursing staff. 6000 samples were collected from 5000 sites around intensive care units, examining the hands, gloves and gowns of medical workers before and after the interaction with a patient and thoroughly investigated. More than a third of healthcare workers had bacteria with multiple drug resistance *Staphylococcus aureus* (MRSA), *Enterococcus faecium*. Approximately 70% of the subjects (blood pressure cuffs, call buttons, bed rails) in contact



**Fig. 1.** Photogram of patient L., 46 years, thoracoscopy of fibrinous-purulent pleurisy, growth of connective tissue collagen layer



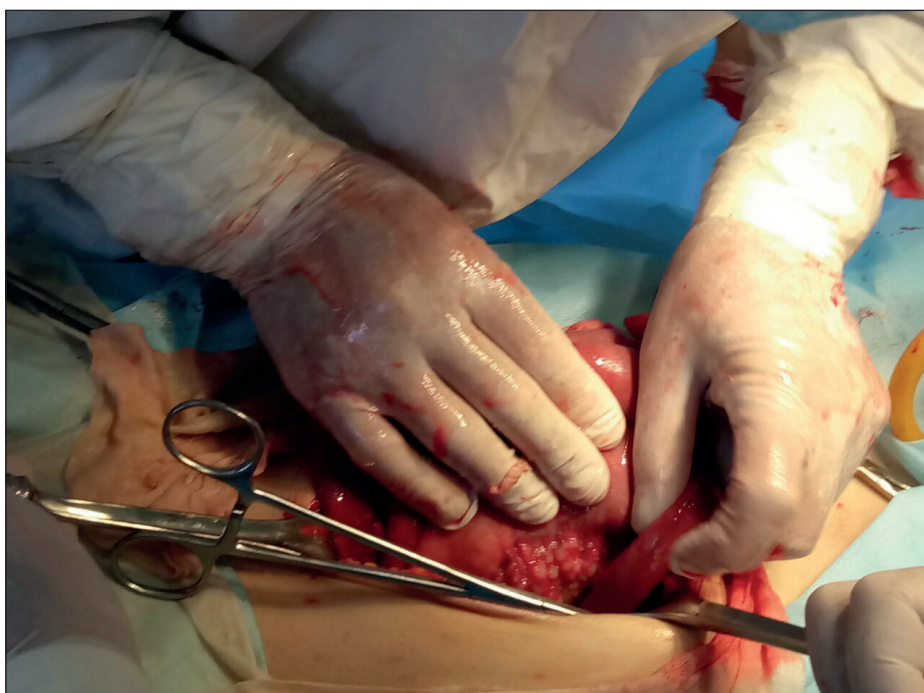
**Fig. 2.** Histophotogram of patient G., 56 years, tuberculous empyema of the pleura (in the center of Pyrohov-Langhan giant cells, along the periphery of the growth of connective granulation tissue). Hematoxylin and eosin staining. X200.

with patients were infected with superbacteria. According to the authors of the report, errors of medical staff were easy to eliminate due to the introduction of strict control over the means of daily disinfection of wards, intensive care units [4].

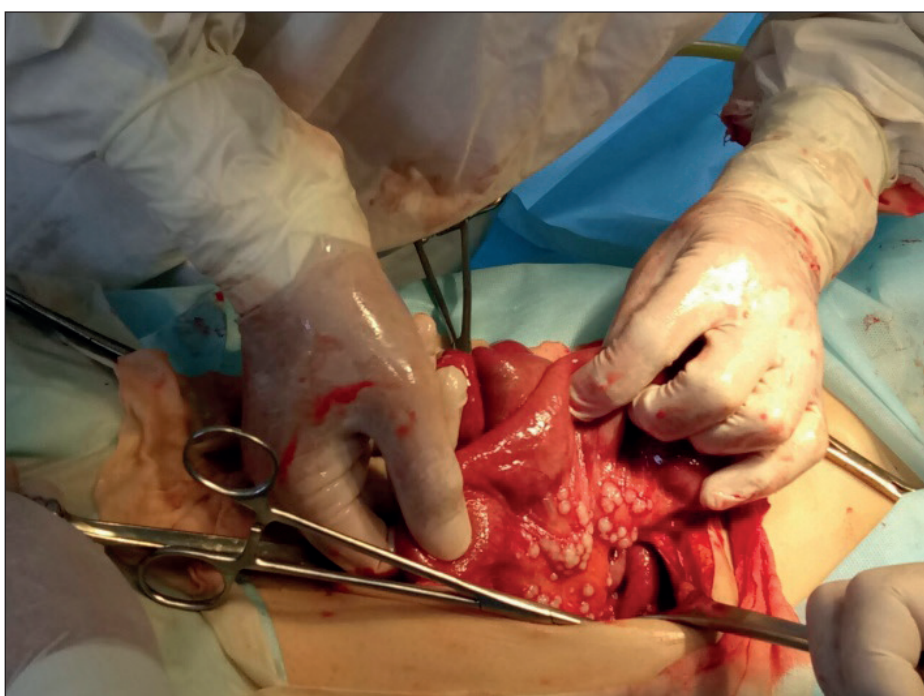
2) a form of resistance: the misuse of any antibiotics can develop not only the resistance of the pathogen, but drug dependence on the prescribed antibiotic. It is proved that under the action of antibiotics the composition and nature of the microflora in the human body changes. Some pathogens died, some become resistant, while others become addicted to drugs. That is, strains of bacteria, the growth of which is stimulated by one or another antibacterial drug. And, in some cases, bacteria can only grow in the presence of this dependent chemotherapy. Thus, it is possible to

explain in parallelly the malignant variant of resistance of internal hospital microbial strains, fungi and viruses. The drug dependence of the mycobacterial cell to which the doctor directs the antibacterial drug becomes an agent that stimulates metabolism and the basic functions of reproduction of an already persistent form of bacterial infection. Unfortunately, medicine today has little research about the drug dependence of bacteria, mycobacterium TB, and so it seems that it rarely develops. The most dangerous form of treatment for patients is their treatment after a break, ineffective treatment after the end of the main course of therapy, abacillarity and progression during treatment, inadequate use of drugs with proved resistance, interruption of the course of treatment, alcohol abuse and compatibility of co-infection TB/HIV/hepatitis. Then in the body of the





**Fig. 3.** Photogram of patient, I., 54 year-old male, tuberculous peritonitis with gastric perforation



**Fig. 4.** Photogram of patient, 37 year old female, diffuse purulent-fibrinous peritonitis with multiple perforations of the small intestine.

patient there is a “selection of superbacteria” of the most aggressive species that survived after chemotherapy, and now they begin to multiply rapidly and give birth to “deadly strains”. Drug addiction syndrome must be differentiated with conventional primary antibiotic resistance reactions. The dependence of mycobacterium tuberculosis on antibacterial drugs is a negative factor, which dramatically reduces the effectiveness of treatment of TB patients and complicates the course of the tuberculosis process.

3) form of resistance: bacterial mutations occur during treatment. Under the influence of antibiotics, the morphological, cultural and biological properties of bacteria change. An antibiotic that acts on a bacterium, affects

metabolism and causes gene mutations. In a certain region of the DNA molecule, there is a so-called secondary resistance. For example, tuberculosis mycobacteria can persist in cocoon, granular and L-form, keeping their pathogenicity for decades. Returning them from persistence to pathogens is called reversion. It is in the reversal of the MBT to the chaotic use of antibacterial drugs for the last 70 years that the secondary drug resistance of Koch's bacillus to streptomycin, fluoroquinolones, aminoglycosides, carbapenems and others has emerged. It is believed that drug resistance occurs by “adaptation” of MBT to drugs of the first class (isoniazid + rifampicin). Under conditions of antimycobacterial therapy, point chromosomal mutations



**Fig. 5.** Electron microscopy of bacteriophage that captured the bacterium

occur with changes in the ribosomal matrix system of mycobacteria. Genes whose mutation causes resistance to certain anti-TB drugs were identified. On the other hand, in each population of MBT sensitive to drugs populations, there are 25 % of resistant mutants that continue to multiply in the course of treatment and their population is steadily increasing. In case of ineffective treatment, there is an accumulation of mutations to certain drugs, which are fixed in 2-3 genes. Thus, the secondary acquired resistance develops, which manifests itself in 1-2 months of intensive basic course of treatment. It is only after one month of treatment for the first diagnosed tuberculosis patient that extensive studies on secondary resistance should be made, as 75 % of treated chronic patients proved the emergence of such resistance.

4) form of resistance: the fact of mixed specific-nonspecific infection (*M.tuberculosis* + *St.aureus*; *M.tuberculosis* + *Str.pneumoniae*; *M.tuberculosis* + *Aspergillus*; *M.tuberculosis* + *Acinetobacter*; *M.tuberculosis* + *Pseudomonas* + *Pseudomonas* + proved *M.tuberculosis* / HIV1-2 and others), which are now deadly to humanity. Patients become defenseless from a mixed specific-nonspecific, fungal and viral infection. Superbacteria are not more aggressive than their relatives of a certain kind, they are mostly in intensive care and palliative care wards. It is where patients with open forms, wounds or pressure sores are treated, physically impaired and immunologically depleted. There are many examples of superbacteria being transmitted from one patient to another because of non-sanitary control, the so-called "borrowed super-bacterium". Nowadays, doctors have almost exhausted the arsenal of antimicrobials and are forced to use reserve drugs, such as linezolid and carbapenems. Reserves are increasingly being prescribed for the treatment of gram-negative infections. Over the last decade, we have seen the emergence of fluoroquinolone-resistant strains, a sharp increase in the number of tuberculous pleurisy and pleural tuberculous empyema. Exudative pleurisy is associated primarily with an undetermined cause of complications of a variety of diseases by the etiopathogenesis: 1) specific (*tuberculosis*, *actinomycosis*,

*acquired immunodeficiency*, *HIV infection*), 2) nonspecific (*parapneumonic*, *paracancrosis*, *septic*, *virological*), 3) post-traumatic (*convoluted hemo-* and *pneumothorax*), 4) congestive (*cardiac*, *postoperative*) and others. The low level of diagnosis of pleural tuberculous empyema in these categories generates even greater increase in lung tuberculosis resistance. "Specificity" of tuberculous empyema of the pleura is a fairly conventional term, because according to the clinical course, a mixed bacterial gram (-/+) microflora is always present in the suppuration cavity.

The rational choice of diagnosis and surgery significantly reduce the formation of suppuration during pleural empyema. Detection of pathomorphological features of pleural empyema, acquired bronchial fistulas to the stage of purulent inflammation requires strict systematization and individualization of the terms of conservative or surgical treatment, its nature and volume. The clinical picture of reactive infectious pleural empyema usually begins gradually, and fluid in the pleural cavity often coincides with the manifestations of pneumothorax or is revealed during radiological examination. Exudate is often serous in nature with little protein. The degree of thickening of the pleura indicates the remoteness of exudative pleurisy. Special studies allow diagnosing MBT bacillus in exudate. Thoracoscopy reveals fibrin loss, sometimes tuberculous tubercles appear on the surface of the pleura. Minor pleural lesions by Koch bacilli (MBT), absence of pleuro-bronchial fistula, with massive AB therapy (fluoroquinolones, aminoglycosides – indirectly affect the MBT), usually ends with resorption of exudation and pleural obliteration. However, the activity of the tuberculous process in the pleura remains high, and the process often recurs into tuberculous empyema of the pleura. By the nature of the course, tuberculous empyema is divided into: 1) asymptomatic, cold suppuration type, no tuberculous changes in the lungs, and broncho-pleural fistulas do not develop; 2) progressive empyema with marked changes in the lungs and the presence of broncho-pleural fistula; 3) recurrences occupy the middle position between asymptomatic and progressive, fistulas when recurrent, as a rule, are not observed, and the exacerbation of the condition



will depend on the received conservative treatment and the sanitation of the cavity of empyema; 4) mixed tuberculous empyema, which manifest as a severe course due to the adherence of a secondary putrefactive infection. In case of mixed empyema, dystrophic changes of the internal organs develop very quickly. The prognosis for these forms is very serious and can go into septic conditions. This is actually a superinfection, which we are now increasingly finding in generalized destructive forms. Treatment of local suppuration with broad-spectrum antibiotics in septic conditions is ineffective, which is manifested by the rapid development of resistance of the pathogen with the phenomena of severe purulent-resorptive fever. Clinically it is manifested by general exhaustion, the patient quickly loses weight, lack of appetite, he is often feverish  $T\ 39-40^{\circ}C$ , there is massive sweating, diarrhea, cough with hemoptysis, often bloody feces, chest pain, shortness of breath. At first the leukocytosis is not significant, but expressed lymphopenia, thrombocytopenia and accelerated ESR 50–70mm/h. The process of supertuberculosis can be delayed from 6 to 10 weeks. Among 10 patients we observed, only 2 survived due to the prescription of Tienam antibiotic with antituberculosis drugs in one patient and human immunoglobulin, plasma transfusions, and parenteral antituberculosis drugs otherwise.

Most treated patients are doomed patients who are unable to undergo surgery to eliminate suppuration in the chest or abdominal cavity. Local changes in the abdominal cavity were manifested by diffuse serous-purulent peritonitis, breaking of granulations, perforation of caseous-necrotic ulcers of the intestine, tubercular rash on the mesentery. Such patients die very quickly despite the surgery and the massive combined antibiotic therapy. More often, in these patients, we observed videothoroscopically (VTS) the manifestations of suppurative tuberculous pleurisy, which required their separate presentation.

**The stage of fibrinous-purulent pleurisy** (stage I-II) begins when the exudate in the pleura becomes purulent. During this period, the inflammatory processes develop in the vascular elastic-collagenous layer of the pleura: collagen fibers are stratified by a protein fluid that is rich in fibrin. There is a mass of segmented leukocytes on the surface of the pleura, among the fibrinous masses there are accumulations of lymphocytes and erythrocytes. In this stage, the surface and even the deep elastic layer are often broken. The process of inflammation at this stage is strictly limited to the pleural layer and does not pass to the lung tissue, which is shown in Figure 1.

The stage of fibrinous-purulent inflammation always indicates the maximum degree of inflammatory response and depends on the preservation or termination of the harmful agent. Therefore, in the study of the clinical stage of empyema, the decisive factor in the transition of the second stage to the third – reparative is not the time factor, but the state of the main focus of infection.

**Reparative stage** (stage III) is essentially not a reaction of inflammation, it is only a consequence of infection. Usually, the intensity of the regenerative reactions is proportional to the degree of pre-existing tissue destruction and the

appearance of the so-called “dead” substance in the pleural cavity. The reparative stage of empyema is called the stage of granulation tissue design, which often begins at 8<sup>th</sup>–12<sup>th</sup> day. Initially, the granulation tissue has the appearance of a thin layer of round glomerular cells, which are placed between the boundary layer of fibrinous-purulent layers and the preserved layers of the pleura. Then the collagen fibers begin to gather in granulations. Forming granulation tissue creates a pyogenic membrane which produces suppuration on one side and separates it from adjacent tissues on the other. Thus, the body suppresses the growth and reproduction of Superbacteria.

Depending on the stage of the inflammatory process, it is morphologically possible to distinguish a more chronic course of tuberculous empyema of the pleura, which alternates with periods of exacerbation and attenuation. There is no clear criterion for the transition of acute empyema to chronic. The course of an acute empyema usually lasts 1–3 months, but this period can be prolonged for a longer time, but can be shortened. Actually, it is not the timing of inflammation that causes the transition of acute pleural empyema to chronic, but the condition of its walls. If alternative inflammatory processes predominate in the inflammatory zone, then even with long-term course of pleural empyema disease, it should be considered as acute.

Chronic inflammation is always characterized by residual tissue production, mainly due to scar granulation tissue. These signs of chronic inflammation are especially characteristic of chronic pleural empyema. In this stage, the thickness of the walls of the empyema reaches 2–3 cm, with the side of the parietal pleura wall is 2 times thicker than the side of the visceral pleura. The walls of the empyema sac are rigid, compacted, and layers of fibrin and suppuration up to 5 mm thick are found on the inner surface. In the wall of chronic empyema there are three layers: pyogenic, scar and a layer of pleural tissues. The study of the morphological features of pleural adhesions, depending on the timing of their formation, as well as the possibility of local conservative influence on the pleural joints and walls of the empyema cavity still remain relevant, which is showed in Figure 2.

**Fistula formation.** In practice, pleuro-pulmonary fistulas are more common, rarely pleuro-thoracic. It should be noted that we observed 2 mechanisms of formation of pleuro-bronchial fistula: 1) break of parietal tuberculous lesion or cavern from the lung to the pleura; 2) at a caseous necrosis of a tubercle on a pleura in a lung. As a result of the rupture of the tubercle, a narrow gap is formed between the pleura, alveolar passages and small bronchi. At deep caseous necrosis, the expressed lesion of the pleura, wider fistulas are formed between the pleura and the larger bronchi. The largest fistulas are formed when the cavern opens into the pleural cavity, thus draining the bronchial cavern is transformed into the actual bronchial fistula. Very often, causal masses of the cavern close the mouth of the fistula, but when lysis of caseous necrosis, the fistulas continue to work. In the tissues of the fistulous passages, there are almost always specific tuberculous elements in

the form of epithelioid-cell granulomas. These granulomas are always inflammatory, only mechanical surgical removal contributes to the process of scarring and healing.

The risk of lethal tuberculosis superbacteria can be demonstrated on the examples of the development of tuberculous peritonitis:

**Case 1.** 54 year-old male patient I., who for 3 months received intensive phase of treatment with 4 antimycobacterial drugs (H+R+E+Z) in the department of regional TB dispensary concerning: Clinical diagnosis: Pulmonary TB (disseminated) first diagnosed Destr +, MBT +, M +, K +, Resist (-) complicated by diffuse purulent-fibrinous peritonitis, perforation of the antral gastric region, hist. + (2019).

At the end of the intensive phase of treatment (3 months) the patient underwent P-o control of the treatment of pulmonary tuberculosis and revealed free gas under the domes of the diaphragm. The patient was urgently operated on by a surgeon for diffuse tuberculous peritonitis, suturing of perforated gastric ulcer. A photo-drug of tuberculous lesion of the stomach is shown in Figure 3.

The postoperative period proceeded with the complication of bilateral exudative pleurisy, which was eliminated by additional punctures. The patient received massive venous anti-tuberculosis therapy with fluoroquinolones and macrolides, and the wound healed with primary anaplerosis. The patient was discharged to the outpatient home care phase (isoniazid 0.3; rifampicin 0.6) and then died within 1 month from the progression of supertuberculosis. At the 2nd month of treatment, the patient showed a second form of drug resistance to the prescribed treatment, which I described previously. The patient under the DOT program was extended an intensive phase to 90 doses with the same drugs and received a progressive result of total resistance in the form of diffuse peritonitis with perforation of the stomach.

**Case 2.** 37 year old female patient O., was transferred from City Hospital No. 2 after surgery of the inferior laparotomy for diffuse purulent tuberculous peritonitis, resection to 60 cm of the small intestine with removal of enterostomy. Clinical diagnosis: AIDS co-infection stage 4, severe CD4 immunosuppression (<50 µl/ml) / first diagnosed (2018) intestinal tuberculosis with small intestine perforation Destr +, MBT +, M +, Hist. + Resist. – Surgery: resection of the small intestine with enterostomy. A photo-drug of tuberculous bowel disease is shown in Fig. 4.

The postoperative period proceeded up to 14 days favorably, the postoperative wound healed with primary anaplerosis against the background of receiving meronem, (fluoroquinolones, isoniazid, inbutol) intravenously, kanamycin intramuscularly, pyrazinamide orally. From day 15, tuberculosis progressed in the form of purulent peritonitis, diffraction of the wound, organ failure, progression of hepatic and renal failure, and cardiovascular disease. The patient died despite massive antibiotic therapy, anti-tuberculosis and immunocorrective therapy from the progression of HIV/TB/AIDS superinfection, the so-called fourth mixed form of resistance, which I described in detail above.

## DISCUSSION

In Ukraine, as we can see from the above examples, we have for a long time different types of hidden tuberculosis superinfection. The simple question is: how to do what to do and how to treat it? Neither WHO nor the UN can clearly answer these questions. GLASS Global Antimicrobial Resistance Surveillance System – first joint effort to observe resistance of selected bacteria causing acute infections: *Acinetobacter spp.*, *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Salmonella spp.*, *Shigella spp.*, *Neisseria gonorrhoeae* launched by WHO in 2015, and as of December 2018 already involved 71 countries. There is a growing awareness in the world of the need to carry out the common actions of the states of Europe and America to overcome antibiotic resistance. The most recent document focusing on the issue of combating resistance was the UN Secretary-General's report released in April 2019. The main point of this document is: "... as factors that enhance resistance to antimicrobial drugs associated with humans, animals, plants, food and the environment, there is a need to implement sustainable measures within the framework of a "single concept of healthcare", that will be aimed at involving all stakeholders and their union based on a common vision and goals [5, 6, 12]. The situation with tuberculosis resistance in Ukraine, unfortunately, is the equation with many unknowns due to the lack of confident, correct, standardized and analyzed data. Therefore, we have not yet become a participant in this WHO global GLASS process [1, 2].

Today, alternatives to traditional antibiotics are several promising strategies for combating infectious diseases: bacteriophages and phagotherapy, antimicrobial peptides and bacteriocins, monoclonal antibodies, vaccines, probiotics, and biologically active additives. In my opinion, the way out for the treatment of tuberculosis should be sought in the epidemic process of vaccination and revaccination of BCG. Vaccination of patients with tuberculosis with the bacillus Calmet-Geren (BCG) has historically become popular. The vaccine contains live mycobacteria of the vaccine strain *M. Bovis*. Propagated in the body after vaccination, mycobacteria form a long and lasting protection against tuberculosis. The production of antibodies to resistant strains of mycobacteria is carried out as long as the mycobacterial superantigen is released to the immune system, and sometimes it lasts for years. Modern vaccines contain recombinant antigens that, when introduced into the body with an adjuvant (plasmid), induce an immune response to a specific pathogen. In addition, DNA vaccines can have live recombinant microorganisms, such as (*M. tuberculosis* + *St.aureus*; *M.tuberculosis* + *Str.pneumoniae*; *M.tuberculosis* + *Aspergillus*; *M.tuberculosis* + *Acinetobacter*; *M.tuberculosis* + *Pseudomonas*; *M.tuberculosis*/HIV1-2 and others) into genome of which a gene encoding several resistant antigens will be inserted. Thus, mixed DNA vaccines (IC43, GSK2392105A, SA3Ag) against several aggressive combinations of superbacteria can be created [7]. The development of a new vaccine of total resistant tuberculosis (XXDR) in the world remains less and less.

A second alternative to antibiotics is a group of endogenous polypeptides that includes of 50–100 amino acids, which are encoded by single genes and are called aminopolypeptides (AMPs). Of particular interest to the AMP is the amphipathic site of hydrophobic and hydrophilic domains, which is involved in the destruction of membranes of several types of bacteria, fungi and viruses. Recent studies have shown antimicrobial activity against bacteria with multiple drug resistance, stimulation of humoral and cellular immunity (stimulation of neutrophils, macrophages and lymphocytes), especially in generalized septic infections.

In the pharmaceutical market the most popular are polymyxins B and E (colistin, cubicin) producing *Streptomyces roseosporus* with bactericidal activity to grams (+) of bacteria: staphylococci (*S. aureus*, *S. Haemolyticus*); streptococci (*S. agalactiae*, *S. Pyogenes*), group G, (*Cl. perfringens*, *Peptostreptococcus spp*), which are used to treat infections resistant to methicillin, vancomycin and linezolid [10]. Most recently, J.H. Lui (Ed) (2016) isolated the texobactin peptide from a new species of gram (-)  $\beta$ -proteobacteria, called the *Eleftheria terrae*. This lipopeptide in very low concentrations has been found to exhibit high activity against multidrug-resistant strains of *Staphylococcus aureus*, inhibiting the growth of *Mycobacterium tuberculosis*, *Clostridium difficile* and *Bacillus anthracis* [8]. Perhaps the mechanism of antimicrobial action of this peptide is associated with the destruction of the initial stages of synthesis of bacterial wall molecules. Other studies, to identify new antibacterial agents, use the HAMLET protein-lipid complex against *Streptococcus pneumoniae*, which includes human breast milk and  $\alpha$ -lactalbumin, which have been found to be lethal to tumor cells [9]. This natural complex killed *Streptococcus pneumoniae* and did not cause the development of any resistance [15].

The third alternative to antibiotics is bacteriophages, which are viruses that kill bacteria, they are also called “devourer of bacteria” [14]. At the same time, this name is misleading, bacteriophages do not devour and parasitize on bacteria and can transmit molecular genetic information to superbacteria, destroying them and turning them back into non-pathogens, which is shown in Fig. 5.

Their ability to digest the MBT, and especially the resistant ones, should depend on the state of the macro-organism, age, sex, hereditary factors, virulence, and number of births. Predisposition to tuberculosis is correlated with such antigens of the major histocompatibility complex (HLA), DR2, B7, B14 [11,16]. The role of humoral immunity in tuberculosis has not yet been fully elucidated. So, the future of humanity must depend on DNA vaccination, genetic engineering and immunology.

## CONCLUSIONS

1. In the course of humanity’s development, the population in the 21st century has met with deadly bacteria that are capable of combining with other resistant microbial strains and exhibiting aggressive resistance to chemically

synthesized antibiotics, and are now used in medical institutions.

2. Due to the widespread and total resistance of microorganisms over the next 5 years, there is a real threat to the human population with the emergence of pandemic infections of generalized tuberculosis, atypical pneumonia, hepatitis B, C and HIV/TB/AIDS co-infections.
3. The Global GLASS Antimicrobial Resistance Surveillance System needs to be implemented worldwide and alternative antimicrobial strategies widely developed.
4. Social prevention and hard steps to cure patients with all forms of tuberculosis will reduce the source of resistant infection; categorical cancellation in Ukraine of the DOT-program of outpatient uncontrolled self-treatment of the patient with antituberculosis drugs.
5. Strict assignment of pharmacy antibiotics with controlled prescriptions, behavioral motivation of the population, and refusal to use antibiotics in livestock and agriculture.

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## ORIGINAL ARTICLE

# NEUTROPHIL ACTIVITIES IN ADOLESCENTS WITH TYPE I DIABETES MELLITUS DEPENDING ON PERIODONTAL STATE

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## ABSTRACT

**The aim:** To estimate the neutrophil activities in adolescents with type 1 diabetes mellitus (T1DM) depending on periodontal state.

**Materials and methods:** A total of 93 individuals aged 12-16 years, including 62 T1DM patients and 31 healthy (H) controls, were included. Both groups were categorized into subgroups depending on their periodontal state. Phagocytic activity of neutrophils (PAN) the index of neutrophil activation (INA), and the percent of formazan-active neutrophils were evaluated using the spontaneous and the induced nitroblue tetrazolium (sNBT and iNBT) tests into oral rinses.

**Results:** PAN was significantly higher in the healthy (H) controls with gingivitis compared with the individuals with gingival health ( $p < 0.0001$ ). This parameter decreased significantly in the T1DM subjects, especially with periodontitis, compared with the H controls ( $p < 0.0001$ ). The percent of formazan-active neutrophils and INA in the sNBT test increased in the T1DM patients with gingival health and continued to raise as periodontal state of adolescents with T1DM worsened ( $p < 0.0001$ ). The parameters of the iNBT test in the T1DM adolescents decreased with the periodontal disease development ( $p < 0.0001$ ) that may demonstrate that superoxide production exhausts in diabetes, especially associated with periodontal disease.

**Conclusions:** The sNBT test in studied adolescents showed that both periodontal disease and T1DM increase the rate of activated neutrophils ( $p < 0.05$ ).

**KEY WORDS:** periodontitis, diabetes mellitus, phagocytosis, neutrophils, gingivitis

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## INTRODUCTION

The effect of systemic diseases on the periodontal state has been studied in a number of researches [1-3]. Type 1 diabetes mellitus (T1DM) has long been called “juvenile diabetes” and today is considered the most common type of endocrine diseases in adolescents [4]. Recent studies showed that T1DM patients are at higher risk of developing periodontal diseases than non-T1DM individuals [5], and periodontal disease is one of the early manifestations of diabetes in oral cavity of children [6].

Neutrophils have a lot of activities to form the initial response against pathogenic insults: adherence and migration, degranulation and release of inflammatory mediators, phagocytosis and apoptosis [7]; also they represent the most of leukocytes of gingival crevice [8]. Clinical studies have reported that the neutrophil activities impaired in blood [9] and crevicular fluid [10] in the adults with periodontitis. Furthermore, impaired chemotaxis, phagocytosis and increased production of free radicals were confirmed in blood of the diabetic patients [11].

Increased level of glucose in saliva and crevicular fluid in diabetes strengthens proliferation of periodontopathic bacteria. To phagocytize bacteria of dental biofilm neutrophils can use both oxygen-dependent and oxygen-independent means [12]. The recent review points to the strengthening of superoxide generation in the patients with periodontitis associated with

diabetes mellitus due to both hyperglycemia and periodontitis [13]. However, the overproduction of intracellular superoxide not only defends, but attacks periodontal tissues [14].

The nitroblue tetrazolium test is one of the methods to find release of superoxide radicals by stimulated and non-stimulated neutrophils. Nitroblue tetrazolium is easily phagocytized by cells and reduced to formazan with the hexose monophosphate shunt activation [15]. Unstimulated neutrophils from diabetic patients produce a great amount of superoxide anions and reduced NBT more efficiently [16]. Neutrophils migrate from crevicular fluid or periodontal pocket into the oral cavity and can be evaluated in oral rinses. To the best of our knowledge, no report about the evaluation of the phagocytic activity of neutrophils and the NBT test in oral rinse of adolescents with periodontal disease associated with T1DM.

## THE AIM

To estimate the neutrophil activities in adolescents with T1DM depending on periodontal state.

## MATERIALS AND METHODS

A group of adolescents was examined who were patients of the endocrinological ward of Paediatric community

hospital in the town of Poltava (Ukraine). The study was conducted in accordance with the ethical standards of the Helsinki Declaration (1975, revised in 2002). Ethical approval was obtained from the Bioethical Committee of Ukrainian medical stomatological academy (renamed to Poltava state medical university in 2021), and informed consent was obtained from all the study subjects (their parents).

The study comprised of 62 subjects with severe manifestation of T1DM (30 males and 32 females) aged 12-16 years. Duration of the disease was under 1 year in 18 subjects, from 1 to 5 years in 26 subjects, and over 5 years in 18 ones. Periodontal examination was performed in compliance with the guidelines for periodontal screening [17]. Patients with T1DM were categorized into three subgroups: 18 subjects with gingival health, 23 subjects with gingivitis, 21 ones with periodontitis. Healthy (H) controls included 16 adolescents with gingival health and 15 ones with gingivitis (15 males and 16 females). Exclusion criteria were history of any systemic disease other than diabetes for the patients with T1DM, presence of smoking, and history of antibiotic therapy within 6 months prior to study.

Phagocytic activity of neutrophils (PAN) and the nitroblue tetrazolium (NBT) test were examined into oral rinses. The oral rinse samples were obtained from all adolescents before the breakfast and the insulin injection. Three preliminary oral rinses with 10 ml of physiological saline for 30 s (PS, 0.9% NaCl) were repeated with 5 min break. Phagocytic activity of neutrophils was examined in the first and the second rinses which immediately delivered to the laboratory. The rinses centrifuged at 2000 rpm for 10 minutes. The *Candida albicans* cells in the yeast phase only were mixed with the neutrophil sediment and kept for 30 min at 37°C. The suspension was centrifuged at 1500 rpm for 5 min. Then the supernatant was removed and smears from the sediment were fixated in the methanol for 5 min, air dried, and stained with 10% Giemsa solution. The percentage of phagocytic neutrophils was calculated from the observations of 100 viable cells under the light microscopy.

The third oral rinses with PS weren't used and the forth oral rinses were taken for the NBT test evaluation. The samples (2 ml) were placed in the disposable tubes, immediately delivered to the laboratory, and PS was added to bring the level to the 10 ml. INA and the percent of formazan-active neutrophils among 100 cells were evaluated by the spontaneous and the induced NBT (sNBT and iNBT) tests [15] with modification of Saiapina L.M. [18]. The index of neutrophil activation (INA) was calculated the formula:  $a+2b+3c+4d/100$ , where a, b, c, d – number of active neutrophils in 0, I, II, III grades activation accordingly. The grade of activation is evaluated by the square of formazan granules (0 – cells without formazan, 1 – 25% of cell's square was stained, II – 75%, III – 100%).

Taking in consideration groups comparisons in the studied groups, P-value below 0.0125 was calculated with Bonferroni coefficient (0,05/4 where 4 – number of group comparisons) and considered significant for neutrophil activities. Two-way analysis of variance (ANOVA) and the

F-test were used for determining whether factory-to-factory variation was significant for diabetes and the periodontal state, where P-value below 0.05 accepted.

## RESULTS

Analyzing the levels of the phagocytic activity of neutrophils in oral rinses the authors found that PAN was significantly higher in the H individuals with gingivitis than in group of individuals with gingival health ( $p < 0.0001$ ) (Table I). Significantly lower PAN in oral rinses were demonstrated in all groups of the T1DM subjects than in the H controls with gingival health ( $p < 0.0001$ ). A similar trend was found for the T1DM subjects with gingivitis, the median of which was significantly lower PAN compared with the diabetic subjects with gingival health ( $p < 0.0001$ ). When the T1DM subjects with periodontitis were compared with the T1DM subjects with gingival health and gingivitis differences between comparisons continued ( $p < 0.0001$ ).

Table II shows comparisons of the sNBT and iNBT test parameters between the healthy controls and the patients with T1DM. The percent of formazan-active neutrophils in sNBT test was lower in the H individuals with gingival health as compared with the H individuals with gingivitis ( $p < 0.0001$ ). This parameter was higher in the T1DM subjects with gingival health than in the H controls with same state ( $p < 0.0001$ ). The T1DM subjects with gingivitis had higher percent of formazan-active neutrophils compared with the H controls with gingival health and gingivitis ( $p < 0.0001$ ), and the T1DM subjects with gingival health ( $p = 0.0005$ ). Also, the percent of formazan-active neutrophils in the T1DM subjects with periodontitis was significantly higher from that in the H controls ( $p < 0.0001$ ), the T1DM subjects with gingival health ( $p < 0.0001$ ) and gingivitis ( $p = 0.0122$ ).

INA in sNBT test was higher in the H individuals with gingivitis compared with the individuals with gingival health ( $p < 0.0001$ ). The T1DM subjects with gingivitis had higher INA in this test compared with the H controls ( $p < 0.0001$ ) and the T1DM subjects with gingival health ( $p < 0.001$ ). INA in the sNBT test in the T1DM subjects with periodontitis was higher from those in the H controls, the T1DM subjects with gingival health ( $p < 0.0001$ ), but similar to the T1DM subjects with gingivitis ( $p = 0.08$ ).

The percent of formazan-active neutrophils in the iNBT test was similar in the H controls with gingival health and gingivitis ( $p = 0.13$ ) and significantly decreased in the T1DM individuals with gingival health compared with the H controls with same status ( $p = 0.0001$ ). The T1DM individuals with gingivitis had lower percent of formazan-active neutrophils compared with the H controls and the T1DM individuals with gingival health ( $p < 0.0001$ ). Same trend was found in the T1DM individuals with periodontitis ( $p < 0.0001$ ). In addition, there was significant difference ( $p < 0.0001$ ) regarding the percent of formazan-active neutrophils in the iNBT test between the T1DM subjects with gingivitis and periodontitis.

**Table I.** Comparison of the phagocytic activity of neutrophils in oral rinses between the healthy controls and the patients with T1DM

Phagocytic activity	H controls with gingival health (n=16)	H controls with gingivitis (n=15)	T1DM patients with gingival health (n=18)	T1DM patients with gingivitis (n=23)	T1DM patients with periodontitis (n=21)
Mean±SD	30.08±0.96	35.02±1.28 <sup>A</sup>	27.21±1.13 <sup>A</sup>	21.23±0.97 <sup>A,B,C</sup>	17.68±1.07 <sup>A,C,D</sup>

\*Categorical variables are presented as n (%). The p-values were computed with the t independence test. A: significant difference at  $p < 0.0125$  compared with the H controls with gingival health; B: significant difference compared with the H controls with gingivitis; C: significant difference compared with the T1DM patients with gingival health; D: significant difference compared with the T1DM patients with gingivitis.

**Table II.** Comparisons of the NBT test parameters between the healthy controls and the patients with T1DM

Parameter	H controls with gingival health (n=16)	H controls with gingivitis (n=15)	T1DM patients with gingival health (n=18)	T1DM patients with gingivitis (n=23)	T1DM patients with periodontitis (n=21)
Formazan-positive cells in the sNBT test (%)	28.26±2.51	38.96±3.45 <sup>A</sup>	42.17±3.73 <sup>A</sup>	51.06±4.11 <sup>A,B,C</sup>	54.35±4.22 <sup>A,C,D</sup>
INA in the sNBT test (%)	1.59±0.08	1.82±0.08 <sup>A</sup>	1.92±0.09 <sup>A</sup>	2.16±0.09 <sup>A,B,C</sup>	2.21±0.10 <sup>A,C</sup>
Formazan-positive cells in the iNBT test (%)	66.9±2.81	68.67±3.61	63.20±3.19 <sup>A</sup>	59.42±3.12 <sup>A,B,C</sup>	57.32±2.21 <sup>A,C,D</sup>
INA in the iNBT test (%)	2.60±0.08	2.67±0.09	2.58±0.09	2.42±0.10 <sup>A,B,C</sup>	2.29±0.10 <sup>A,C,D</sup>

\*Categorical variables are presented as n (%). The p-values were computed with the t independence test. A: significant difference at  $p < 0.0125$  compared with the H controls with gingival health; B: significant difference compared with the H controls with gingivitis; C: significant difference compared with the T1DM patients with gingival health; D: significant difference compared with the T1DM patients with gingivitis.

INA in the iNBT test was not significantly higher in the H controls with gingivitis compared with the individuals with gingival health ( $p = 0.04$ ), and T1DM did not lead to a difference in INA between the individuals with gingival health ( $p > 0.05$ ). The T1DM individuals with gingivitis had lower INA in this test compared with the H controls and the T1DM individuals with gingival health ( $p < 0.0001$ ). The T1DM subjects with periodontitis showed significantly lower INA in comparison with the H controls ( $p < 0.0001$ ), the T1DM subjects with gingival health ( $p < 0.0001$ ), and the subjects with gingivitis ( $p = 0.002$ ).

Healthy control groups of patients with and without gingivitis and T1DM groups of patients with and without gingivitis were statistically treated with two-way ANOVA. Data was not statistically significant in this test using the phagocytic activity of neutrophils related the gingival state ( $F = 0.009$ ,  $p > 0.1$ ) and existence of diabetes as measurement variable ( $F = 2.32$ ,  $p > 0.1$ ). Similar not significance was found for the percent of formazan-positive cells in sNBT test related to the gingival state ( $F = 117$ ,  $p = 0.05$ ), but dates were significant related to existence of diabetes as measurement variable ( $F = 206$ ,  $p < 0.05$ ). Data was statistically significant in this test using INA in the sNBT test related to the gingival state ( $F = 4489$ ,  $p < 0.01$ ), and related to existence of diabetes as measurement variable ( $F = 2209$ ,  $p = 0.013$ ). Formazan-positive cells and INA in the iNBT test do not present statistically significant data related to studied factors (all  $p > 0.1$ ).

## DISCUSSION

Hyperglycemia caused by diabetes mellitus can alter periodontal tissues in many ways. A high level of glucose in saliva and crevicular fluid stimulates growth of cariogenic

and periodontopathic bacteria [19]. Dental plaque induces chronic activation of neutrophils to gingival crevice, and neutrophils use superoxide production to phagocytized bacteria. However, oxygen radicals, cytokines, bactericidal proteins, and matrix-degrading enzymes released by neutrophils could not only protect periodontal tissues against damage, but could activate the damaging pathways [7]. Apart from this, the periodontopathic gram-negative bacteria are able to induce the cytokines which causes insulin resistance [20, 21]. Thus, periodontitis can block glycemic control and hamper glycemic control can further stimulate periodontal disease.

NBT test and phagocytosis assay are indirect markers of oxygen-depend antimicrobial activity of neutrophils. An increased superoxide production in blood was found in localized juvenile periodontitis [22]. The adult diabetic subjects with chronic generalized periodontitis demonstrated a lower phagocytosis degree and an increase in the NBT test in blood [23]. However, the response of peripheral neutrophils, which was analyzed in those studies, may be different from neutrophils of crevicular fluid or periodontal pocket.

In the present study, PAN in oral rinse increased in the healthy adolescents with gingivitis as the host immune response to microorganisms. Impaired neutrophil activities such as phagocytosis, superoxide generation, and chemotaxis in the diabetic patients with periodontitis [24] are considered as their hyperactivity [25]. Genetic polymorphism related to the neutrophil function may help periodontal pathogens to evade the neutrophil response or may lead to the neutrophil hyperactivity [12]. The reduced PAN in the T1DM patients with gingival health in the current study may have caused by diabetes and predisposed to the periodontal disease. PAN decreased as periodontal

state of subjects with T1DM worsened and had the lowest value in the subjects with periodontitis, so these results correlate with previous findings [24]. The findings of a two-way ANOVA suggest that diabetes did not have a greater impact on PAN than gingival state.

The percent of formazan-active neutrophils and INA in the sNBT test were used in this study for screening of the activated neutrophils. These parameters increased in the healthy patients with gingivitis and continued to raise as periodontal state of adolescents with T1DM worsened, which was in accordance with previous study [24]. In addition, the findings of a two-way ANOVA suggest that diabetes had a greater impact on the percent of formazan-active neutrophils than gingival state, and both diabetes and gingival state impacted on INA in the sNBT test.

The iNBT test assesses the functional ability of neutrophils to finish phagocytosis. The authors found significantly lower parameters of the iNBT test in the T1DM patients as compared with healthy controls, and these parameters worsened with periodontal disease development. Obtained results correlate with the study of Ahkamova et al. [25], where higher parameters of the iNBT test decreased in the adult patients with the severe stage of chronic periodontitis. This impact could be explained by prohibition of superoxide release of neutrophils by bacteria of periodontal pockets. Also a high extracellular glucose concentration reduces oxygen production from activated neutrophils [26]. However, our findings of a two-way ANOVA test suggest that diabetes did not have a greater impact on the iNBT-test than gingival state.

Obviously, periodontopathic bacteria and hyperglycemia both modify the neutrophil activities. Finally, the presence of T1DM is related to impaired phagocytic activity and superoxide production. Future researches may develop a novel pathogenic strategy in the patients with T1DM associated with periodontal disease based on the neutrophil activities' regulation.

## CONCLUSIONS

PAN decreases with development of periodontal disease both in healthy adolescents and adolescents with T1DM ( $p < 0,0125$ ). Parameters of the sNBT test increased in healthy adolescents with gingivitis and patients with T1DM who had different periodontal state ( $p < 0,0125$ ), showing the reactive oxygen overproduction of neutrophils. The decreased parameters of the iNBT test in subjects with T1DM and gingival health ( $p < 0,0125$ ) may demonstrate that superoxide production exhausts in diabetes, and these indices worsened with development of periodontal disease ( $p < 0,0125$ ). The sNBT test in studied adolescents showed that both periodontal disease and T1DM increase the rate of activated neutrophils ( $p < 0,05$ ).

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*The Authors declare no conflict of interest.*

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## ORIGINAL ARTICLE

# METABOLIC AND HORMONAL FEATURES OF CHRONIC HEART FAILURE IN PERSONS WITH POST-INFARCTION CARDIOSCLEROSIS WITH TYPE 2 DIABETES MELLITUS AND OBESITY

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**ABSTRACT**

**The aim:** To determine the role of lipid metabolism and fractalkin and clusterin in the progression of chronic heart failure in patients with postinfarction cardiosclerosis with concomitant type 2 diabetes and obesity.

**Materials and methods:** A retrospective analysis of a comprehensive examination of 67 patients with postinfarction cardiosclerosis with concomitant type 2 diabetes and obesity. All patients were divided into 3 groups depending on the functional class (FC) of CHF: 1 group (n = 22) – patients with CHF II FC; Group 2 (n = 23) – patients with CHF III FC; Group 3 (n = 22) – patients with CHF IV FC. All patients were examined clinically, they were instrumental, biochemical and hormonal examination.

**Results:** With the progression of CHF from FC II to FC III there is a deterioration of lipid metabolism: a significant increase in cholesterol levels by 5.5%, TG – by 15.7%, LDL cholesterol – by 74.4%, VLDL cholesterol – by 15, 9%, reduction of HDL cholesterol – by 27.6% (p < 0,05). An analysis of the fractal equation showing that ailing on CHF is advised by FC; riven clusterin – decrease.

**Conclusions:** Classical changes in patients with postinfarction cardiosclerosis with CHF and concomitant type 2 diabetes mellitus and obesity, which are the formation of atherogenic lipid metabolism disorders associated with body weight, as well as changes in the latest indicators such as fractalkin and clusterin, indicating the role of these molecules in the progression of CHF.

**KEY WORDS:** chronic heart failure, postinfarction cardiosclerosis, type 2 diabetes, fractalkin, clusterin, obesity

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**INTRODUCTION**

Diseases of the cardiovascular system remain the leading cause of overall morbidity and mortality [1, 2]. Over the past three decades, some reduction in cardiovascular mortality has been associated with improved control of risk factors such as cholesterol (cholesterol), hypertension (AH), and smoking [3]. At the same time, there is an increase in obese people, type 2 diabetes mellitus (DM), the severity of metabolic shifts in which leads to vascular accidents [4].

The leading etiological factor in the development of chronic heart failure (CHF) is coronary heart disease (CHD), which according to many studies develops in almost 70% of cases [4, 5].

The pathogenesis of CHF is multifactorial and very complex, which includes the impact on the cardiovascular system of etiological factors and the activation of a complex of compensatory mechanisms. Risk factors for CHF include: left ventricular myocardial hypertrophy, type 2 diabetes, obesity [6-9].

It should be noted that the evolution of views on the pathogenesis of CHF resembles a spiraling movement – at each new round of obtaining new knowledge there is a return to old truths with modern analysis and combining them with the current paradigm.

To identify the stage of CHF, it is important to search for non-invasive methods of early differential diagnosis, risk assessment, disease prognosis, treatment dynamics.

Clusterin and fractalkin are among the biomarkers that indicate a predisposition to the development of CHF and early diagnostic indicators of the disease, especially in the asymptomatic course.

The role of clusterin in lipid transport and inhibition of inflammation has now been proven, making this molecule a potential candidate as a marker of cardiovascular disease, diabetes, and obesity [10-12].

There is evidence of fractalkin – the only chemokine that exists in soluble and fixed forms in the pathogenesis of cardiovascular disease [13-16], which allows us to consider it as a marker of activation of the inflammatory process associated with chemotaxis of various leukocytes, primarily monocytes and lymphocytes in the area of inflammation. It is possible that the results of further studies will prove the possibility of using fractalkin as a target for therapeutic effects in patients with CHF. Global studies demonstrate the uncertainty of a number of issues regarding the progression of CHF in patients with postinfarction cardiosclerosis and concomitant metabolic disorders and dictate the need to find new modern markers.

## THE AIM

To determine the role of lipid metabolism and fractalkin and clusterin in the progression of CHF in patients with postinfarction cardiosclerosis with concomitant type 2 diabetes and obesity.

## MATERIALS AND METHODS

In accordance with the purpose of the work, a retrospective analysis of a comprehensive examination of 67 patients with postinfarction cardiosclerosis with concomitant type 2 diabetes obesity. All patients were divided into 3 groups depending on the functional class (FC) of CHF: 1 group (n = 22) – patients with CHF II FC; Group 2 (n = 23) – patients with CHF III FC; Group 3 (n = 22) – patients with CHF IV FC. All patients were examined clinically, they were instrumental, biochemical and hormonal examination. Echocardiographic examination was performed according to the standard method of H. Feigenbaum on an ultrasound device Radmir (Ultima Pro 30, Ukraine). Biochemical examination was performed by the peroxidase method using the Cholesterol Liquicolor reagent kit from Human (Germany), which included the determination of total cholesterol (CHC), high-density lipoprotein (HDL) in heparin-stabilized serum. The level of triglycerides (TG) in the serum was determined by enzymatic colorimetric method using a set of reagents «Triglycerides GPO», company «Human» (Germany). The coefficient of atherogenicity (CA) was calculated by the formula of AM Klimov: firm «Human» (Germany). The coefficient of atherogenicity (CA) was calculated by the formula of AM Klimov: firm «Human» (Germany). The coefficient of atherogenicity (CA) was calculated by the formula of AM Klimov: The level of very low density lipoproteins (VLDL cholesterol): in mmol / l. The level of low-density lipoproteins (LDL): LDL cholesterol = CHD - (LDL cholesterol + HDL cholesterol) in mmol / L. Body weight index (BWI) – Kettle index – was determined by the formula:

The clusterin level was determined by enzyme-linked immunosorbent assay using the Human Clusterin Elisa test system manufactured by Bio Vender (Czech Republic).

Serum concentration of fractalkine was performed by enzyme-linked immunosorbent assay using a set of reagents Human Fractalkine Elisa Kit «RayBio®» (Georgia).

The research was carried out in the biochemical department of the Central Research Institute of Kharkiv National Medical University of the Ministry of Health of Ukraine on the enzyme-linked immunosorbent assay «Labline Go» (Austria).

The obtained results are presented as the mean  $\pm$  standard deviation from the mean ( $M \pm m$ ). Statistical processing of the obtained data was carried out using the statistical software package Statistica, 8.0 (Stat Soft Inc, USA), Microsoft office Excel-2003. Evaluation of differences between groups in a distribution close to normal was performed using Pearson's test. Differences at  $p < 0.05$  were considered statistically significant.

## RESULTS

The analysis of the obtained data (Table I) shows that with the progression of CHF from FC II to FC III there is a deterioration of lipid metabolism: a significant increase in cholesterol levels by 5.5%, TG – by 15.7%, LDL cholesterol – by 74.4%, VLDL cholesterol – by 15.9%, reduction of HDL cholesterol – by 27.6% ( $p < 0.05$ ). These results demonstrate atherogenic progression of the blood lipid spectrum in patients with postinfarction cardiosclerosis and type 2 diabetes. At the same time, an increase in BWI by 8.8% was noted, which indicates a more pronounced obesity in the group of patients with CHF III FC.

It should be noted that the level of Hb1Ac was the same in all three groups of patients.

At the same time, with the progression of CHF III FC to FC IV there is a decrease in CHD – by 8.3%, TG – by 27.4%, LDL cholesterol – by 12.2%, VLDL cholesterol – by 21.16% and an increase HDL cholesterol – by 15.7% ( $p < 0.05$ ), which is accompanied by a decrease in body weight – BWI decreases by 23.1%.

Analysis of the level of fractalkin showed that in patients with CHF with an increase in its FC, it increases. On the contrary, the level of clusterin decreased: in patients with CHF II FC it was 14.0% and 33.2%, respectively, higher than in the groups of CHF III FC and CHF IV FC ( $p < 0.001$ ).

## DISCUSSION

The study shows the classic changes in patients with postinfarction cardiosclerosis with CHF and concomitant type 2 diabetes and obesity, which are the formation of disorders of lipid metabolism of atherogenic nature, which are associated with body weight, as well as changes in new indicators such as fractalkin and clusterin, indicating the role of these molecules in the progression of CHF.

The striking effect of type 2 diabetes on the development and prognosis of CHF is due to a whole set of interrelated mechanisms. First of all, these are factors of high cardiovascular risk that make up the syndrome of insulin resistance: dyslipidemia, hypertension, obesity, inflammation [17]. Hyperglycemia is a leading link in heart disease and the presence of type 2 diabetes contributes to the development of coronary atherosclerosis and realizes its negative impact on the progression of CHF due to the occurrence and severity of coronary heart disease [18-19].

This paper reflects the concept of the effect of chemokines on myocyte contractility, as evidenced by the results of other researchers who demonstrate that fractalkin causes a decrease in cardiomyocyte contractility through the CXCR4 receptor [20-21].

The obtained results suggest that type 2 diabetes, obesity, dyslipidemia, inflammation have common pathogenetic mechanisms of development and progression of cardiovascular complications in patients with comorbid pathology, lead to summation and potentiation of cardiovascular risk, which is consistent with the results obtained by other studies. reflected in subsequent works [22-24].

**Table I.** Indicators of lipid metabolism, clusterin, fractalkin and HbA1c levels in the examined groups of patients

Indicator	CHF II FC, n = 22	CHF III FC, n = 23	CHF IV FC, n = 22	p
ZHS, mmol / l	5.44 ± 0.08	5.76 ± 0.08	5.28 ± 0.09	p1-2<0.05 p1-3 <0.05 p2-3 <0.05
LDL cholesterol, mmol / l	3.59 ± 0.05	3.85 ± 0.05	3.41 ± 0.05	p1-2<0.05 p1-3 <0.05 p2-3 <0.05
LDL cholesterol, mmol / l	1.51 ± 0.05	1.79 ± 0.06	1.41 ± 0.05	p1-2<0.05 p1-3 <0.05 p2-3 <0.05
HDL cholesterol, mmol / l	0.62 ± 0.04	0.45 ± 0.04	0.54 ± 0.03	p1-2<0.05 p1-3 <0.05 p2-3 <0.05
TG, mmol / l	1.78 ± 0.10	2.12 ± 0.09	1.54 ± 0.09	p1-2<0.05 p1-3 <0.05 p2-3 <0.05
CA	3.43 ± 0.07	3.55 ± 0.07	3.42 ± 0.08	p1-2>0.05 p1-3 > 0.05 p2-3 > 0.05
Fractalkin, pg/ml	987.25 ± 5.35	1004.49 ± 6.44	1030.74 ± 6.97	p1-2<0.05 p1-3 <0.05 p2-3 <0.05
Clusterin, pg / ml	41.49 ± 0.36	37.56 ± 0.26	27.40 ± 0.31	p1-2<0.001 p1-3 <0.001 p2-3 <0.001
BWI, kg / m2	33.60 ± 0.98	36.16 ± 1.01	28.34 ± 1.06	p1-2<0.05 p1-3 <0.05 p2-3 <0.05
Hb1Ac,%	7.2	7.4	7.1	p1-2-3<0.05

P – is the probability of difference between groups

## CONCLUSIONS

1. Due to the progression of chronic heart failure in patients with postinfarction cardiosclerosis and concomitant type 2 diabetes and obesity, an increase in all fractions of lipoproteins at stage III functional class was diagnosed, and then their decrease (at stage IV functional class), which may indicate a deterioration in this category of patients, due to the progression of metabolic shifts, stagnation, dysfunction of the main parenchymal organs.
2. Increased circulatory levels of fractalkin and decreased clusterin content in patients with postinfarction cardiosclerosis with concomitant type 2 diabetes mellitus and obesity is accompanied by an increase in the functional class of chronic heart failure.
3. Fractalkin and clusterin play a significant role in the progression of chronic heart failure in patients with postinfarction cardiosclerosis with concomitant type 2 diabetes and obesity, so they can be used as biomarkers of the severity of heart failure.

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## ORIGINAL ARTICLE

# MEDICAL AND SOCIAL JUSTIFICATION OF THE FINANCIAL AND ECONOMIC CONDITION OF THE «HORODENKA NON-COMMERCIAL CENTER OF PRIMARY MEDICAL CARE» BEFORE AND AFTER THE INTRODUCTION OF THE HOSPITAL DISTRICT

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**ABSTRACT**

**The aim** of the work is to identify the peculiarities of medical and social justification of the financial and economic condition of «Horodenka non-commercial center of primary medical care» before and after introduction of a hospital district. To achieve the goal, the following are defined task: to conduct an analysis of the main indicators of «Horodenka non-commercial center of primary medical care»; to determine the problems of inefficient work of the «Horodenka non-commercial center of primary medical care».

**Materials and methods:** When conducting research, they were used general scientific and special methods of research, in particular the system approach and system analysis – to carry out a comprehensive study of the identified objects and systems in their external and internal relationships, as well as determination of approaches to identifying and analyzing problems and developing ways to solve them solution; process approach – for the study of various types of activities in the existing management system of the health care facility before and after implementation of the hospital district; medical and statistical – for statistical processing of received data; analytical methods.

**Results:** The efficiency of health care facilities and the quality of the provided medical services are considered as the main target functions of the health care system. In many countries, programs for ensuring the quality of medical care have been implemented and are operating. The activities of Ukrainian medical institutions and the health care system as a whole are often harshly criticized by patients and the public for the low quality of providing medical services. The quality of medical services, medical care and medical infrastructure definitely depends on the principles of building the Ukrainian medical system and the development of the national economy. Because without a financial basis, it is very difficult to build an effective health care system and ensure proper medical care and the work of all medical institutions.

**Conclusions:** Thus, after the introduction of the hospital district in the «Horodenka non-commercial center of primary medical care», it is proposed to carry out a number of measures to increase the effectiveness of the implementation of financial management. In order to increase the efficiency of management, including financial resources, it is important to improve personnel management.

The main emphasis in the management is the formation of the personnel potential of the «Horodenka non-commercial center of primary medical care» the involvement of qualified specialists in the field of medicine, the motivation of various directions for the support and improvement of the qualifications of management personnel. It is also important to use the system of financial planning of a budget institution, to ensure expenses for its life activities. In particular, the main direction of cost control is targeted use of funds, strict control over this use, formation of an effective internal audit system of a medical institution.

**KEY WORDS:** Health care institution, hospital district, financial and economic condition, management efficiency, interaction

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**INTRODUCTION**

Thanks to the implementation of the medical reform in 2017, medical care in Ukraine has already moved significantly towards European standards. A number of medical institutions have been merged and reformed. Almost all medical organizations faced some form of organizational change, which certainly affected their financial situation. At the same time, there is a problem in building an adequate financial system in the conditions of reform. The concept of reforming the health care system provides for the creation of hospital districts. This is a system for medical institutions that provide secondary medical care and specialized medical care. The Resolution of the Cabinet of Ministers of Ukraine «On some issues of the formation of hospital districts» dated 27.11.2019 No. 1074 [1]

states that hospital districts are located in certain places and consist of medical institutions of secondary and emergency medical care and are defined as a functional association. The doctor's work mode is determined in accordance with the tasks assigned by the medical institution or its structural unit, taking into account the doctor's duties defined by the job description.

**THE AIM**

The aim of the work is to identify the peculiarities of medical and social justification of the financial and economic condition of «Horodenka non-commercial center of primary medical care» before and after introduction of a hospital district.

To achieve the goal, the following are defined task: to conduct an analysis of the main indicators of «Horodenka non-commercial center of primary medical care»; to determine the problems of inefficient work of the «Horodenka non-commercial center of primary medical care».

## MATERIALS AND METHODS

When conducting research, they were used general scientific and special methods of research, in particular the system approach and system analysis – to carry out a comprehensive study of the identified objects and systems in their external and internal relationships, as well as determination of approaches to identifying and analyzing problems and developing ways to solve them solution; process approach – for the study of various types of activities in the existing management system of the health care facility before and after implementation of the hospital district; medical and statistical – for statistical processing of received data; analytical methods.

## RESULTS AND DISCUSSION

In the course of the study, it is advisable to consider the main trends in the activity of doctors in the Collective Enterprise «Horodenka non-commercial center of primary medical care» (Table I). Thus, in the period 2014-2018, the largest number of admitted patients was in 2018, while the lowest number of calls was observed in this year.

Analysis of the financial condition of a medical institution should, first of all, confirm that its activity is financially stable. It allows to organize staff, make the necessary purchases, pay bills, pay taxes and pay off debts. The simplest condition and simultaneous requirement of financial stability and solvency is the need for profitable activity of the organization, receiving income from any type of activity that exceeds the costs of carrying out the activity [3].

The existence of non-profit, so-called non-profit medical organizations, does not mean that their profitability does not need to be analyzed. First, medical institutions that provide medical and preventive services should not be economically profitable. Secondly, non-profit organizations are called organizations that fully use their income to support and develop their activities. Complex indicators of financial activity, characterizing the financial condition of a health care institution, are determined on the basis of simpler single indicators, which themselves can also determine the success of financial activity. Such financial indicators include: organizational profit, cost price, total income, fixed assets, working capital, assets, liabilities,

equity; accounts receivable, accounts payable[4].

Let's consider the main indicators characterizing the financial condition of the Collective Enterprise «Gorodenka Non-Commercial Center of Primary Medical Care» (Table II).

We will also analyze the changes in the main indicators characterizing the financial condition of the Collective Enterprise «Horodenka non-commercial center of primary medical care» (Table III).

As can be seen from Table III, the amount of fixed assets is growing every year, with the largest increase observed in 2016

when, compared to 2015, the total value of fixed assets increased by 112,9%. In 2018 there was a significant decrease in the amount of liabilities (by 37,1%) since before this period it was declining but not at such a pace. It should be noted a significant increase in equity and financial result for 2018 compared to 2017, which was 65,5%. Also, during the study, an assessment of financial stability of Collective Enterprise «Horodenka non-commercial center of primary medical care for 2017-2018 was carried out (Table IV).

As can be seen from the table 4, the coefficient of autonomy of the enterprise increased in 2018, while at the same time there is a decrease in the coefficient of efficiency of the use of own capital and financial stability, which is a negative trend. The analysis of financial stability allows us to understand how well a medical institution manages its financial resources. Insufficient financial stability can lead to bankruptcy and lack of funds for the development of production, therefore, the state of financial resources must meet the requirements of the modern market and the needs of the development of a medical institution [9].

Based on the results of the analysis and taking into account the information presented in [10], it is appropriate to note that the introduction of a hospital district in the Collective Enterprise «Horodenka non-commercial center of primary medical care» requires the implementation of the following recommendations in practice, which will make it possible to significantly improve the financial and economic activity of the «Horodenka non-commercial center of primary medical care»:

- renewal of fixed assets, sale of outdated equipment and purchase of new equipment (high-quality and new equipment significantly increases the level of diagnosis and treatment, which improves the level of competitiveness of the organization and attracts the attention of patients when choosing a place for examination and treatment);
- stabilization of liquidity indicators (high liquidity indicators are positively perceived by creditors, but free funds can bring additional income, therefore it is advisable to optimize the level of non-current monetary assets in order to achieve the required level of liquidity without losing alternative opportunities);
- increase in incoming cash flows, primarily due to an increase in income, for example, by concluding contracts for corporate service of enterprises, developing a marketing program with the aim of increasing consumer loyalty and forming a positive image of the institution, improving the qualifications of personnel;
- expanding the channels of receiving cash income: participation in grant programs, attracting targeted loans, the use of leasing or renting, attracting the help of patrons, investing temporarily free funds;
- implementation of personnel policy (revision of the composition and capacities of units, strengthening of interaction between them, investment in personnel capital, increase in productivity and efficiency of work by optimizing the technical and technological component);
- review of the main items of expenditure (use of energy-saving technologies, search for the most optimal suppliers of goods, works and services).

In addition, we note that it is possible to increase the efficiency of personnel management in the Collective Enterprise

**Table I.** Outpatient reception and calls for 2014-2018 at the Collective Enterprise «Horodenka non-commercial center of primary medical care»

Years	Reception	Calls	In All	Change in % compared to the previous year
2014	149334	38857	188191	-
2015	157398	41024	198422	5,44
2016	151859	42255	194114	-2,17
2017	154745	40166	194911	0,41
2018	159035	36831	195866	0,49

Source: compiled by the author based on the reporting data of the Collective Enterprise «Horodenka non-commercial center of primary medical care» [2].

**Table II.** Indicators of the financial condition of the Collective Enterprise «Horodenka Non-Commercial Center of Primary Medical Care» for 2015-2018, thousand hryvnias.

Indicator	2015	2016	2017	2018
Fixed assets	700068,0	1490240,0	2239516,0	3816090,0
Equity and financial result	1821483,0	2214582,0	2474278,0	4094925,0
Obligations	210241,0	205263,0	183073,0	115181,0

Source: compiled by the author based on the reporting data of the Collective Enterprise «Horodenka non-commercial center of primary medical care» [5-8].

**Table III.** Changes in the financial condition of the Collective Enterprise «Horodenka Non-Commercial Center of Primary Medical Care» for 2016-2018, %

Indicator	2016	2017	2018
Fixed assets	112,9	50,3	70,4
Equity and financial result	21,6	11,7	65,5
Obligations	-2,4	-10,8	-37,1

Source: compiled by the author based on the reporting data of the Collective Enterprise «Horodenka non-commercial center of primary medical care» [5-8].

**Table IV.** Evaluation of financial stability indicators of Collective Enterprise «Horodenka Non-commercial Center of Primary Medical Care» for 2017-2018

Indicator	Formula	2017	2018	Absolute deviation	Growth rates, %
Coefficient of autonomy	$K_{(avt)} = \frac{Sh.equity}{Balance}$	0,011	0,018	0,007	63,64
Coefficient of financial stability	$K_{fin.stab} = \frac{Sh.equity}{borrowed\ funds}$	0,415	0,358	-0,057	-13,73
Financial risk factor	$K_{fin.risk} = \frac{borrowed\ funds}{Sh.equity}$	4,012	4,541	0,529	13,19
Coefficient of efficiency of use of own capital	$K_{eff.own\ cap} = \frac{net\ profit}{Sh.equity}$	4,215	3,425	-0,790	-18,74

Source: compiled by the author based on the reporting data of the Collective Enterprise «Horodenka non-commercial center of primary medical care» [5-8].

«Horodenka Non-Commercial Center of Primary Medical Care» due to the introduction of logistics services institutes, as was effectively carried out in the Collective Non-Commercial Enterprise «Rubizhne Central City Hospital» [11].

The efficiency of the use of reserves is characterized by a system of absolute and relative indicators. Absolute indicators are used in the practice of accounting and are used for analysis. Based on the data of the balance sheet of the Collective Enterprise «Horodenka non-commercial center of primary medical care», it is possible to draw a conclusion regarding the minimum number of material and technical means available in the hospital to ensure the uninterrupted operation of the hospital.

Absolute inventory balances on the balance sheet date may change as a result of two factors: quantity and value. Quantitative impact calculations and cost factors for the change in stocks for each type are calculated by the method of absolute differences. To determine the reasons for the increase in inventory balances, they are analyzed according to the same type of group in quantitative (natural) and value values.

Based on the information presented in [12], a new effective mechanism of interaction between the Collective Enterprise «Horodenka non-commercial center of primary medical care» and general practice clinics of family medicine is: the creation of educational centers for permanent post-graduate training of



medical workers (doctors, secondary level institutions); creation and organization of work on the basis of regional hospitals; improving the qualification level of medical and secondary medical personnel.

Increasing the level of qualification of the medical staff, together with the use of the latest clinical technologies and resource provision of the Collective Enterprise «Horodenka non-commercial center of primary medical care» fully determines the quality and efficiency of the provision of medical care. Therefore, first of all, it is necessary to ensure the selection of medical personnel who work in the Collective Enterprise «Horodenka non-commercial center of primary medical care». It is necessary to hire a doctor who has practical experience and has the 2nd qualification category and above. It is possible to provide an open competitive selection of medical workers to fill vacant positions. Secondly, to increase the level and importance of middle-level medical workers in the process of providing medical care to patients, to transfer part of the functions of doctors to them, to attract junior medical workers to provide medical care. The key element of optimizing the personnel policy of the hospital is the use of a differentiated approach to the payment of medical workers, taking into account real needs, and not strict centralized standards.

## CONCLUSIONS

Thus, after the introduction of the hospital district in the Collective Enterprise «Horodenka non-commercial center of primary medical care» to increase the effectiveness of the implementation of financial management, it is proposed to carry out a number of measures. The goal of increasing the efficiency of management, including financial resources, is the improvement of personnel management.

The main emphasis in the management is the formation of personnel potential of the Collective Enterprise «Horodenka non-commercial center of primary medical care», the involvement of qualified specialists in the field of medicine, the motivation of various directions to support and improve the qualifications of the management staff. It is also important to use the system of financial planning of a budgetary institution, to ensure expenses for its life activities. In particular, the main direction of cost control is the targeted use of funds, strict control over this use, and the formation of an effective internal audit system of a medical institution.

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## REVIEW ARTICLE

# CONTRAST INDUCED ACUTE KIDNEY INJURY IS NOT A SITUATION TO BE AFRAID OF

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**Bartłomiej Maciej Anton<sup>1</sup>, Sławomir Nazarewski<sup>1</sup>, Jolanta Malyszko<sup>2</sup>**<sup>1</sup>DEPARTMENT AND CLINIC OF GENERAL, VASCULAR AND TRANSPLANT SURGERY, MEDICAL UNIVERSITY OF WARSAW, WARSAW, POLAND<sup>2</sup>NEPHROLOGY, DIALYSIS AND INTERNAL MEDICINE, WARSAW MEDICAL UNIVERSITY, WARSAW, POLAND**ABSTRACT**

Acute kidney injury (AKI), formerly called acute renal failure (ARF), is clinically manifested as a reversible acute increase in nitrogen waste products, as measured by blood urea nitrogen (BUN) and serum creatinine levels. Contrast induced acute kidney injury (CI-AKI) is a potentially fatal complication of angiographic procedures caused by the use of contrast media (CM). It is the third most prevalent cause of hospital acquired acute renal damage, accounting for around up to 30% of cases. Contrast induced nephropathy (CIN) is defined as a greater than 25% or 0.5 mg/dl (44 µmol/l) increase in serum creatinine (Scr) from baseline within 3 days. More sensitive indicators of renal damage are sought, hence numerous tubular injury biomarkers are being studied. Multiple risk factors may lead to the development of CIN; these risk factors are classified as patient-related and procedure-related. Treatment of CIN is primarily symptomatic and consist firstly of careful fluid and electrolyte management, although dialysis may be necessary in some cases. With available treatment options, prevention is the cornerstone of management.

**KEY WORDS:** acute kidney injury, contrast induced acute kidney injury, contrast media

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**INTRODUCTION**

Contrast induced acute kidney injury is third most common hospital-acquired AKI basing on data from US hospitals [1, 2]. It occurs in even to 30% patients who receive iodinated contrast media injection and it is associated with renal failure and following high risk of mortality. The European Society of Urogenital Radiology defined CI-AKI as an increase in serum creatinine to 44 µmol/L, 0.5 mg/dL, or of 25% from baseline within 3 days of contrast media injection, with exclusion of any other alternative aetiology [3]. CI-AKI is a major health-care problem. With more than 2 million cardiac catheterizations performed [4] and over 30 million doses of iodinated contrast medium administered annually [5], the overall harm to patients' health and the public costs associated with CI-AKI are vast [6]. Omitting contrast medium, however, often leads to suboptimal diagnostic information, thus compromising overall therapeutic outcome. The need to minimize risk of CI-AKI must, therefore, be balanced against the need to obtain optimum imaging.

Most patients with normal kidney function who receive contrast will not experience any renal complications; however, those with volume depletion or chronic kidney disease (CKD) are at increased risk for CI-AKI.

Nonetheless a constant fear of CI-AKI leads to withholding of contrast. It is reducing diagnostic and therapeutic effectiveness in vast of clinical cases but is also misleading episodes of acute kidney injury from connecting them with their real causes. It pursues to a reduction of the effective management in the future.[7]

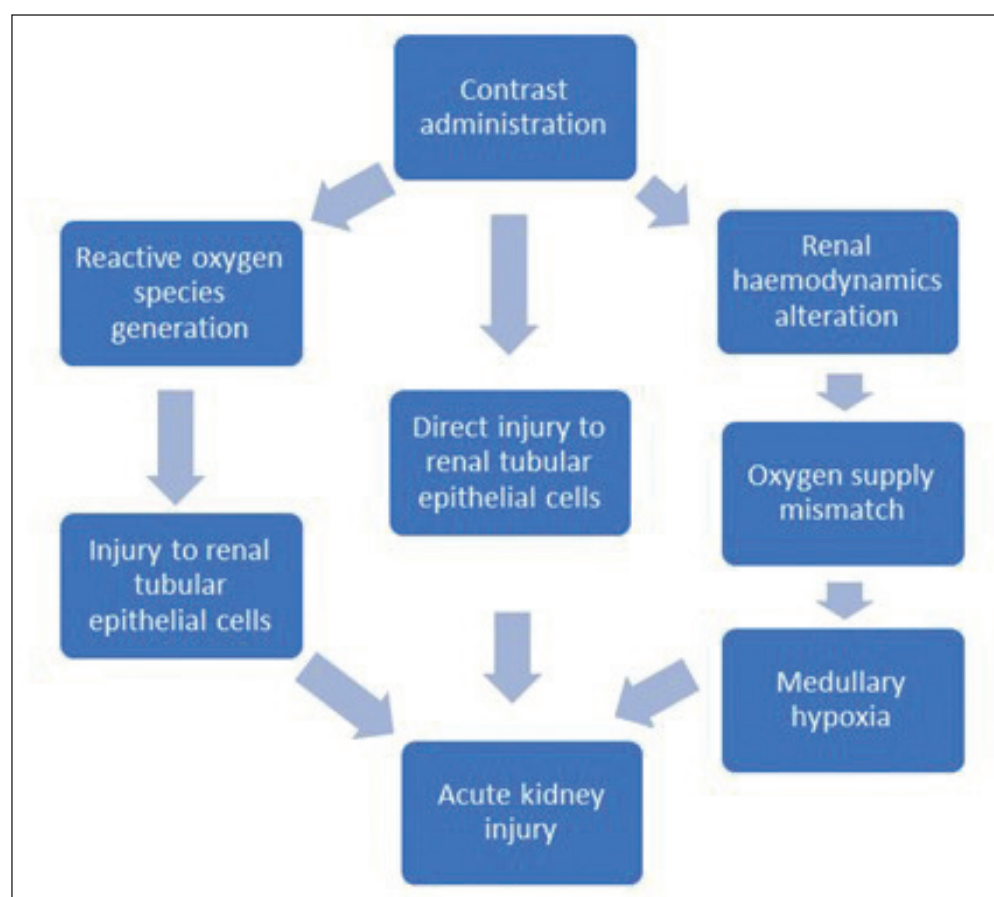
**THE AIM**

The aim of this paper is to review the current literature on the pathophysiology, prevalence, risk factors and prevention of contrast induced acute kidney injury.

**REVIEW AND DISCUSSION****PATHOPHYSIOLOGY**

In different studies, it is significantly proven that variety of contrast agents reduce renal blood flow in medulla, trigger free oxygen radicals and also induce apoptosis of renal tubular cells (Fig. 1) [8].

Previous work on the physiological effects of intravascular contrast on the kidney supports the nephrotoxicity of iodinated contrast. In variety of animal models, intravascular administered of iodinated contrast resulted in decreased renal blood flow and decreased partial oxygen pressure in the part of the external medulla, a segment of kidney that is particularly susceptible to disrupted oxygen provision [9]. A hemodynamic adverse effect of this contrast agent has also been observed in studies in healthy humans using blood oxygen level-dependent MRI, in which the use of iodinated contrast agent reduced the blood flow to the renal medulla. In animals, contrast administration has also been shown to increase the generation of free oxygen radicals, an effect that is associated with a decrease of glomerular filtration[8]. Although in vitro studies demonstrated that the iodinated contrast



**Fig. 1.** Pathophysiology of contrast induced nephropathy.

has an impact on mitochondrial enzyme activity and leads to renal tubular epithelial cell apoptosis [10]. Even though results from animal models do not necessarily reflect to humans, and those studies surely have different limitations, many studies provide pathophysiologic basis for the nephrotoxicity of iodinated contrast.

## RISK FACTORS

Basing on van der Molen et al [11] and European Society of Urogenital Radiology different group of patients are exposed renal complications after contrast administration.

### I LEVELS OF EGFR AT WHICH THERE IS A RISK

The risk of PC-AKI in patients with  $\text{eGFR} \geq 30 \text{ ml/min/1.73m}^2$  after intravenous and intra-arterial CM administration with second-pass renal exposure is very low, but there is conflicting evidence on the risk for intra-arterial CM administration with first-pass renal exposure

Level of Evidence: B

Preventive measures are recommended for patients with  $\text{eGFR} < 30 \text{ ml/min/1.73m}^2$  before intravenous and intra-arterial CM administration with second-pass renal exposure

Level of Evidence: C

Preventive measures are recommended for patients with  $\text{eGFR} < 45 \text{ ml/min/1.73m}^2$  if they are in ICU or if they will receive intra-arterial CM administration with first-pass renal exposure

Level of Evidence: C

Recommendations for prevention of PC-AKI in adults may also be used in children and adolescents

Level of Evidence D

## II RISK FACTORS

The principal risk factor for PC-AKI is impaired renal function. Most other published patient-related risk factors are risk factors for the presence of chronic kidney disease or AKI, and are not specific for PC-AKI

Level of Evidence B

There is no difference in PC-AKI risk between IOCM and LOCM. The use of ionic, high-osmolar CM and repeated CM injections in a short period (48–72 h) should be avoided

Level of Evidence C

When CM are injected intravenously, there is insufficient evidence that CM dose is a risk factor. When CM are injected intra-arterially, the ratio of CM dose (in gram Iodine) / absolute  $\text{eGFR}$  (in  $\text{ml/min}$ ) should be kept below 1.1 or the ratio of CM volume (in  $\text{ml}$ ) /  $\text{eGFR}$  (in  $\text{ml/min/1.73m}^2$ ) should be kept below 3.0 when using a CM concentration of 350  $\text{mg/ml}$

Level of Evidence C

## PREVENTION

It should be realized that various groups of patients have different risk of developing CIAKI while exposed to contrast media.

For out-patients who undergo a single contrasted procedure, contrast media seems to be the only reason of developing AKI, while in ICU patients contrast media is one of several other factors leading to renal impairment. An important fact is that the group of patients who undergo contrast-enhanced CT scan is the one with a greater risk for AKI.

Definitely prevention should be adapted to patients with greater risk for CI-AKI. One of the crucial factors is proper hydration. The others but also very important are use of: bicarbonates, N-acetylcysteine, vitamins and finally dialysis [12].

Proper hydration pre- and post-contrast CT is so far known to be the most important preventive strategy. It is obvious that hydration results in lowering of blood contrast agent concentration that leads to reduction of interaction of contrast with kidneys. [13] For a more than a decade hydration with isotonic saline has been the standard in coronary angiography [14]. Nowadays use of bicarbonates as hydration that eliminates free reactive oxygen species is very valuable [15, 16]. ON opposition to that some authors suggest that bicarbonates have no higher value than oral hydration [17, 18]. Marenzi et al [19] described that some strategies which used forced diuresis basing on furosemide combined with a device that provides constant intravenous fluid compensation of the urine produced. This way helped to prevent CI-AKI and fluid overload in groups of patients who underwent angiography [20].

Additionally to sodium bicarbonate prehydration, N-acetylcysteine has also been studied as a prevention of CI-AKI effecting on reactive oxygen species [21]. As an antiapoptotic drug it helps in preservation and protection of renal tissue [22]. Because its low bioavailability and accumulation recent studies suggest using N-acetylcysteine with ultrasmall gold nanoclusters as a nanozyme-based antioxidant to prevent AKI [23].

Vitamins C and E as known anti-oxidants have been assessed as preventive agents for CI-AKI. Vitamin E was evaluated positive to lower the risk of CI-AKI [24]. Vitamin C in combination with N-acetylcysteine was also effective at reducing the risk of CI-AKI [25].

Haemodialysis being able to remove 70-80% of injected low-osmolar contrast media dose [26] could be used in group of patients exposed to renal impairment [26]. Due to its limitations it cannot be used in every single case and it is not a proper preventive method.

## DISCUSSION

According to Fahling et al. [2] the prevalence of contrast-induced acute kidney damage (CIAKI) remains debatable, however clinically significant CI-AKI is less common than previously supposed [27]. Individual patient risk variables dictate the processes through which contrast media causes kidney injury [28]. Pre-existing decreased renal tissue perfusion increases the cytotoxic effects of contrast agents, exacerbating renal hypoxia [29]. The rheological features of contrast media are harmful, especially in dehydrated individuals. Contrast medium causes apoptosis by disrupting cell membranes, which raises intracellular  $Ca^{2+}$  levels, stimulates the pro-apoptotic unfolded protein response and lowers ATP levels [30]. Volume expansion has been shown to be useful in avoiding CI-AKI [31].

Oral hydration gives immediate short-term renal protection, but intravenous treatment of isotonic saline provides long-term protection but must be initiated hours before contrast agent exposure [32]. Loop diuretics such as furosemide paired with servo-controlled volume infusion may give the best renal protection against CI-AKI. In this case, urine excretion or central venous pressure can be employed as set points [33]. In opposition of the whole idea of contrast induced acute kidney injury stands Ehrmann et al. [34] who write that the lack of randomized controlled trials casts a doubt on the theory. For more than five decades the idea of CIAKI that was emerged through observation and presumed causation even without rigorous hypothesis testing and has been implemented in therapeutic practice [35].

## CONCLUSIONS

Contrast Induced Acute Kidney Injury may be a serious complication in the group of high-risk patients. The idea should be still discussed and tested. What is known that the proper hydration and individual patient's assessment is the crucial factor in the therapeutic process. Citing Paracelsus "Omnia sunt venena, nihil est sine veneno. Sola dosis facit venenum" we should put more effort on understanding the use of proper dose of contrast media and adequate patient's preparation.

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## REVIEW ARTICLE

# ARTICULATOR APPLICATION IN ORTHODONTIC DIAGNOSTICS

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**ABSTRACT****The aim:** The purpose of the study was to determine the indications for the use of articulator in orthodontics based on the results of the analysis of scientific and foreign literature.**Materials and methods:** 23 scientific sources were selected, from more than 11 countries over the past 39 years (1981 – 2020) devoted to the use of orthodontics articulators. As a result of processing 23 key international scientific papers on the use of articulators in orthodontics, we can talk about three areas of opinion regarding this problem.**Conclusions:** To sum up 30.34% of authors consider the articulator to be an obligatory orthodontist's tool for correct diagnosis. The idea of 34.78% of specialists is that the articulator cannot simulate the physiology of the TMJ – therefore, it is more a source of additional errors than an assistant in the orthodontic diagnostics. The rest of the scientists (34.78%) agree that it is advisable to use the articulator selectively – namely, if the doctor deals with “complex” orthodontic pathologies.**KEY WORDS:** dental articulators, dental occlusions, temporomandibular joint

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**INTRODUCTION**

The masticatory organ is a complex system of structural and functional interrelations. Unfortunately, doctors often neglect its thorough functional diagnostics. With the rapid development of dental technologies and unlimited possibilities for a treatment method choice orthodontist faces the problem of functional diagnostics as an integral part of modern orthodontics as never before [1].

One of the reasons for this situation is probably the lack of consensus regarding diagnostic methods. The divergence of opinions causes a feeling of uncertainty and even anxiety among practicing orthodontists before using systematic functional diagnostics in their daily practice. Without competent functional diagnostics, orthodontic treatment will be unpredictable, which leads to an increase in the number of patients with various complications [2].

The search for the causes of functional disorders requires the introduction of systematic methods that must be adapted to each specific clinical situation.

The purpose of examination procedures is diagnosis while the purpose of diagnosis is a flawless treatment plan. More information we have about the patient – more competent the diagnosis and more predictable the treatment will be. The final treatment plan is drawn up after a functional, structural and aesthetic analysis of the patient's oral cavity, X-ray examination, investigation of plaster models of the jaws, which are subsequently cast in an articulator along the facial arch.

When it comes to a complex and lasting orthodontic treatment, patients have a number of fears. One of them is the fear of undesirable and unpredictable functional changes. This anxiety is not in vain as improperly per-

formed treatment not only causes discomfort and impaired masticatory function, but also more serious problems as persistent headaches, TMJ dysfunction, musculoskeletal diseases in the skull and cervical spine area, etc. To minimize the risk of such unpleasant consequences, it is necessary to consider not only the peculiarities of the mutual arrangement of the jaws, but also the trajectory of movement of the mandible, which is a very individual indicator. This is where the articulator comes in.

**ARTICULATOR IMPLEMENTATION HISTORY**

In 1858 while examining a skull Bonneville found that the average distance between the condylar processes and the incisal point is 10 cm. By connecting these points, a triangle is obtained which is called Bonneville's triangle. It serves as one of the basic building blocks of many anatomical articulators to determine the spatial position of models in the articulator [3].

One of the first gnathological societies was founded by the German doctor B. McCollum back in 1926. Together with Harlan, they developed the first effective method for localizing the transverse (transversal) horizontal axis and transferring the registration impression into the articulator using the Snow facial arch. McCollum and Stewart published a research community in which they formulated the principles of mandibular movements in the transversal and horizontal axes, the ratio of the maxilla to the mandible in an articulator that was designed to reproduce the movements of the dentition. Before the emergence of new modern articulators, many stages of their improvement passed [4, 5]. The purpose of articulator usage is to ac-



**Fig. 1.** Simple hinge articulator

curately simulate the relationship of the upper and lower jaw, including the registration of the parameters of the occlusal surfaces with further reproduction of the boundary movements of the mandible. The new development of functional diagnostics is associated with the name of the Swiss physician Alfred Gizi. He suggested that the problem of articulation could be solved by recording the individual movements of the mandible and reproducing them technically. According to Gizi, it is the TMJ that directs the movements of the mandible. He created an articulator which consisted of elements that repeated the anatomy of a human joint. Earlier, Dr. Gizi began using the articulator to model the occlusal surfaces of artificial teeth. [6]

In 20th century dentistry, the concept of balanced occlusion prevailed. However, Stewart denied the validity of this concept. Uneven erasure of the buccal and lingual cusps of teeth with the formation of deflecting occlusal contacts leads to a change in the nature of jaws closing according to his opinion. These patients complain of frequent biting on cheeks and tongue, which indicates a loss of masticatory efficiency. It is the articulator (from the Latin *articulus* – joint, articulation) that makes it possible on the patient's working model to reproduce the movements characteristic of the mandible, to study the mutual arrangement of the jaws during mastication, the amplitude of mouth opening and closing, protrusive, retrusive and lateral movements of the mandible.

Back in 2002, Rudolf Slavichek spoke about the rationality of using articulators in the diagnosis and choice of a treatment strategy for pathological types of occlusion; planning complex types of dental treatment; manufacturing of various orthodontic structures; the manufacture of dentures of any complexity (removable and fixed); diagnostic grinding of teeth; when planning orthognathic operations; [1, 7]. And this is far from all the manipulations

in which modern dentists cannot do without an articulator.

Orthodontists constantly ask the question if they really need an articulator and a facial bow in their daily orthodontic appointments. Is it always needed to mount models to the articulator? Therefore, the issue of use is not a definite “yes” or “no”, but a matter of rational use.

Before searching for an answer to the question posed, the awareness about the articulators types is needed.

#### TYPES OF ARTICULATORS

There are five main types of articulators: simple hinge articulators; Mean value or fixed condylar path articulators; semi-adjustable; fully-adjustable or versatile; virtual articulators.

In a simple hinge articulator (Fig. 1), only hinge movements are possible, any lateral movements are excluded. The opening / closing path of the articulator frame does not match the opening / closing path of the mouth. Articulated articulators only reproduce the pathology of the bite, and do not help to eliminate it.

In mean value articulators (Fig. 2), the value of the condylar and incisal angle is fixed. You can change the relationship of the incisors, but there is no way to adjust lateral shift. Benet's angle is fixed – 15° and horizontal condylar path – 35°.

Mean-value articulators can be used for the manufacture of single crowns and, if necessary, for the manufacture of a complete removable denture with edentulous jaws [8].

Semi-adjustable articulators (Fig.3) have mechanisms for reproducing the condylar and incisal paths, which are regulated both according to the average data and according to the individual angles of these paths. Their significant advantage is the ability to adjust the angle of the sagittal condylar path. This type of articulator is convenient for use in most clinical cases [9].

There are two types of semi-adjustable articulators:

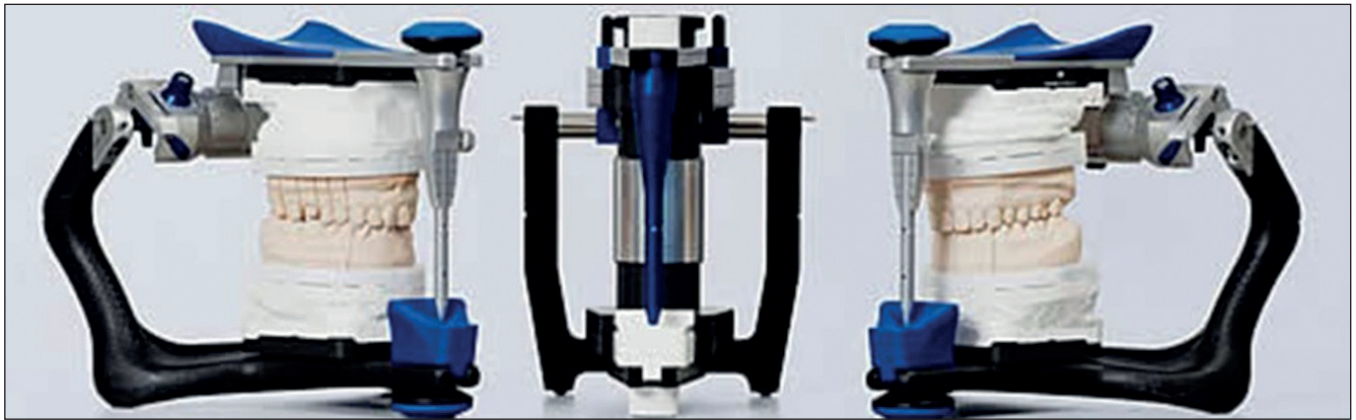
ARCON (arc) – the condylar ball is located below the guiding surfaces, its movements depend on the relief of the occlusal surface of the teeth (“sliding” articulator). It models the condylar process. The system is made in the form of a movable ball moving along the hole. This type is used in multi-purpose facial arch articulators.

Non-ARCON (arcess) – the condylar ball is located in a rut, which limits its movement within certain limits, the movements of the mandible depend on the established angles (“condylar” articulator). An immediate simulator, which repeats all the movements of the joint, is located at the top of the mechanism.

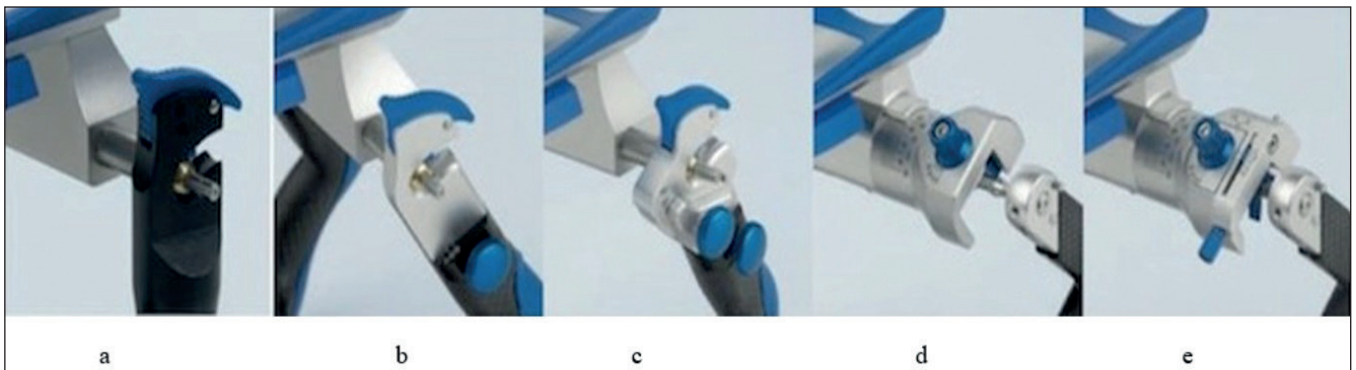
Each variant has its own advantages and disadvantages. The first option (arcon) is most often used. It is more in line with the human anatomy, it is quite easy to customize and has replaceable elements. All this makes the work on the arcon system convenient and accurate enough. The only drawback of this system, which is inherent in old articulator models, is the need for constant pressure on the upper frame.

The Non-arcon system allows you to work with securely fixed models. However, it does not provide for a change in the shape of the heads, there are inaccuracies from an ana-





**Fig. 2.** Mean Value or Fixed Condylar Path Articulators



**Fig. 3.** Semi-adjustable articulators «Non-arcon» (a, b, c) and «Arcon» (d, e)



**Fig. 4.** Fully-adjustable or universal articulator

tomical point of view. Therefore, usually all the necessary measurements are made using the Arcon articulator, which in this respect is more accurate and more convenient, and then the results are transferred to a more reliable in terms of data fixation – Non-Acorn [10]

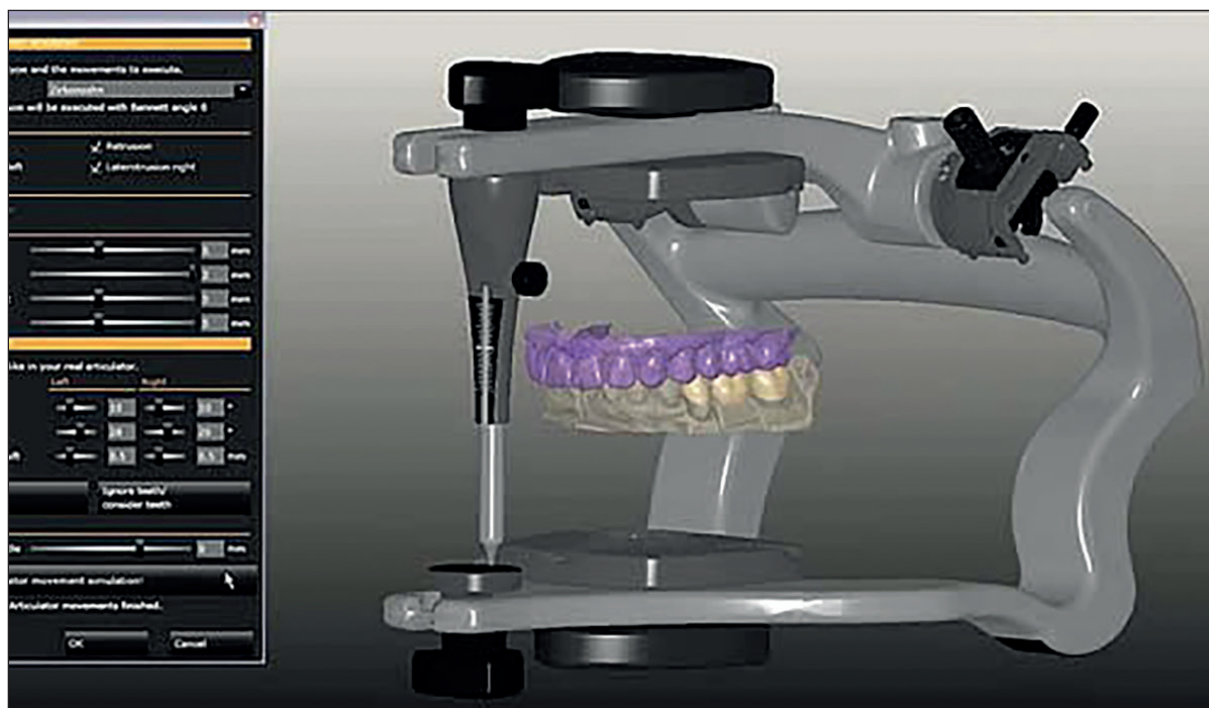
Fully adjustable or universal articulators (Fig.4) adjust the angles of the lateral condylar path and horizontal con-

dylar path as well as the lateral shift. This device reproduces the movements of the mandible with the greatest accuracy and is used to draw up a treatment plan for complex orthodontic pathologies and in the manufacture of the most complex prosthetic constructions that require careful occlusal analysis. This type of articulator is adjusted according to the individual data of the position of the jaws using the facial bow. In universal articulators, it is also possible to adjust protrusion up to 6mm and retrusion up to 2mm [11].

Digital (virtual) articulator (Fig.5) – In modern programs for modeling dentures there is a place reserved for the function of a virtual articulator. Complex and relatively expensive mechanical systems can be eliminated. They are being replaced by computer systems. The function of the virtual articulator is implemented in such a way that the models of the jaws are scanned using a 3D scanner and placed in the 3D space of the dental scanner in the position they occupy relative to the articulator frames [12]. To work in virtual space, taking into account the individual parameters of the patient, it is necessary to have computed tomography (CBCT), virtual models, trajectories of movement and the correct orientation of virtual models of both jaws [13].

CAD – Computer-Aided Design is a software that allows to identify the spatial position of the virtual dentition relative to the hinge axis. Then, the program uses the averaged characteristics or sets the individual characteristics of the angles of movement of the mandible, predetermined in advance with axiography [14,15].





**Fig. 5.** CAD software or virtual articulator concept

Correct mount of the model into the articulator will not be possible without the preliminary mount of the facial arch to the patient. The data gained with the facial arch is transferred to the articulator. The arc is not used in all cases, but its application allows to get more accurate individual indicators. For the most accurate reproduction of the parameters of the position of the maxilla relative to the mandible hinge rotation axis it is necessary to perform the following manipulations in the articulator:

- to obtain an occlusal impression (wax plate, silicone);
- to fix the face bow;
- to mount models into the articulator.

## THE AIM

The aim of the study was to determine the indications for the articulator usage in orthodontics based on the results of the analysis of scientific literature.

The study involved 37 scientific sources over the past 39 years (1981 – 2020).

## MATERIALS AND METHODS

We have selected 23 scientific (Table I) sources from more than 11 countries of the world (South Korea, Japan, USA, Australia, Czech Republic, Italy, Spain, Austria, PRC, Germany, UK, etc.) over the past 39 years (1981 – 2020) dedicated to the application of orthodontics articulators. The remaining 14 publications allowed us to present a historical picture of the appearance of the articulator in dentistry in general, its technical improvement over time and the expansion of indications for use, but were not included in the number of analyzed ones.

## REVIEW AND DISCUSSION

Having reviewed the scientific publications, it should be noted that there is no unequivocal opinion of scientists.

Regardless of time and geographic location, scientific researchers splitted into three main groups, namely, for the use of the articulator, against, and those who are for selective usage (depending on the pathology). Almost a third of researchers 30.34% (7 out of 23) were included in the “For” group, i.e. recommend to use of an articulator in orthodontics.

Theodore D. Freeland (2012) emphasizes that the articulator is an integral part of the orthodontic practice. According to him, it is possible to reveal hidden occlusal problems, especially in cases when it comes to the vertical position of the jaws only with the help of an articulator [33].

Domingo Martin and Renato Cocconi (2012) insist on the importance of using an articulator in clinical practice in order to improve the productivity of the orthodontist in complex multidisciplinary cases. The authors provide a list of significant reasons for mounting models into the articulator: assistance in determining the centrally oriented occlusion mismatch in 3 planes, assistance in determining the first point of contact in the central relationship, assistance in studying the patterns of abrasion and inclination of the maxillary incisors in patients with periodontal disease, assistance in determining the need for “trial” treatment, in drawing up plans for orthognathic surgery patients, assistance in the manufacture of joint splints [32]. The next group of researchers, voting for the “selective usage of articulators,” that is, according to direct indications. This group is 34.78% (8 out of 23), in other words, 3.44% more than the “For” group, which was 34.78%.

American orthodontics represented by D. Rinchuse, S. Kandasamy and co-authors in a series of articles [26-29,31] devoted to the problem of using articulators, explain their position that articulators are important when planning complex measures of

**Table I.** Publications on the use of articulators in orthodontics

Nº	Author	Article title	Year	Country	Position
1	Roth RH	Functional occlusion for the orthodontist. [16]	1981	Germany	Selective (depending on pathology)
2	Rinchuse DJ	The impact of the American Dental Association's guidelines for the examination, diagnosis, and management of temporomandibular disorders on orthodontic practice. [17]	1983	Czech	Against
3	Mohl ND	Temporomandibular disorders: role of occlusion, TMJ imaging and electronic devices—a diagnostic update. [18]	1991	USA	Against
4	Lindauer SJ, Isaacson RJ, Davidovich M.	Condylar movement and mandibular rotation during jaw opening. [19]	1995	USA	Against
5	Frank E. Cordray	Centric relation treatment and articulator mountings in orthodontics. [20]	1996	USA	Position for
6	J. R. Clark	Functional Occlusion: II. The Role of Articulators in Orthodontics. [21]	2001	United Kingdom	Against
7	Alpern MC, Alpern AH.	Innovation in dentistry: the polycentric occlusal system. [22]	2003	Czech/ USA	Selective (depending on pathology)
8	Alex Jacobson	Does articulating study casts make a difference to treatment planning? [23]	2003	United Kingdom	Against
9	C.Gartner/ B.Korda	The Virtual Articulator: Development and Evaluation. [13]	2003	Germany	Position for
10	Robert Kelleher	Common sense, open mind needed. [24]	2006	USA	Against
11	Richard M.Port	Articulators and gnathology: better care is the real goal. [25]	2006	USA	Selective (depending on pathology)
12	Donald J.Rinchuse Sanjivan Kandasamy	Articulators in orthodontics: An evidence-based perspective. [26]	2006	USA/ Australia	Selective (depending on pathology)
13	Donald J.Rinchusea Sanjivan Kandasamy	Word of mouth—articulators in orthodontics: chewing the facts. Part 1. [27]	2007	USA/ Australia	Against
14	Donald J.Rinchusea Sanjivan Kandasamy	Word of mouth—articulators in orthodontics: chewing the facts. Part 2. [28]	2007	USA/ Australia	Against
15	Donald J.Rinchusea Sanjivan Kandasamy	Myths of orthodontic gnathology. [29]	2009	USA/ Australia	Selective (depending on pathology)
16	J. Michael Hudson	Articulators in orthodontics. [30]	2012	USA	Position for
17	Donald J.Rinchusea Sanjivan Kandasamy	Articulators in orthodontics. [31]	2012	USA/ Australia	Selective (depending on pathology)
18	Domingo Martin. Renato Cocconi	Orthodontic dental casts: The case for routine articulator mounting. [32]	2012	Spain/ Italy	Position for
19	Theodore D. Freeland	Articulators in Orthodontics. [33]	2012	USA	Position for
20	Claudia Aichinger	Orthodontic dental casts: The case against routine articulator mounting. [34]	2012	Austria	Position for
21	Wang Li; Han Xianglong; Bai Ding	The controversy of routine articulator mounting in orthodontics. [35]	2013	China	Selective (depending on pathology)
22	1.Kaoru Inami 2.Yuri Higashi 3.KuoChiang	Prediction of dentofacial changes induced by an articulator intended for orthognathic surgery. [36]	2019	Japan	Selective (depending on pathology)
23	1.Park JH 2.Lee GH 3.Moon DN 4.Kim JC 5.Park M 6.Lee KM	A digital approach to the evaluation of mandibular position by using a virtual articulator. [37]	2020	Korea	Position for

orthodontic treatment with orthognathic surgery for establishing the correct vertical ratio at the laboratory stage of treatment. The authors also include here orthodontic cases with multiple tooth edentulousness, in which it is impossible to fix stable occlusal contacts, when there is a significant discrepancy ( $> 2$  mm) between the fissure-cusp ratio. The authors recommend mounting of models in the articulator in persons with TMJ dysfunction.

This group includes the publications of researchers [12, 14, 15] who use a digital diagnostic protocol in their practice, based on the individual parameters of the patient, 3D scanning of the jaws, CAD programs, computed tomography, axiography. This method allows to compare these diagnostic data in a virtual space – in a virtual articulator. This program, as a more reliable method, allows you to identify the spatial position of the dentition relative to the hinge axis, eliminates the human error factor as in an analog articulator. In 2003, in Germany, C. Gärtner, B. Kordass [13] presented the results of comparative characteristics of two types of articulators: mechanical and virtual. In their opinion, the virtual articulator is not only a valuable addition to functional and occlusal analysis, but can also be used as a tool for planning and modeling complex orthodontic cases.

In general, both groups, ideologically close in spirit which are “For” and “For the selective usage of the articulator,” make up the overwhelming majority – 65.12%, i.e. in 15 articles out of 23 a scientifically substantiated point of view was expressed for the need for an adequate clinically justified use of an articulator, as another way to improve functional orthodontic diagnostics, which positively affects the correctness of treatment, quality and effectiveness.

The third orthodontic group “Against” are the opponents of articulators application in orthodontics, which is 34.78% (8 out of 23). One of the representatives of this position R. Kelleher [24] in 2006 made a conclusion in his article about the importance of adhering to sound principles of functional stability of occlusion. However, the idea that we can control the health of the temporomandibular joint using rigid measurements, manipulations and equipment, in his opinion, distracts from the real task of comprehending and collecting a unique anamnesis, conditions and response of each patient to treatment. Therefore, we must not rely on the devices which application may cause mistakes.

Thus, based on the data obtained as a result of processing 23 key international scientific publications devoted to the advisability of using articulators in orthodontics, we can talk about three areas of opinion regarding this problem. Supporters of the first opinion “For” claim about the expediency and mandatory use of articulators in the treatment of orthodontic pathology, motivating this with high diagnostic accuracy, physiology (considering the masticatory component) and improving the productivity of the orthodontist in multidisciplinary and complex cases [20, 13, 30, 32–34].

In this group of publications special attention should be paid to studies devoted to digital articulators – a modern method of digital diagnostics in orthodontics. Its application in combination with 3D imaging obtained with CBCT, as well as a digital scan of the oral cavity, is especially valuable, since it allows you to determine with maximum accuracy the physiological position of the patient's jaws relative to the base of his skull [13–15, 37].

However, there are also many scientists who support the opinion that articulator application in the treatment of orthodontic

pathologies is not advisable, motivating their point of view by the unreasonable expenditure of time and money, risk of making mistakes at various stages of working with the device, which can have an accumulation effect and ultimately negatively influence the result of treatment. The articulator, in their opinion, cannot accurately simulate the physiology of TMJ movements and the trajectory of movements of the mandible [17–19, 21, 23, 24, 27, 28].

The most logical, according to the results obtained, is the opinion of the second group of authors, who consider selective usage of articulators only in certain cases, in particular, when planning orthognathic surgeries in cases with multiple adentia, with TMJ dysfunction. In “simple” cases, the articulator may not be used to eliminate the risk of possible errors at various stages of its usage [16, 22, 25, 26, 29, 31, 35, 36].

## CONCLUSIONS

Thus, after analyzing all above-mentioned publications devoted to the usage of articulators for diagnosis of orthodontic pathology, we may conclude that scientists from the USA were most involved in this issue. To summarize, 30.34% of authors consider the articulator to be an obligatory tool of an orthodontist for correct diagnosis. The idea of 34.78% of specialists is that the articulator cannot simulate the physiology of the TMJ, therefore, it is more likely a source of additional errors than an assistant. The rest of the scientists (34.78%) agree that it is advisable to use the articulator selectively, namely, if the doctor deals with “complex” orthodontic pathologies.

Two groups, “For” and “for selective use of articulators” make up the overwhelming majority – 65.12%, they support the necessity of mounting models in the articulator. We can conclude that the usage of this diagnostic method definitely has its pros and cons, but in current realities, it is a modern and improved diagnostic method that has not lost its relevance for the past 39 years.

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## REVIEW ARTICLE

**ON SOCIAL NETWORKS, DIGITAL MEDIA, AND MENTAL HEALTH**

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**Tetiana Danylova<sup>1,2</sup>, Svitlana Storozhuk<sup>3</sup>, Viktor Vus<sup>1</sup>, Vitalii Shmarhun<sup>4</sup>, Nataliia Kryvda<sup>3</sup>, Olena Pavlova<sup>3</sup>, Svitlana Kholodynska<sup>5</sup>**<sup>1</sup>INSTITUTE OF SOCIAL AND POLITICAL PSYCHOLOGY, NATIONAL ACADEMY OF EDUCATIONAL SCIENCES OF UKRAINE, KYIV, UKRAINE<sup>2</sup>THE GRADUATE SCHOOL FOR SOCIAL RESEARCH, INSTITUTE OF PHILOSOPHY AND SOCIOLOGY OF THE POLISH ACADEMY OF SCIENCES, WARSAW, POLAND<sup>3</sup>TARAS SHEVCHENKO NATIONAL UNIVERSITY OF KYIV, KYIV, UKRAINE<sup>4</sup>NATIONAL UNIVERSITY OF LIFE AND ENVIRONMENTAL SCIENCES OF UKRAINE, KYIV, UKRAINE<sup>5</sup>PRYAZOVSKIY STATE TECHNICAL UNIVERSITY, MARIUPOL, UKRAINE**ABSTRACT****The aim:** The paper attempts to investigate the impact of social networks and digital media on mental health and psychological well-being.**Materials and methods:** The data collection was carried out using PubMed, Scopus, Web of Science, Google Scholar databases. Research papers were identified according to the search terms “cyberspace”, “cyberpsychology”, “digital media”, “virtual reality”, “social networks”, “mental health”, “psychological well-being”, “beauty”, “beauty standards”. The authors used integrative anthropological approach, interpretive research paradigm, and multidisciplinary analysis.**Conclusion:** The digital world is a relatively recent phenomenon, therefore, the potential relationship between its use and mental health and psychological well-being has not yet been adequately explored. Most of the research has been conducted over the last decades; and until now the scientific community has not been able to fully interpret the obtained results and come to final conclusions. As Timpano and Beard rightly pointed out, the future research should be focused on types and motivations for the use of social networks; identify potential mechanisms linking the social media use and mental health symptoms; reveal an impact of social networks on declines in face-to-face social interactions; understand the link of social networks with maladaptive coping strategies and health behaviors. The relationship between social networks, digital media use and psychological functioning is not straightforward and depends on many factors. However, we should not forget that there is nothing new under the sun. The same people act in both digital and physical spaces. And the psychological problems that are revealed in the digital universe are the same that “accompany” the actors of the digital communication process in the real, physical world. Anyway, we cannot avoid cyberspace, so we have to learn how to interact within this challenging world.**KEY WORDS:** cyberspace; cyberpsychology; digital media; virtual reality; social networks

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**INTRODUCTION**

Nowadays, psychology has entered a new era of its development. This is directly related to such a phenomenon as cyberspace – the space in which the digital dimension of modern human life has found its expression. As of April 2022, there were five billion active Internet users worldwide (63 % of the global population) and about 93 % of them were social media users [1]. Social media has become a platform for the exchange of information and ideas; and new technologies have had a significant impact on social interaction, learning, and behavior patterns of the users of the digital space [2]. The widespread access to information associated with always-on devices has led to the fact that technology has penetrated all spheres of life including the private sphere. The rapid development of the information space has paved the way for greater political participation, emergence of a large number of personal and professional networks, and flattening of hierarchy in many countries [3; 4]. As Friedman emphasizes, in the future the success will be determined by education, abilities, ingenuity, and access to the global communication system. This situation will

radically change the conditions for the activities of those who live on the border with the “flat world” (the world permeated with horizontal structures that is more in line with the model of equal cooperation) [5]. In general, the ways we consume information and communicate with each other have fundamentally been changed [6; 7].

The worldwide spread of new technologies laid the foundation for a new branch of psychology – cyberpsychology, which aims to understand the psychological processes associated with all aspects and features of human behavior in cyberspace [8]. New science explores Internet personality and behavior; social media and psychological functioning of an individual; video games; telepsychology; virtual reality; artificial intelligence, etc. [9]. Among the many challenges cyberpsychology faces is the impact of social networks and digital media on mental health and psychological well-being.

The complex and dynamic interaction of the individuals in the digital world requires thorough analysis and deep understanding. Numerous social contacts and virtual groups, exchange of opinions, self-expression on the In-

ternet where it is possible to present any ideal self-image have created a unique space that has become a “home” for billions of inhabitants of the planet Earth. There is a popular saying “Only love makes a house a home”, however, hoping to find warmth and comfort in the digital world, the users often encounter not love but demons there, demons such as stress, depression, anxiety, low self-esteem, aggression, that is, everything they have tried to avoid in the real world [10]. Brooks [11], Cole, Nick, Varga, Smith [12], Nabi, Prestin, So [13], Boers, Afzali, Newton, Concord [14], Twenge, Joiner, Rogers, Martin [15], Wellman [16] have made a significant contribution to research. An ambiguous influence of social networks and digital media on the users has led to discussions among scientists. While some researchers consider its positive impact on mental health and psychological well-being, others emphasize the dangers of cyberspace.

## THE AIM

The paper attempts to investigate the impact of social networks and digital media on mental health and psychological well-being.

## MATERIALS AND METHODS

The data collection was carried out using PubMed, Scopus, Web of Science, Google Scholar databases. Research papers were identified according to the search terms “cyberspace”, “cyberpsychology”, “digital media”, “virtual reality”, “social networks”, “mental health”, “psychological well-being”, “beauty”, “beauty standards”.

The authors used integrative anthropological approach, interpretive research paradigm, and multidisciplinary analysis.

## REVIEW AND DISCUSSION

In recent years, the rapid spread of social networks has caused profound changes in the patterns of communication and interaction providing access to a unique platform for the exchange of ideas and connection with the whole world. This has contributed to the emergence of a “unified information space”, in which individuals have the opportunity to find themselves, realize their potential, and expand the boundaries of knowledge and communication. Undoubtedly, it has provided many advantages to individual members and the world community, but, like everything in the world, the new mode of communication has also revealed its reverse side manifested in the growth of depression, stress, anxiety, and a decrease in self-esteem among the active users of social networks. Recent research suggests that the long-term use of social networking sites such as Facebook may be associated with symptoms of depression, anxiety, and stress. Pantic et al. stated that the frequent use of Facebook and other social networks led to specific behavioral changes that negatively affected mental health. They established a relationship between the con-

stant use of social networks and an increase in the level of depression [17]. According to Twenge and colleagues, time spent on screen activity correlates with increased severity of symptoms of depression and risk of suicide, although the positive correlation between social media use and mental disorders is significant only for girls [15].

Boers et al. stressed that the excessive use of social networks and TV by adolescents could increase symptoms of depression [14]. Brooks found that the frequent use of social networks “led to lower performance on the task, as well as higher levels of technostress and lower happiness” [11, p. 26]. Although it seems that social networks can satisfy an individual’s need for social connections, research conducted by Kross and colleagues suggests that Facebook use can undermine young people’s sense of well-being [18]. Lin et al. agree upon the idea that the widespread use of social media increases depression, especially among young people [19].

The researchers emphasize the influence of social networks on the spread of a negative body image, especially among women [20]. An acute problem related to traditional gender stereotypes is the ideal image, artificial beauty imposed by advertising media [21]. Cover girls act as ideal face and body models. Although users/consumers understand that these women are professional models who are paid to maintain physical shape, and modern technologies provide opportunities to “conceal” any flaws, they still try to achieve the ideal. The cult of beauty is as old as the world. Myths and fairy tales of our childhood continue to resonate with the perception of our adult personalities. Nowadays, the beauty myth, which has replaced the myths of home, motherhood, women passivity and obedience, is associated with the institutions of power that represent the male world and is used in a counterattack on the females. Wolf states, “Beauty is a currency system like the gold standard. Like any economy, it is determined by politics, and in the modern age in the West it is the last, best belief system that keeps male dominance intact. In assigning value to women in a vertical hierarchy according to a culturally imposed physical standard, it is an expression of power relations in which women must unnaturally compete for resources that men have appropriated for themselves” [22, p. 12]. Beauty takes women out of the structures of power and return them to the social space where men prefer to see them. Beauty is put on the market dominated by capitalism and patriarchy – they make money out of beauty and use it to maintain the status quo [20; 22].

The beauty ideal presented in advertisements send the message that an attractive appearance is the only aspect that matters in life. All achievements outside this sphere are insignificant. This leads to the fact that women who do not meet the standards consider themselves inferior, suffer from social anxiety, prejudice, and inequality [23]. Stress, inferiority complex, and envy towards conventional beauties negatively affect the psychosomatic health of women [24]. The influence of visual media, which portrays idealized faces and bodies, leads to a distortion of one’s own image [25]. The researchers demonstrated the

powerful sociocultural influence of cyberspace on the lives of young women [26], as well as the connection between the active use of the Internet, especially social networks, and growing body dissatisfaction among teenage girls [27; 28]. The emphasis on the specific parts of the body leads to dehumanization, female objectivation, and self-objectivation [29]. Such trends are a real concern.

The world's obsession with the unattainable beauty standards over the past few decades has been accompanied by an increase in body dissatisfaction among both women and men [30], which entails dysfunctional eating behavior, distress, and depression [31]. Men also suffer from the imposed stereotypes – they should be “real men”. Hegemonic masculinity identifies itself with “common sense” and dominant ideology [32]. The images of men in media correspond to many aspects of traditional masculinity: physical strength, brutality, aggressiveness, dominance [33; 34].

Giaccardi and colleagues found that sports and reality TV viewing by college males was a predictor of adherence to the traditional masculinity ideology and its manifestation in interpersonal relationships [35; 36]. Depiction of athletic men and sports broadcasting confirm the paradigm of hegemonic masculinity and convince men that they must always fight for themselves and always win. In sports programs, strength, status, and endurance are primarily associated with masculinity. These programs glorify the athlete-superhero and justify his physical and verbal aggression. Strong and aggressive men are praised, while weak and passive representatives of the same sex do not “fit” into the category of heroes [37]. These “standards” are transferred to social networks. In male-dominated space including cyberspace, the standards of the alpha male do not allow a man to admit his real problems and be vulnerable; instead, he should represent a flawless Superman who would rather die than reveal his true feelings [38]. Prevailing cultural stereotypes of masculinity are directly related to the high level of depressive disorders and suicidal behavior of men who are not in line with the traditional system of expectations [39; 40].

Many people today are depressed due to the conflicts with others, layoffs, or serious illnesses, and excessive use of social media is a common factor that exacerbates this negative state. Active users of social media are most often exposed to the bad news, as far as news related to wars, natural disasters, terrorist acts, political crises, and deaths are always top news. It negatively affects mental health and leads to the development of increased anxiety and symptoms of depression. Lyall et al. found that the use of Facebook around bedtime increased the likelihood of developing major depressive disorder by 6% and decreased subjective happiness by 9% [41]. Aldao notes that such a situation locks us into a vicious circle of negativity: “the more time we spend scrolling, the more we find those dangers, the more we get sucked into them, the more anxious we get” [42]. Such trends are alarming because through this lens the world appears to be a very bleak place that makes consumers of negative information feel like they are in danger every day of their lives.

The other voices are also heard in the cyberspace discourse. Jelenchick and colleagues stressed that they did not find sufficient evidence that supported a relationship between social networking site and depression: “counseling patients or parents regarding the risk of “Facebook Depression” may be premature” [43, p. 130]. Seabrook, Kern, and Rickard showed that the use of social media under the conditions of positive interaction, social support, and social connection reduced depression and anxiety, increased self-esteem and life satisfaction, while negative interactions and social comparisons were associated with higher levels of depression and anxiety [44]. Cole et al. found that the use of Twitter in the context of social support could reduce depression. In particular, a larger number of contacts and high activity on Twitter are especially useful for people who are limited in personal communication. The researchers note that the use of social networks has greater benefits for people with less social support [12].

## CONCLUSION

The digital world is a relatively recent phenomenon, therefore, the potential relationship between its use and mental health and psychological well-being has not yet been adequately explored. Most of the research has been conducted over the last decades; and until now the scientific community has not been able to fully interpret the obtained results and come to final conclusions. As Timpano and Beard rightly pointed out, the future research should be focused on types and motivations for the use of social networks; identify potential mechanisms linking the social media use and mental health symptoms; reveal an impact of social networks on declines in face-to-face social interactions; understand the link of social networks with maladaptive coping strategies and health behaviors [45]. The relationship between social networks, digital media use and psychological functioning is not straightforward and depends on many factors, such as the way it is used, communication patterns, emotional state, comorbidities, self-awareness, values, motivation, attitudes, and preferences. However, we should not forget that there is nothing new under the sun. The same people act in both digital and physical spaces. And the psychological problems that are revealed in the digital universe are the same that “accompany” the actors of the digital communication process in the real, physical world. Anyway, we cannot avoid cyberspace, in which a significant part of our lives takes place. Our health and well-being depend on our ability to interact effectively within this challenging space.

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## REVIEW ARTICLE

# VOLUNTEER MOVEMENT OF UKRAINIAN STUDENTS IN THE FIELD OF MEDICAL AID, EDUCATION, HEALTH CARE FOR CHILDREN AND ADULTS (END OF THE XIX – 30S OF THE XX CENTURY)

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## ABSTRACT

**The aim** is to analyze the leading directions of volunteer activity of Ukrainian students in the field of medical, social assistance, education of children, youth and adults in Galicia (end of the 19th – 30s of the 20th century).

**Materials and methods:** The study uses a number of scientific methods: chronological, historical, specific search, content analysis – provide selection, analysis of the source base, allow to identify general trends, directions of development, achievements and gaps of the Ukrainian student movement in Galicia in the field of medical, social care, education and enlightenment of children and adults in the late XIX – 30s of the XX century; extrapolation and actualization – focus on creative thinking, adaptation and use of this historical experience under the current conditions.

**Conclusions:** Voluntary activity of Ukrainian students (end of the 19th – 30s of the 20th century) is an interesting peculiar phenomenon not only in national, but also in European history, which has real achievements and deserves a scientific and theoretical understanding from the standpoint of today. Student volunteer experience in the field of social and medical protection of children and adults, education, cultural development, promotion of a healthy lifestyle, dissemination of sanitary and hygienic knowledge, medical counseling can be useful and instructive now, when Ukraine is fighting against the Russian aggressor. We outline the volunteer activity of students who belonged to the “*Medychna hromada*” society (1910-1944) as a national phenomenon of the organization of public medical care of the population of Galicia, which has no analogue in the history of Ukrainian medicine. It is primarily about a high degree of civic self-awareness, patriotism, self-sacrifice for the benefit of the Ukrainian people, the provision of medical services to low-income sections of the population, widows, orphans, disabled people, veterans of the Great War, medical care of children and youth, etc. – all this inspires modern doctors who provide assistance to soldiers of the Armed Forces of Ukraine, wounded in hospitals, internally displaced persons, etc.

**KEY WORDS:** Ukrainian student societies, medical assistance, volunteering, social care, training of the future doctor

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## INTRODUCTION

During the second half of the 19th and 20th centuries the organized activity of Ukrainian students was part of the nationwide movement, an important factor in cultural and educational work, medical and social care of children, youth and adults, the movement for the preservation of the health of Ukrainians, the formation of national consciousness, etc., the struggle for the independence of Ukraine, etc. Nowadays, armed Ukrainian students defend the integrity of Ukraine's borders, fight against the Russian enemy for the independence of the state, provide sanitary and medical assistance, actively participate in the volunteer movement, and engage in charitable activities. The forms and methods of public activity of Ukrainian students changed at different stages of the historical development of Ukraine, depending on socio-political, socio-economic, cultural and educational conditions, state power, government systems, etc., but

the key basis of the Ukrainian student movement during the 19th-21st centuries is that it always had a nationally oriented character, was a component of national revival, an active participant in the cultural, educational, social, medical, and health care movement of Ukrainians, went beyond narrow professional problems.

Despite the fact that scientists (I. Bilavych [1], K. Brozek [2], Y. Ganitkevych [3], I. Gurak [4], R. Kovalyuk [5], B. Savchuk [6], etc.) are actively researching the activities of Ukrainian public associations in Galicia, in particular the development of the student movement in the 19th-20th centuries, but in science there has not been a comprehensive study of the participation of Ukrainian students in the volunteer movement regarding the medical and social care of children, youth and adults, the development of public education in Galicia at the end of the 19th – in the 30s of the 20th century.

## THE AIM

The aim is to analyze the leading directions of volunteer activity of Ukrainian students in the field of medical, social assistance, education of children, youth and adults in Galicia (end of the 19th – 30s of the 20th century).

## MATERIALS AND METHODS

The study uses a number of scientific methods: chronological, historical, specific search, content analysis – provide selection, analysis of the source base, allow to identify general trends, directions of development, achievements and gaps of the Ukrainian student movement in Galicia in the field of medicine, of social care, education and enlightenment of children and adults in the late XIX – 30s of the XX century; extrapolation and actualization focus on creative thinking, adaptation and use of this historical experience under the current conditions.

## REVIEW

Organized forms (clubs, societies, organizations, associations, etc.), participation of students in public, socio-political, cultural and educational life, struggle for the sake of Ukraine by the parts of military formations, etc. are considered to be the Ukrainian student movement [4; 5; 6]. During the studied period, studentship played the role of a generator of the national idea, a catalyst of national and social processes; factors that influenced the development of the Ukrainian medical and educational movement, the struggle for the health of children, youth and adults, etc.

In Galicia, an organized student movement was formed in the middle of the 19th century and was caused by the Ukrainian national revival, the spread of the ideas of romanticism, developed in the legal constitutional field of the Austrian/Austro-Hungarian Empire, which gave grounds for the legal activity of numerous Ukrainian student societies, created the basis for their active involvement in the Ukrainian national movement. The tradition of the student movement, created in the “Austrian” period, determined its important role in subsequent periods, when state policy was less favorable [5]. The spirit of service to national ideals, self-sacrifice, readiness for armed struggle for Ukraine’s independence was cultivated in student centers. Naturally, an important area of activity of Ukrainian students was cultural, educational, medical, health-preserving, social ones; they organized cultural events, educational courses, organized lectures, created dramatic and musical groups, as volunteers worked in the “*Narodna Lichnytsia*” in Lviv, in hospitals, together with doctors they worked benevolently in educational institutions, consulted villagers, provided medical care to children, engaged in medical education, etc.

All this contributed to the active development of the national and public life of Ukrainians not only in Lviv and county towns, but also in the province, in the mountainous regions of the region. Ethnographic expeditions, traveling lectures of Ukrainian students, organized by students who belonged to the societies (“*Sich*” (1867), “*Prosvita*”

(1868), *Naukove tovarystvo named after Shevchenko* (1873), *Rus’ke tovarystvo pedagogichne / Ukrai’ns’ke pedagogichne tovarystvo* (1881), “*Akademichna besida*” (1870), “*Akademichnyj kruzhok*” (1871), “*Druzhnij lyhvar*” / “*Akademichne bratstvo*” (1871), “*Vatra*” (1892), “*Akademichna gromada*” (1896), “*Osnova*” (1897), “*Rus’ka akademichna pomich*” / “*Ukrai’ns’ka akademichna pomich*” (1902), *Tovarystvo pryhylnykyv ukrai’ns’koi nauky, literatury i shtuky* (1904), *Tovarystvo naukovykh vykladiv named after P. Mogily u Lvovi* (1908), “*Ukrai’ns’kyj students’kyj sojuz*” (1909), “*Medychna gromada*” (1910), “*Profrus*” (1921), *Tovarystvo pryhylnykyv knygy in Prague* (1927), etc. Active educational work among the population, especially rural ones, took place during vacations and holidays, when students went home, where they distributed the publication “Enlightenment”, held classes and conversations with villagers, launched large-scale cultural and informational activities, organized libraries, convened meetings, parties, gatherings, organized festive events in honor of T. Shevchenko, I. Franko, historical figures of Ukraine, dedicated to national and religious holidays, carried out anti-alcohol propaganda, created sobriety circles, arranged lectures aimed at sanitary and hygienic education, etc.

Voluntary activity of Ukrainian students of the studied period is an interesting peculiar phenomenon not only in national, but also in European history, which has real achievements, deserves a scientific and theoretical understanding from the standpoint of today, the experience and practice of Ukrainian students in the field of educational, medical, social volunteering can be useful and instructive now, when Ukraine is fighting against the Russian aggressor.

Retrospective analysis of the process of organizational and ideological formation and development of Ukrainian student associations in Galicia at the end of the XIX – in the 30s of the XX century showed that most of them imitated the corresponding models of youth associations of other nations (usually Austria-Hungary, Poland), but under the conditions of the Austrian and later Polish state-political regimes, Ukrainian student associations maintained their autonomy, created numerous centers in the region, developed their own ideological principles and forms and methods of work that corresponded to the possibilities and needs of the social development of Ukraine, its urgent needs.

Therefore, the Ukrainian student body organized its activities on the principle of “national autarky”, which provided for the provision of national social, cultural, educational, material and financial needs based on the Ukrainians’ own resources, it sought to compensate for the gaps in state policy in solving important social problems, in particular in the fields of medical and social care, education, culture, health care and sports. Ukrainian students who belonged to various organizations contributed to the improvement of medical and sanitary-hygienic services for the population, launched a broad struggle for a healthy lifestyle among children, youth and adults, especially regarding the spread of hygienic knowledge, anti-alcohol and anti-nicotine education, promotion of active types sports, etc. In this

sense, they in a certain way compensated, supplemented, and in some cases replaced the activities of the state in the field of public health protection, opposed the policy of increasing alcoholism. Together with other Ukrainian public associations, Ukrainian students contributed to the mass educational movement for the formation of knowledge on the basics of hygiene and sanitation. These facts, as well as the fight against drunkenness, work on maternal and child health care, the ability to consolidate the will and aspirations of Ukrainians in a mass movement for a healthy lifestyle, became not only a real contribution to the preservation of the gene pool of the Ukrainian people, but also demonstrated to European nations and to the whole world the scale of the Ukrainian public movement, which was carried out on a volunteer basis.

Ukrainian medical students wrote a bright page in the development of the public medical movement in Galicia, medical and sanitary-hygienic service and the struggle for a healthy lifestyle for children, youth and adults. During the interwar period, the level of medical care in Galicia remained extremely low. Due to the high cost of services, it was inaccessible to the majority of the Ukrainian population, who, however, lacked elementary knowledge of the basics of hygiene and sanitation. Gaps in the state health care system were compensated by national public institutions (*ULT*, *UHT*, "*Medychna hromada*", "*Vidrodzhennia*"), which directed efforts to help the least protected groups, in particular the peasantry, students, children, mothers, etc. The same applies to the struggle for a sober lifestyle, anti-alcohol propaganda, because the state pursued a policy of increasing the level of alcohol consumption, especially in "crossroads". The magazine "*Vidrodzhennia*" as an organ of the anti-alcohol society "*Vidrodzhennia*" (1909) claimed that in the 1930s, Galicia rose to one of the first places in Eastern Europe in terms of anti-alcohol propaganda. The reasons for this were given by the fact that in 1931-1937 Ukrainian magazines published more than 2,000 articles of relevant content [7]. The publication cites numerous facts of the "sobering" of dozens of rural communities, which, in turn, contributed to the improvement of the moral climate and the criminogenic situation in the region. The number of crimes committed on the basis of "drunkenness" decreased; the popularity of "alcohol-free" weddings grew, the practice of holding cultural and educational events without alcohol, etc., strengthened.

The most prominent national public institution of Ukrainians, which provided medical care to the poorest strata of the population, was the "*Narodna Lichnytsia*" (1903), which not only occupied a special place in the structure of the public national movement of Galicia, but was also a kind of evidence of volunteerism at the European level. Thus, during the 1920s, 14-18 Ukrainian doctors worked at the institution on a charitable basis, they treated from 6.5 to 8.3 thousand people on an outpatient or inpatient basis every year. Many of them underwent complex free surgical operations and provided medical treatment. With the participation of medical students, 17-25 thousand examinations and consultations were carried out each year.

Such volunteer activity of doctors of the Lviv "*Narodna Lichnytsia*" in the field of qualified medical care received wide resonance and recognition among the population of Western Ukraine, as well as Eastern Europe: sick people came here for help not only from all over Galicia, but also from Volyn, Kholmshk region, etc. The activities of "*Narodna Lichnytsia*" had a supranational character as in 1924, among the patients of the institution, Ukrainians made up 54.5%, Poles – 33%, Jews – 10%, etc. [8; 9; 10].

Medical students actively contributed to the fight against tuberculosis. Due to the low level of socio-economic life and the lack of a system of sanitary and medical care, various infectious diseases, especially tuberculosis, spread among the population of the region on a threatening scale. During the interwar period, the number of patients with this disease in Ukrainian lands ranged from 200,000 to 250,000, and this figure was 1.5 to 2 times higher than in the central voivodeships of Poland. Every year, 7-10% of patients died, and mostly they were people aged 25-45, and among young Ukrainian children, tuberculosis spread two to three times more actively than among Polish or Jewish children [10].

Therefore, the problem of disease prevention and the formation of the basics of sanitary and hygienic knowledge among children, youth and adults gained special relevance. The fight against tuberculosis was led by the *Ukrayins'ke Hygienichne Tovarystvo (UHT)* (1929), which took as an example the organizational models of European nations, in particular the Poles. Its founders were Ukrainian doctors and medical students, who aimed to promote the basics of hygienic knowledge and popularize the prevention of epidemics and, in particular, tuberculosis. Thanks to the efforts of the well-known doctor, scientist, public figure M. Panchyshyn, as of 1933, the *UHT* grew from 195 people (1929) to 309 people, a third of whom were medical students and specialists with a medical education [11; 12]; and thanks to other public associations ("*Prosvita*", "*Ridna Shkola*"), branches of *UHT* began to be opened for Greek-Catholic clergy in regional cities, but only six of them were officially active. Ukrainian students worked as volunteers in 6 sections: organizational and propaganda, tuberculosis, anti-alcohol, venereology, physical education, editorial. They actively participated in the examination of more than three dozen villages, whose inhabitants were most affected by infectious diseases. The results of the examinations showed that the main reason (8-10 cases out of ten) of the spread of epidemics was the failure to isolate sick people from healthy people. Important measures were taken by the members of the *UHT* in 1929-1930, when with the help of 35 radiographs purchased by the society, they examined almost a third of the rural communities of Galicia [11; 12]. The educational work of student-volunteers of *UHT* was especially intensified in December, when, on the initiative of the International Anti-tuberculosis Union, it was declared the month of fighting tuberculosis in many European countries.

The next step in the fight against infectious diseases was the organization of mass educational work among children



and adults on the prevention of diseases and the formation of elementary hygienic knowledge. Only during 1929–1931, the members of the *UHT*, mostly medical students, prepared about 80 thematic abstracts, delivered more than 300 lectures, held dozens of lectures that lasted three days. About 40,000 villagers were covered by this massive educational work [8; 11; 12].

## DISCUSSION

It is important to emphasize that Ukrainian students did not have a separate institution of their own, like representatives of other nationalities, until 1905 they belonged to the Polish society “Library of Medicine Listeners” (*Biblioteka słuchaczy medycyny*). Since in 1905 Polish students created their own organization – the Medical Society (“Koło medyków”), it is natural that Ukrainians followed their example. As of 1910, 30 Ukrainian youths were studying medicine at Lviv University. On June 22, 1910 17 people from them (O. Barvinskyi, V. Bilozor, Yu. Bozheyko, M. Vavryk, S. Hasiuk, M. Kozak, O. Kordasevych, T. Krokhmalnyi, E. Oleksiy, I. Posmytyukh, I. Ryhlo, M. Sapelyak, D. Sprys, K. Stupnytskyi, L. Shust, V. Shchurovskyi) created their own institution “*Medychna Hromada*” at the founding meeting in the premises of the student society “*Academichna hromada*” [13].

Ivan Horbachevsky, a world-class Ukrainian scientist (chemist, biochemist, hygienist, epidemiologist, terminographer), candidate for the Nobel Prize in Physiology and Medicine in 1911, a socio-political leader (during his studies at the University of Vienna, head of the student society “*Sich*” (1875–1877) which was patronized by “*Medychna Hromada*”; together with the outstanding scientist Ivan Puliyu, he organized the “*Ukrayinska Hromada*” society in Prague, created a fund to help students; since 1910, he was the honorary president of *ULT*, an educational figure (during 1883–1917 – a Professor of the Department of Medicinal Chemistry at Charles University in Prague; 1902–1903 (or 1903–1904 [14]) – the rector of Charles University; Dean of the Faculty of Medicine in 1889–1890, 1894–1895, 1904–1905, 1911–1912; since 1919 – a professor at the Ukrainian Free University in Vienna, Prague (since 1921); since 1924 – the rector of the Ukrainian Free University), statesman (in 1906–1917, a member of the Supreme State Sanitary Council in the Czech Kingdom, from 1908 – an adviser to the imperial court, in 1917–1918 – the Minister of Health of Austria-Hungary) [1; 14], willingly “accepted the protectorate over the society”, always materially and morally supported it [13]. According to the charter, which was approved on May 31, 1910, in addition to providing moral and material assistance to medical students, creating and maintaining a medical library, running a cheap kitchen for students, student sanatoriums-prophylactics, etc., the emphasis was placed on the public educational work of the members of the “*Medychna hromada*”: giving lectures, organizing concerts, etc. The aim of the association was to train and educate as many true social doctors, citizen doctors, with high principles of med-

ical ethics and the desire to selflessly work for the native people [13]. For this purpose, separate commissions were organized, among them the lecturer’s commission, which initiated a wide educational activity of students [13]. Ahead of the time, long before the beginning of the Great War, in 1912, the “*Medychna hromada*” independently arranged “Samaritan courses”, which were common in Europe, medical students arranged a series of lectures devoted to first medical aid, special attention was paid to medical aid to the wounded; in addition to theoretical information, they also organized practical exercises: forming practical skills of the trainees in applying bandages, artificial respiration, etc. These courses lasted several weeks, had a large number of participants (about 80, mostly women), were popular and had recognition from the public [1; 14]. It is expedient to use this experience in Ukraine today, when active military actions are being conducted on its territory, caused by the invasion of Russia on February 24, 2022.

There are two trends which are peculiar for the development of the Ukrainian student movement at the end of the 19th and the beginning of the 20th centuries: students who studied at Lviv Polytechnic and Lviv University left Polish societies and created their own, such as, for example, “*Osnova*” (at the Polytechnic) and “*Medychna hromada*” at Lviv University, which had “educational and scientific” and “professional credit” direction [6; 15; 16]; nationwide student structures are created to coordinate work. In the pre-war period, these functions were performed by the societies “*Vatra*” and “*Academichna hromada*”, from September 1909 by the *Ukrayinskyi students’kyi soyuz* (*Ukrstudsoyuz*), which aimed to become a center of “scientific and social life” for “moral and material assistance”, as by the middle of 1913, it consisted of 38 sections and 500 people [15; 16].

During the interwar period, the student volunteer movement was revived in the field of social and medical protection of children and adults, education, cultural development, promotion of a healthy lifestyle, and dissemination of sanitary and hygienic knowledge. *Ukrayinske Likarske Tovarystvo* (*ULT*), *Ukrayinske Hygienichne Tovarystvo* (*UHT*), “*Medychna hromada*”, Union of Ukrainian Women, “*Prosvita*”, “*Vidrozhennia*”, “*Ridna Shkola*” and others lead this important direction of the national movement of Ukrainians. The increase of this authority was facilitated by the first Ukrainian medical congress in Galicia held in Lviv in November 1924. In addition to important scientific and medical problems, it actively discussed the public vocation of a medical worker and involving him in extensive educational work in the areas of hygiene and disease prevention [9].

Despite all the difficulties, Ukrainian studentship remained the most organized social state of Ukrainians in Galicia, primarily thanks to its local professional associations. Thus, in the second half of the 1930s, 33 Ukrainian academic societies operated, 22 of them were in Lviv. Active volunteer activities were carried out by such societies as “*Osnova*” (numbered 212–130 people), “*Medychna hromada*” (99 people), “*Vatra*” (70–18 people), the “*Obnova*” society of the Catholic orientation, created in 1934 (71

persons) etc. [17; 18], as well as many student organizations that operated under the patronage of “*Prosvita*”, “*Ridna Shkola*”, other mass societies as their “sections”, “clubs”, although in fact they remained independent associations.

Ukrainian students actively worked in the field of social welfare of pupils and preschoolers. Such public associations as “*Ukrayinska zahoronka*”, UKTODOM, “*Ridna shkola*”, *Tovarystvo vacacynych osel*, etc. thanks to patrons, the help of the public, the clergy, nuns, especially Metropolitan Andrey Sheptytskyi, a network of institutions for the rehabilitation of children was created [19].

Members of ULT, UHT, “*Medychna hromada*” on a volunteer basis conducted examinations of the state of health of students who attended educational institutions of “*Ridna Shkola*”. For example, in 1926, according to the results of surveys of the state of health and social and living conditions of students of Lviv educational institutions, it was found that 34% of children were not eating enough, 20% had enlarged glands, and 25% had bad teeth; 12% of them had vision problems and the same number had skin diseases. Every tenth person was found to have an unsatisfactory lung condition, and 5 and 4% of people suffered from anemia and other ailments, respectively [20]. Depending on the opportunities and needs, preschoolers, as well as students of public schools, teacher’s seminars, gymnasiums, and specialized institutions of the “*Ridna Shkola*” received targeted assistance to improve the social and domestic conditions of life and health (free lunches, additional food, provision of warm clothes of children from poor families and orphans, etc. Since 1927, they have introduced a system of general mandatory medical examination, which made it possible to detect diseases, primarily tuberculosis, in the early stages and take preventive measures to prevent them. During school and after-school hours, children mastered the basics of sanitary and hygienic knowledge and a healthy lifestyle. For this purpose, special circles and sections were created [20].

It is important to note that the Ukrainians of Halychyna were aware of the important charity work in the field of education, culture, social, and medical protection carried out by the student body, so they actively supported it materially. The pages of periodicals of that time are full of calls for help to “academics” and testify to the consequences of such actions. Urban and rural branches of “*Prosvita*”, “*Ridna shkola*”, “*Sokola*”, “*Plast*”, “*Luga*”, “*Soyuz Ukrayinok*”, “*Silskyi gospodar*”, and other Ukrainian public associations systematically organized collections of donations for the benefit of students on various occasions, which on average brought from 5 to 80 zlotys, and in some places much larger sums.

We outline the volunteer activity of students who belonged to “*Medychna hromada*” (1910-1944) as a national phenomenon of the organization of public medical care of the population of Galicia, which has no analogue in the history of Ukrainian medicine. It is primarily about a high degree of civic self-awareness, patriotism, and self-sacrifice for the sake of serving the Ukrainian people. This generation of the Ukrainian medical elite during the

interwar period of the 20th century showed Europe an example of great courage and self-devotion, volunteerism, benevolence, affirmed the high ideals of humanism and Christian morality. This work of Ukrainian young men and women was duly evaluated and recognized by their Polish colleagues who were advanced doctors and public figures.

The experience of volunteer activities of the members of the “*Medychna hromada*” society is of great importance for today, as there are numerous examples of selfless work in the field of health care and medical provision of the Ukrainians of the region, medical assistance to soldiers in the ranks of the Legion of Ukrainian Sich Riflemen and the Ukrainian Galician Army, the organization of anti-alcohol and anti-nicotine activities, propaganda among the rural population of the basics of sanitary and hygienic knowledge, mass medical education, selfless (free) work in the “*Narodna Lichnytsia*”, provision of medical services to low-income sections of the population, widows, orphans, disabled people, veterans of the Great War, medical care of children and youth etc. Nowadays they inspire medics who provide assistance to soldiers of the Armed Forces of Ukraine, wounded in hospitals, internally displaced persons, etc.

The experience of organizing mass medical education among the population by Ukrainian students during the studied period is instructive for the professional training of future medical workers. Thus, from the beginning of its existence, “*Medychna hromada*” systematically carried out medical education of Ukrainians. The essay “Tasks of a Ukrainian medical student in social work” delivered by the student Sakhno on December 20, 1927 at the 3rd General Meeting, which clearly outlined the content and directions of public advisory and educational work [13], is emblematic for current students. The results of the study of the source base prove that only during 1927-1928, thanks to the volunteer activity of students who belonged to the cultural and educational commission, 40 popular lectures of “anti-tuberculosis content” were organized; during 1928-1929, “medical students Migotskyi, Kozhynskyi, Klyufas, Voronka read 120 abstracts on medical topics” [5; 13; 17].

## CONCLUSIONS

So, Ukrainian public student associations really contributed to the improvement of medical and sanitary-hygienic services for children, youth and adults of Galicia, launched a large-scale struggle for a healthy lifestyle, established mass medical education in the field of providing first aid, combating ailments, and preventing infectious diseases, medical examinations of the population, treatment and rehabilitation of people, mother and child health care. In all these areas, they worked selflessly and free of charge in the field of protecting people’s health (“*Narodna Lichnytsia*” in Lviv), acted as volunteers, benefactors (served vulnerable sections of the population, children, mothers, disabled people, widows, orphans, veterans, etc.), relied on their own strength. The lack of state subsidies, limited own resources (medical equipment, professional

personnel, etc.) prevented the establishment of Ukrainian “*narodna lichnytsias*” by the cities of Galicia. Thanks to the volunteer work of Ukrainian students, primarily doctors, tens of thousands of Ukrainians were involved in large-scale educational work led by UHT, ULT, “*Medychna hromada*”, “*Vidrodzhennia*” and others. It became an important factor in the formation of mass knowledge on the basics of hygiene and sanitation, preventing the occurrence and spread of various diseases, primarily tuberculosis. Voluntary educational activities of Ukrainian students in the field of the mass movement of Ukrainians for a healthy lifestyle, anti-alcohol and anti-nicotine propaganda had great success (even in the general European context). The volunteer work of Ukrainian students has become a kind of social educational and medical phenomenon, the uniqueness of which can be seen primarily in the fact that the “*Medychna hromada*” gathered Ukrainian students-“ascetics”, the young generation of the future Ukrainian medical elite, who demonstrated to Europe an example of great courage and self-devotion, benevolence, affirmed the high ideals of humanism and Christian morality. This institution became a source for the training of specialists for volunteer work in the field of public medical care of the Ukrainians of the region, this experience must be actively used today in the complex realities of wartime, the provision of first medical aid under war conditions, the spread of pandemics and other infectious diseases, and the rehabilitation of those who suffered as a result Russian invasion, mass knowledge of the basics of hygiene and sanitation, etc. The training of a national doctor should become a priority task of medical education institutions.

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*The Authors declare no conflict of interest.*

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## REVIEW ARTICLE

# HOMOCYSTEINE AND CARDIOVASCULAR DISEASE – A CURRENT REVIEW

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## ABSTRACT

Cardiovascular diseases remain the leading cause of death worldwide for the past 20 years. Of these, ischemic heart disease has the highest mortality rate. In over 98% of cases it is caused by atherosclerosis of the coronary arteries. Homocysteine is an amino acid, containing a sulfhydryl group, which is formed as a result of the metabolism of the amino acids methionine and cysteine, which is supplied with protein-containing foods. A small amount of it is necessary for the proper functioning of the body, however, an increased concentration in blood plasma, which hyperhomocysteinemia, negatively affects blood vessels leading to the development of atherosclerosis and thrombotic complications. The adverse effect on blood vessels results from various mechanisms, such as: excessive activation of Toll-like 4 receptor, activation N-methyl-D-aspartate receptors, increased production of reactive oxygen species, and impairment of nitric oxide synthesis. Elevated levels of reactive oxygen species are associated with increased expression of proinflammatory cytokines such as IL-1 $\beta$ , IL-6, TNF- $\alpha$  (tumor necrosis factor), MCP-1 and intracellular adhesion molecule-1. Another factor contributing to hyperhomocysteinemia is mutation of the MTHFR gene, which in normal conditions is responsible for maintaining homocysteine levels within the normal range. People with MTHFR mutation are more prone to develop atherosclerosis and the following complications: myocardial infarction, stroke, thrombotic episodes and coronary artery disease. The aim of this paper is to present evidence supporting the role of homocysteine in the development of many cardiovascular diseases.

**KEY WORDS:** homocysteine, atherosclerosis, cardiovascular disease, hyperhomocysteinemia

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## INTRODUCTION

Homocysteine is an amino acid having a sulfhydryl group (-SH), which is formed as an intermediate product during the metabolism of the amino acid methionine, which is supplied to the body with the proteins consumed. The name derives from its similarity in chemical properties to cysteine, hence HOMOCysteine. Normal serum concentrations are in the range of 5 – 15  $\mu$ mol/L. The term “hyperhomocysteinemia” refers to its elevated levels and is associated with various cardiovascular and neurodegenerative disorders [1]. In this review, we discuss current reports on the effects of hyperhomocysteinemia on the cardiovascular system.

Homocysteine exists in plasma in four forms: about 1% circulates as a free thiol, 20-30% binds to itself (forming dimers) or to other thiols, and 70-80% is bound to plasma proteins, mainly albumin [2]. As mentioned earlier, homocysteine is synthesized by demethylation of methionine, which binds to ATP and undergoes S-adenosylation to form S-adenosylmethionine (SAM). The methyl group attached to the sulfur of SAM can be transferred to other substances, making S-adenosylmethionine the main methyl donor in cellular reactions. Demethylation of SAM leads to the formation of SAH (S-adenosylhomocysteine), which then undergoes a process of hydrolysis, ending in the formation of homocysteine and adenosine [3, 4].

Further metabolism of homocysteine depends on the current needs of the body. In the case of methionine deficiency, homocysteine can be re-methylated under the influence of the enzyme methionine synthase, in the presence of vitamin B12 and methyltetrahydrofolate, leading to the formation of methionine. In addition, this process requires the presence of 5,10-methylenetetrahydrofolate reductase (MTHFR) and an adequate supply of folic acid.

In the second reaction occurring with the participation of cystathionine  $\beta$ -synthase, which uses vitamin B6 (pyridoxine) as a cofactor, homocysteine is converted to cysteine.

Under normal conditions, homocysteine synthesis and breakdown are in balance. Excessive consumption of products providing methionine, disorders of its metabolism, as well as deficiencies of vitamins and enzymes involved in homocysteine metabolism lead to an increase in plasma homocysteine concentrations, which has a negative impact on human health [3, 5].

## THE AIM

The aim of this study is to present evidence supporting the role of homocysteine in the development of many cardiovascular diseases.

## REVIEW AND DISCUSSION

### CAUSES OF HYPERHOMOCYSTEINEMIA

Hyperhomocysteinemia is a condition characterized by abnormally high levels of homocysteine in the blood (above 15  $\mu\text{mol/L}$ ). Depending on the concentration of homocysteine, it can be classified as mild (15–30  $\mu\text{mol/L}$ ), intermediate (30–100  $\mu\text{mol/L}$ ) and severe (>100  $\mu\text{mol/L}$ ). Severe hyperhomocysteinemia is rare and usually results from severe genetic mutations of enzymes involved in homocysteine metabolism, which manifests itself in the form of such disorders as mental retardation, osteoporosis, and vascular incidents due to thrombosis [6]. Genetically determined hyperhomocysteinemia can result from defects in the following enzymes: methionine synthase, 5,10-methylenetetrahydrofolate reductase (MTHFR) or cystathionine  $\beta$ -synthase. The most common disorder, detected worldwide, is MTHFR single nucleotide polymorphisms, which are associated with mild to moderate hyperhomocysteinemia. It has been shown that in most cases with moderately elevated homocysteine levels, the cause is a point mutation in the coding region of the MTHFR gene, resulting in the resulting enzyme having half the activity [2, 7]. In contrast, severe congenital hyperhomocysteinemia and classic homocystinuria are thought to be caused by homozygous deficiency of cystathionine  $\beta$ -synthase, which increases blood homocysteine levels by up to 40-fold [2, 8].

Mild and moderate forms of hyperhomocysteinemia, which are associated with environmental factors, are far more common. It is known that levels of folic acid, vitamin B12 and, to a lesser extent, vitamin B6 vary inversely with total homocysteine concentration. Since the chemical reactions necessary for homocysteine metabolism require the presence of folic acid, vitamins B6 and B12, elevated homocysteine levels are influenced by an inadequate diet, resulting in an insufficient supply of these compounds. In addition, hyperhomocysteinemia can be caused by physiological factors such as older age, male gender and menopause. Cigarette smoking, coffee consumption, abnormal lipid profile and high blood pressure are other environmental factors [9]. Certain conditions including kidney failure, diabetes, liver disease, thyroid disease, psoriasis, rheumatoid arthritis and some cancers are also believed to contribute to mild to moderate increases in homocysteine levels. Since the kidneys are the main route of homocysteine elimination, when their function is impaired, homocysteine builds up and blood levels increase. Other factors that cause hyperhomocysteinemia are various drugs that interfere with folic acid, vitamin B12 and B6, such as methotrexate, metformin, estrogen-containing contraceptives, some antiepileptic drugs and diuretics [2, 10]. Recent studies have shown that there is a close relationship between hyperhomocysteinemia and cardiovascular disease [11].

### HOMOCYSTEINE AND CARDIOVASCULAR DISEASE

Cardiovascular diseases have remained the leading cause of death worldwide for about 20 years. Of these, ischemic

heart disease has the highest mortality rate. In more than 98% of cases, its underlying cause is atherosclerosis of the coronary arteries. The association of elevated plasma homocysteine levels with various cardiovascular pathologies has been observed in a large number of studies. Accumulating evidence suggests that hyperhomocysteinemia leads to endothelial cell damage, reduces vascular elasticity and alters the hemostatic process, causing the development of atherosclerosis and complications such as heart attacks and strokes. Homocysteine can aggravate myocardial damage both in the pathway of atherosclerotic vascular changes, but also in a direct way, which in its clinical form can manifest as dilatation. In addition, it exacerbates the adverse effects of cardiovascular risk factors and leads to the promotion of inflammation [4, 12, 13].

One of the main symptoms of hyperhomocysteinemia is thromboembolic incidents. Hasan et al. showed that among oncology patients, venous thromboembolism (VTE) is the most common complication and the second most common cause of death due to elevated homocysteine levels. They observed that patients with advanced stage cancer have both hyperhomocysteinemia and CVD. In contrast, in a group of patients with early-stage cancer, both hyperhomocysteinemia and CVD were absent [14]. Unfortunately, the mechanism underlying thrombosis caused by elevated homocysteine levels is not well understood. However, it is thought to be caused by homocysteine-mediated free radical formation, which leads to endothelial dysfunction. It is also known that homocysteine can form a more reactive compound, homocysteine thiolactone, which binds permanently to the terminal groups of lysine or arginine, causing protein denaturation and the formation of insoluble toxic protein aggregates (known as N-Hcy proteins) or amyloids. This mechanism, referred to as “protein homocysteinylolation,” leads to the accumulation of these compounds in the heart, for example, which can lead to cardiovascular dysfunction [15]. Jakubowski et al. observed that homocysteinylated proteins lose their original biological functions, acquiring cytotoxic, pro-inflammatory, autoimmune and pro-thrombotic properties, which contribute to the development of cardiovascular disease, diabetes, Alzheimer’s disease, kidney disease, neural tube defects and male infertility [16]. The above data were confirmed in a study conducted by Pearl-Kaján et al. who, analyzing myocardial and aortic samples taken from patients undergoing cardiac surgery, proved that N-Hcy protein, accumulates in diseased tissues of the human heart [17]. A number of studies have proven a positive correlation between the level of N-Hcy protein, a product of thiolactone homocysteinylolation, and an increased risk of atherosclerosis. It has been shown that the higher the level of N-Hcy protein, the greater the number of coronary arteries involved in the atherosclerotic process [16, 18].

The large WENBIT cohort study by Borowczyk et al. evaluated the chemically reactive homocysteine metabolite thiolactone as a risk marker for acute myocardial infarction. The analysis included more than 2,000 urine samples of patients with suspected stable angina who were tested for

thiolactone activity. During a follow-up of 4.7 years, 183 patients (8.9%) developed acute myocardial infarction. Based on the results, the thiolactone/creatinine ratio was shown to be significantly associated with the risk of myocardial infarction during follow-up [19]. It follows that urinary homocysteine thiolactone can be used as a marker of acute myocardial infarction. Similarly, Gurda et al. proved that thiolactone is associated with the development of cardiovascular disease. The above results indicate a significant role for homocysteine thiolactone in the development of atherosclerosis and vascular disease [18].

Paraoxonase 1 (PON1) and homocysteine thiolactonase (HTase) are enzymes involved in the breakdown of homocysteine thiolactone via the hydrolytic pathway to homocysteine. A prospective study involving 315 Japanese patients evaluated the significance of (PON1)/(HTase) activity and serum homocysteine levels for mortality in patients after percutaneous coronary intervention for acute coronary syndrome or stable ischemic heart disease. During a median follow-up of 10.5 years, 73 patient deaths (24.5%) were recorded. Based on the results, low HTase activity and high homocysteine levels have been shown to be associated with a high risk of death after percutaneous intervention [20].

The adverse effects of homocysteine on the cardiovascular system are thought to be due to various mechanisms, such as excessive activation of Toll-like receptor 4 [21], activation of N-methyl-D-aspartate receptors, increased production of reactive oxygen species and impaired nitric oxide synthesis. Elevated levels of reactive oxygen species are associated with increased expression of pro-inflammatory cytokines such as IL-1 $\beta$ , IL-6, TNF- $\alpha$  (tumor necrosis factor), MCP-1 and intracellular adhesion molecule-1. The above processes contribute to the development of atherosclerosis and its associated complications: myocardial infarction, stroke, aortic aneurysms and Alzheimer's disease [22, 23].

In a study by Tang et al. it was observed that high plasma homocysteine levels correlate with the existence of atherosclerosis in the cerebral vasculature and the occurrence of transient ischemic attack (TIA). The role of homocysteine in the development of TIA is most likely due to the production of free radicals during homocysteine oxidation. In turn, free radical toxicity to endothelial cells promotes the synthesis of oxidized low-density lipoproteins, inducing plaque instability and progression of atherosclerosis [24].

On the other hand, Cao et al. showed that patients with atherosclerotic carotid or intracranial artery stenosis have significantly higher homocysteine levels. This is due to homocysteine's involvement in the inflammatory response, which leads to atherosclerotic plaque instability within the arteries and an increased risk of ischemic stroke [25].

With cardiovascular disease being the most common cause of death worldwide, there is an urgent need to find reliable biomarkers associated with disease progression to identify patients at increased risk. It appears that routine determination of homocysteine may be helpful in screening and preventing serious complications, as recent evidence

suggests that high levels significantly increase the incidence of vascular damage and atherosclerosis.

## THERAPEUTIC OPPORTUNITIES

There is evidence that regular physical activity, smoking cessation, and an adequate supply of folic acid and vitamin B12 are associated with lower serum homocysteine levels. Foods rich in folic acid include baker's yeast, cauliflower and green vegetables such as spinach, lettuce, broccoli, Brussels sprouts and asparagus. On the other hand, the most vitamin B12 is contained in animal products, namely: fish, offal, eggs and dairy products. Sources of vitamin B6 include: meat, fish, grains, cabbage, legumes, peppers, Brussels sprouts, carrots, and bananas [26]. It follows that people on a vegan diet are at risk of vitamin B12 deficiency, and thus elevated homocysteine levels and associated complications [2].

In a large prospective study, daily folic acid supplementation of 0.5 to 5.0 mg was shown to reduce serum homocysteine levels by about 25%, and an additional supply of 0.4 mg of vitamin B12 by another 7%. It is clear, however, that the bioavailability of preparations depends on the type of supplement, age, gender and health status. Bioavailability is negatively affected by gastrointestinal diseases and many medications, such as metformin, oral contraceptives, and antiepileptic drugs [27].

In the U.S. and Canada, Yang et al. observed that enrichment of cereal products with folic acid results in a decrease in plasma homocysteine levels, and thus a reduction in stroke mortality has been reported [28]. It is believed that a reduction in homocysteine levels by 3  $\mu\text{mol/L}$  would be associated with a 16% decrease in the risk of coronary heart disease, and the risk of stroke by up to 24%. The protective effect of folic acid on coronary artery disease may be due to both improved endothelial function by lowering homocysteine levels, as well as its antioxidant potential and interaction with endothelial nitric oxide synthase (eNOS), which exhibits antiatherogenic effects [29, 30].

Higher plasma homocysteine levels have been shown to correlate with the risk of recurrent stroke, while vitamin therapy with folic acid, vitamin B6 and B12 has been shown to reduce the frequency of such incidents. Therapy, consisting of taking 2.5 mg of folic acid, 50 mg of pyridoxine (vit. B6) and 1 mg of cobalamin (vit. B12) for 5 years, has been found to reduce the risk of stroke, while it does not mitigate the effects of stroke after it has occurred [31].

There are also studies that have observed little benefit from folic acid supplementation in reducing the risk of vascular disease and stroke. Zeng et al. analyzing 14 randomized trials involving a total of 39,420 patients showed a slight response to folic acid supplementation for hyperhomocysteinemia [32]. Such a condition is thought to be due to the body's scarce supply of folate and vitamin B12. For, treating a vitamin B12 deficient patient with folic acid and vice versa, taking vitamin B12 by a folic acid deficient person can exacerbate the neurological consequences of both deficiencies. Therefore, correctly, before including

follic acid, it would be wise to rule out and correct cyanocobalamin deficiencies [33].

To prevent the development of cardiovascular disease, it is also important to control traditional risk factors, such as smoking cessation, lowering blood pressure, eating an adequate and well-balanced diet, regular physical activity and weight loss.

## CONCLUSIONS

Despite advances in medicine, cardiovascular disease has long caused a heavy toll and burden on health care worldwide. Therefore, it is necessary to find reliable markers of cardiovascular disease risk factors. There is a view that one potential biomarker of thromboembolic incidents may be homocysteine, whose elevated levels have a negative effect on the cardiovascular system. The prevalence of hyperhomocysteinemia varies in the population and depends on age, diet, genetic conditions. Factors associated with increased homocysteine levels include, in addition to genetic susceptibility, increasing age, male gender, smoking, coffee consumption, faulty diet, unfavorable lipid profile and associated high blood pressure, and elevated creatinine levels. Hyperhomocysteinemic patients are believed to develop various cardiovascular complications, such as vascular damage, thromboembolic disease, coronary artery disease and atherosclerosis. There is evidence that the hyperhomocysteinemic state is also an underlying cause of neurodegenerative diseases (including dementia, Alzheimer's and Parkinson's diseases), diabetes, megaloblastic anemia and some cancers [14]. The reactive homocysteine metabolite thiolactone in urine has also been shown to be a predictor of acute myocardial infarction in patients with ischemic heart disease [19].

Although there are data in the literature supporting the beneficial effects of folic acid, vitamin B6 and B12 supplementation in the treatment of hyperhomocysteinemia, their supply may not be effective in some cases. Nevertheless, more studies are needed to prove the importance of homocysteine in identifying high-risk patients, which would allow them to receive early medical care and prevent premature death from vascular complications.

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## REVIEW ARTICLE

# GENITALS MUTILATING SURGERIES (FEMALE CIRCUMCISION): LEGAL MEASURES OF COUNTERACTION

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## ABSTRACT

**The aim:** To highlight legal measures of counteraction to genitals mutilating surgeries (female circumcision) and the perspectives for their possible improvement.**Materials and methods:** International regulatory legal acts, acts of "soft" law, statistical data, research results are the materials of the research. Comparative, comparative and legal, system analysis, formal and logical, other methods were applied during the research.**Conclusions:** It has been proved that although there are four different types of female circumcision, any such surgery performed without medical grounds is female genitals mutilating surgery. The authors have offered to enshrine a direct ban on performing genitals mutilating surgeries (female circumcision) in the norms of national legislation with appropriate measures of legal liability for those involved.**KEY WORDS:** legal counteraction, women's health, mutilation, violence, circumcision

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## INTRODUCTION

There have recently been many comparative and legal studies focused on various types of liability in the healthcare sector and affecting both human rights and the interests of the state in the whole [1]. Publications related to global healthcare and international cooperation, imposing international responsibility on states for internationally illegal actions in the healthcare sector are especially relevant in the context of the COVID-19 pandemic [2]. At the same time, there are still problems that certain communities (states, cultures, ethnic, social groups) prefer to keep silence, thus diminishing the destructive impact of such problems on the level and completeness of human rights protection.

We must agree that every social group in the world has specific traditional practices and beliefs, some of them are beneficial to all members of society, while others are detrimental to specific groups, such as women. Dangerous traditional practices include female genitals mutilating surgeries; forced feeding of women; early marriages; various prohibitions or practices that do not allow women to control the birth of children themselves; dietary restrictions and traditional childbirth practices; preference for male children and its implications for girls; killing of newborn girls; early pregnancy, dowry and ransom [3].

In terms of multiculturalism and globalization, certain negative phenomena can be spread in those places where they have not been observed before. It is their greatest danger, which requires timely and decisive measures to

find measures of counteraction. One of such extremely important problems is genitals mutilating surgeries (female circumcision) also known by its abbreviation (FGM – Female genital mutilation).

## THE AIM

The aim of the article is to highlight legal measures of counteraction to genitals mutilating surgeries (female circumcision) and the perspectives for their possible improvement. The following objectives have been defined in accordance with the aim: to analyze the historical experience of counteracting the practice of female circumcision, to determine the directions of legal counteraction to this phenomenon in the modern world, to suggest the ways to concretize and improve legal measures of counteraction to genitals mutilating surgeries (female circumcision).

## MATERIALS AND METHODS

The empirical basis of the presented research was the provisions of international regulatory legal acts, acts of "soft" law, as well as national regulatory acts in the field of human rights protection. Theoretical basis of the article is research focused on the medical and legal aspects of combating violence against women, ways to prevent and minimize cases of female genital mutilation, and improvement of measures to counteract to female circumcision as a ritual practice.

The choice of research methods is determined by the aim, objectives and subject matter of the scientific article. At the same time, both general scientific and special research methods were used in the course of scientific research. For example, the comparative method made it possible to compare the extent of spreading the researched problem in certain countries and regions. The comparative and legal method contributed to the consideration of international and national norms related to counteracting religion-based violence against women. The method of system analysis made it possible to identify the main causes and factors contributing to the preservation of the tradition of female circumcision. The historical and legal method allowed us to consider the problem in its retrospective. The formal and legal method was used while studying international documents and analytical reports on the implementation of counteraction to religion-based violence against women. Methods of formal logic were used in determining the main measures of legal counteraction to genitals mutilating surgeries (female circumcision) in modern conditions.

## REVIEW AND DISCUSSION

Despite the latent nature of the problem, the issues of the nature, manifestations, as well as measures of counteraction of female genital mutilation were the subject matter of research by many scholars and specialists in various fields. At the same time, the history of counteraction to female genital mutilation is significantly shorter than the history of the application of the violent procedure itself. The increase in the number of migrants from Asian and African countries in the European region, together with the existence of such a practice in certain regions of the North Caucasus, creates new precedents related to the facts of the ritual practices of female circumcision. In this regard, human rights and freedoms, human dignity and gender equality, as well as other democratic values are irreparably harmed, while organizing the fight against female genital mutilation requires effective legal instruments and coordinated actions of entities at all levels – starting from local to international levels.

According to the World Health Organization, there are four main types of female genital mutilation practices: 1) partial or complete amputation of the glans of clitoris (the outer visible part of the clitoris – the sensitive part of the female genitalia) and / or prepuce of clitoris (skin folds around the glans of clitoris); 2) partial or complete amputation of the glans of clitoris and labia minora (internal folds of the vulva) with amputation (excision) or without amputation of the labia majora (external skin folds of the vulva); 3) narrowing the vaginal orifice by forming a dense covering ferrule (infibulation). The ferrule is formed as a result of excision and movement of the labia minora or labia majora, sometimes by suturing, with or without amputation of the prepuce of clitoris and the glans of clitoris, like in the first type; 4) all other harmful non-medical surgeries on the female genitals (for example, paracentesis, piercing with objects, making incisions, curettage and cautery

of the genital area). Deinfibulation should be considered as a separate form – the practice of discission of artificially narrowed women vaginal orifice previously subjected to infibulation; it is often necessary to increase the health and well-being levels, as well as to enable sexual intercourse and sedation of childbearing [4].

According to the president of the California Institute of Health and the author of the book “Circumcision”, D. Gollacher, the practice of female genital mutilation is widespread in the world and is typical both for the natives of Australia and for many African peoples. The main purpose of the ritual is control over female sexuality, as well as initiation into adulthood. According to one of the versions, this practice was also subjected to slaves in the Ancient Rome to suppress their “natural instincts to reproduction”. According to some researchers, this custom existed in the Ancient Egypt. It is indirectly evidenced by the works of the Roman historian and geographer Strabo. It is noteworthy that even today one of the variants of this practice of mutilating female genitals is called “pharaonic circumcision” [5].

There are assumptions that surgeries on female clitoris were performed back in the days of the Inca Empire, and even in Cuzco, the capital of the Incas. There was an Amarukancha temple, where the image of a dragon-serpent devouring a scorpion referred to the rite of cutting off the clitoris in girls, since it symbolized the masculine principle in women [6].

Studying the problem of female circumcision in Côte d'Ivoire, researcher V. Sarkisova-Kuame points out that attributing this tradition to Islam is erroneous. This misconception is due to the fact that the ritual practice of female genital mutilation occurs in countries where the majority of the population is Muslim, while religious texts do not contain prescriptions for the necessity and admissibility of the corresponding ceremony [7, p. 61].

R.A. Orekhov also mentions this in his study by noting that female genital mutilation is associated exclusively with local pagan customs, and not with the requirements of Islam. The scholar notes that female circumcision currently takes place in such countries as Egypt, Sudan, Saudi Arabia, South Yemen, Bahrain, Oman, Malaysia, Pakistan, Indonesia, Philippines. At the same time, such practices are absent in Iran, Iraq, Algeria, Libya, Morocco and Tunisia. Based on this fact, the author makes a conclusion about the pre-Islamic period of the emergence of customs associated with female circumcision [8, p. 131].

Based on the foregoing, it is advisable to support the point of view about the connection of female genital mutilation practices with certain religious cults, common mainly in certain states of Africa, the Middle East and Asia. At the same time, there is concern about the cases of such violence in those regions and places, where such offenses have not been recorded before.

Regarding the organization of the counteraction to the practice of female circumcision, it should be noted that any countermeasures appeared much later. S.F. MacDonald in his work titled “Female circumcision” back in 1958

claimed that removal of the clitoris “eliminates irritability, masturbation, excitability... and preputial calculus, which contributes to “painful intercourse and frigidity” [9]. Thus, pseudoscientific position related to the expediency and admissibility of female genital mutilation was support back in the middle of the XX century.

In turn, legal counteraction to female genital mutilation originates from the adoption of the Convention on the Elimination of All Forms of Discrimination against Women (UN General Assembly Resolution 34/180 of 18 December 1979). It is important that the content of the said Convention has no direct references to female circumcision; however, the international document affirms the equality between men and women, as well as the inviolability of human rights and the dignity of the human person [10].

Specific measures aimed at eradicating the practice of genitals mutilating surgeries (female circumcision) have been taken since 1997 at the international level, when a joint statement on this issue was published following the results of the work of international organizations as World Health Organization, the United Nations Children’s Fund (UNICEF) and the United Nations Population Fund (UNFPA). According to the World Health Organization, progress in solving the problem of female circumcision consists of: increased international participation in the movement for stopping this practice; the creation of international monitoring agencies and the drafting of resolutions condemning this practice; reviewing the regulatory legal framework and strengthening political support for stopping the practice of female genital mutilation (including the adoption of a law against this practice in 26 countries of Africa and the Middle East, as well as in 33 other countries, where migrants from countries practicing female genital mutilation surgeries live); support for stopping the practice of female circumcision by men and women themselves who live in practicing communities [4].

The United Nations Population Fund (UNFPA) together with the United Nations Children’s Fund (UNICEF), has been implementing the largest global program to accelerate the termination of female genital mutilation since 2008. This program currently covers 17 countries in Africa and the Middle East, as well as supports regional and global initiatives [11].

The Third Report of the Committee A (draft) to the 61st World Health Assembly was published in the same year, suggesting the adoption of the resolution including counteraction to female circumcision among other things. It is noteworthy that it was offered to “adopt and enforce legislation to protect girls and women from all forms of violence, in particular female genital mutilation, and to enforce laws prohibiting female genital mutilation by any person, including medical professionals” [12, p. 9]. We can give an example of the tragic death of 13-year-old Egyptian Suher al Bataa due to genitals mutilating surgery performed in the summer of 2013. As a result of her death in Egypt, there was the first trial in the history of the country over the physician who performed the circumcision of the girl caused her death and over the father who brought her to a medical institution [13].

The next period is the UN General Assembly’s adoption of resolutions regarding the intensification of the global efforts of UN Member States aimed at eradicating the practice of female genital mutilation. Not studying each of these documents in details, we will consider some of the main ones. Thus, the Resolution (A/67/450 and Corr.1) was adopted on December 20, 2012, which among other things approved February 6 as the International Day of Zero Tolerance for Female Genital Mutilation [14]. The Resolution (A/69/481) was adopted on December 18, 2014 aimed at supporting women and girls subjected to such violence, as well as at stepping up efforts to eliminate female genital mutilation [15]. Paragraph 13 of the Resolution (171/68) dated from December 19, 2016 indicated the States on the need to develop policies and norms that ensure the effective implementation of national laws that provide for the elimination of discrimination and violence against women and girls, in particular female genital mutilation, and that establish appropriate accountability mechanisms at the national and local levels to monitor how these laws are respected and implemented [16].

The Report of the UN Secretary-General dated from July 27, 2018 on the root causes of the practice of female circumcision and the factors contributing to it, the scale of its worldwide spread and its consequences for women and girls should be considered as the next important step in counteracting female genital mutilation. In addition, the Report provides relevant evidence and data, analyzes progress made to date by the Member States and the UN system in this regard. Besides, the Report includes conclusions and recommendations for future activities [17].

Noting the coordination of the actions of international organizations, namely the UN and WHO in counteracting female genital mutilation, we should also pay attention to the fact that such efforts are largely focused on “soft” law methods, explanatory work and recommendations.

Other legal measures to counteract to genitals mutilating surgeries (female circumcision) belong to the functioning of the judicial system at the state (national courts) and international (European Court of Human Rights) levels. We emphasize that, D. Westley, while studying female circumcision and infibulation in Africa in 1999, pointed to the emergence of lawsuits aimed at obtaining asylum by African migrants in order to avoid the corresponding mutilating surgeries. The researcher also pointed out the persistence of this illegal tradition, which created significant obstacles to overcoming this form of violence against women [18]. However, it is necessary to take into account two polar points: firstly, migrants are interested in finding reasons for obtaining the refugee status, when not entirely honest methods are often used. Secondly, the ECHR in its judgements, leaving complaints unsatisfied, often underestimates the threat of female genital mutilation to the life and health of women and girls.

For example, the ECHR in its judgement No. 8969/10 of September 20, 2011 “Omeredo v. Austria” considered the complaint inadmissible (the complaint was declared manifestly baseless). Notably, the applicant fled Nigeria in May



2003 and applied for asylum in Austria on the grounds that she was threatened by the female circumcision procedure in her homeland [19, p. 11]. Similar judgements were taken by the ECHR in relation to *Enita Pamela Izevbeekhai and others v. Ireland* of May 17, 2011 No. 43408/08 [20], as well as *Sow v. Belgium* of January 19, 2016 No. 27081/13 [21].

The third type of measures of legal counteraction to genitals mutilating surgeries (female circumcision) we correlate with legal liability. We are talking about the creation of national legislation norms, whose certain provisions should contain a direct ban on female genital mutilation. On the other hand, the effectiveness of such measures is leveled by the intimate aspect of the problem, when law enforcement agencies just do not have information about many cases of such violence. As an example, we point out that the prohibition of genitals mutilating surgeries (female circumcision) is provided in the Constitutions of Ghana (the Art. 39) and Somalia (paragraph 4 of the Art. 15), laws on the protection of children's rights (Mauritania, Kenya, Liberia), the provisions of the Criminal Code (Djibouti, Congo, Ethiopia, the Netherlands). Serious organs damage as an aggravating circumstance is provided by criminal law in Cameroon, Sudan. The special FGM Act is in effect in Benin, Italy, Sweden, UK. The Juvenile Sexual Offenses Act has been passed in Tanzania and combinations of the above legal strategies are in force in Ghana [22].

Regarding the perspectives for specifying and improving measures of legal counteraction to genitals mutilating surgeries (female circumcision) we consider it necessary: 1) to clearly define the ways on improving national criminal legislation and to introduce criminal liability for genitals mutilating surgeries (female circumcision) and to prevent such forms of violence against women and girls; 2) to legally consolidate the strategy and information programs aimed at the formation of traditional democratic values; 3) that religious, cultural, political figures and popular media people to condemn the existing practices of genitals mutilating surgeries (female circumcision); 4) to create an appropriate legal framework for the operation of places of protection ("shelters") and counseling centers, ensuring the confidentiality of victims of such encroachments and illegal actions, as well as persons in need of protection.

## CONCLUSIONS

Female genital mutilation surgeries have many forms, but are most commonly associated with female circumcision. The specified problem is gradually reaching the international level, which is related to globalization and active migration processes in the modern world. A lot of women, as noted by Yu.A. Antonova and S.V. Sirazhudinova in the report on the results of a qualitative study in the Republic of Dagestan "Genitals mutilating surgeries of girls", as of 2016, remain victims of various forms of discrimination both in their private and family life, and in connection with their status in society. Researchers point out that the practice of female genital mutilation by certain individuals

and communities, which is detrimental to the health and legal status of women, their general condition, is still in demand [23]. Despite the existence of four different types of female circumcision, any such surgery performed without medical grounds is female genital mutilation. We single out three measures of legal counteraction to genitals mutilating surgeries (female circumcision): a) soft law, b) judicial protection, c) legal liability. The enshrinement of a direct ban on performing genitals mutilating surgeries (female circumcision) in the norms of national legislation with appropriate measures of legal liability for those involved should be considered as the most effective measure.

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The Authors declare no conflict of interest.

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## REVIEW ARTICLE

# MEDICINAL HERBS AND PLANTS IN MEDIEVAL MEDICAL PRACTICE (BASED ON THE LATIN POEM “DE VIRIBUS HERBARUM” BY MACER FLORIDUS)

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**Marta J. Petryshyn<sup>1</sup>, Halina M. Zahajska<sup>2</sup>, Oxana V. Liubimova<sup>2</sup>, Veronika H. Todoshchuk<sup>2</sup>**<sup>1</sup>VASYL STEFANYK PRECARPATHIAN NATIONAL UNIVERSITY, IVANO-FRANKIVSK, UKRAINE<sup>2</sup>YURIY FEDKOVYCH CHERNIVTSI NATIONAL UNIVERSITY, CHERNIVTSI, UKRAINE**ABSTRACT**

**The aim:** The aim of our research is to make an inventory and systematize prescriptions for the use of medicinal plants during the early Middle Ages, based on Macer Floridus' original Latin text “De viribus herbarum”, to develop awareness of the role of phytotherapy in medieval medicine and the possibility of integrating herbal medicine with modern conventional methods of prevention and treatment.

**Materials and methods:** The material for this study is a medieval Latin didactic poem by the 11th-century French physician and researcher Odo of Meung-sur-Loire (pseudonym Macer Floridus), the extant manuscripts of which are known in the history of medicine as “De viribus herbarum” or “De natura herbarum”. The medical-pharmacological treatise (published in 1831 by Ludwig Choulant) describes the medicinal properties of seventy-seven plants of peasant gardens, grasses of meadows and fields of Europe, medicinal herbs of medieval apothecary gardens as well as aromatic plants and spices of the East.

**Conclusions:** Medicinal plants and herbs were successfully used to treat diseases of the gastrointestinal tract, spleen, hepatobiliary system, urinary and respiratory organs and were also applied in gynecology, dermatology, ophthalmology and dentistry.

**KEY WORDS:** history of medicine, phytotherapy, Odo Magdunensis, herbal text

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**INTRODUCTION**

For thousands of years, mankind has accumulated information about the medicinal properties of plants and learned how to use them to treat various diseases. The centuries-long experience of peoples provided the basis for the development of phytotherapy. At present, the rapid development of synthetic organic chemistry and the pharmaceutical industry has somewhat diminished interest in the use of medicinal plants in medical practice, but herbal medicines often have undeniable advantages over synthetic drugs, as they provide a pronounced therapeutic effect with minimal risk of side-effects. Given this, phytotherapy should have its rightful place in clinical practice, and medical and pharmaceutical professionals should be interested in learning more about herbal medicine in the Middle Ages and how it can be adapted to modern conditions.

**THE AIM**

The aim of our research is to make an inventory and systematize prescriptions for the use of medicinal plants during the early Middle Ages, based on Macer Floridus' original Latin text “De viribus herbarum”, to develop awareness of the role of phytotherapy in medieval medicine and

the possibility of integrating herbal medicine with modern conventional methods of prevention and treatment.

**MATERIALS AND METHODS**

The material for this study is a medieval Latin didactic poem by the 11th-century French physician and researcher Odo of Meung-sur-Loire (pseudonym Macer Floridus), the extant manuscripts of which are known in the history of medicine as “De viribus herbarum” or “De natura herbarum” [1]. The medical-pharmacological treatise (published in 1831 by Ludwig Choulant) describes the medicinal properties of seventy-seven plants of peasant gardens, grasses of meadows and fields of Europe, medicinal herbs of medieval apothecary gardens as well as aromatic plants and spices of the East.

We used a combination of general scientific and special research methods that complement each other in order to achieve the aim. Theoretical analysis and synthesis of the professional literature were chosen to explore various views on the selected issues. To interpret the factual material, a descriptive method was used. The method of contextual analysis enabled textual fragments to be selected and the healing properties of various plants to be inquired into.

## REVIEW AND DISCUSSION

A survey of the scientific literature shows that the didactic poem “De viribus herbarum” has rarely been the subject of study. Some references to the influence of Macer Floridus on the development of herbal therapy in the early medieval medicine can be found in the monograph by I. Eisenmann-Tappe and J. Mayer [2]. The authors have attempted to adapt medieval prescriptions for herbs and plants to the requirements of modern medicine. Bruce P. Flood, Jr. examines the influence of Pliny the Elder (c.79 AD) on the generalization of the recommendations and prescriptions for herbal medicines, particularly mint, given in the text of Macer Floridus’ poem [3]. The subject of T. Niedenthal’s research interests is the comparative analysis of the use of seventy-seven plants described in the poem, in the Middle Ages and in modern medicine [4]. Thoughts on the role of Macer Floridus in the development of phytotherapy can be found in the research of J. Mayer [5, 255]. The story of the origin of the poem “De viribus herbarum” and its influence on medical practice and didactic poetry of subsequent centuries are covered in the heritage of F. Voronov [6]. It was found that medicinal plants were mostly used for the treatment and prevention of diseases of the gastrointestinal tract, ENT organs and respiratory system, and eyes [6, 5]. The aim of D. Stehlíková’s research is to study the experiences with the use of wormwood (*Artemisia abrotanum*) described in the poem as well as in four student commentaries on the early 15th century work “De viribus herbarum” and the manuscript from Znojmo, deposited in the Czech National Library [7].

Despite previous work, we believe that the prescriptions for the use of medicinal plants and herbs for the treatment and prevention of diseases in the medieval medical practice, contained in the original Latin text, should receive more extensive coverage, as the principles of modern phytotherapy are grounded in the centuries-old experience of the ancestors.

Medicinal plants have long been used in medicine in natural and processed form as an effective natural remedy for treating and increasing the body’s resistance due to the complex of biologically active substances (alkaloids, saponins, glycosides, phytoncides, vitamins, etc.). The analysis of the text of the poem “De viribus herbarum” revealed that medicinal herbs and plants were used in the Middle Ages to treat diseases of the gastrointestinal tract, spleen, hepatobiliary system, respiratory organs, kidneys, and urinary tract. Prescriptions collected by the French physician promoted recovery from burns, wounds, injuries, were used to treat gynecological pathologies, and were effective in dermatology, ophthalmology, and dentistry.

The use of medicinal plants against digestive diseases has a long history, as in medieval cities, unlike the ancient world, there were practically no water supply and sewage systems, no conditions for food storage. People lived in overcrowded houses with poor ventilation, unsanitary conditions prevailed, and deaths from epidemics, infectious diseases and digestive diseases were the scourge of the times.

The therapeutic effect of herbal treatment of digestive disorders was based on anti-inflammatory, antispasmodic, antimicrobial, enveloping, laxative, carminative, stomach and intestinal motility stimulating and choleric properties of plants. Our analysis of the poem reveals that forty-three plants, namely wormwood, nettle, rue, celery, dill, fennel, leek, chamomile, cabbage, oregano, iris, caraway, sorrel, pepper, etc., were used in medieval medical practice to treat gastrointestinal disorders, diseases of the spleen and hepatobiliary system. These medicinal herbs and plants have almost all biologically active components in their arsenal, which allows their prolonged use without addiction.

An imbalance of beneficial and pathogenic microorganisms caused intestinal disorders. If useful bacteria cannot neutralize the toxins produced by putrefactive bacteria, constipation, diarrhea, increased flatulence, and stomach pain occur. To restore the micro-flora of the gastrointestinal tract, medieval medicine recommended not only food rich in dietary fiber, but also medicinal plants. The analysis of the text of the poem revealed that celery, rue, lovage, caraway, dill, and garlic have a carminative effect, that is, through the spasmolytic effect, stimulate passage of flatus formed in the intestines as a result of the processes of fermentation and putrefaction, and eliminate the feeling of bloating. For example, medieval physicians recommended applications of crushed garlic and pork fat as a remedy for bloating: *Porcino iungens adipi si conteris ipsum / Non modicos reprimes superaddens saepe tumores* (V, 191–192)<sup>1</sup>. Decoction of green rue in spicy vinegar was regarded an effective remedy against flatulence: *compescit talis decoctio tormina ventris* (VII, 273). For the syndrome of excessive gas and dyspepsia, warm water with pounded dill: *Cum tepida tritum patiens si potet Anethum, / Intastinorum curat ventrisque dolorem* (X, 405–407), wine with chamomile: *Tormina sic sedat stomachique inflatio potu* (XIV, 569), or decoction of lovage root: *Non modicum colicis prodest haec mansa vel hausta, / vel si radicis elixatura bibatur* (XXV, 894–895) were recommended.

Wood betony, dill, cabbage, oregano, mint, pepper, caraway, galangal, and cinnamon were used to facilitate digestive processes. For example, a pinch of wood betony powder with honey helped to improve digestion: *Pondere vero fabae pulvis cum melle voratus / Post coenam stomachum iuvat, ut bene digerat escam* (XI, 480–481). Use of oregano with white wine also had a therapeutic effect: *Evenit, ex vino prodest, si sumitur albo; / Cum calida morsus stomachi lenire probatum est* (XXXVIII, 1313–1314). Peppermint was found to have a stimulating effect on the secretion of digestive glands: *Illius potu vis digestive iuvatur* (XLVII, 1570). Raw or boiled black pepper with honey was also used to treat sluggish bowels: *Crudum vel coctum sumptum, vel melle iugatum, / Vim digestivam stomachi iecorisque iuvabit* (LXVI, 2066–2067). Functional activity of the stomach and bowels was stimulated by car-

<sup>1</sup> The Roman numeral stands for the number of the chapter, the Arabic numeral – for the number of the verse line.



away seeds: *Et digestivum stomachi iecorisque calorem excitat* (LXIX, 2115) and cinnamon: *Humores stomachi siccant, corroborat ipsum, et facit acceptas ut digerat ocus escas* (LXXIII, 2151). Cabbage that is rich in vitamins and fiber was considered one of the best remedies to improve motility and facilitate excretion of digested food from the body: *Atque iuvat stomachum sumptas ut concoquat escas* (XXXVI, 1229). For therapeutic purposes, as a means to regulate the functioning of the gastrointestinal tract, gruel of boiled garlic was recommended: *Adiuvat incoctum puti sumptumque tenesmon* (V, 190). To prevent and treat constipation, decoction of the white base of the leaves of leeks was used as a mild laxative: *Sic ut aquam primam mutes, addasque secundam / Utilis eius aquae duro fit potio ventri* (XIII, 526–528). Lettuce, which since ancient times has been known both as a food plant and as a medicinal plant, when cut, was also prescribed as a laxative: *Utilis est stomacho, ... mollit et alvum, / Omnibus his melius prodest decocta comesta* (XX, 767–768). The seeds of watercress, a herbaceous salad plant of the cabbage family, mashed with a drachma of warm water, was considered an effective remedy against constipation: *Semen cum tepida contritum pondere dragmae / Et bibitum, dicunt quod durum molliat alvum* (XXX, 1013–1014), half-raw cabbage: *At semicrudus solet illam solver sumptus* (XXXVI, 1231). Onions had a similar effect on gut motility: *Et dicunt illas mollire salubriter alvum* (XXXIII, 1098).

Caraway, lettuce, leeks, mint, sorrel, poppy, cabbage, galingale and cinnamon were used by medieval physicians as soothing and astringent agents for the gastrointestinal tract. For example, leek with wine was recommended to treat diarrhea: *... cum vino stringere ventrem* (XIII, 529), as well as lettuce seeds: *Cum vino bibitum fluxum quoque reprimit alvi* (XX, 771–772), seeds of black poppy: *Sperma nigri bibitum cum vino stringere ventrem* (XXXII, 1056), or caraway seeds: *Et fluxum ventris in aceto stringere coctum* (LXIX, 2117). As a treatment for diarrhea boiled cabbage: *Si multum coquitur, restringere dicitur alvum* (XXXVI, 1230), sorrel herbs: *Sumptaque sicut olus fertur restrigere ventrem* (LXIII, 1999), cinnamon: *Humores stomachi siccant, corroborat ipsum* (LXXIII, 2150) were used. For pain and spasms in the intestines, it was recommended to use nettle seeds with honey: *Illius semen colicis cum melle medetur* (IV, 120), decoction of rue in spicy vinegar: *Si coquis hanc in aqua, cui vinum iunxeris acre, / Compescit talis decoctio tormina ventris* (VII, 272–273), wine with camomile: *Aut si cum vino potetur saepius illa; tormina sic sedat* (XIV, 569), or the common tormentil: *Vim digestivam iuvat hoc colicisque medetur* (LXX, 2129). Pennyroyal and fennel were believed to have medicinal properties that could help relieve heartburn resulting from digestive and gastric motility disorders. Pennyroyal was used with diluted vinegar: *Cum pusca sumpta vel aceto quod sit aquosum* (XVI, 647), and fennel – with water: *Fervorem stomachi cum lymphā mitigat hausta* (XVII, 700). Also, fennel and wine were used to stop bouts of nausea: *Nausea cum vino sumpta sedabitur illa* (XVII, 699). To stop vomiting, garden chervil soaked in strong

vinegar: *Intictum valido si manducetur aceto / saepe solet vomitum ventremque tenere solutum* (XXVII, 940–941) or pennyroyal decoction: *... vomitum quoque detinet hausta* (XLVII, 1571) were prescribed.

Plants regulating bile formation and bile secretion such as: wormwood, garlic, mint, parsnip, iris, pepper, verbena, etc. proved to be therapeutic for liver and biliary tract diseases. For example, garlic with coriander and wine: *...fuit usus cum coriandro / Et vino, causas sic curans ictericorum* (V, 180–181), camomile decoction: *Icteris prodest eius decoctio sumpta / Et mire prodest iecoris potato querelis* (XIV, 573–574), which stimulated the movement of bile and liver function, verbena with wine, which in the Middle Ages was considered a panacea for various diseases: *Pestiferos morsus curat superaddita trita / cum vino* (LVIII, 1863) were recommended for treating various kinds of jaundice. Absinthin, a bitter glycoside of wormwood, combined with celery, stimulated bile secretion and increased its outflow: *Icteris crudam dabis hanc apio siciatam* (III, 73). Liver diseases were also healed by parsnip roots with honey water: *Mulsa si decoquis eius / radices, multum decoctio proderit eius, Si potanda datur, splenis iecorisque querelis* (XXXVII, 1265–1267), raw or boiled black pepper with honey: *Crudum vel coctum sumptum, vel melle iugatum, / Vim ... iecorisque iuvabit* (LXVI, 2066–2067). In medieval medicine, the cholokinetic properties of iris rhizome powder with honey water were known: *Cum mulsa bibitus choleram depellit* (XLIII, 1469), which in modern medicine is mainly used as an analgesic, antitumor and sedative means.

The spleen is an important organ in the human body, performing the function of protection against infections, participating in blood purification, as well as being one of the main storages of blood. To treat diseases of the spleen, nettle, celery, cabbage, parsnip, birthwort, iris, peony, and sorrel were used. The simplest remedies for spleen diseases during the Middle Ages were cabbage soaked in vinegar: *Assumptus crudus, sic ut tingatur aceto, / Splen reprimit tumidum* (XXXVI, 1232–1233), iris powder and wine mixture: *Spleneticis et contractis et frigore laesis / Cum vino bibitus pulvis medicabitur eius* (XLIII, 1472–1473), peony mixture with honey water and crushed almonds: *splen ... cum mulsa sumpta iuvabit / Si iungatur ei violenter amygdala trita* (XLIX, 1607–1608). Splenomegaly was treated with applications of decoction of mashed sorrel root in strong vinegar on the spleen area: *Decoctas valido redices eius aceto / Et tritas spleni superaddito saepe tument* (LXIII, 2008–2009), and consumption of grated celery with dill juice: *Hydropicos et splen tumidum iuvat ille ... / Si cum feniculi succo contrita bibatur* (VIII, 360–362). For splenosclerosis it was advised to use decoction of birthwort, which modern folk medicine applies in gynecology and dermatology: *Splenis duriciam solvit laterisque dolorem, / si mixta potatur aqua* (XLI, 1413).

In the arsenal of medicinal plants used to cure respiratory and ENT diseases, the text of the poem mentions thirty-four plants, namely wormwood, nettle, plantain, garlic, rue, leek, thyme, poppy, onion, violet, elecampane,

melissa, etc., most of which are also applied by modern medicine. Therapeutic value of the mentioned herbs and plants is due to their antimicrobial, mucolytic, antipyretic, hypersensitizing, and anti-inflammatory properties. Since cough is the most common symptom of diseases of the bronchopulmonary system, a significant part of the recommendations of the poem is devoted to the treatment of this very symptom. One of the best cures for coughs, pneumonia, and pleurisy was nettle seed powder with honey: *Illius semen ... cum melle ... et tussim veterem curat, si saepe bibatur* (IV, 120–121). Due to phytoncides with antiviral, bactericidal and fungicidal properties, garlic decoction was considered effective for suffocation and coughing: *Elixum tussim iuvat et suspiria sedat* (V, 187). In medieval phytotherapy, the powder of buckeye with honey was used to treat congestive cough with profuse mucus, suffocation, and pleurisy: *Betonicae pulvis cocto cum melle iugatus / Empicos, tussimque iuvat, suspiria sedat* (XI, 437–438). Pennyroyal powder with honey was considered one of the best expectorants: *Illius pulvis cum melle vel haustus / Pectoris humores viscosos extenuare* (XVI, 644–646), and warm wine with this herb provided a soothing effect in case of cough: *Tussim compescit cum vino sumpta tepenti* (XVI, 666). Powder of summer savory with honey or wine was used as a cure for the respiratory system diseases: *Illius pulvis cum cocto melle subactus / Et mansus (vel cum vino si sumitur idem) / Pectoris humorem pellit per sumpta tenacem* (XXIII, 847–849). Oregano powder with honey was considered effective in the treatment of bronchitis and other respiratory diseases due to its expectorant properties: *Illius pulvis tussim cum melle repellit* (XXXVIII, 1297). Curative properties were also found in the fresh stems, leaves and flowers of violets, rich in saponin and alkaloid Violin, which have expectorant effects and are good against lung diseases in children: *Mollibus in costis aut in pulmonibus ipsis, / Sedat si mixto potabitur amne recenti; / Infantum tussim sic et suspiria ssedat* (XL, 1372–1374). Iris root decoction with wine was also recommended as a remedy against upper respiratory diseases: *Cum vino sedat tussim* (XLIII, 1466). Cough and shortness of breath were helped by the powder of elecampane with honey: *Eius radicum cum melle voratus / Tussim compescit* (XLIV, 1499–1500), decoction of fresh melissa: *Eius si viridis decoctio saepe bibatur / ... Asthmaticis eandem prodest orthopnoicisque* (L, 1655–1657).

Various herbs and plants were as well applied in otology and laryngology. For example, wormwood steam was used to relieve blocked ears, and a mixture of honey, sodium bicarbonate, and wormwood was recommended for angina: *Decoctaeque vapor obstrusas liberat aures, / Si manant sanie cum melleterens superadde. / Subvenit angina melli nitroque iugata* (III, 81–83). Earache was treated with goose lard and garlic drops: *Anseris huic adipem iungas tepidumque dolenti / Infudas auri, praeclare subvenit illi* (V, 185–186), wood betony juice with rose oil: *Illius succus roseo commixtus olivo / Auribus infuses varios fugat dolores* (XI, 435–436), warm leek juice with goat's bile or honey water: *Auris compescit cum caprae felle*

*dolorem / Praesta idem pariter cum mulsa mixtus et auri / infusus tepidus* (XIII, 538–539), plantain juice: *Et dolor hoc auris sedabitur* (VI, 220), horeground juice with rose oil: *Auriculaeque gravem dicunt curare dolorem, / Hunc mixtum roseo si fundas intus olivo* (XLII, 1452–1453), onion juice with breast milk: *Femineo lacti commixtus succus earum / Pellit saepe graves infusus ab aure dolores* (XXIII, 1105–1106), and nightshade juice: *Dicitur auriculae mire sedare dolorem / Illius succus, si sensim funditur intus* (LX, 1920–1921). To restore hearing, ear drops of sour sorrel juice were recommended: *Auribus expressus si succus funditur eius / Adiuvat auditum mire* (XVIII, 737–738). To regain voice in case of laryngitis, an infusion of poppy leaves was applied to the throat: *De foliis eius tritis factum cataplasma / Eximie fauces dicunt curare tumentes* (XXXII, 1062–1063), and green cabbage juice – to sip: *Affirmat raucae multum succurrere voci* (XXXVI, 1258). Wool soaked in centaury juice, which modern medicine uses mainly to stimulate appetite and increase gastrointestinal activity, has been applied to treat polyposis: *Hoc succo lanam madidam si naribus addas, / Compesces morbum, qui polypus est vocatus* (LIII, 1738–1739).

Inevitable concomitants of medieval man were wounds, which could cause malfunctions of the body up to and including death. Therefore, one of the primary tasks of surgery was care and healing of wounds. Forty-four of the 77 plants described in the poem, namely: nettle, plantain, marshmallow, dill, juniper, sleek, rose, soft rush, centaury and others were used by doctors to treat tissue damages and wound infections, to cure burns and to stop bleeds. These plants contain biologically active substances (plant antibiotics, phytoncides, essential oils, resins, tannins, organic acids, alkaloids, glycosides) that inhibit the growth of pathogenic microorganisms. To treat infected wounds, plants that produced both curative and antimicrobial effects were used. For example, to accelerate the process of purification and granulation of wounds, poultices with nettle and salt: *Cum sale de foliis eius factum cataplasma / Ulceribus prodest et sordida vulnera purgat* (IV, 125–126), applications of plantain with honey: *nimis humida vulnera siccant, / Si superaddatur cum melle, et sordida purgat* (VI, 204–205), bandages with celery juice, spelt and egg white: *Illius succus farris cum polline mixtus / Atque ovi lacrymo vulnus bene purgat et ulcus, / Si superaddatur emplastri more frequenter* (VIII, 363–365) were recommended. A pronounced bactericidal effect against pathogenic microflora was observed when juniper with honey: *Vulnus cum melle vel ulcus / Fortiter exsiccate, et sordes purgat eorum* (XII, 494–495), rose oil with vinegar: *Permiscendo sibi si forte iugatur acetum / Expurgat sordens vulnus repletque profundum* (XXI, 794–796), white horehound with honey: *Si mel Marrubio iungatur vulnera purgat* (XLII, 1445), grass of wall germander mashed with honey: *Sordens purgabit vulnus, licet inveteratum, / Si cum melle teras et tritam desuper addas* (LIX, 1912–1913), or aloe powder: *Vulnus quodque recens putredine purgat ab omni / Illius iniectus pulvis siccandoque sanat* (LXXVII, 2240–2241) were put onto wounds. Applications of mashed plantain:

Stringit manantem superaddita trita cruorem (VI, 210), a mixture of aloe, egg white, and frankincense: Huic aloë iungens lacrymumque albuminis ovi, / Sic ut sit spisum, sectae superaddito venae, / Aut his vulneribus nimioque sanguine manant (LXXVI, 2220–2225) were applied on wounds as hemostatic agents. Medieval doctors treated canker sores with applications of grated parsnips: Appositum cancris tritum cum melle medetur (XXXVII, 1282), chervils with honey: Appositum cancris tritum cum melle medetur (XXVII, 929) or cabbage: Vulnera non tantum curare recentia Caulem / ipsemet affirmat, sed quamvis inveterata, / Et canculos etiam (XXXVI, 1208–1210). Some plants were used in proctology to treat hemorrhoids. For instance, it was recommended that flowers of marshmallow mashed with wine: Vel si cum vino tritum florem superaddas, / ... anumque iuvare dolentem (IX, 371–372), roasted seeds of dill: Apponas haemorrhoidis si semen Anethi / Ustum, curat eas (X, 423–424), chopped onions: Hasque iubent haemorrhoidis superaddere tritas (XXXIII, 1123), mallow decoction: ... et ani / Haec eadem mire prodest (LXII, 1992) should be put on piles. To treat anal fissures, a wax ointment based on the decoction of the herbaceous part of violets was used: Ani fissuras, quas appellant ragadias, / Addita cerotis medicatur saepe perunctas (XL, 1361–1362).

To treat burns, plantain with egg white: Ovi cum lacrymo mire medicatur adustos (VI, 212), an ointment of lily root bulb, plums and olive oil: Eius radices bulbis, quae Lilia profert, / Sub prunis tectus iuncto quoque tritus olivo / Prodest usturis mire superadditus ignis (XXII, 812–814), poultices of mallow leaves boiled in olive oil: Decoctis eius foliis si iungis olivum, / ... Et combusturis illo bene subvenis ignis (LXII, 1987–1989) were administered. Animal bites were cured with poultices of marshmallow leaves boiled in olive oil: Decoctis oleo foliis factum cataplasma / Quosvis pestiferous morsus ... curat (IX, 393–394), or decoction of oregano in wine: Illius in vino curat decoctio sumpta / Quosvis pestiferous morsus, si saepe bibatur (XXXVIII, 1287–1288). To fix fractures, medieval doctors used leek juice: Fracturas solidat cito, duritiasque relaxat (XIII, 545). Poultices made of betony leaves were used to treat skull fractures: Tritaque fracturae capitis haec sola medetur (XI, 444).

In gynecology, herbal remedies have been successfully used in the treatment of uterine hemorrhage, menstrual disorders, other diseases of the female reproductive organs. The analysis revealed that forty-three plants were utilized in gynecological practice. To relieve menstrual symptoms, women would take decoctions of wormwood: menstrua solvit (III, 60), catnip: Appositu potuque suo cito menstrua purgat (XV, 603), and lemon balm: decoctio menstrua purgat (I, 1660), wine with wild thyme: Cum vino ... solet producere menstrua potus (XXXIX, 1340–1341). Polymenorrhea was treated with sour sorrel leaves and wine: Cum vino potato ... manatia menstrua sistit (XVIII, 728–730). It was believed that in case of metrorrhagia, peony seeds with wine: Seminis illius ter quinque rubentia grana / Cum vino fluxum matricis sumpta coërcent (XLIX, 1630–1631), as well as a mixture of the seeds of henbane and poppy seeds with honey water: Seminis illius obolus cum semine

mixtus / Miconis pariter cum mulsa sumitur illis / Utiliter, fluxum matricis quae patiuntur (LXI, 1952–1953) would be helpful. Local effect on the area of the uterus was applications of wool with the juice of plantain: Succus cum lana matrici subditus eius / Stringit manantem nimium siccando cruorem (VI, 229–230). Powdered leaves of betony with honey water were recommended for genital prolapse and ruptures of the uterus: Cum mulsa bibitus prodest pulvis foliorum / Ruptis atque steras potus levat iste cadentes (XI, 474–475). Based on empirical experience, specific plants were used to induce spontaneous miscarriage. The abortifacients included wormwood, rue, juniper, camomile, pennyroyal, salvia, galingale, elecampane and wall germander. The abortive effect of the mentioned plants is caused either by the content of alkaloids or essential oils, or by a pronounced hemostatic action. For example, the alkaloid-rich salvia with honey water: Cum mulsa ... pellit abortivum (XXIV, 871–872) and rue: Si saepe bibatur, / Expellit partum potu (VII, 270) were used as abortifacients. According to medieval physicians, leek juice helped restore women's reproductive function: Reddit fecundas mansum persaepe puellas (XIII, 519). To stimulate labor and accelerate placenta delivery, decoctions of horehound seeds: Accelerat partus eadem pellitque secundam (XLII, 1444), mint: Haustaque cum sapa partum solet accelerare (XLVII, 1478), and fumigations with darnel: Parturiens mulier si se subfumiget illa, / Asseritur citius ventris deponere pondus (LXIV, 2027–2028) were considered helpful.

In medieval medicine, wormwood, garlic, rue, and catnip were successfully applied against helminth infestations. For example, the smell of grated garlic or boiled garlic in vinegar and honey fought various types of parasitic worms: In mulsa coctum commixtum cui sit acetum / Et bibitum vermes ventris tineasque repellit (V, 166–167).

Medieval doctors successfully applied twenty-three plants to treat eye diseases, namely wormwood, plantain, celery, wood betony, fennel, sour sorrel, purslane, onion, cabbage, violet, henbane, horeground, centaury, white hellebore, black false hellebore, greater celandine, wall germander, mallow, cowbane, black pepper, cinnamon, nard, aloe. It was believed that the eye ointment made of wormwood and honey restored visual acuity: Et claros oculos reddit cum melle peruncta (III, 79). The roots of violet, saffron and myrrh: Radices Violae cum mirrha tunde crocoque, / Hoc inflammatis oculis apponito nocte (XI, 1355–1356), the juice of henbane seeds: Viscosum calidumque potest compescere rheuma, / si fuerint oculi patientis saepe peruncti (LXI, 1949–1950) or cowbane: Vel si sint eius circumlita lumina succo; / Haec quoque pellentur sacer ignis et herpeta cura (LXV, 2043–2044) were ointmented over the eyes when treating conjunctivitis.

In the medical treatise we also find advice on herbal treatment of the diseases of the oral cavity. Plantain juice of the was a remedy for gum disease: Gingivas reprimet tumidas et sanguine plenas (VI, 226) and stomatitis: Sordida purgabit bene vulnera quaelibet oris, / Ore diu tentus si succus volvitur eius (VI, 217–218), pennyroyal podwer strengthebed the gums: Gingivas sicci pulvis



confirmat et usti (XVI, 657), while rose: Ore diu tentum dentis sedare dolorem (XXI, 797) and lemon balm: Ore retenta solet dentis sedare dolorem (L, 1661) were used to relieve toothache.

Various herbs and plants have been widely known in dermatology and cosmetology. Since ancient times, women used fruits, flowers, and leaves of plants to care for their skin, nails, and hair. For example, an ointment of wormwood ashes and wax was used as a hair dye: Denigrat crines cinis eius, si bene mixtus / Ceroto fuerit et eo sint saepe peruncti (III, 93–94), freckles were removed with crushed celery greens: Haeque superposita turpis lentigo fugatur (VIII, 361), and to improve the condition of the skin, honey and camomile masks were recommended: Squamas de vultibus aufert, / Si tritam apponas solam mellive iugatam (XIV, 570–571). Washing with warm decoction of pennyroyal was considered an effective cure for itching: Elixatura si quisquam saepe tepenti / Illius abluitur, pruritus non patientur (XVI, 663–664). As a local remedy for parasitosis caused by small mites *Sacrotres scabiei*, patches of saltbush (*Atriplex*) were very efficient: Hocque superpositum scabros cito detrahit unguis (XXVIII, 951).

## CONCLUSIONS

The plant world is not only a priceless treasure trove of nature, but also man's long-time friend. The use of plants in medicine has been sanctified by centuries of humankind's experience, for in nature's laboratory there is a cure for every disease. A poem by Macer Floridus, an 11th-century French scholar and physician, is a jewel of medieval phytotherapy, combining the antique legacy with the author's own experience. Healing plants and herbs from apothecary gardens, orchards, fields and meadows of Europe, as well as aromatic spices of the East were successfully used to treat and prevent diseases of the gastrointestinal tract, spleen, hepatobiliary system, respiratory organs, kidneys, urinary system.

The prescriptions collected by the French doctor promoted recovery from burns, wounds, injuries, gynecological pathologies, were also effective in dermatology and cosmetology, ophthalmology, and dentistry. The historical sources testify that medicinal plants have not lost their positions over the centuries; in fact, they continue to attract attention, since the preparations of herbal origin have many advantages over synthetic drugs due to their low toxicity and the possibility of prolonged use. Therefore, although modern pharmacy is rapidly progressing, it is necessary not to forget the valuable experience of using herbal remedies at various stages of the society's development.

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The Authors declare no conflict of interest.

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## CASE STUDY

## AGGRESSIVE COURSE OF DIFFERENTIATED THYROID GLAND CANCER IN A PATIENT WITH INSULIN RESISTANCE: CASE ANALYSIS FROM PRACTICE

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### ABSTRACT

Differentiated thyroid cancer (DTC) is usually characterized by a harmless clinical behavior but in some cases it can manifest itself as a metastatic damage to the bone system. The authors reported case from their practice of an aggressive DTC course in a patient with insulin resistance, accompanied by the development of metastases in the bone system. The main goals of the patient's treatment at each stage of radioiodine therapy were to reduce the foci of metastatic bone damage in terms of their number and volume as well as insulin resistance as a risk factor affecting insulin-like growth factors.

A clinical case analysis found that radioiodine therapy might be useful for disease control in cases with potentially aggressive variants of DTC. This category of patients requires careful monitoring of insulin resistance, insulin-like growth factors and appropriate antitumor treatment.

**KEY WORDS:** differentiated thyroid cancer, insulin resistance, metastatic bone damage

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### INTRODUCTION

At present, thyroid cancer (TC) is the fourth most common malignancy in women. However, by 2030, TC is expected to rank third among the most common cancers in women [1]. DTC, which includes papillary and follicular TC, is the most wide spread (over 90 %) of all types of TC [2–4]. Annual mortality from TC, which is 0.38 cases per 100,000 population (0.35 in men, 0.39 in women), remains relatively stable [3]. For the most part, DTC is characterized by harmless clinical behavior and significant survival rate, despite a significant risk of recurrence [4–9]. However, a small proportion of DTC may manifest a very aggressive clinical behavior at the stage of initial diagnosis of the disease, which significantly increases the mortality rate [5].

Usually, in the case of DTC, the most frequent metastatic localization is the regional cervical lymph nodes [6], however, the development of metastatic damage of the bone system is quite rare.

The treatment problem of malignant neoplasms of the thyroid gland and their metastasis relates not only to the morphological, clinical, and molecular-biological features of the tumors themselves, but also to the insufficient study of the influence of factors that can aggravate the course of the disease. Such factors today include insulin resistance,

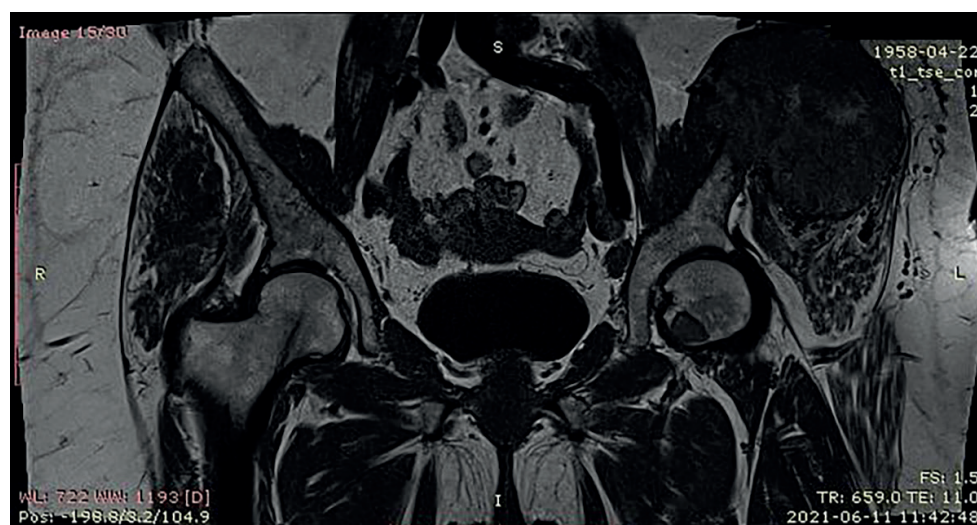
which can affect the main components of the signaling system of insulin-like growth factors [15].

### THE AIM

The aim of the work was to analyze the authors' own case from the practice of diagnosis and treatment of a woman with DTC with insulin resistance and generalization of the tumor process into the bone system.

### MATERIALS AND METHODS

The material of this study was information on the results of comprehensive diagnosis and treatment of a patient with DTC with generalization of the tumor process into the bone system and insulin resistance. The patient underwent MRI, CT, trepan biopsy followed by morphological examination, osteoscintigraphy, laboratory tests (TG, TSH, ATGA, calcium in the blood; blood analysis for carbohydrate metabolism (Homa-IR index, glycosylated hemoglobin, C-peptide, IGF-1, IGF-2), planar scintigraphy of the whole body on the «residual» activities of <sup>131</sup>I sodium iodide after issuing therapeutic activities of <sup>131</sup>I- NaI.



**Fig. 1.** MRI of the patient M. with DTC and metastatic damage of the pelvic bones and the head of the left femur.

## CLINICAL CASE

Patient M., 64 years old, has considered herself sick since April 2021, when she noticed «thickening of the neck», pain in the left part of the pelvic area. She sought medical help at the Poltava Regional Oncology Dispensary (Ukraine). She was diagnosed with DTC after the examination. According to the treatment protocol for the identified disease, the first stage of treatment was a thyroidectomy on May 18, 2021.

Fragments of thyroid gland tissue with invasive complexes of tumor cells were found during the morphological examination of the operative material. The tumor cells had somewhat variable pale eosinophilic cytoplasm and indistinct borders, the cell nuclei were elongated, luminal with marginal chromatin condensation and irregular outline of the nuclear membrane. Some nuclei had intranuclear «grooves». The phenomenon of accumulation and overlapping of nuclei was noted. The tumor also had signs of lymphovascular invasion. The conducted morphological study made it possible to establish a diagnosis: papillary TC, invasive follicular variant.

On June 10, 2021, the patient applied to the State Organization «Grygoriev Institute for Medical Radiology and Oncology of the National Academy of Medical Sciences of Ukraine» (Kharkiv, Ukraine), where she underwent further examination and treatment. Taking into account the patient's complaints of pain in the left half of the pelvic region and the suspicion of extrathyroidal spread of the disease, she underwent an MRI of the hip joints without contrast enhancement on June 11, 2021 (Fig. 1). During the session, formations were found at the level of the wing of the left iliac bone measuring up to 95×80 mm and up to 12 mm, in the body of the left iliac bone measuring up to 25 mm, in the head of the left femur measuring up to 17 mm, in the sacrum on the left measuring up to 166 mm, in the right part of the sacrum up to 10 mm, in the right ischium – up to 10 mm. Thus, the performed MRI revealed a metastatic damage of the bones of the pelvis and the head of the left femur.

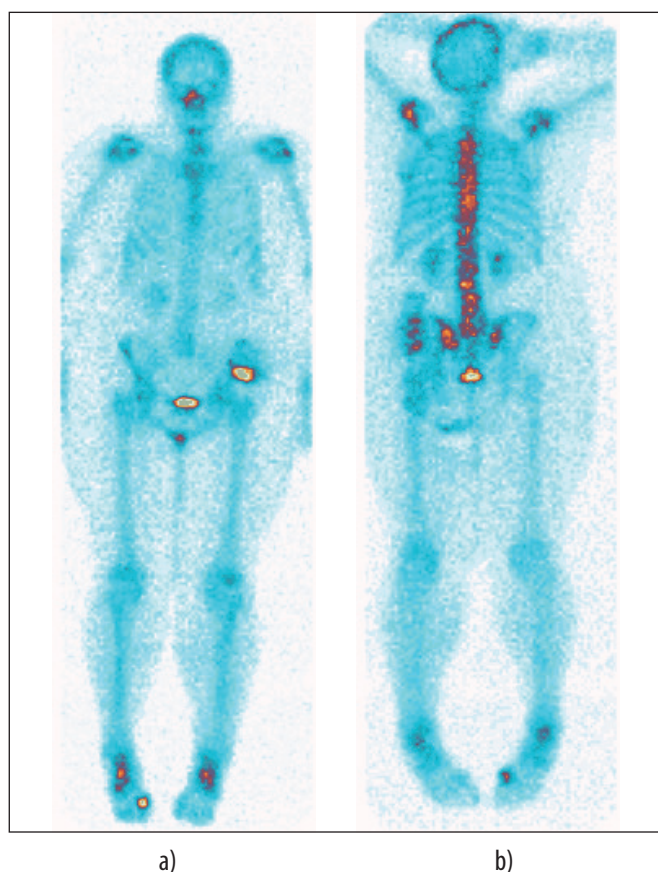
On June 17, 2021, the patient underwent a CT scan of the chest, abdominal cavity and pelvis with intravenous

tomohexol-350 contrast to diagnose possible damage of other organs and systems. CT scan revealed moderately enlarged lymph nodes of the retroperitoneal group up to 9 mm, general and external iliac lymph nodes up to 24×11 mm. The described enlarged lymph nodes corresponded to a metastatic damage in terms of structure and shape. A locus of destruction with a large heterogeneous soft tissue component measuring 85×90×100 mm was found in the wing of the left ilium. The tumor had clear signs of neo-angiogenesis, was supplied with blood from the external iliac artery. Foci of destruction were also found in the wing measuring 11 mm, in the right scapula measuring 17 mm, in the neck of the humerus on the right measuring 20 mm. CT-tomograms did not find any metastatic damage of internal organs.

On June 30, 2021 the patient underwent a trepan biopsy of the soft tissue formation of the left iliac bone for morphological verification of the detected multiple metastatic changes. The cytological examination of the obtained material established metastases of the TC. Morphological examination of the biopsy revealed tumor tissue, the structure of which corresponded to metastasis of follicular TC.

In view of the pronounced extrathyroidal spread of the TC in patient M., planar osteoscintigraphy was performed on July 13, 2021 for a more detailed diagnosis of the spread of metastatic bone damage after intravenous administration of 600 MBq 99mTc-MDP (Fig. 2). During the latter, numerous foci of hyperfixation of the osteotropic radiopharmaceutical drug were found in different areas of the bones (maximum fixation – 522% compared to the contralateral area).

A number of laboratory tests were also performed on patient M. to confirm that the established pathological changes in the bones and soft tissues were related to TC. Thus, the level of TG in the patient's blood serum was more than 1000 ng/ml (reference values 0.7–75.8 ng/ml); ATGA – 1495 U/ml (reference values – up to 115 U/ml); TSH – 23.079 mU/ml (reference values 0.4–3.6 mU/ml). The level of total calcium in blood serum was 2.3 mmol/l (reference values 2.20–2.55 mmol/l). In the clinical tests of



**Fig. 2.** Osteoscintigrams of patient M. SPECT images in anterior rectus (a) and posterior rectus (b) projections after 3.5 hours of intravenous administration of radiopharmaceutical drug ( $^{99m}\text{Tc}$ -MDP).

blood and urine, we found no significant differences from normal values. In addition, we determined the parameters of carbohydrate metabolism: insulin – 16.3 mU/ml (reference values 2.6–24.9 mU/ml); NOMA index – 3.52 (reference values up to 2.77); C-peptide – 3.52 ng/ml (reference values 0.9–7.1 ng/ml); blood sugar – 5.19 mmol/l (reference values 4.56–6.38 mmol/l); glycated hemoglobin – 6.1% (reference values 4.0–6.0%); IGF-1 – 92.3 ng/ml; IGF-2 – 1257.1 ng/ml.

On August 11, 2021 the patient underwent radioiodine therapy using 5995 MBq of  $^{131}\text{I}$  sodium iodide per os according to the treatment program. After the completion of external dosimetry, a planar scintigraphy of the whole body was performed 7 days later on the so-called «residual» activities of  $^{131}\text{I}$  sodium iodide (Figs. 3, 4).

Patient M. also underwent SPECT-CTAC (Fig. 5). The patient's condition after the treatment was satisfactory. Then she was prescribed suppressive hormone therapy at a dose of 200 µg per day with a control determination of TSH level in blood serum 1 month after the start of L-thyroxine. A further course of treatment is scheduled after 8–9 months. During the period between the first and second courses of radioiodine therapy, the patient took 200–150 µg of L-thyroxine while maintaining suppressive TSH levels and a drug from the biguanide group – metformin at a dose of 850 mg twice daily in order to overcome insulin

resistance. To inhibit osteoclast activity, cell proliferation and induction of apoptosis of metastatic cells in bone tissue, the patient was prescribed zoledronic acid – a drug from the group of bisphosphonates at a dose of 4 mg every 28 days. We also applied accompanying therapy: calcium gluconate, dexamethasone, dexalgin.

On 05/22/2022, the patient again turned to the clinic of the the State Organization «Grygoriev Institute for Medical Radiology and Oncology of the National Academy of Medical Sciences of Ukraine» (Kharkiv, Ukraine). She had similar complains of pain in the left part of the pelvic area. In the process of laboratory research after the four-week cancellation of hormone therapy with L-thyroxine, the following data were obtained: the level of TG in blood serum was more than 1000 ng/ml; ATGA – more than 4000 U/ml; TSH – 83.989 mU/ml; the level of total calcium in blood serum – 2.23 mmol/l; NOMA index – 1.57; C-peptide – 2.5 ng/ml; blood sugar – 5.24 mmol/l; glycated hemoglobin – 5.9%; insulin – 6.75 mU/ml; IGF-1 – 43.7 ng/ml; IGF-2 – 629.7 ng/ml. Clinical tests of blood and urine, biochemical analysis of blood were without significant changes.

On May 25, 2022, patient M. underwent radioiodine therapy using 5995 MBq of  $^{131}\text{I}$  sodium iodide per os. After the completion of external dosimetry, a planar scintigraphy of the whole body was performed 7 days later on the «residual» activities of  $^{131}\text{I}$  sodium iodide (Fig. 6, 7).

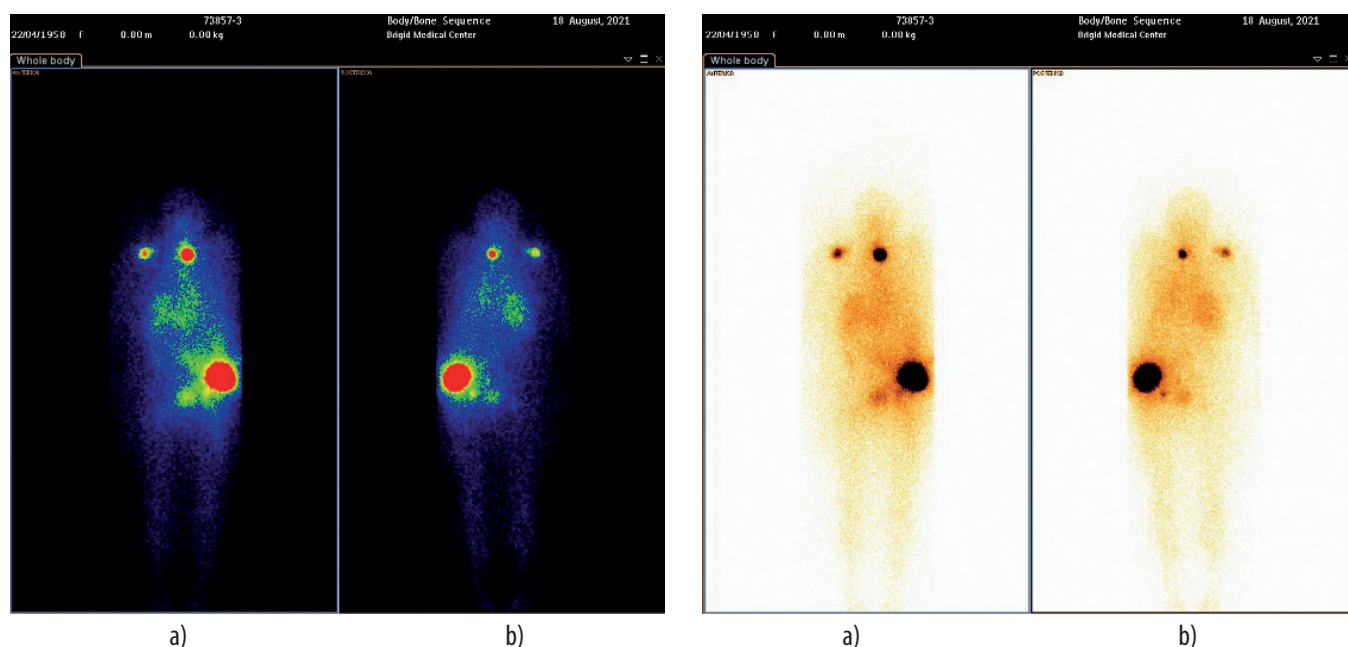
During planar scintigraphy of the whole body, on the «residual» activities of  $^{131}\text{I}$  sodium iodide after the release of the therapeutic activity of radioiodine, foci of radioiodine fixation were established in the projections of the right humerus, in the bones of the pelvis on the left, doubtful fixation of the radiopharmaceutical drug in the right lung. That is, we obtained the image of functionally active metastases of the TC, achieving radionuclide ablation of the «final» thyroid tissue in a typical location. The patient tolerated the treatment satisfactorily. She was also prescribed suppressive hormone therapy with L-thyroxine to maintain the TSH level in blood serum at 0–0.1 mU/ml. In addition, the patient was prescribed intravenous administration of 4 mg of zoledronic acid. The next course of radioiodine therapy is planned in 6–8 months with the aim of further treatment of functionally active TC metastases of the extrathyroidal localization.

## DISCUSSION

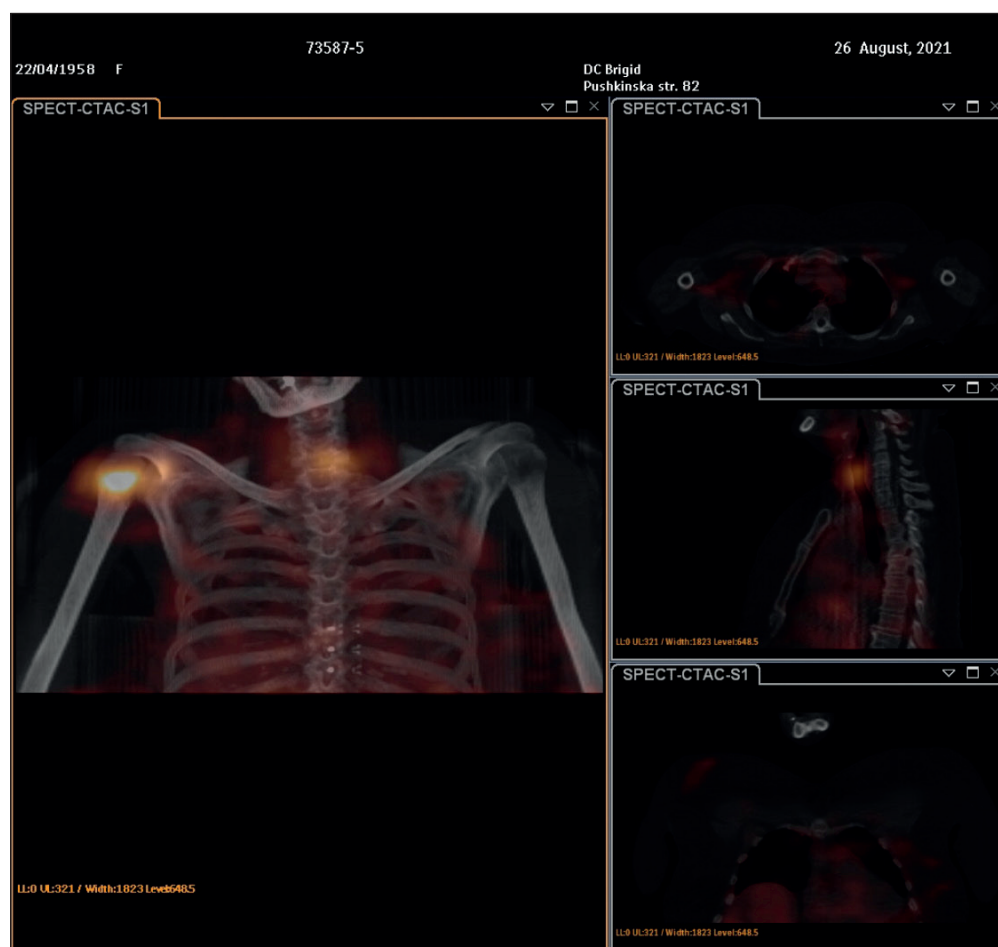
DTC is one of the five types of cancer that most often metastasizes to bones [7]. After the lungs, the bones are the second most frequent localization of distant metastases of DTC [8]. Bone metastases of DTC are usually osteolytic damage with secondary bone formation in response to bone destruction and involvement of soft tissue [9].

Any type of DTC can metastasize to bone structures. Frequency of bone metastases is three times higher for follicular TC (7–28%) compared to papillary TC (1–7%) [10]. A likely explanation is that follicular DTC spreads more easily through the bloodstream to distant organs due to its greater tendency to invade blood vessels [11].



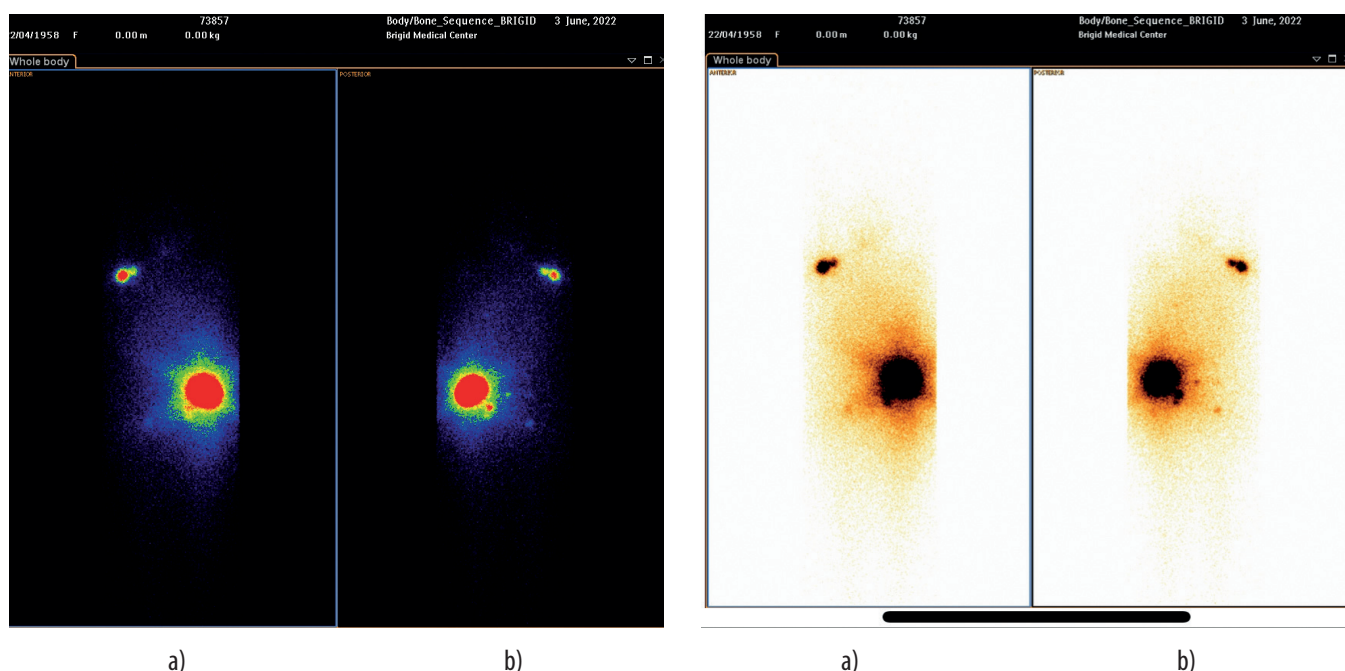


**Fig. 3, 4.** Response to radioiodine therapy of patient M. with TC after the first course. On the scintigrams in the front (a) and back (b) projections, several «hot spots» of fixation of the radiopharmaceutical drug are visualized on the neck and shoulder on the right, pelvic bones, in the left and right lung by post-therapy whole-body scans on day 7 after the initial administration of 5995 MBq  $^{131}\text{I}$  sodium iodide. The SPECT image shows functionally active metastases of the TC (in the color image – Fig. 3, in the black and white image – Fig. 4).



**Fig. 5.** Patient M.'s response to radioiodine therapy (after the first course of radioiodine therapy). SPECT-CTAC-scans show foci of  $^{131}\text{I}$  fixation (a, b) in the projection of the location of the «residual» tissue of the thyroid gland, in the projection of the shoulder joint on the right, which were performed 7 days after taking 5995 MBq of  $^{131}\text{I}$  per os.





**Fig. 6, 7.** Patient M.'s response to radioiodine therapy after the second course on May 25, 2022. On the front (a) and back (b) projections, several «foci of fixation» are visualized on the neck and shoulder on the right, pelvic bones. SPECT-scans show functionally active metastases of the DTC.

The axial skeleton, especially the spine and pelvis, is the most common site of involvement. Cancer cells easily reach the red bone marrow of these bone segments because the blood flow is known to be high. In addition, the predominant connection between the thyroid gland and the axial skeleton is provided by Batson's vertebral-venous plexus, which plays a significant role in the drainage of the head and neck area through indirect connections with the inferior veins of the thyroid gland [12].

Bone-related events, including pathologic fractures, spinal cord compression, the need for bone irradiation or surgery and malignant hypercalcemia, can be a major complication in patients with DTC with bone metastases, leading to impaired quality of life and shortened survival [10]. Radioiodine therapy is one of the effective methods of patients' treatment with metastatic bone damage in the case of DTC. With the ability to accumulate  $^{131}\text{I-NaI}$ , metastases decrease in number and volume. This method of treatment is a salvation for this category of patients.

The prognosis of patients with DTC with bone metastases is generally poor, and survival rates are lower than those observed in cases of other distant localizations [13]. The overall 10-year survival varies from 13 to 21% [14].

Although TC is mostly non-aggressive, it is necessary to take into account the possibility of metastatic damage of the bone system. In a practical sense, serial evaluation of bone damage, using diagnostic radiological methods, is primarily intended to ensure absence of progression. Patients with TC with previous metastatic damage of the bones have a higher risk of additional metastases in the bone system, so they should undergo active and timely screening for this pathology.

## CONCLUSIONS

The authors have analyzed a case of an aggressive course of differentiated thyroid cancer in a patient with insulin resistance from their practice which was accompanied by the development of metastases in the bone system. The given scintigrams are the responses on the «residual activities» of  $^{131}\text{I-NaI}$  to radioiodine therapy. The main goals of treatment at each stage of radioiodine therapy were to reduce the foci of metastatic bone damage in terms of their number and volume and to reduce insulin resistance as a factor affecting insulin-like growth factors.

A clinical case analysis has found that radioiodine therapy may be useful for disease control in cases with potentially aggressive variants of differentiated thyroid cancer. This category of patients requires careful monitoring of insulin resistance, insulin-like growth factors and appropriate antitumor treatment.

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# Conflict of interest:

The Authors declare no conflict of interest.

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