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Effect of deficiency of natural anticoagulants on clinical heterogeneity of rheumatoid arthritis

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ABSTRACT

Aim: To investigate the concentration of natural anticoagulants protein C (PC) and tissue plasminogen activator (t-PA) in patients with rheumatoid arthritis (RA) and to evaluate the effect of their concentration on the course of the disease.

Materials and Methods: We examined 74 patients with RA. There were 15 men and 59 women and control group consisted of 27 subjects. The average age of the patients was 47.3±1.12 years. Laboratory (PC, t-PA, C-reactive protein, TNF-alpha, von Willebrand factor, and lipid profile) and instrumental (ultrasonography and Doppler) examinations were performed.

Results: PC deficiency was significantly more common among men with RA (26.6%), but did not depend on the age and the duration of the disease ($p<0.05$). PC deficiency correlates with the degree of activity of the inflammatory process ($r = -0.27$) and TNF-alpha ($r = -0.37$). The levels of PC and t-PA were associated with the level of cholesterol ($r = -0.25$), LDL-C ($r = -0.31$), and HDL-C ($r = 0.31$). In patients with PC and t-PA deficiency, significantly higher (by 19% and 12%, respectively) serum levels of Von Willebrand factor were recorded than in patients with normal levels ($p<0.05$).

Conclusions: The study of PC and t-PA revealed a deficiency of important natural anticoagulants, that helps us to expand the understanding of the mutually aggravating effect of the deficiency of these compounds and changes in known biologically active substances on the course of RA, and to supplement the pathogenetic picture of RA with certain links.

KEY WORDS: rheumatoid arthritis, protein C, tissue plasminogen activator

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INTRODUCTION

Difficulties in the timely diagnosis of rheumatoid arthritis (RA), which is considered one of the models of chronic inflammatory diseases, are not always due to the pathognomonic symptoms of the initial period of the disease, which may remain unnoticed by specialists for some time [1]. In the context of understanding the clinical heterogeneity of the disease, the search for new links in the pathogenesis of both RA and its systemic manifestations continues. Studies of immunologic disorders in RA open up significant prospects for improving the methods of diagnosis and treatment of various clinical variants of this disease.

In particular, the mechanisms of hyperproduction of interleukin-2, interferon γ, interleukin-17; imbalance between proinflammatory (tumor necrosis factor alpha (TNF-alpha), interleukin-1β, interleukin-6 (IL-6), interleukin-8, etc.) and anti-inflammatory cytokines (interleukin-4, interleukin-10, soluble interleukin-1β antagonist, etc.) with the production of the former over the latter are understood. It has been established that the synthesis of TNF-alpha predominates in the onset of the disease, and its uncontrolled synthesis underlies the chronicization of the pathological process and progressive bone destructive changes. TNF-alpha is involved in the development of clinical signs of inflammation, induces the expression of adhesion molecules, stimulates neoangiogenesis, fibroblast proliferation, which play a key role in the formation of the rheumatoid pannus [2].

However, the increase in the number of vascular systemic manifestations in the population of patients with RA, especially among male patients, indicates the need to identify the nature of changes in both

the vascular wall and the hemostatic system and to determine the role of these changes in the multidirectional pathways of RA progression. One of the important components of systemic hemostasis, which is also involved in the regulation of the human immune system in response to vascular or inflammatory damage, is the protein C (PC) system. Its activated form has strong anticoagulant activity. Also, activated PC has cytoprotective and anti-inflammatory effects on vascular endothelial cells, neuronal cells, and various cells of the human immune system [3, 4].

Since 1960, when PC was discovered by Professor Seegers, scientists have described numerous properties of this substance. There are about 20 genes known to be upregulated by activated PC and 20 genes known to be downregulated by activated PC. The former include genes with anti-inflammatory and anti-apoptotic activity, and the latter - with pro-inflammatory and pro-apoptotic activity. Another important function of activated PC is the ability to accelerate plasminogen-dependent thrombus lysis, which is a direct manifestation of its angioprotective properties [3, 5].

AIM

The aim of our study was to investigate the concentration of natural anticoagulants PC and tissue plasminogen activator (t-PA) in patients with RA and to evaluate the effect of their concentration on the course of the disease.

MATERIALS AND METHODS

We observed 74 patients with RA. There were 15 men (20.3%) and 59 women (79.7%). The control group consisted of 27 people of comparable age and gender. The average age of patients was 47.3±1.12 years.

RA was diagnosed based according to the American College of Rheumatology (ACR)/European Alliance of Associations for Rheumatology (EULAR) 2010 criteria [6].

Exclusion criteria: Pregnancy, patients with liver disease, vitamin K deficiency, metastatic tumors, ICE syndrome, fresh thrombosis, severe bacterial infection at a young age, nephrotic syndrome, homocysteinuria, patients treated with warfarin and other indirect anticoagulants, prolonged antibiotic therapy with insufficient food intake, oral contraceptives.

The content of C-reactive protein (CRP), TNF-alpha and Von Willebrand factor (VWF) in the blood was determined by enzyme-linked immunosorbent assay using standard kits from «Diagnostic Automation Inc.», USA, "Calbiotech", Germany and "Shield diagnostics", England.

The activity of PC was determined using a PC activator derived from the venom of the common scabies (Agkistrodon halys) using the chromogenic substrate S236 [7, 8]. The potential of the fibrinolytic system was assessed by the activity of t-PA [9].

Serum levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were measured according to standard methods. The value of low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula: LDL-C=TC-HDL-C-0.45*TG.

To study endothelial function, high-resolution echolocation and Doppler ultrasonography of the brachial artery were used as described by Celermajer D. et al. (1992). The thickness of the intima-media complex (IMC) was determined by scanning the common carotid artery (CCA) in B-mode echolocation at a distance of 2 cm from the bifurcation in the diastolic phase at maximum magnification. The degree of atherosclerotic vascular lesions and the presence of atherosclerotic plaques were assessed by Wendelhag I. et al. (1993) [10].

Data analysis was performed in SPSS Statistics v.23. Summary statistics of mean, standard deviation and percentiles were used for quantitative measurements. The association between measures was assessed using the correlation test and t-test. The probability value was estimated at 0.05 confidence level (P=0.05).

RESULTS

A comparative analysis of the frequency of certain disorders in the hemostatic system in patients with RA and practically healthy subjects revealed some differences (Table 1). In particular, the average level of the natural anticoagulant PS in patients with RA was 10.6% lower compared with controls, and the proportion of patients with PS deficiency was 2.5 times higher, respectively. Analysis of the state of the fibrinolytic system in patients with RA revealed a significant suppression of its activity, which was manifested by a decrease in the content of t-PA. In particular, the average level of t-PA in patients with RA was 17.4% lower than in the control group, and the proportion of people with its deficiency was 36%, while in the control group it was only 11.1%. No gender differences were found in the frequency of disorders and mean values of t-PA levels, while men were significantly more likely to have a PC deficiency compared with women.

A comparative analysis of the studied hemostatic parameters in patients with RA depending on age

Table 1. Ranking of PC and t-PA levels in patients with RA and control group, their relationship with gender, age, disease duration and BMI

		PC		t-PA	
		Abs. value, (M±m)	**PC deficiency ≤70 %**	**Abs. value, (M±m)**	**t-PA deficiency ≤ 1,7 IU/ml**
1	Control group, n=27	94,6±1,39	2 (7,4%)	2,02±0,03	3 (11,1%)
2	Patients with RA, n=74	85,53±1,47$^{\beta}$	14 (18,9%)$^{\beta}$	1,72±0,05$^{\beta}$	27 (36%)$^{\beta}$
	Including				
3	Women with RA, n=59	85,52±1,63	10 (16,9%)	1,69±0,06	21 (35,6%)
4	Men with RA, n=15	84,53±3,51	4 (26,6%)*	1,85±0,10	6 (40%)
	Connection with the age of patients with RA				
5	≤ 35 years, n=6	90,17±5,54	1 (16,6%)	1,86±0,19	2 (33,3%)
6	36-50 years, n=31	87,59±2,35	5 (16,2%)	1,77±0,08	9 (29,0%)
7	> 50 years, n=37	84,76±1,93	8 (21,6%)	1,66±0,08	16 (43,2%)
	Dependence on the duration of the disease				
8	< 5 years, n=25	87,80±2,57	5 (20,0%)	1,66±0,08	6 (24,0%)
9	5-10 years, n=15	85,73±3,43	4 (26,6%)	1,88±0,10	7 (46,6%)
10	>10 years, n=34	83,76±2,12	5 (14,7%)	1,66±0,07	14 (41,2%)
	Connection with BMI of patients with RA				
11	BMI<30, n=55	86,04±1,72	9 (16,4%)	1,76±0,06	18 (30,9%)
12	BMI>30, n=19	84,05±1,47	5 (26,3%)§	1,60±0,06	9 (52,6%)§

Note: p<0.05, * - significant difference in women with RA, § - in patients with RA with BMI <30, β - compared to the control group.

Table 2. Ranking of PC and t-PA levels in patients with RA depending on the indicators of lipid metabolism

	Lipid levels	**PC**		**t-PA**	
		(M±m)	**r**	**(M±m)**	**r**
TC, mmol/l	Optimal, n=23	90,04±2,36	-0,27$^{\pi}$	1,85±0,09	-0,25$^{\pi}$
	Extremely elevated, n=28	86,93±2,44		1,74±0,09	
	High, n=23	79,30±2,44*§		1,57±1,10*	
HDL-C, mmol/l	Normal, n=19	87,68±2,50	0,19	1,85±0,08	0,31$^{\pi}$
	Subnormal, n=11	89,36±3,51		1,96±0,11	
	Low, n=44	83,64±2,06		1,60±0,07$^{\#\alpha}$	
LDL-C, mmol/l	Normal, n=33	87,55±2,04	-0,26$^{\pi}$	1,78±0,07	-0,31$^{\pi}$
	Extremely elevated, n=12	92,17±3,61		1,84±0,16	
	High, n=29	80,48±2,28$^{¥£}$		1,60±0,09	
TG, mmol/l	Normal, n=15	89,33±3,11	-0,21	1,84±1,11	-0,24
	Extremely elevated, n=31	84,94±1,14		1,73±0,08	
	High, n=37	84,65±2,18		1,68±0,08	

Note: p<0.05, * - significant difference compared to the group "optimal TC level"; § - to the group "extremely elevated TC level"; # - to the group "normal HDL-C level"; α - to the group "subnormal HDL-C level"; ¥ - to the group "normal LDL-C level"; £ - to the group "extremely elevated LDL-C level"; π - significant values of the correlation coefficient.

revealed that PC and t-PA deficiency is inherent in all age categories. Disorders in the hemostatic system in patients with RA also had no relationship with the duration of the disease, as indicated by the almost equal proportion of patients with PC and t-PA deficiency among patients with a disease duration of up to 5 years and more than 10 years. At the same time, PC and t-PA levels were associated with body mass index (BMI). In particular, in the group of patients with a BMI > 30, the proportion of patients with PC and t-PA deficiency was 1.6 and 1.7 times higher, respectively, than in patients with a BMI < 30.

The analysis showed that the levels of PC and t-PA were associated with the lipid spectrum of serum in patients

Table 3. Serum levels of PS and t-PA in patients with RA depending on inflammatory process activity

			PC		t-PA	
			(M±m)	r	(M±m)	r
1	CRP, mg/l	≤ 25 percentile, n=20	88,30±2,51	-0,34#	1,92±0,07	-0,32#
		>25≤75 percentile, n=30	88,03±2,29		1,73±0,09	
		>75 percentile, n=24	80,08±2,60*§		1,54±0,10*	
2	TNF-alpha, pg/ml	≤ 25 percentile, n=16	89,88±2,62	-0,37#	2,22±0,07	-0,40#
		>25≤75 percentile, n=33	85,67±2,24		1,68±0,08*	
		>75 percentile, n=22	80,77±2,83*		1,54±0,11*	
3	ESR, mm/h	≤ 25 percentile, n=18	88,56±2,50	-0,27#	1,91±0,06	-0,30#
		>25≤75 percentile, n=30	88,90±2,29		1,80±0,08	
		>75 percentile, n=26	79,54±2,42*§		1,50±0,10*§	
4	DAS_{28}, points	≤ 25 percentile, n=20	87,55±2,38	-0,14	1,88±0,06	-0,28#
		>25≤75 percentile, n=25	84,08±2,85		1,68±0,10	
		>75 percentile, n=29	84,62±0,72		1,64±0,09*	

Note: p<0.05, * - significant difference compared to the group "<25 percentile"; § - to the group ">25≤75 percentile"; #- significant values of the correlation coefficient.

Table 4. Levels of PC and t-PA depending on subclinical manifestations of atherosclerotic vascular lesions and serum levels of VWF in patients with RA (M±m)

		PC		t-PA	
		Normal level, n=60	Low level, ≤70 %, n=14	Normal level, n=47	Lower than 1,7 IU/ml, n=27
IMC CCA (mm)		0,81±0,03	0,95±0,08	0,76±0,03	0,98±0,05*
Number of subjects with IMC CCA>0,90 mm, n (%)		31 (51,7%)	8 (57,1%)*	14 (29,8%)	17 (62,9%)*
EDVBA, %		5,31±0,67*	2,47±1,46*	6,05±0,81	2,53±0,80*
Число осіб з EDVBA ≤8,0%, n (%)		40 (66,6%)	11 (78,6%)	27 (57,4%)	24 (88,8%)*
Presence of atherosclerotic plaques		18 (30%)	8 (57,1%)*	12 (25,5%)	14 (51,9%)*
Lesions of CCA by Wendelhag scale, n (%)	0	42 (70%)	6 (42,8%)*	35 (74,5%)	13 (48,1%)*
	1	15 (25%)	6 (42,8%)*	9 (19,2%)	12 (44,4%)
	2	3 (5%)	1 (7,2%)*	3 (6,3%)	1 (3,7%)
	3	0 (0%)	1 (7,2%)	0 (0%)	1 (3,7%)
VWF		150,4±6,83	178,6±6,68*	157,6±5,91	175,9±9,58*

Note: p<0,05, *- significant difference compared to subjects with normal level of PC and t-PA.

with RA (Table 2). It was found that the levels of PS and t-PA were associated with the levels of TC, LDL-C and HDL-C and had no significant relationship with the levels of TG in the blood serum. They were 13-18% lower in patients with high TC, LDL-C, and low HDL-C levels than in those with normal levels of lipid metabolism markers. At the same time, the levels of hemostatic markers had virtually no relationship with serum TG concentration. The correlation analysis also did not reveal close relationships between the concentration of PC and t-PA with TG levels (r = -0.21 - 0.24), while indicators of the antithrombotic and fibrinolytic links of the hemostatic system were associated with the levels of TC, LDL-C and HDL-C (r = -0.25 - 0.31).

We found that the activity of the inflammatory process also significantly affected the severity of hemostatic disorders (Table 3). It turned out that as the disease activity increased, the average values of PC and t-PA significantly decreased and the proportion of patients with disorders in the antithrombotic and fibrinolytic links of the hemostatic system increased. Thus, in the group of patients with high levels (>75th percentile) of inflammatory markers CRP and ESR, the mean values of PC and t-PA were not only significantly 10% lower than those in the group of patients with minimal (≤25th percentile) activity, but also significantly lower compared with the group of patients with moderate inflammatory activity.

Similar patterns were found in the level of TNF-alpha and the total index of joint syndrome DAS28. Correlation analysis confirmed the relationship between hemostatic system parameters and disease

activity. The closest correlation (r = -0.32, r = -0.40) was recorded between the levels of TNF-alpha and CRP with the concentration of PC and t-PA, less close (r = -0.27, r = -0.30), but also significant with the value of ESR and the total DAS28 score. TNF-alpha can affect the expression of all major components of the fibrinolytic system.

Regarding the involvement of hemostatic disorders in the development of structural and functional changes in the cardiovascular system, it was found that in patients with RA, atherosclerotic changes in the vessels (decreased endothelium-dependent vasodilation of the brachial artery (EDVBA) in response to a reactive hyperemia test, thickening of the IMC CCA), as well as the intensity of atherosclerotic vascular lesions are closely associated with PC and t-PA deficiency; compared with the group of patients with normal levels, patients with reduced EDVBA and thickening of the IMC CCA were found 1.2 and 1.5 times more often (Table 4).

Serum levels of VWF in patients with RA showed a close relationship with disorders in the antithrombotic and fibrinolytic components of the hemostatic system. In particular, patients with PC and t-PA deficiency had significantly higher serum levels of VWF (by 19% and 12%, respectively) than patients with normal levels of PC and t-PA.

DISCUSSION

The results of the study revealed a deficiency of both PC and t-PA in patients with RA. Similar correlations of decreased levels of PC and t-PA in the blood were demonstrated in a study of infectious and septic conditions [11]. In the work of Mosnier, it was proved that the release of t-PA by endothelial cells is stimulated by PC [12]. Apoptosis inhibition and blockade of inflammation by activated PC were also found due to changes in the gene expression profile in endothelial cells.

This leads to a decrease in the formation of proinflammatory cytokines by activated monocytes, and protection of endothelial barrier function. Activated PC has an anti-inflammatory effect on endothelial cells and leukocytes. During inflammation, endothelial cells lose their antithrombotic properties due to the more intense expression of adhesive molecules, while the expression of nitric oxide and thrombomodulin, on the contrary, decreases [13]. In another study, the authors demonstrated that the levels of thrombin-activated fibrinolysis inhibitors were higher in conditions of increased inflammatory activity (CRP>10 mg/L) than in less severe inflammatory changes (CRP<10 mg/L) [14].

Inflammation leads to a shift in the balance of the hemostatic system towards a prothrombotic state. The PS system prevents thrombus formation by inhibiting the release of proinflammatory mediators and reducing vascular endothelial adhesion molecules [5]. This reduces leukocyte adhesion, tissue infiltration, and limits the focus of destruction of surrounding tissues, and reduces chemotaxis. Under conditions of inflammation, the above mechanisms are suppressed. K. Liang and co-authors [15] have established a link between the development of severe extra-articular manifestations of RA (including vasculitis) and the involvement of peripheral vessels in the process. A number of scientific studies have shown that inhibition of the reactions of activation of the PC causes a sharp increase in the production of IL-6, interleukin-8, TNF-alpha and other cytokines and reduces the body's tolerance to various endotoxins. Evidence has been obtained that PC not only blocks leukocyte activation but also regulates the activity of matrix metalloproteinases, which causes degradation of the extracellular matrix and localized ulceration [4, 13, 16]. It is in this pathogenetic plane that the mechanisms of pathogenic effects of TNF-alpha, IL-6, and a decrease in the concentration of PC and, accordingly, t-PA, which are destructive for both articular surfaces and the vascular wall, intersect.

The association of PC and t-PA deficiency with BMI confirms the multidirectional role of obesity in the formation of inflammation. White adipose tissue produces a number of peptides, including those that control fibrinolysis (plasminogen activator inhibitor-1). Changes in the volume of white adipose tissue in obesity lead to a disruption in the production of this signaling molecule. Adipocytes are able to produce plasminogen activator inhibitor-1, so in obesity, an increase in the production of this protein, respectively, reduces the level of t-PA. In addition, plasminogen activator inhibitor-1, which is synthesized in excessive amounts in obese individuals, stimulates the migration of macrophages, interleukin-8 (IL-8), and IL-6, i.e., proinflammatory cytokines [17]. In this situation, the role of obesity in the formation of inflammation is realized both directly, through the formation of TNF-alpha and IL-6, and indirectly through the stimulation of proinflammatory agents by plasminogen activator inhibitor-1.

PS deficiency was significantly more common in men with RA. This is associated with epidemiologic studies on the prevalence of systemic vascular manifestations, especially rheumatoid vasculitis, in the male population [18].

In other epidemiological studies, Weiler, 2011, demonstrated that in patients of older age groups, PC deficiency is 25-40% [19]. In our study, in this age group, there was only a tendency to increase the proportion of patients with coagulation disorders.

In terms of detailing the impact of dyslipidemia on the formation of vascular damage in patients with RA, an interesting result was obtained regarding the lack of correlation

between the levels of natural anticoagulants and their serum TG concentration. Obviously, this phenomenon is associated with the biochemical structure of TG, which does not contain phospholipids, unlike LDL-C and HDL-C. Although the role of antibodies to phospholipids in RA remains open today, a number of researchers describe cases of antiphospholipid syndrome in patients with RA [20-22]. Antiphospholipid antibodies have the ability to inhibit the PC system in several ways: through inhibition of thrombin formation, through the effect on thrombomodulin, through inhibition of the construction of PC complex proteins on the anionic surfaces of phospholipid matrices, and through inhibition of cofactors Va and VIIIa, etc. Antibodies to phospholipids also affect the levels of PC and/or protein S (acquired deficiency) [23].

The results obtained on the decrease in the level of natural anticoagulants logically fit into the general picture of endothelial dysfunction in patients with RA, since the deficiency of PC and t-PA initiate a cascade of biochemical changes, which, in turn, affect the development of structural and functional changes in the vessels, accelerating the development of the atherosclerotic process. The frequency of detection of atherosclerotic plaques in the CCA, as well as the severity of atherosclerotic vascular lesions according to the Wendelhag scale, were also higher in the group of patients with t-PA and PC deficiency.

CONCLUSIONS

1. Significantly more frequent deficiency of PC was found among men with RA, deficiency of both natural anticoagulants (PC and t-PA) - among patients with a BMI over 30, high serum levels of VWF, high levels of total cholesterol due to its atherogenic fractions and among patients with high intensity of atherosclerotic vascular lesions, but did not depend on the age group of patients and the duration of the disease.
2. Moderate strength of correlations between natural anticoagulants (PC and t-PA) deficiency, the degree of activity of the inflammatory process, TNF-alpha and the total index of the manifestation of the joint syndrome was revealed.
3. The study of PC and t-PA revealed a deficiency of important natural anticoagulants, which will expand the understanding of the mutually aggravating effect of the deficiency of these compounds and changes in known biologically active substances on the course of the disease, clinical heterogeneity of RA, and supplement the pathogenetic picture of RA with certain links. Compensation of PC deficiency may be a promising area of therapy aimed at restoring endothelial function, reducing the systemic inflammatory response, and inhibiting the atherosclerotic process in patients with RA.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Nurses' attitudes towards mental illness and home health care quality as a means of managing psychiatric patients' relapses and re-admissions: a quasi -experimental study

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ABSTRACT

Aim: This study aims to assess the impact of an educational intervention on nurses' knowledge and attitudes regarding home health care, relapse management, and psychiatric hospital admissions.

Materials and Methods: A quasi-experimental study was conducted with 106 nurses at Erada for Mental Health and Addiction. Standardized assessment tools, including a knowledge scale for home health care quality and a mental illness devaluation scale, were used.

Results: Most participants were female 78.4% with a bachelor's degree 77%, and the average age was 2.06±.954. The analysis revealed a significant improvement in knowledge levels post-intervention P<0.000, however, there was a slight non-significant increase in devaluation scale scores after the intervention (pre-24.55±2.96, post-25.18±3.45).

Conclusions: Targeted interventions to enhance nurses' knowledge of home health care quality and promote positive attitudes toward mental illnesses can lead to improved patient care, potentially reducing relapses and readmissions among psychiatric patients. Future research should explore the long-term impact and sustainability of such interventions in mental health care settings**.**

KEY WORDS: nursing, mental health, attitudes, home health care, psychiatric patients, readmissions, stigma reduction

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INTRODUCTION

Home health care (HHC) is integral to modern healthcare systems, providing cost-effective care that eases hospital strain and supports patients, particularly those unable to access in-person care due to chronic conditions or mental health issues [1]. Mental health disorders are especially prevalent in young adults, with barriers like stigma, under-reporting, and societal influences contributing to underutilization of mental health services [2]. These disorders impact patients' cognitive, emotional, and physical quality of life [2]. In Saudi Arabia, HHC programs have evolved with government support to address various health needs, starting with a cancer-focused program at King Faisal Specialized Hospital in 1991 and expanding to a national initiative by 2008 [3]. These services are provided by multidisciplinary teams, including psychiatric nurses, who offer support and promote patient independence [4]. Mental health disorders have escalated post-COVID-19, highlighting the need for long-term care models. The global burden of mental disorders, contributing 7% to disability-adjusted life years (DALYs), underscores the necessity of structured, quality HHC for serious mental illness (SMI) patients [5]. Research has shown that mental illness-related stigma within healthcare systems and among providers impedes patient recovery. Stigmatizing attitudes from healthcare providers can result in poor care management and social marginalization [6, 7]. Nurses, especially those trained in psychiatric care, are pivotal in addressing this issue. Studies indicate that advanced education and specialized training in psychiatric nursing positively influence attitudes towards mental health [8], although factors such as cultural, demographic, and religious beliefs may also shape these attitudes [9]. Research from other countries underscores the potential of HHC in enhancing patient quality of life. Studies from Brazil and China found HHC programs improved overall quality of life and patient satisfaction [9]. Yet, limited studies in Saudi Arabia have examined the impact of HHC programs on mental health patient outcomes. Only one study in

Riyadh documented improvements in patient quality of life following HHC interventions in government hospitals [10, 11]. For mental health patients in Saudi Arabia, HHC usually begins post-hospitalization, with regular home visits by multidisciplinary teams. Best practices suggest that psychiatric nurses providing home care need substantial mental health training, ideally at the master's level, to deliver comprehensive services like cognitive-behavioral therapy, symptom management, and family education [12, 13]. Despite this, there are gaps in standardized training and the extent of skills necessary for effective home-based psychiatric care.

SIGNIFICANCE OF THE STUDY

Records indicate that nurses at the Erada Institution for Mental Health and Addiction lack the qualifications and licensure required for registered psychiatric nursing. While some hold associate or bachelor's degrees, few possess master's degrees, and most are involved in administrative roles rather than direct patient care. At Erada, the selection of home health care (HHC) patients is conducted by rotating staff, yet limited research has examined the hospital's HHC program since its introduction three years ago. Studies on attitudes toward mental illness generally focus on the public or medical students, with minimal insight into the attitudes of primary healthcare providers (PHCs) towards mental illness in our cultural context. An educational intervention for nurses could help enhance their understanding of quality HHC services and foster positive attitudes toward psychiatric patients and their families. This intervention aims to improve nurses' knowledge and attitudes, ultimately reducing relapse and readmission rates for psychiatric patients at the Erada Psychiatric and Addiction Institution, part of the Ministry of Health (MOH) in Jeddah, Saudi Arabia.

AIM

The study aims to assess the impact of an educational intervention on improving nurses' attitudes towards psychiatric patients and enhancing their knowledge of home health care for managing relapses and readmissions at the Erada Complex for Mental Health and Addiction, affiliated with the Ministry of Health in Jeddah, Saudi Arabia.

The study addresses three primary research questions:

1. What are the participants' knowledge and attitudes toward home health care quality before and after the intervention?
2. How do nursing staff's knowledge and attitudes influence their capability to manage patient relapses and readmissions?
3. What associations exist between participants' demographic characteristics and their knowledge and attitudes in both pre- and post-assessment phases?

HYPOTHESIS

- Null Hypothesis (H_0): The educational intervention does not improve nurses' attitudes and knowledge.
- Alternative Hypothesis (H_1): The educational intervention significantly impacts nurses' attitudes towards psychiatric patients and their knowledge of home health care.

MATERIALS AND METHODS

DESIGN

A quasi-experimental design with pre-test and post-test assessments, time series analysis, and a nonequivalent control group was used to evaluate the intervention's impact without randomization. This approach is common in educational and healthcare research where random assignment is impractical, allowing for tracking changes over time and comparing outcomes across groups with similar characteristics [14, 15].

SETTING AND PARTICIPANTS

The study took place at the Erada Complex for Mental Health and Addiction in Jeddah, Saudi Arabia. A convenience sample of 106 nurses (from a pool of 145) with experience in home health care visits and a willingness to participate was selected. The hospital has 200 nurses across inpatient, outpatient, and emergency units. To minimize selection bias, participant matching, and a nonequivalent control group were employed. Pre- and post-assessments allowed for within-subject comparisons, and assessors were blinded. Statistical tests, including t-tests and chi-square tests, were used to control for potential biases and ensure that the effects observed were due to the intervention [16].

SAMPLE SIZE CALCULATION

The sample size was determined using the Roe-soft software program, considering a 95% confidence level and a margin of error of ±5%. With 145 nurses having experience in home health care visits, a minimum sample size of 106 was calculated based on a response distribution of approximately 50% using a convenient purposive sampling method.

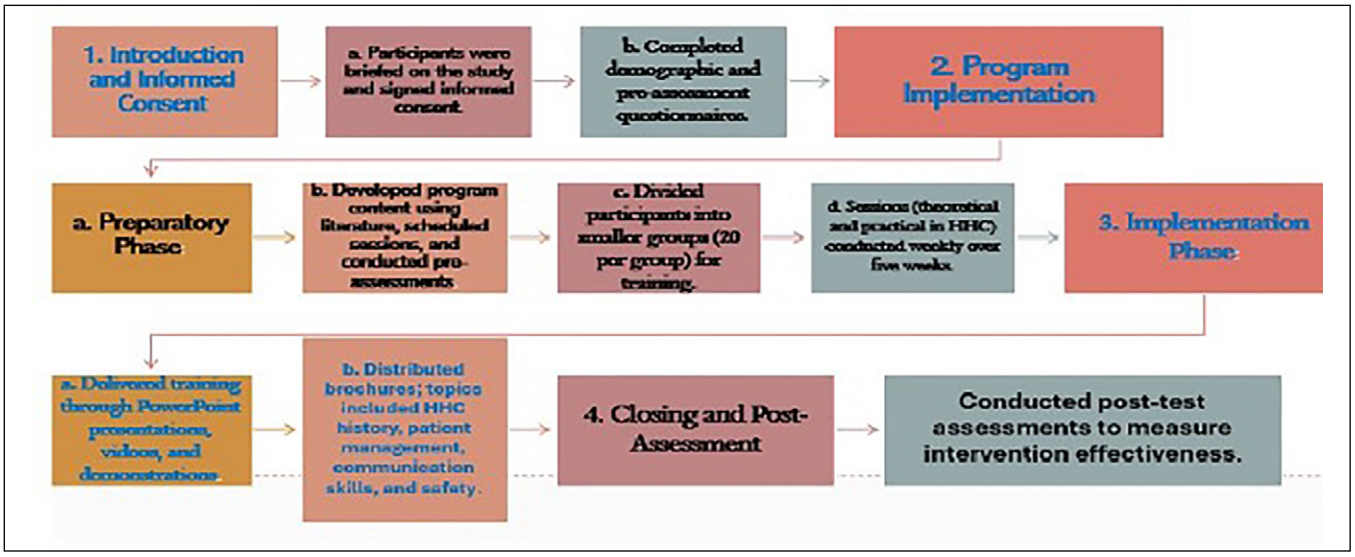

Fig. 1. Program phases summary flowchart.

TOOLS OF STUDY

1. **Demographic and Personal Data**: This tool collected basic information about participants, such as their gender, age, years of experience, previous attendance at Home Health Care (HHC) workshops, how they were selected to work in HHC services, and their educational background.
2. **Perceived Discrimination and Devaluation (PDD) Scale**: The PDD scale, created by sociologist Bruce G. Link [17] assesses the perceived devaluation and discrimination faced by individuals with psychiatric disorders. It includes 12 items that measure two subdomains:

Perceived devaluation (expectations about how others view individuals with mental disorders).

Perceived discrimination (expectations about how others will treat individuals with mental disorders). The scale uses a five-point response format and has been validated in previous studies. The Chinese version of the PDD was used in this study and includes a response option called "not sure." The items were categorized into three levels of agreement: "completely agree," "mostly agree," and "completely disagree."

3. **Knowledge about Home Health Care (HHC) Scale**: This self-reporting questionnaire, developed by Almoajel et al. [6], evaluates the knowledge of home healthcare professionals on various aspects of HHC. It includes questions on 11 dimensions such as satisfaction, perception of patients and institutions, training, knowledge, continuity, access, complaints, working hours, respect, incentives, standards, and effectiveness. The validity of the instrument was confirmed by experts from various sectors as per the main developer's evaluation.

DATA COLLECTION PROCEDURE

The study received ethical approval from the CONJ Research Unit, KAIMRC, IRB, and the Manager of the Erada Mental Health and Addiction Complex, Ministry of Health, Jeddah, KSA. Informed consent was obtained from each participant after providing detailed information about the study's purpose.

DATA COLLECTION PHASES

Data collection occurred in three main phases as it is shown by flowchart (Fig.1) as following:

Introduction and Informed Consent: After study approval, participants were contacted and asked to complete a demographic questionnaire. They were provided an overview of the study and required to sign an informed consent form. Participants also completed pre-assessment questionnaires (pre-test) that included demographic data, perceived discrimination (PDD), and knowledge of home healthcare (HHC).

IMPLEMENTATION OF THE PROGRAM

Preparatory Phase: The program's content was developed based on relevant literature. The planning phase included creating a timetable for strategies, teaching methods, and participant assignments. Pre-test assessments were conducted to evaluate participants before the program began. Participants were divided into smaller groups of 20 individuals for effective training. The training consisted of two main sessions: a theoretical session and a practical session on HHC, each lasting two hours. The sessions were conducted

Table 1. Distribution of the sample studied according to demographic background (N = 106)

		Gender			
		Female		Male	
		Count	Column N %	Count	Column N %
Age	less than 25	39	52.7%	0	0.0%
	from 25 to 35	16	21.6%	12	37.5%
	from 36 to 45	15	20.3%	18	56.3%
	from 46 to 55	4	5.4%	2	6.3%
Total mean =2.06 standard deviation 0.954					
Education level	Diploma	15	20.3%	18	56.3%
	Bachelor	57	77.0%	11	34.4%
	Master	2	2.7%	3	9.4%
	PhD	0	0.0%	0	0.0%
Experience in Home Health Care (HCC)	no experience	49	66.2%	10	31.3%
	from 1 to 3	10	13.5%	1	3.1%
	from 4 to 10	1	1.4%	11	34.4%
	more than 10	14	18.9%	10	31.3%
Previous HHC workshops	No	43	58.1%	16	50.0%
	Yes	31	41.9%	16	50.0%
How did you elect to work at HHC Services?	voluntary	10	13.5%	5	15.6%
	elected by co-worker	3	4.1%	8	25.0%
	elected by the hospital manager	2	2.7%	4	12.5%
	others	29	39.2%	15	46.9%
	do not work at the hospital	30	40.5%	0	0.0%
Do you think that HHC visits impact reducing the number of readmissions and relapses in psychiatric patients?	No	3	4.1%	2	6.3%
	Yes	71	95.9%	30	93.8%
As a nurse, have you been harmed or assaulted by the psychiatric patient or his family during an HHC visit?	No	66	89.2%	23	71.9%
	Yes	8	10.8%	9	28.1%
Did you miss any of the patients' visits?	No	59	79.7%	21	65.6%
	Yes	15	20.3%	11	34.4%
What do you think is the reason for missing visits?	0	2	2.7%	0	0.0%
	Poor in organization	23	31.1%	8	25.0%
	There is no integrated team	13	17.6%	7	21.9%
	Lacl of transportation	8	10.8%	0	0.0%
	Busy medical team	6	8.1%	5	15.6%
	Others	22	29.7%	12	37.5%
Does your hospital have accreditation for HHC from an international organization?	No	59	79.7%	24	75.0%
	Yes	15	20.3%	8	25.0%

during working hours, with one group attending per day. Training was repeated weekly over five weeks to accommodate all participants.

Implementation Phase: Various instructional methods were used, including PowerPoint presentations, brainstorming sessions, demonstrations, and examples.

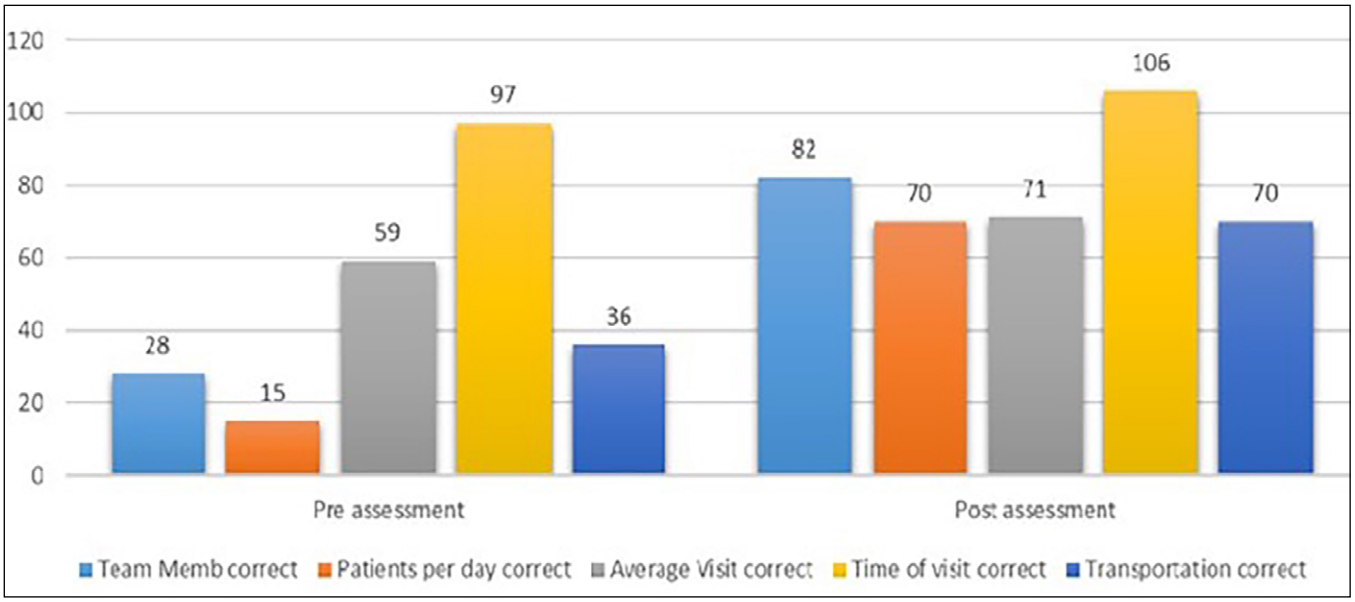

Fig. 2. Comparison between knowledge of the participants in pre / post assessment (N=106).

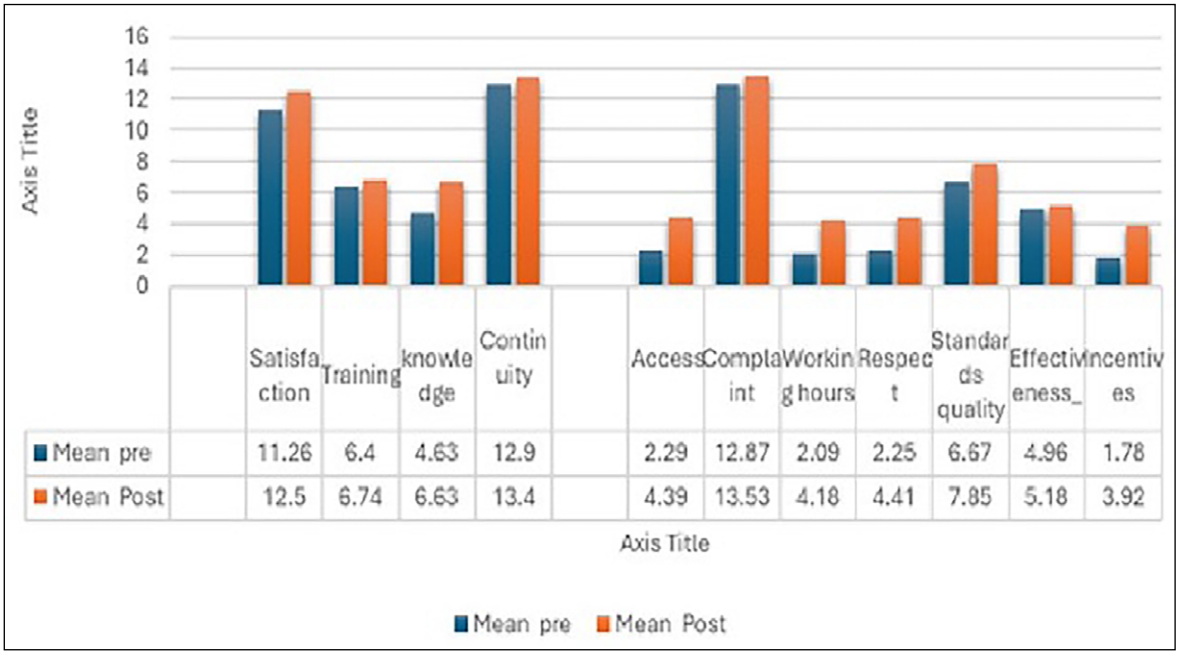

Fig. 3. Comparison among target participant in pre / post assessment of subdomains of knowledge of home health care quality standards scale (N=106).

Media, videos, and brochures were incorporated into the sessions. Participants received brochures containing training materials, and each session was followed by a summary of the skills learned. Topics covered in the program included the history of home health care, its role in managing relapse and readmission of mentally ill patients, communication skills with families and patients, assessing mental status, psychosocial and environmental hazards, patient rights, and maintaining safety.

Closing and Post-Assessment Measurement: After the intervention, participants completed a post-test to evaluate the effectiveness of the program using the same research instruments.

DATA MANAGEMENT AND ANALYSIS PLAN

The data analysis employed SPSS version 24. Descriptive statistics, including mean and standard deviation, summarized inventory and scale variables. A paired t-test assessed score changes before and after interventions, evaluating the interventions' impact. Cohen's d effect sizes contextualized observed differences. Additionally, ANOVA was conducted to examine score variations across demographic subgroups, with post-hoc tests identifying significant differences. Pearson's correlation was used to explore relationships between scores and demographic traits, with statistical significance set at $P < 0.05$. Effect sizes provided further insight into the magnitude of the findings.

Table 2. Comparison between knowledge about the standard quality of HHC and the perceived devalued attitude towards mental illness among study participants before / after the evaluation (N=106)

variable	N	M±SD	Paired Differences	Std. Error Mean	95% Confidence Interval of the Difference		t-test	One side pair	2-sided pair
					Lower	Upper			
Pre PPD	106	24.54±2.9	-.63±4.5	.446	-1.5	.252	-1.4	.080	.159
Post PPD	106	25.17±3.45							
Pre knowledge	106	2.31±1.00	-1.04±1.28	0.125	-1.29	-0.800	-8.4	<0.001	<0.001
Post K	106	3.36±.938							

Table 3. Correlation between before and after perceived devaluation and discrimination scale and knowledge of the quality of home health care standards among study participants (N= 106)

Correlations					
		Pre-Pdd	post_pdd	PRE_HHCQ	POST_HCCQ
pre perceived devaluation	Pearson Correlation	1	-.016	.283**	-.161
	Sig. (2-tailed)		.869	.003	.099
	N	106	106	106	106
post perceived devaluation	Pearson Correlation	-.016	1	.072	.060
	Sig. (2-tailed)	.869		.464	.544
	N	106	106	106	106
PRE knowledge HHCQ	Pearson Correlation	.283**	.072	1	-.097
	Sig. (2-tailed)	.003	.464		.321
	N	106	106	107	106
POST knowledge HCCQ	Pearson Correlation	-.161	.060	-.097	1
	Sig. (2-tailed)	.099	.544	.321	
	N	106	106	106	106

Notes: ** – the correlation is significant at the 0.01 level (2-tailed).

RESULTS

The sociodemographic profile of individuals working in home health care (HHC) services is summarized in Table 1. The data shows that most participants are female, accounting for 95.9% of the sample, with males being less represented. Most participants are under 25 years old, with the largest percentage falling within the 36 to 45 age group. In terms of education, the majority hold bachelor's degrees (77.0%), followed by those with a Diploma (20.3%). Individuals with master's or Ph.D. degrees are fewer in number. Regarding experience in HHC, a significant percentage have no prior experience (66.2%), while experienced individuals typically have over 10 years in the field. Attendance at previous HHC workshops is evenly split between attendees and non-attendees. Reasons for missed visits include issues such as poor organization (31.1%), lack of an integrated team (17.6%), and other factors like a busy medical team and transportation challenges. The majority strongly believe that HHC visits have a positive impact on reducing readmissions and relapses (95.9%). In terms of safety, most participants report not experiencing harm or assault during HHC visits (89.2%), although a notable percentage (10.8%) have encountered such incidents. Additionally, a significant proportion (20.3%) of respondents admit to missing patient visits.

The bar graph shows the differences in incorrect responses regarding basic knowledge of home health care before and after assessments (Fig.2). Significant variations are evident in key knowledge areas such as team members (28 before, 82 after), daily patient visits (15 before, 70 after), average visit count (59 before, 71 after), correct timing for patient visits (97 before, 106 after), and type of transportation (36 before, 70 after).

Table 2 compares the scores of participants before and after assessments regarding their understanding of the standard quality of home health care (HHC) and their attitude towards mental illnesses (Tabl.2). The mean score for perceived devalued attitude (pre-PPD) in the pre-assessment is 24.54±2.9, which slightly decreases to 25.17±3.45 in the post-assessment (post-

Table 4. Correlation between Demographic Background with Perceived Discrimination and Devaluation Scale (PDD)

		Pre-assessment			Post-assessment		
		Mean Square	F	Sig.	Mean Square	F	Sig.
Gender	Between Groups	.253	1.229	.266	.318	1.650	.069
	Within Groups	.206			.193		
Age	Between Groups	1.063	1.201	.286	.931	1.027	.438
	Within Groups	.886			.907		
Qualifications	Between Groups	.540	2.157	.014	.365	1.315	.202
	Within Groups	.250			.277		
Experience years	Between Groups	1.971	1.290	.225	1.989	1.314	.203
	Within Groups	1.527			1.513		
Previous workshop	Between Groups	.377	1.657	.075	.228	.902	.573
	Within Groups	.228			.253		
Choice of services	Between Groups	2.267	1.247	.253	1.565	.805	.683
	Within Groups	1.817			1.943		
Effect relapses	Between Groups	.060	1.385	.172	.053	1.215	.270
	Within Groups	.043			.044		
Exposed to assault	Between Groups	.132	.963	.500	.235	2.012	.019
	Within Groups	.137			.117		
Describe the assault	Between Groups	.263	1.100	.368	.307	1.330	.194
	Within Groups	.240			.231		
Missing visit	Between Groups	.194	1.045	.418	.244	1.389	.161
	Within Groups	.186			.176		
Reason for missing the visit	Between Groups	1.889	.607	.862	2.628	.877	.602
	Within Groups	3.110			2.995		
Accreditation	Between Groups	.090	.484	.943	.177	1.042	.423
	Within Groups	.185			.170		

PPD). However, this difference is not statistically significant (t (105) = -1.4, p = 0.080). In contrast, there is a significant improvement in knowledge scores, with participants scoring 2.31±1.00 in the pre-assessment (pre-knowledge) and 3.36±0.938 in the post-assessment (post-knowledge). The paired difference is -1.04±1.28 (t (105) = -8.4, p < 0.001).

The bar graph compares average scores for pre- and post-assessments in different subdomains of the Knowledge of Home Health Care Quality Standards Scale (Fig.3). Overall, there was an improvement in all subdomains after the evaluation, with higher average scores in the post-assessment. The satisfaction subdomain consistently had the highest average scores, indicating a high level of participant satisfaction with home healthcare quality standards (M = 11.26 in pre-assessment, increasing to 12.5 in the post-assessment). Conversely, the Access/Complaint subdomain consistently had the lowest average scores, suggesting limited participant access or awareness of complaint mechanisms (2.29 in pre-assessment, rising to 4.39 after intervention). The most significant difference between pre- and post-assessments was observed in the effectiveness/incentive subdomain (4.96 in pre-assessment compared to 5.18 in the post; 1.78 in pre-assessment compared to 3.92 in the post), indicating a substantial increase in knowledge in this area.

Table 3 shows the Pearson correlation coefficients between variables: pre-assessment perceived devaluation (Pre PDD), post-assessment perceived devaluation (Post PDD), pre-assessment knowledge HHCQ (PRE_HHCQ), and post-assessment knowledge HCCQ (POST_HCCQ). A positive correlation exists between pre-assessment perceived devaluation and pre-assessment knowledge HHCQ (r = 0.283, p = 0.003), indicating higher devaluation is linked to greater pre-assessment knowledge. However, a negative correlation is seen between pre-assessment perceived devaluation and post-assessment knowledge HCCQ (r = -0.161, p = 0.099), suggesting that as devaluation increases, post-assessment knowledge tends to decrease, though not significantly at the 0.05

Table 5. Correlation between Demographic Background with Home Healthcare Quality Dimensions Scale

		Pre-assessment HHQD			Post-assessment HHQD		
		Mean Square	**F**	**Sig.**	**Mean Square**	**F**	**Sig.**
Gender	Between Groups	.489	2.460	.038	0.265	1.426	.103
	Within Groups	.199			0.186		
Age	Between Groups	3.152	3.945	.003	1.258	1.722	.027
	Within Groups	.799			0.73		
Qualifications	Between Groups	.085	.281	.923	0.353	1.362	.135
	Within Groups	.302			0.259		
Experience years	Between Groups	2.857	1.871	.106	2.000	1.453	.092
	Within Groups	1.527			1.377		
Previous workshop	Between Groups	.534	2.275	.053	0.226	0.865	.678
	Within Groups	.235			0.261		
Choice of services	Between Groups	5.687	3.363	.008	1.720	0.875	.664
	Within Groups	1.691			1.966		
Effect relapses	Between Groups	.066	1.496	.198	0.056	1.404	.133
	Within Groups	.044			0.040		
Exposed to assault	Between Groups	.178	1.332	.257	0.203	2.001	.007
	Within Groups	.134			0.101		
Describe the assault	Between Groups	.330	1.383	.237	0.277	1.229	.229
	Within Groups	.239			0.225		
Missing visit	Between Groups	.262	1.430	.220	0.163	.820	.739
	Within Groups	.183			0.199		
Reason for missing the visit	Between Groups	1.801	.602	.699	2.45	.771	.801
	Within Groups	2.992			3.18		
Accreditation	Between Groups	.209	1.229	.301	0.173	1.015	.468
	Within Groups	.170			0.171		

level. There are no significant correlations between post-assessment perceived devaluation and knowledge assessments (Table 3).

Table 4 displays the results of an analysis of variance (ANOVA) comparing pre-assessment and post-assessment data for various variables. In the pre-assessment, a significant difference in group means was observed based on qualifications ($p = 0.014$), but not on factors such as sex, age, years of experience, previous workshop attendance, choice of services, effect of relapses, exposure to assault, description of the assault, missing visit occurrences, reasons for missing visits, or accreditation (Table 4).

In the post-assessment, a significant difference in group means was found based on exposure to assault ($p = 0.019$), while factors like gender, age, qualifications, years of experience, previous workshop attendance, choice of services, effect of relapses, description of the assault, missing visit occurrences, reasons for missing visits, or accreditation did not show significant differences.

Furthermore, no statistically significant interactions were observed between any of these factors in the pre-assessment or post-assessment.

Table 5 shows correlations between demographic variables and Home Healthcare Quality Dimensions scores before and after assessment. Gender was correlated with pre-assessment scores but not post-assessment. Age was correlated in both phases. Service choice was correlated in the pre-assessment only. Exposure to assault was correlated with post-assessment scores. Other variables did not show significant correlations with HHQD scores (Table 5).

DISCUSSION

Home healthcare services in Saudi Arabia are expanding due to the aging population and rising chronic mental health disorders, though quality varies across institutions. This study aimed to evaluate nurses' knowledge and attitudes toward home healthcare services, partic-

ularly for managing psychiatric patients, and to explore factors that could help reduce relapse rates and hospital readmissions. The intervention program showed improvements in nurses' knowledge and attitudes, which could lead to better patient care and increased safety. This finding aligns with previous studies that highlight the challenges and skills needed by nurses in home healthcare settings. For instance, Andersson et al. [18] emphasized the need for nurses to be attentive, flexible, and continually supported in home care environments. However, Matarazzo et al., [19], found that intensive home therapy did not significantly reduce hospital days for psychiatric patients, while some patients preferred hospitalization. This suggests that there are limitations in the implementation of home-based psychiatric care, including resource constraints and lack of coordination, as noted by study [20]. Cornelis et al. [21] examined the impact of intensive home therapy (IHT) versus standard care for patients in severe psychiatric crises, finding that some patients preferred hospitalization and that IHT did not significantly reduce hospital days. Siqeca et al. [22] conducted a study on the feasibility of home-based psychiatric palliative care, reporting that some patients and families opted out due to resource limitations, lack of coordination, and teamwork issues among providers. In this study, there was no notable shift in nurses' perceptions pre- and post-assessment. Similarly, Pintar Babi et al. [23] found that nurses' caring and positive attitudes toward young adults with non-suicidal self-injury (NSSI) remained consistent across various settings and educational backgrounds. The study further explored the complex interplay between knowledge and perceived devaluation. A positive correlation was observed between pre-assessment knowledge and perceived devaluation, which changed to a negative correlation post-assessment, indicating that knowledge may diminish as perceived devaluation increases. Additionally, demographic factors influenced perceived discrimination and devaluation scores (PDD). Post-assessment results aligned with McRae et al.'s [24] findings, showing a significant association between PDD scores and exposure to assault among mental health practitioners, suggesting that experiences of assault heighten perceived discrimination. Arsat et al. [25] noted that qualifications were initially linked to PDD scores, indicating that educational background initially influences these perceptions. Certain demographic factors do not show a statistically significant relationship with PDD scores. Gender, as per Hadera et al. [26], does not show any significant correlation, while Mora-Ros & Ortega-Ortega [27] also find no significant correlation between age and gender. In contrast to previous findings, the importance of qualifications diminishes in the post-assessment period [28]. Additionally, there is no noticeable relationship between accreditation status, years of experience, missed visits, and PDD scores. These findings suggest that although some criteria are important, factors such as gender, age, experience, attendance, and status may not have a major influence on how people perceive prejudice in mental health settings. This systematic approach contributes to a more nuanced understanding of the variables influencing perceptions of discrimination and devaluation by highlighting key findings and distinguishing between variables with significant and nonsignificant correlations. Table 5 shows that age has been identified as a reliable indicator of how people perceive the quality of home healthcare. Significant correlations were observed during both the pre-assessment and post-assessment, aligning with previous research that emphasized the influence of age on perceptions of healthcare services [29, 30]. The results of the pre-assessment indicate a strong connection between initial expectations and service selection. However, the post-assessment results show a weaker correlation, suggesting that the initial impressions may change based on actual care experiences. The post-assessment results demonstrate a significant relationship between perceived treatment quality and exposure to assault, which is consistent with research indicating that safety concerns negatively impact patient satisfaction and perceived care quality [30]. However, other factors, such as gender, experience, and missed visits, showed no significant relationship with perceived devaluation or quality of care, indicating that these variables may not play a critical role in shaping nurses' perceptions of home healthcare services [31]. In conclusion, the study underscores the importance of focusing on age-related factors and safety concerns in home healthcare service delivery while acknowledging the complexities of attitude and knowledge changes in nursing practice. Future research should continue to explore these relationships to better understand and improve home healthcare for psychiatric patients.

LIMITATIONS OF THE STUDY

The study highlights the advantages of home health care (HHC) for patients with mental illness and the role of nurses in delivering this care. However, there are limitations to consider. The sample size is small compared to the total number of HHC nurses, potentially not fully capturing the views of the broader nursing community. Relying on self-reported data may introduce bias, and the absence of a qualitative component limits the depth of information on nurses' attitudes and challenges.

CONCLUSIONS

The study indicates a significant improvement in nurses' knowledge of HHC basics and quality standards following the intervention program. This highlights the potential for targeted interventions to enhance nurses' understanding of essential HHC components. The results also suggest a relationship between perceived devaluation and knowledge of HHCQ before the intervention, though the latter is not statistically significant. These findings emphasize the complex interplay between attitudes and knowledge, necessitating further investigation to develop effective strategies for reducing relapses and readmissions among psychiatric patients. Tailoring interventions to nurses' qualifications, age, and exposure to assault may enhance their knowledge and attitudes toward HHCQ. Additional research is required to identify optimal methods for implementing and evaluating HHC for mental health patients and address any existing challenges within the current framework.

RECOMMENDATIONS

- Develop customized training programs to enhance the quality of home health care services provided by nurses in Saudi Arabia.
- Incorporate trauma-informed care training into nursing education and professional development programs.
- Provide ongoing professional development opportunities for nurses, focusing on home health care quality.
- Offer workshops, seminars, and training sessions to keep nurses updated on best practices in mental health care and home services.
- Monitor and address knowledge gaps through regular assessments and targeted support.

NURSING IMPLICATIONS

- Promote trauma-informed nursing practices and support nurses in navigating emotional challenges related to assault exposure.
- Encourage team collaboration for comprehensive patient care and advocate for lifelong learning and professional development among nurses.
- Facilitate knowledge sharing among nurses with different qualifications and experience levels.
- Implement individualized care plans based on patients' unique needs and preferences.

BROADER HEALTH POLICY IMPLICATIONS

- To improve patient outcomes and reduce relapse and readmission rates, healthcare policies should integrate mental health services into home healthcare (HHC) by equipping providers to address both mental and physical health needs.
- Policies should mandate continuous training in mental health care, interpersonal skills, and psychosocial assessment to improve provider-patient interactions.
- Emphasizing cultural competence in healthcare education ensures providers are responsive to diverse backgrounds, especially in HHC.
- Community-centered care models focused on prevention and early intervention should be prioritized to prepare local providers for managing chronic conditions at home.
- Policymakers should also implement systems to track and evaluate the impact of home-based care and training programs, supporting policy enhancements and achieving optimal outcomes.

ETHICAL CONSIDERATION

The study received approval from the research unit at the College of Nursing, Jeddah, followed by official approval from KAIMRC and IRB (IRB/1640/23). The approval letter was then reviewed by the Ministry of Health to obtain their consent for conducting the study at the Erada complex for mental illness and addiction. Subsequently, permission was granted by the hospital manager to involve nurses in the study. The study participants were contacted to discuss the objectives and methodology. They were informed of their right to withdraw from the study at any time without facing any consequences, and that their participation was voluntary. Participants were assured that their responses would remain anonymous, and that the information provided would be treated as confidential in both the NGHA office and the archives.

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All participants provided written informed consent after receiving a thorough explanation of the study objectives and procedures. No adverse effects or unintended consequences were reported during the study.
Further data is available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Psychological and social aspects of the life of patients with periodontal and maxillofacial diseases

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ABSTRACT

Aim: To analyze the impact of concurrent periodontal and maxillofacial diseases on the mental state and quality of life of patients aged 18–60 years for future comprehensive rehabilitation.

Materials and Methods: A total of 61 patients (aged 28-60 years) with periodontal diseases and concomitant maxillofacial disorders were studied. All patients had a dental examination, accompanied by assessments of anxiety, depression, and self-esteem with the Hospital Anxiety and Depression Scale (HADS), the Dembo-Rubinstein self-assessment method, and the Chaban Quality of Life Scale (CQLS).

Results: The analysis of the HADS A score revealed that 31.1% of patients had a clinical level of anxiety, whereas 41% of patients had a subclinical level of anxiety. Based on the HADS D score, 21.3% of patients had a clinical level of depression, whereas 50.8% had a subclinical level of depression. The aspects of appearance, health, and confidence are highly correlated with patients' mental well-being. The quality of life assessment indicates that the majority of patients with concurrent periodontal and maxillofacial diseases experience a low or moderate quality of life.

Conclusions: Patients with periodontal and maxillofacial diseases require not only dental care but also comprehensive rehabilitation, since appearance and physical health have a major impact on their quality of life.

KEY WORDS: Generalized periodontitis, diseases of the maxillofacial region, emotional state, quality of life, social adaptation, self-perception

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INTRODUCTION

Oral health, being an integral component of overall health, undoubtedly influences an individual's well-being and quality of life [1]. The correlation between periodontitis and oral health-related quality of life (OHQoL) has been shown. D. Kutsal et al. (2021) investigated the impact of periodontitis on OHQoL using the Oral and Dental Health-Related Quality of Life Scale in the UK (OHRQoL-UK). All four indicators were reduced in patients with periodontitis compared to healthy individuals [2]. The relationship between self-reported periodontitis assessed with the newly created modified periodontal screening scale (mPESS) and OHRQol was studied. mPESS ≥5 demonstrates the highest specificity (79.5%) and sensitivity (71.3%) for the identification of individuals with severe periodontitis. OHRQoL was assessed using the Oral Health Impact Profile (OHIP-14). The association between mPESS ≥5 and deteriorating OHRQoL was validated. Furthermore, mPESS ≥5 was independently correlated with a 3.4-fold increase in the probability of deteriorating OHRQoL [3].

The OHIP-14 is one of the most widely-used OHRQoL assessment questionnaires [4]. It is a shortened version of the OHIP-49 that assesses how various aspects of oral health affect a person's physical, mental, and social well-being [5]. The OHIP-14 measures functional limitation, physical pain, psychological discomfort, physical disability, psychological disability, and social disability using 14 self-report items grouped into 7 domains. The cumulative OHIP-14 score ranges from 0 (best OHRQoL) to 56 (worst OHRQoL) and is calculated by summing the values of the 14 items. Higher OHIP-14 scores indicate worse OHRQol, whereas lower OHIP-14 scores suggest better OHRQol [3]. Researchers have demonstrated a significant relationship between OHRQoL, the stage of periodontitis, and symptoms such as bleeding gums, gum pain, gum swelling, bad breath, and loose teeth [6].

Maxillofacial injuries have garnered the attention of the global medical community due to their increasing incidence and variety of associated injuries, as well as their correlation with appearance, loss of function, economic consequences,

and postoperative quality of life issues. These problems can negatively impact a person's overall well-being, and if not detected and treated, they can persist for a long time. There are several methods for measuring patients' well-being and QOL. P. S. Klonoff et al. developed the Profile of the Impact of the Disease [7]. K. A. Atchison and T. A. Dolan presented the General Oral Health Assessment Index [8]. C. R. Webb et al. described the Life Satisfaction Index [9]. M. Findler et al. constructed the Short Form 36 (SF-36) [10].

The WHOQOL-100 test and its shortened version, WHOQOL-BREF, which may be used in a variety of situations, are the most commonly used tests. The WHOQOL-BREF comprises 26 items, each rated on a five-point scale. The WHOQOL-BREF combines dimensions 1 and 3 as well as 2 and 6 of the WHOQOL-100 to develop an instrument for assessing: (1) physical health, (2) mental issues, (3) social relationships, and (4) the environment. The WHOQOL-BREF is popular because it is brief, reducing patient burden and making it easier to use together with other measures [11].

Research indicates that oral maxillofacial disorders and malocclusion can result in numerous mental health issues, including increased social anxiety and social avoidance, along with a decline in quality of life. This necessitates the implementation of standardized and reliable psychological and clinical methods for assessing treatment outcomes [12]. In addition to other methodological issues, they emphasize the inconsistency of psychometric scales and the constructs employed, the inadequate validity and reliability of many scales, the limited sample size, and the absence of a subcategory for abnormalities [13].

The Derriford Appearance Scale [14] and the Psychological Impact Assessment of Dental Esthetics Questionnaire (PIDAQ) [15] are justified for use in patients with craniofacial abnormalities since they were specifically designed to assess psychological adaptability in people with apparent changes in appearance. The Pain DETECT Questionnaire (PD-Q) was used in the screening phase to measure the relationship between pain, mood disorders (anxiety, depression, and sleep disorders), and quality of life in order to better understand the most relevant predictors of pain perception [16].

L. Piedra-Hernández et al. (2023) examined the incidence of dental anxiety (DA) and OHRQOL before and after nonsurgical periodontal therapy (NSPT) and found a relationship between the two indicators. The use of specific questionnaires for DA and OHRQoL may be more appropriate for demonstrating psychological and quality of life differences caused by periodontitis and NSPT [17].

AIM

The aim of the study was to analyze the impact of concurrent periodontal and maxillofacial diseases on the mental state and quality of life of patients aged 18–60 years for future comprehensive rehabilitation.

MATERIALS AND METHODS

A total of 61 patients (aged 28-60 years) with concurrent periodontal and maxillofacial diseases participated in the study: 36 men and 25 women. All patients had a dental examination that included thorough periodontal probing and radiographic assessment. The patients were tested for anxiety, depression, and self-esteem with modern techniques, namely the Hospital Anxiety and Depression Scale (HADS), the Dembo-Rubinstein self-assessment method, and the Chaban Quality of Life Scale (CQLS). The findings will help determine key aspects of psychological rehabilitation, including self-esteem, anxiety, depression, and patients' social adaptation. The HADS technique facilitates the assessment of anxiety levels (HADS A) and depression levels (HADS D). This is crucial for patients with concurrent periodontal and maxillofacial diseases, as they frequently endure psychological pressure caused by their appearance, physical limitations, and challenges in social interactions.

Data were analyzed with the statistical package IBM SPSS Statistics Base (version 22) and EZR. All results were considered statistically significant at a value of $p<0.05$.

RESULTS

A wide range of clinical conditions influenced the research group's complaints. The most common responses were about refusing to smile because they were embarrassed about the swelling of the gums' mucous membrane, its cyanoticity, visible dental deposits that were difficult to remove due to pain during dental hygiene, visible gum bleeding, or, for example, numerous gum recessions. Patients reported difficulty eating, chewing, and biting solid foods, as well as controlling their distance when communicating, due to bad breath, which might have been induced by therapy-related factors such as splinting in certain cases. Patients with generalized periodontitis II B and inflammatory maxillofacial pathology had a different set of symptoms than those with periodontal disease associated with secondary occlusive trauma, bite collapse, an incomplete number of opposing tooth pairs, or a combination of maxillofacial trauma and temporary external defects in the maxillofacial region. According to the HADS A score analysis, 31.1% of patients had clinical anxiety (severe anxiety requiring therapy), 41% had subclinical anxiety (mild or moderate anxiety that may require some medical attention), and 27.9% had no anxiety. According to the HADS D score analysis, 21.3% of patients had clinical depression (treatment required), 50.8% had subclinical depression (moderate symptoms that might exacerbate the overall state), and 27.9% did not have de-

Table 1. Statistical analysis of anxiety/depression severity by gender

Method	Gender (%)		Statistical probability
	m	f	(Chi-square test)
HADS A			0.482
No reliable signs of anxiety	33.3%	20.0%	
Subclinical level of anxiety	36.1%	48.0%	
Clinical level of anxiety	30.6%	32.0%	
HADS D			0.398
No reliable signs of depression	33.3%	20.0%	
Subclinical level of depression	50.0%	52.0%	
Clinical level of depression	16.7%	28.0%	

pression. More than 30% of patients had clinical anxiety, and more than 20% had clinical depression, demonstrating the significant psychological impact of concurrent generalized periodontal and maxillofacial diseases. There is no statistically significant difference between men and women ($p > 0.1$). The findings indicate that both men and women need psychological assistance to overcome anxiety and depressive disorders (Table 1).

The Dembo-Rubinstein self-assessment method is used to measure various parameters, including personal characteristics, health, confidence, abilities, physical appearance, and interpersonal interactions with family and surroundings.

Self-assessment surveys showed that patients had lower rates in the relevant domains. The average health score for women was 5.28, while for men it was 4.67, suggesting that patients have a poor perception of their health. The personal characteristics score was 5.72 for men and 6.72 for women, indicating that women evaluate their personality traits more positively.

Self-assessment of beauty and appearance is an essential factor. The average score for men was 6.14, while for women it was 5.92, suggesting that patients of both sexes do not perceive their physical appearance as ideal, but the difference in rating between men and women is negligible. Self-assessment results suggest that low self-esteem in periodontitis and maxillofacial diseases impacts patients' overall quality of life. It is also crucial to note that factors of appearance, health, and confidence have a substantial correlation (0.40) with patients' mental well-being. This report highlights the need to work on patients' self-esteem throughout rehabilitation.

The Chaban Quality of Life Scale was used to determine patients' overall level of life satisfaction. According to the findings, 1.6% of patients had a very low quality of life because they were unemployed, lacked friends and support, communicated poorly, and felt socially isolated. 54.1% reported a low quality of life, which might be attributed to communication issues that prevent full involvement in social activities. 29.5% reported a moderate quality of life, with 14.8% rating it as high.

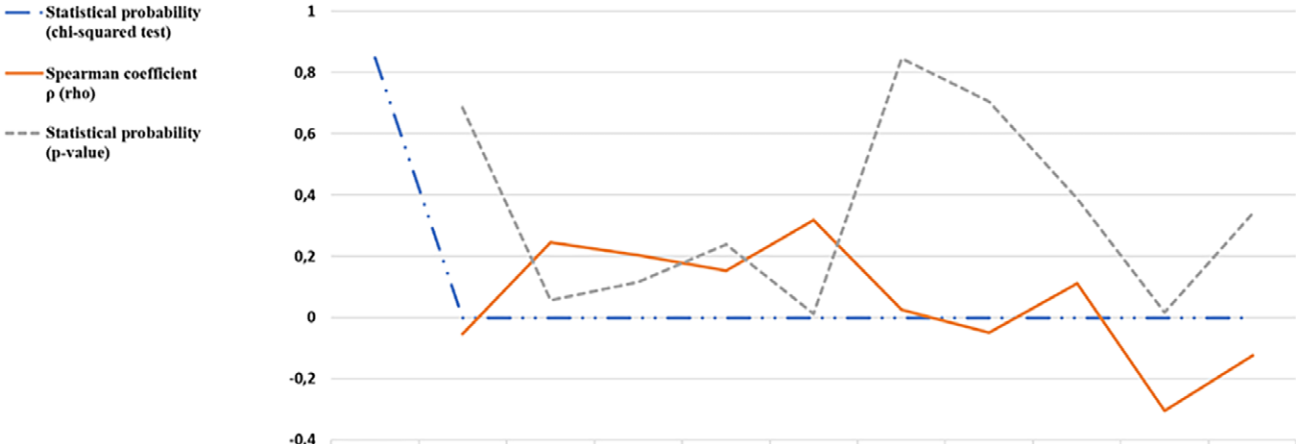
According to the data, the majority of patients with concomitant periodontal and maxillofacial diseases have a low or moderate quality of life. This is due to physical limitations, dental and speech issues, and social challenges. To actively socialize patients with periodontal and maxillofacial diseases and improve their quality of life, physical and psychological rehabilitation must be integrated.

Spearman's correlation coefficient (Fig. 1) was used to examine the relationship between self-esteem, anxiety, depression, and quality of life.

RESULTS OF CORRELATION ANALYSIS

The Dembo-Rubinstein self-assessment scale includes several dimensions, including health, beauty, confidence, relationships, and more factors. The correlation between quality of life levels, as measured by the Chaban scale, indicates the degree to which patients' self-assessment influences their overall quality of life. Individuals who assess their health more positively typically experience a higher quality of life. This implies that physical health significantly influences the perception of overall well-being. Despite almost reaching statistical significance ($p = 0.057$), this indicator substantiates that patients having better overall health often exhibit a superior quality of life (Fig. 1).

Individuals who rate their appearance favourably have a better perception of quality of life. A statistically significant correlation (Spearman's correlation coefficient: 0.319, weak but closer to a moderate correlation; p-value: 0.012, statistically significant correlation) suggests that the self-esteem and overall well-being of patients with periodontal and maxillofacial diseases heavily depend on their appearance. This indicates the need to work with patients on their attitude toward their appearance during psychological rehabilitation.



	Quality of life assessment scale	Nature	Health	Confidence in yourself	Abilities	Beauty, appearance	Relations in the family	Relationships with peers	The level of self-esteem	HADS A	HADS D
Statistical probability (chi-squared test)	0,849										
Spearman coefficient ρ (rho)		-0,053	0,245	0,204	0,153	0,319	0,026	-0,049	0,112	-0,305	-0,125
Statistical probability (p-value)		0,686	0,057	0,115	0,238	0,012	0,845	0,706	0,389	0,017	0,339

Fig. 1. Correlation according to Dembo-Rubinshtein scale; the level of anxiety and depression (HADS) with the level of quality of life according to the quality of life assessment scale (O. Chaban).

There is a weak correlation between confidence and quality of life. It suggests that patients who are more confident may have a slightly better perception of their quality of life, but the difference is not statistically significant enough to make firm conclusions. Patients' data show a statistically significant negative correlation between anxiety level and quality of life (Spearman's correlation coefficient: -0.305, weak negative correlation; p-value: 0.017, statistically significant correlation). It indicates that patients with high levels of anxiety tend to have worse quality of life scores. The correlation is relevant because treating anxiety with psychotherapy can significantly improve a patients' reported quality of life. The scale indicates that personal characteristics have no significant impact on quality of life, as evidenced by the very weak and insignificant correlations (Spearman's correlation coefficient: 0.204, weak correlation; p-value: 0.115, statistically insignificant correlation).

The correlation analysis of patients' self-assessment reveals that appearance and physical health have the biggest influence on their quality of life. This demonstrates the significance of focusing on the perception of one's own appearance and physical condition during rehabilitation. Anxiety also has a big impact on patients' assessments of their quality of life. Therefore, reducing it with psychotherapy treatment can improve patients' emotional states. The findings emphasize the significance of addressing both the physical health and mental health aspects of anxiety in patients with generalized periodontitis and maxillofacial disease.

The study examined gender differences in terms of quality of life and mental health among patients, suggesting the need for differentiated therapeutic techniques for men and women to facilitate a more customized rehabilitation process. We conducted a comparative study using the Dembo-Rubinstein self-assessment method, revealing the factors that could influence the overall self-perception of both men and women. The study identified notable differences between them. Women assessed their personal characteristics more positively than men, with this difference almost reaching statistical significance ($p = 0.056$). This may suggest that women have a more favourable perception of their personal characteristics in comparison to men.

The analysis of the findings indicates that women with periodontitis and maxilofacial diseases assess their health somewhat more positively than men; even so, the difference is not statistically significant. The mean score for men is 4.67. The mean score for women is 5.28. Welch ANOVA statistical probability p-value is 0.243. This could indicate that men tend to be more critical of their physical condition, potentially due to societal norms or stereotypes about male endurance.

The difference in confidence between men and women is negligible and statistically insignificant. The Welch ANOVA statistical probability p-value is 0.833. This suggests that both groups have a comparable level of confidence.

Men with periodontal and maxillofacial diseases evaluate their appearance somewhat more positively than women; nevertheless, the difference is negligible and is not statistically significant. The mean score for males is 6.14. The mean score for women is 5.92. The Welch ANOVA statistical probability p-value is 0.707. This

Table 2. Statistical analysis of the quality of life assessment scale by gender

Method	Group, %		Statistical probability
	m	f	(Chi-square test)
The Chaban Quality of Life Scale			0.849
very low	2.8%	0.0%	
low	52.8%	56.0%	
moderate	30.6%	28.0%	
high	13.9%	16.0%	

may indicate that men are less critical regarding their appearance or prioritize it to a lesser extent.

Analysis of anxiety and depression (HADS A) reveals some gender differences. Women with periodontal and maxillofacial diseases have a bit higher anxiety levels compared to men; however, this difference is not statistically significant. The mean score for men is 8.78. The mean score for women is 9.68. The Welch ANOVA statistical probability p-value is 0.253. This may indicate that women are more predisposed to anxiety, perhaps due to increased emotional sensitivity and a need for external validation, making them more susceptible to stress.

According to the HADS D, women with periodontal and maxillofacial diseases exhibit higher depression levels compared to men; nevertheless, the difference is not statistically significant. The mean score for men is 8.64. The mean score for women is 9.68. The Welch ANOVA statistical probability p-value is 0.175. This may result from women's propensity to express their emotions and focus on internal issues.

The Chaban Quality of Life Scale was administered to both groups. The mean score for men is 12.83. The mean score for women is 12.72. The Welch ANOVA statistical probability p-value is 0.914. The study indicates that the quality of life for both men and women exhibits negligible differences, as evidenced by a significantly high p-value ($p = 0.914$). This suggests that the impact of concurrent periodontitis and maxillofacial diseases on overall quality of life is similarly assessed by both men and women. Patients of both genders have comparable challenges in everyday life, encompassing physical limitations, social barriers, and mental health issues (Table 2).

The analysis of the impact of gender differences on the quality of life of patients revealed that women with periodontal and maxillofacial diseases report higher self-assessments of personal characteristics and health. However, assessments of appearance and confidence are nearly identical between men and women. The women exhibit a somewhat higher level of anxiety and depression compared to the men, albeit without statistical significance. This may suggest that women are more susceptible to anxiety and depressive disorders. Nevertheless, these findings do not significantly impact the overall picture. The quality of life for both men and women is nearly the same, suggesting that both cohorts have comparable challenges associated with their disease.

These findings suggest that the rehabilitation program for patients with periodontal and maxillofacial diseases should acknowledge slight gender differences, particularly in self-assessment and anxiety reduction. However, it is crucial to tailor the therapy strategy universally to meet the overall needs of each gender.

DISCUSSION

V. P. Singh and T. P. Moss assessed patients with craniofacial anomalies using a set of questionnaires, including versions of the DAS and PIDAQ, rated on a Likert scale. There was a significant difference in PIDAQ and DAS59 scores between patients (n=102) and the control group, indicating that patients experienced a more pronounced negative psychological effect on their quality of life regarding appearance (PIDAQ) and greater appearance-related distress (DAS) than the control group [18].

Severe dental anxiety frequently results in poor oral health-related quality of life (OHRQoL). S. D. A. A. Khan et al. (2021) evaluated OHRQoL using a shortened version of the Oral Health Impact Profile (OHIP-14). Researchers found a correlation between dental anxiety, fear, and poor oral hygiene [19].

C. D. Llewellyn and S. Warnakulasuriya aimed to determine the predictive capacity of OHRQoL for anxiety or depression, as well as to investigate the relationship between clinical diagnoses, OHR-QoL, and anxiety or depression. Individuals with dental problems were interviewed face-to-face and assessed using the OHIP-14 for OHRQoL, the Hospital Anxiety and Depression Scale (HADS), and the Visual Analogue Scale for self-reported overall health. Anxiety was observed in 55% of patients, whereas functional limitations and social instability were noted in 54%, according to the OHRQoL domains. Participants had significantly worse OHRQoL scores than the general population across all domains [20].

P. Wiriyakijja et al. (2020) investigated the prevalence of moderate to high levels of comorbid anxiety, depression,

distress, and stress as measured by the HADS and PSS-10 cutoff scores. The HADS and PSS-10 are regarded as reliable measures of psychological distress and stress in patients with aphthous stomatitis [21].

C. Yang et al. (2018) assessed the mental health of patients suffering from oral mucosal diseases such as recurrent canker sores, lichen planus, and burning mouth syndrome using the HADS scale. The OHIP-14 scale was used to assess OHRQoL. That is, the HADS and OHIP-14 scores were employed to examine the relationship between mental health issues and quality of life in these patients. The OHIP-14 and HADS scores were both higher in the study group than in the control group. The OHIP-14 score in recurrent aphthae was the highest among the three patient groups, whereas the OHRQoL was the lowest [22].

Anxiety over anesthesia and surgery is frequent, and many patients perceive it as the worst part of the surgical procedure. L. Eberhart et al. (2020) assessed anxiety with the Amsterdam Preoperative Anxiety and Information Scale (APAIS, range 4-20). The research involved 3,087 patients. 40.5% of patients reported experiencing high levels of anxiety (APAIS >10) [23]. There were statistically significant differences between time points for VAS pain scores, HADS depression scores, SFMP pain scores, and OHIP-14 total scores [24]. The DAI is one of the most comprehensive instruments for assessing the psychometric spectrum of dental anxiety. Although it is believed that patients with the same level of dental anxiety may perceive questionnaire questions differently, the statements formulated in the questionnaire have the same meaning for all patients, regardless of gender, education, or age [25].

CONCLUSIONS

1. According to studies, the majority of patients with periodontal and maxillofacial diseases have clinical and subclinical levels of anxiety and depression due to a variety of complaints such as gingival mucosal swelling, dental deposits, bleeding gums, multiple gingival recessions, secondary occlusal trauma, bite collapse, and an incomplete number of pairs of antagonistic teeth, among others. Periodontitis and maxillofacial diseases have a similar impact on overall quality of life, according to both men and women. Patients of both sexes face comparable everyday challenges, such as physical limitations, social barriers, and mental health concerns.
2. Patients with periodontal and maxillofacial diseases require not only dental care but also comprehensive rehabilitation, since appearance and physical health have a major impact on their quality of life. A high level of anxiety and depression has a substantial effect on the perception of quality of life, necessitating psychotherapeutic support from mental health specialists on one's own appearance and physical condition, which will help stabilize the emotional state, significantly improve patients' quality of life, and promote social integration.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Potential cardioprotective effect of trimetazidine in mice model of endotoxemia: role of AMPK-Nrf2

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ABSTRACT

Aim: To clarify the potential cardioprotective effect of Trimetazidine against experimentally sepsis-caused endotoxic cardiac injury damage in mice.

Materials and Methods: 24 Mice were divided into four groups (n=6): Sham group, CLP group DMSO group, trimetazidine-treated group 50 mg/kg IP, 1hr before CLP, then the animals were sacrificed 24 hr after CLP and tissue sample was taken for measurement of TNF-A, TNF-Ar1, IL-1β, HO-1, MPO, caspase-11, F2-isoprostane and serum troponin by ELISA and gene expression of AMPK-Nrf2 by qpcr and histopathological study.

Results: trimetazidine treated group showed significant changes as compared with clp group regarding TNF-α, TNF-αr1, IL-1β, HO-1, MPO, CASPASE-11, F2-ISOPROSTANE as well as affect tissue mRNA expression of AMPK-Nrf2 genes $p<0.05$.

Conclusions: We evaluate that Trimetazidine has cardio protective effects due to its anti-inflammatory and anti-oxidative action. Also, trimetazidine showed a cardio-protective effect as they affect tissue mRNA expression of AMPK-Nrf2 genes.

KEY WORDS: CLP, sepsis, trimetazidine, TNF-α, TNF-Ar1, IL-1β, Ho-1, MPO, caspase-11, F2-isoprostane

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INTRODUCTION

Sepsis is characterized as a global healthcare concern, stemming from a systemic inflammatory response triggered by bacterial infection [1, 2]. Cardiac impairment stands as a significant outcome of sepsis, contributing to escalated mortality rates. This phenomenon has been linked to heightened inflammation, inhibition of both fatty acid and glucose oxidation, depletion of adenosine triphosphate (ATP), and impairment of the cardiac adrenergic response, which exacerbates cardiac function [3, 4]. In other words, sepsis may decrease cardiac work via a rise expression level of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6, which act as cardio depressant pro-inflammatory mediators resulting in cardiac contractile dysfunction, cardiac hypertrophy, and heart failure [5]. It has been documented findings that there is an increase in the production of these oxidant molecules during sepsis in many cells such as mitochondria and cardiomyocytes [5, 6]. Functioning as a detector of intracellular energy levels, AMPK serves as a crucial focal point for the regulation of inflammation. Emerging insights indicate that activating AMPK can mitigate oxidative stress and counteract inflammation [7]. The mechanistic links between AMPK and inflammation have primarily revolved around their relationship with the NF-κB pathway. Evidence shows that chemical activators of AMPK can diminish NF-κB-mediated transcription and AMPK activation can inhibit NF-κB signaling and consequent inflammation driven by fatty acids in macrophages, even though NF-κB subunits are not direct targets of AMPK [8, 9]. Given the well-established role of the Nrf2 pathway in mitigating oxidative stress and quelling inflammation, the potential for interactions between the Nrf2 and AMPK pathways has been acknowledged [10]. Trimetazidine inhibit β-oxidation of free fatty acid. By selectively inhibiting (LC 3-KAT (Has been found to have anti-apoptosis, anti-inflammatory, anti-oxidative and pleiotropic effects [11].

AIM

The aim of our research is to clarify the potential cardioprotective effect of Trimetazidine against experimentally sepsis-caused endotoxic cardiac injury damage in mice.

MATERIALS AND METHODS

The University of Kufa Department of Pharmacology and Therapeutics was the site of this study. All experiments were approved by Animal Care and Research

committee of the University of Kufa. Animals were housed in the animal house at University of Kufa.

STUDY DESIGN

Twenty-four adult males of Swiss white mice (weighting 20-30 g, aged 4-8 weeks) were purchased from the animal resource center, under conditions of 24°C ± 2°C with alternative 12-hrlight/12-hr dark cycles. The mice were also allowed to a free access to the water and diet until the start of experiment. Mice randomized into 4 groups (n=6): Sham group (laparotomy without CLP), CLP group, DMSO group and trimetazidine treated group (50mg/kg IP, 1hr before CLP, then the animals were sacrificed 24 hr after CLP).

EXPERIMENTAL PROCEDURE

The induction of sepsis was done via the cecal ligation and puncture model (CLP) based on previous studies [7, 8, 12]. In brief, an 18-G needle was employed in conjunction with the double puncture approach to induce organ (cardiac) dysfunction during the early stages of sepsis first 24 hours [9]. A small amount of stool was extracted to ensure the patency of the puncture sites. After this, the abdomen was sutured. All animals have received a subcutaneous resuscitative dose of normal saline (20 mL/kg body weight) to induce organ (cardiac) dysfunction during the early stages of sepsis first 24 hrs.

PREPARATION OF TRIMETAZIDINE

Powder was obtained from MedChem express company, and prepared in diluted DMSO 10%, then was given in a dose of 7.2 mg/kg i.p, 1 hr before CLP [13].

COLLECTION OF A TISSUE SAMPLES

At the end of the procedure (24 hours), mice were re-anesthetized with 20 mg/kg xylazine and 100 mg/kg ketamine. Blood sample was collected immediately from the heart. The blood samples were then centrifuged at 3000 RPM for 10 min. Serum was collected by centrifuging again at 3000 RPM for 1 minute to remove any red blood cells [14, 15]. The heart tissue was rinsed with an ice-cold saline to remove any red blood cells or clots and divided into 3 parts first part was kept at deep freeze for homogenization and ELISA study, second part was kept in RNA later solution at deep freeze until qRT-PCR testing was performed, whereas the remaining portion was formalin fixed until histopathological analysis was performed.

TISSUE HOMOGENIZATION FOR TNF-A, TNFAR, IL-1B, HO-1, CASPASE-11, MPO AND F2-ISOPROSTANE MEASUREMENT

Tissue homogenization technique was performed according to the previous studies [11].

TISSUE PREPARATION FOR HISTOPATHOLOGY

The heart tissue histopathology and scoring were performed according to Zingarelli protocol [16]

EXPRESSION OF AMPK- Nrf2 HEART TISSUE BY QRT-PCR

The mRNA expression of AMPK-Nrf2 is determined using a quantitative real-time PCR, as specified by the manufacturer. Real-time quantitative (RT-q) PCR-total RNA was extracted using special chemicals and instruments [16]. The primer sequences used for qRT-PCR Gene primer sequence is:

AMPKr: 5-GGTCCTGGTGGTTTCTGTTG-3'
AMPKf: 5-CTCTATGCTTTGCTTTGCTGTGTGG-'3
Nrf2r: 5-TGAGAGACTGGTCACACT-3'
Nrf2f: 5-CAGCATGATGGACTTGGA-3'

STATISTICAL ANALYSIS

Statistical analysis was performed using a IBM SPSS 24.0. Data were expressed as mean ± SEM. ANOVA was used for the multiple comparisons among all groups followed by Bonferroni's test. The Mann-Whitney U and Kruskal -Wallis tests were used to assess the histopathological changes, which were determined as, scores from 0 to 4. ($P<0.05$ was considered statistically significant difference).

RESULTS

Effect of trimetazidine on cardiac tissue level of TNF-α, TNFαR, IL-1β, HO-1, caspase-11, MPO and F2-isoprostane, data showed a significant elevation $p<0.05$ in TNF-α, TNFαR, IL-1β, HO-1, caspase-11, MPO and F2-isoprostane, in CLP and DMSO groups when compared with sham group. Additionally, trimetazidine group showed markedly decreased levels of these markers if compared with the CLP & DMSO groups (significant difference, P value <0.05). More interesting, level of serum troponin was significantly higher ($p<0.05$) in sepsis and DMSO groups as compared with the sham group. Additionally, trimetazidine group showed markedly reduced levels ($p<0.05$) of serum troponin when compared with sepsis and DMSO groups (significant difference P value <0.05) (Fig.1-8).

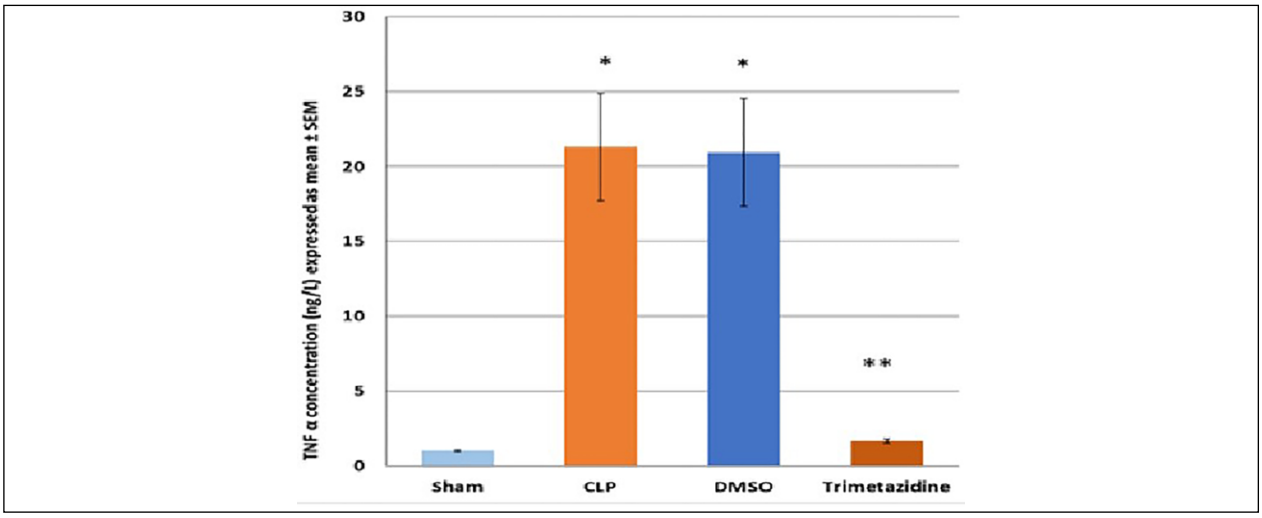

Fig. 1. Mean tissue level of TNF-α (ng/L) in 4 experimental groups 24 hours after sepsis. *: significant difference in CLP & DMSO as compare with the sham group; **: significant difference in pretreated group as compare with CLP group.

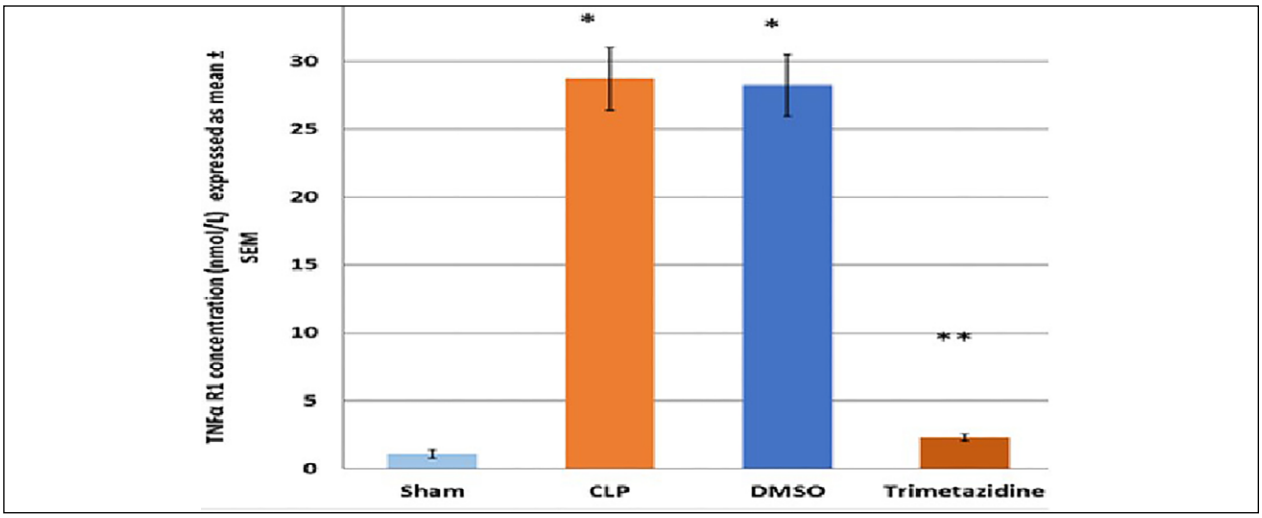

Fig. 2. Mean tissue level of TNF-αR1 (nmol/L) in 4 experimental groups 24 hours after sepsis. *: significant difference in CLP & DMSO as compare with the sham group; **: significant difference in pretreated group as compare with CLP group.

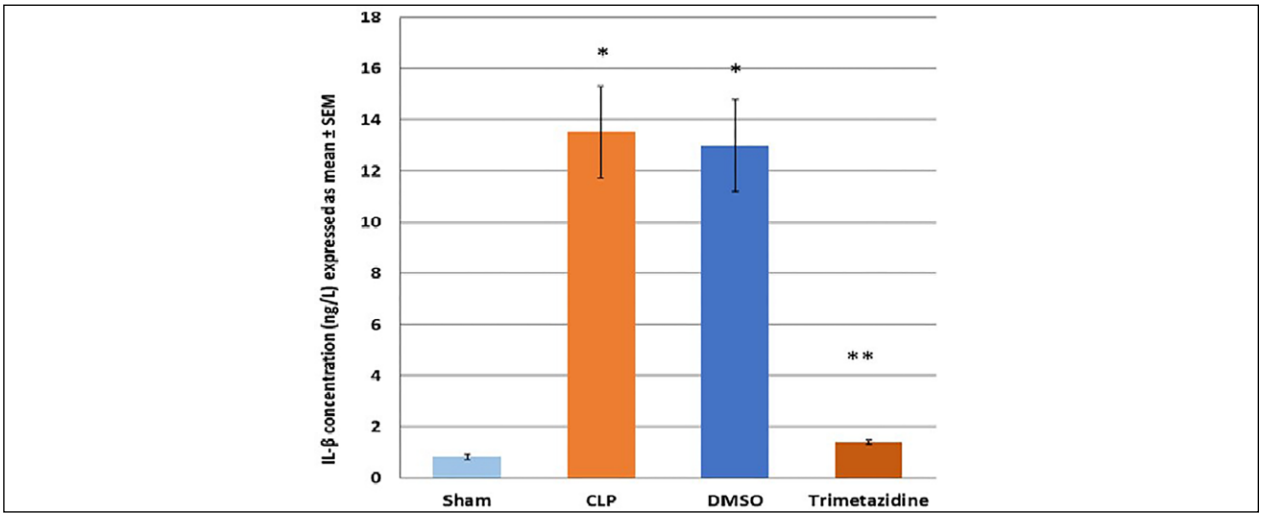

Fig. 3. Mean tissue level of IL- 1β (ng/L) in 4 experimental groups 24 hours after sepsis. *: significant difference in CLP & DMSO as compare with the sham group; **: significant difference in pretreated group as compare with CLP group.

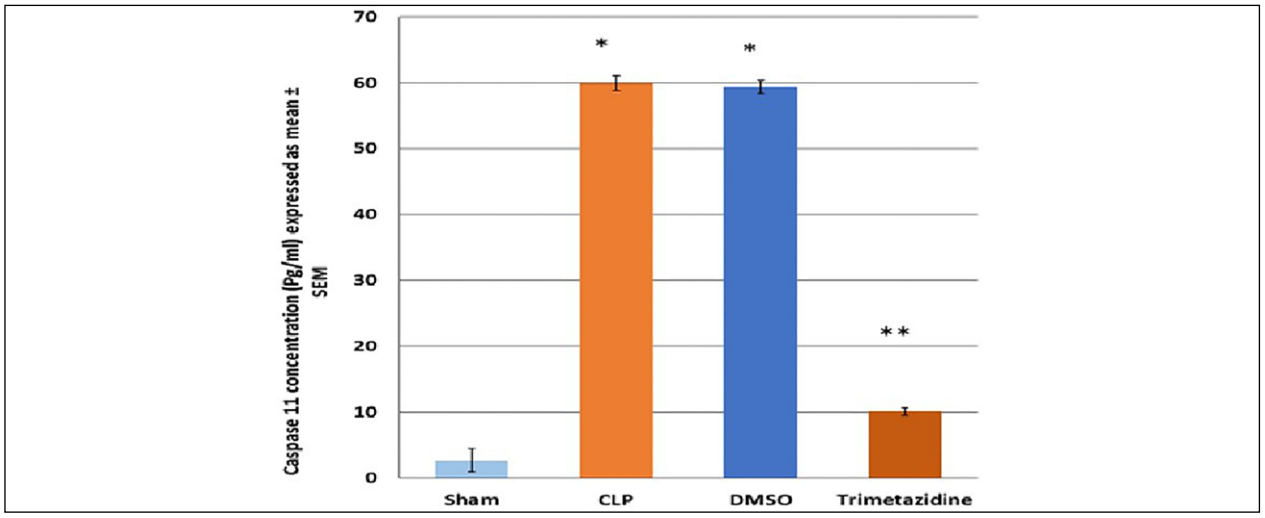

Fig. 4. Mean tissue level of caspase-11 (pg/ml) in 4 experimental groups 24 hours after sepsis. *: significant difference in CLP & DMSO as compare with the sham group; **: significant difference in pretreated group as compare with CLP group.

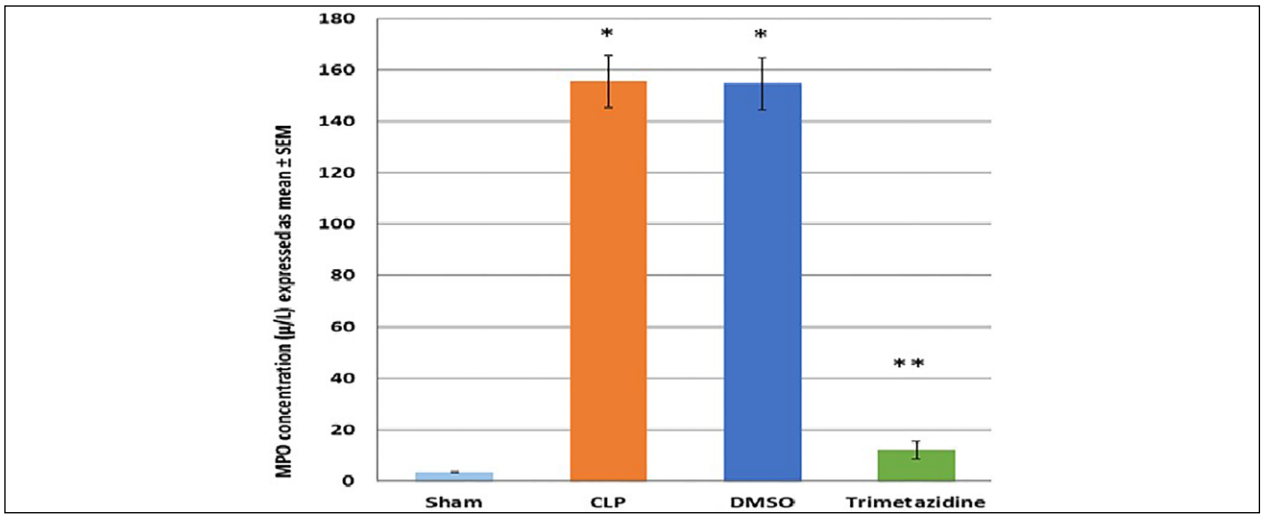

Fig. 5. Mean tissue level of MPO (µ/L) in 4 experimental groups 24 hours after sepsis. *: significant difference in CLP & DMSO as compare with the sham group; **: significant difference in pretreated group as compare with CLP group.

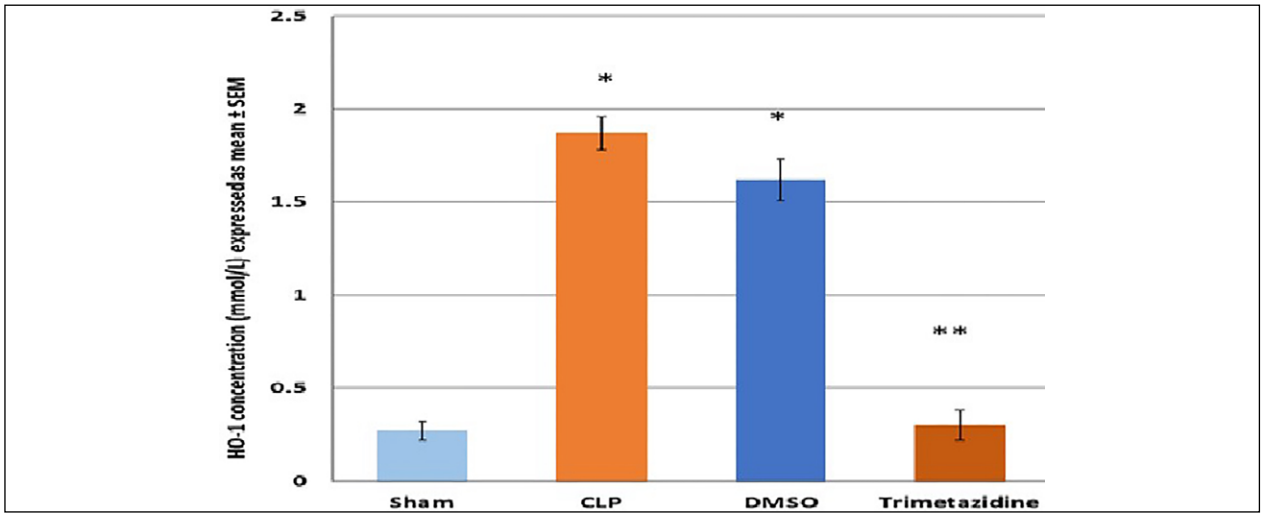

Fig. 6. Mean tissue level of HO-1 (mmol/L) in 4 experimental groups24 hours after sepsis. *: significant difference in CLP & DMSO as compare with the sham group; **: significant difference in pretreated group as compare with CLP group.

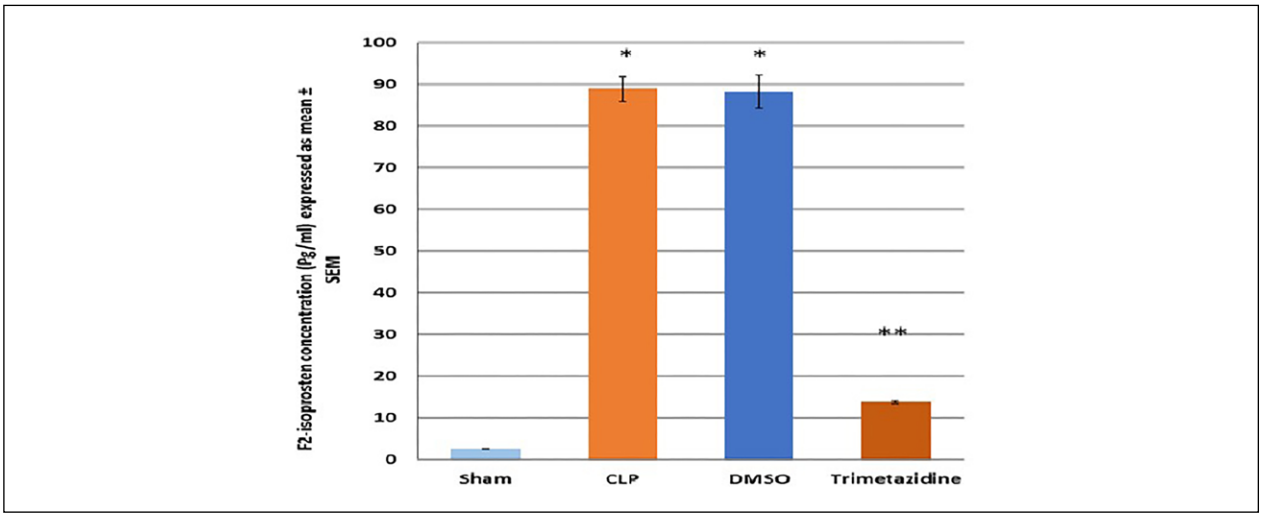

Fig. 7. Mean tissue level of F2- isoprostane (pg./L) in 4 experimental groups 24 hours after sepsis. *: significant difference in CLP & DMSO as compare with the sham group; **: significant difference in pretreated group as compare with CLP group.

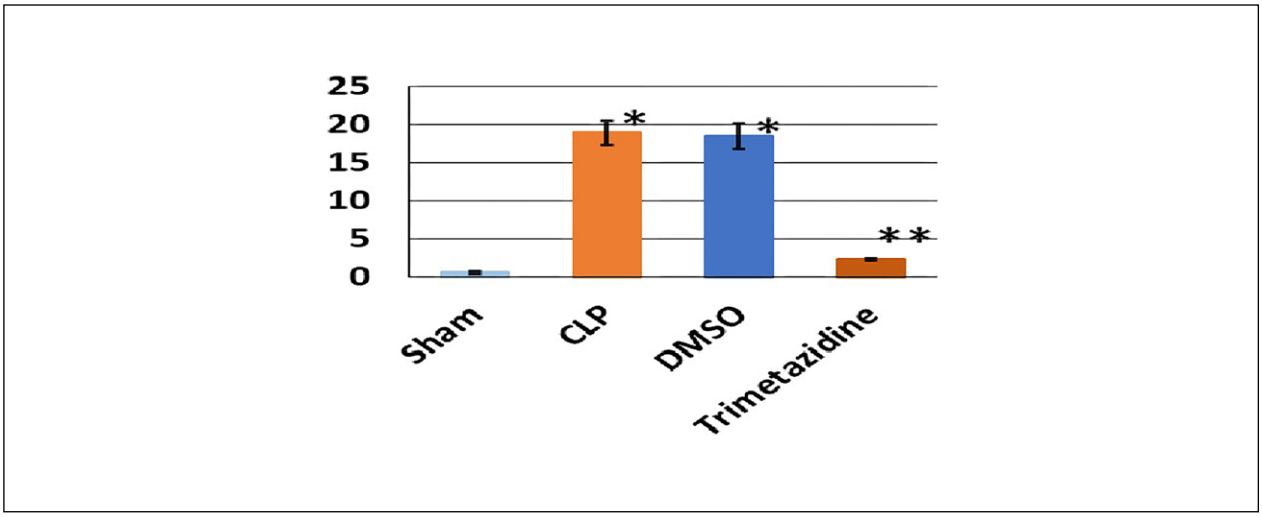

Fig. 8. Mean serum level of troponinn (pg/ml) in 4 experimental groups 24 hours after sepsis. *: significant difference in CLP & DMSO as compare with the sham group; **: significant difference in pretreated group as compare with CLP group.

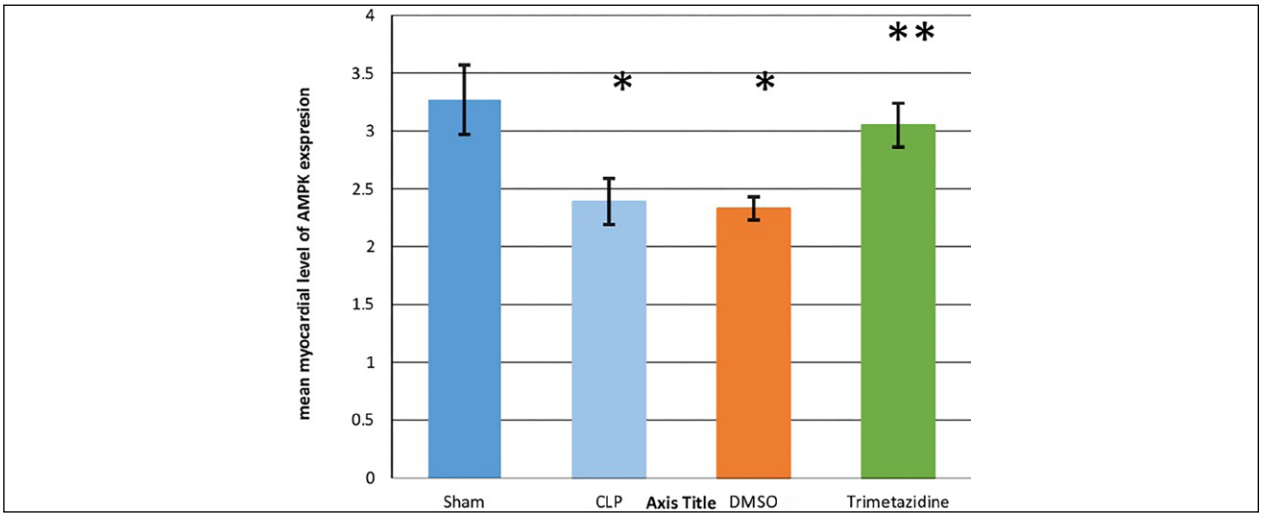

Fig. 9. Mean myocardial level of AMPK expression in the 4 experimental group 24 hours after sepsis. Data are expressed as mean ± SEM.
*: significant difference in CLP & DMSO as compare with the sham group; **: significant difference in pretreated group as compare with CLP group.

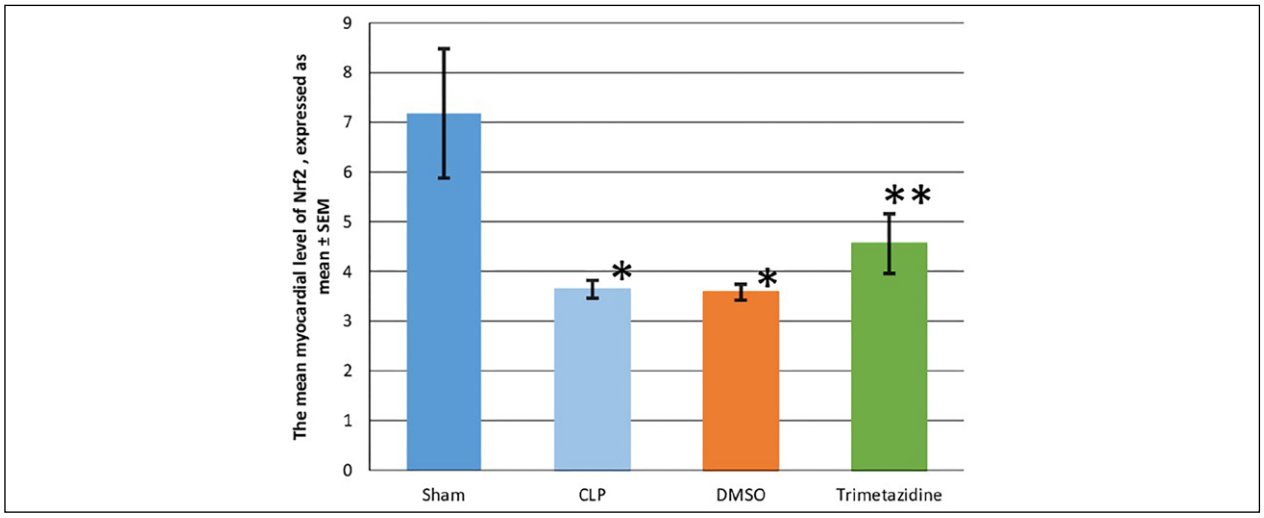

Fig. 10. Mean myocardial level of Nrf2 expression in 4 experimental groups 24 hours after sepsis. Data are expressed as mean ± SEM.
*: significant difference in CLP & DMSO as compare with the sham group; **: significant difference in pretreated group as compare with CLP group.

EFFECT OF TRIMETAZIDINE ON MRNA EXPRESSION OF AMPK-NRF2

Quantitative real-time PCR demonstrated a significant decrement in mRNA expression of AMPK-Nrf2 gene in CLP & DMSO groups when compared with the sham group ($p<0.05$). In comparison to the CLP & DMSO groups, the trimetazidine group had markedly increased levels of AMPK-Nrf2 ($p<0.05$) (Fig. 9-10).

HISTOPATHOTOGICAL FINDINGS

Data showed that CLP caused a significant tissue damage which was represented as scores from 0–4 and characterize a Sham group showing normal cardiomyocyte; CLP& DMSO groups, score 4, show necrotic cardiomyocyte. Trimetazidine group, score 2 show reduction in the tissue damage by reducing the necrosis in the cardiomyocyte.

SHAM GROUP

All animals in this group had normal histopathological findings (Fig.11A)

CECAL LIGATION AND PUNCTURE (CLP) GROUP

Score 4 damaged cardiac tissue (myocardial tissue sections of mice in the CLP group: showed congested blood vessels (black arrow) & extravasation of blood cells (red arrow), H & E, 10X) (Fig.11B).

DMSO GROUP

Score 4 damaged cardiac (tissue myocardial tissue sections of mice in the vehicle DMSO group: showed congested blood vessels (black arrow) & extravasation of blood cells (red arrow), H & E, 10X) (Fig.11C).

TRIMETAZIDINE GROUP

Trimetazidine group histological changes arranged from mild to moderate changes with a different number of mice (myocardial tissue sections of mice in the trimetazidine group: showed, interstitial oedema and focal necrosis (Fig.11D).

DISCUSSION

Sepsis is a critical condition-involving malfunction of organs due to an imbalanced immune response to infection, and it stands as a leading contributor to mortality among hospitalized individuals [17]. Moreover, sepsis is recognized as the foremost factor behind fatalities in intensive care units. Among the significant complications associated with sepsis is myocardial dysfunction, often referred to as sepsis-induced cardiomyopathy or cardiotoxicity, which substantially amplifies the mortality rate [18]. The current study focused on evaluating the prophylactic effects of trimetazidine in order to minimize the cardiotoxicity during polymicrobial sepsis in mice model which was done by cecal we showed that TNF-α, TNFαR, IL-1B, caspase-11, HO-1 MPO, F2-isoprostane levels was significantly elevated in the CLP and DMSO groups as compared with the sham group. This study is compatible with that obtained by Secher and others have highlighted that the TNF signaling pathway plays a central role in activating innate immunity in response to a variety of pathogens. In a polymicrobial sepsis model, evidence suggests a significant impact of TNF-R1 and R2 activation in the disruption of immune responses and the resultant mortality associated with sepsis [17]. This study agrees with a previous study which show that the elevation of TNFα and IL-1β level during the

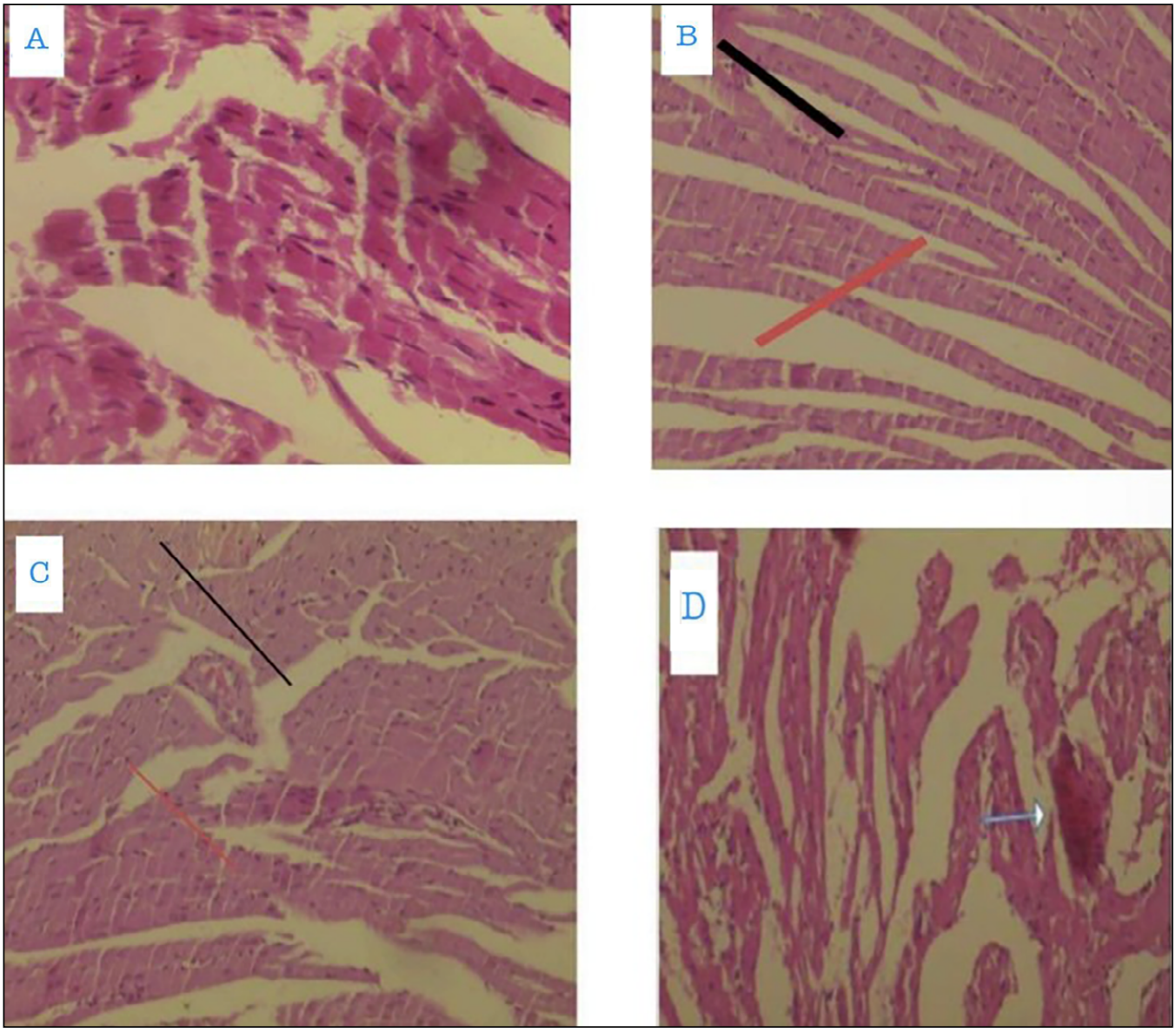

Fig. 11. Myocardial tissue sections of mice: (A) Sham group; (B) CLP group; (C) DMSO group; (D) Trimetazidine group. (H & E, 10X).

inflammatory phase of polymicrobial sepsis can be related to the microbial products activation of inflammasome which in turn will convert the inactive form of IL-1 β converting enzyme (ICE) into the active form that latter one will transform the precursor (pro- IL-1β) into the biologically active form (IL-1β) [19]. The present study reveals that there is a significant decrease in heart level of (IL-1β, TNFα) for trimetazidine pretreated group as compared to control group [20]. Trimetazidine decreased the expression of pro-inflammatory cytokines in cardiac and peritoneal macrophages stimulated by LPS. Trimetazidine-treated macrophage reduced apoptosis in cardiomyocyte that induced by LPS. The anti-apoptosis actions of trimetazidine caused by reduction in pro-inflammatory cytokines, mainly because of normalizing the sirtuins 1 (Sirt1)/ AMPK/Nrf2/ HO-1 and Sirt1/PPARα pathways in macrophages. Cytokine release was also controlled by ROS that were alleviated by trimetazidine through activation of the Sirt1/ AMPK/PPARα [21]. The current investigation demonstrates a substantial reduction in the heart's TNFαR1 levels within the trimetazidine pretreated group, when contrasted with the control group. As far as our current understanding goes, there exists a lack of available data concerning the influence of trimetazidine on TNFαR1 levels in cases of cardiac injury induced by endotoxins. This outcome is likely linked to the antioxidative properties of trimetazidine.

THE EFFEC OF SEPSIS ON CASPASE -11

Study reveals that there is a significant decrease in heart level of caspase- 11 for trimetazidine pretreated group as compared to control group. These findings imply that trimetazidine performs a critical role against cardiac damage. This protection mechanism is mainly due to its anti-inflammatory action by highly protein expression of AMPK.

These data suggest that pretreatment with trimetazidine may decrease apoptosis in the cardiomyocyte. Additionally, trimetazidine may potentiated expression of protein caused to ketogenesis through the activation of AMPK/PPARα signaling pathways [22].

THE EFFECT OF SEPSIS ON MYELOPEROXIDASE

The current investigation reveals that the administration of trimetazidine results in a noteworthy decrease in the levels of MPO within the group treated with trimetazidine, in comparison to the control group. This outcome aligns with findings from prior research suggest that trimetazidine acts main role in decreasing expression of MPO in rat lungs of silica-treated. This effect could potentially be achieved by inhibiting the overproduction of lactate induced by anaerobic glycolysis. Furthermore, the regulation of oxidative stress might contribute to the enhancement of protective effects exerted by trimetazidine [23, 24].

THE EFFECT OF SEPSIS ON THE OXIDATIVE STRESS BIOMARKERS HEME OXYGENASE-1 &F2-ISOPROSTANE

The present study finds out that trimetazidine causes a significant lower tissue level of HO-1 in trimetazidine treated group as compared with the control group. Trimetazidine reduced cardiomyocyte apoptosis and oxidative stress through activation of Nrf2/HO-1 pathway and inhibition of NF- B signaling pathway [23]. The present study finds out that trimetazidine causes a significant lower level of F2- isoprostane in trimetazidine treated group as compared with the control group. data suggested that trimetazidine highly reduced the serum levels of oxidative stress marker F2-isoprostane in patients with stable refractory angina [25].

THE EFFECT OF CLP ON CARDIAC TROPONIN-I

The present study revealed a noteworthy increase in the serum cardiac troponin levels within the CLP and DMSO groups when compared to the sham group. This outcome aligns with the results of a prior study conducted on rabbits to assess the impact of CLP-induced sepsis on cardiac troponin-I levels, where a substantial rise in cardiac troponin-I was detected in the experimental group compared to the control group of rabbits [26]. In this study, trimetazidine exhibited a significant reduction in the serum levels of cardiac troponin-I compared to the control group, indicating the preservation of heart function. This outcome aligns with [7], as their study also indicated a noteworthy decrease in cardiac troponin-I levels within the trimetazidine-treated group Trimetazidine has cytoprotective effect and protected the mitochondria against pressure overload and increased the ATP supply in cardiomyocytes [11].

EFFECT OF ON MRNA EXPRESSION OF AMPK-Nrf2

Our study demonstrated that the mRNA expression of AMPK-Nrf2 was significantly lower in the CLP and DMSO groups as compared with the sham group. This study agrees with a previous study, where they found that AMPK can function as a pivotal upstream target in Nrf2-related processes. Studies suggest that the activation of AMPK can alleviate both inflammation and redox imbalance through the Nrf2 signaling pathway, as demonstrated by Park et al. [27]. In the study conducted by previous study it was proposed that trimetazidine effectively mitigates myocardial impairment induced by CLP in mice [20]. This effect was achieved by enhancing the migration of neutrophils to cardiac tissue through a mechanism dependent on AMPK/Nrf2/CXCR2 signaling. Another independent study also suggested that trimetazidine could potentially reduce cell apoptosis in cardiac tissue [28]. These cardioprotective effects of trimetazidine may related to its anti-inflammatory and anti-oxidative action of AMPK & Nrf2 pathways Nrf2 controls antioxidant genes, leading to elimination of ROS and reduced inflammation [29].

EFFECTS ON MYOCARDIAL HISTOPATHOLOGY

In the present study, the group treated with trimetazidine exhibited a notable decrease in the extent of cardiac tissue injury. When compared with the CLP and DMSO groups the trimetazidine group showed moderate architecture with less degree of histopathological changes such as a moderate degree of inflammation and necrotic area [29]. The current study shows that pretreatment of mice exposed to CLP with trimetazidine improved heart damage suggesting that trimetazidine would have a protective impact against endotoxic cardiac injury [29]. The cardioprotective effects of trimetazidine are associated with its ability to counteract inflammation and oxidative stress through the activation of AMPK and Nrf2 pathways. Nrf2 is responsible for regulating genes involved in antioxidant responses, resulting in the clearance of reactive oxygen species (ROS) and a subsequent reduction in inflammation [20, 29].

CONCLUSIONS

The present study adds to the growing body of research that trimetazidine, has potential ameliorative impact on the mice that were subjected to CLP through its role as anti-inflammatory, antioxidant and anti-apoptotic effects.

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This study was performed in accordance with the recommendation of the Guide and Use of Laboratory animals' association for Laboratory animal science, Kufa University. All animals' considerations and conventions were approved by the Animal Care Committee. All mice used in this study were sacrificed were under xylazine and ketamine mixture anesthesia.

CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Aerobic skin microbiota study in patients with paratraumatic eczema developed as a result of combat injuries

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ABSTRACT

Aim: To establish taxonomic composition, population level and microecological indicators of the "macroorganism-microbiota" ecosystem by determining the expression levels of individual pathogenicity factors of microorganisms isolated from the skin areas of patients with paratraumatic eczema developed as a result of combat injuries.

Materials and Methods: Studied microbiologically eczematous lesions content in 54 military men to determine the qualitative and quantitative composition of microbiocenosis to analyze the taxonomic composition, population-species level, microecological indicators and expression levels of individual pathogenicity factors.

Results: The results indicate that most eczematized areas are contaminated with S.aureus (48.1%) with the highest level of quantitative dominance. The rate of quantitative dominance of other strains is significantly lower: *S.epidermidis* – 2.56 times, *S.haemolyticus* – 4.56 times, *S.pyogenes* – 5.86 times, *E.faecalis* and *K.pneumoniae* – 20.56 times. When determining the sensitivity of S. aureus clinical strains to antibacterial drugs, we established high levels of sensitivity to mupirocin, fusidic acid, and oxazolidinones – 95.1%, 90.2%, and 87.8%, respectively. Moreover, *S. aureus* dominated among the strains with a high ability to form biofilms – 68.2%, with an average ability – *S epidermidis*, 28.8%.

Conclusions: S.aureus is the leading pathogen in the infectious-allergic process with paratraumatic eczema according to the constancy index of each taxon, frequency of manifestation, Margalef's species richness index, Whittaker's species diversity, the value of Simpson's and Berger-Parter's species dominance indices, as well as the population level of each taxon, the coefficient of quantitative dominance and the coefficient of significance. We should consider this fact when prescribing therapy.

KEY WORDS: combat injuries, paratraumatic eczema, aerobic microbiota

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INTRODUCTION

Combat injuries are more complicated and severe compared to peacetime injuries. The microbiology of military wounds changes with the development of both medicine, and warfare. The resulting wounds are mostly characterized by the presence of a large defect area, impaired blood supply, infection, and slow healing. Such injuries require effective medical care for quick recovery [1, 2]. Paratraumatic eczema, which occurs around wounds that do not heal for a long time after injuries, cuts, skin burns or combat wounds, is most often registered among the clinical varieties of microbial eczema [3]. In most patients, dermatosis is asymmetrical, localized on open areas of the skin (hands, forearms, lower legs, face, neck). Lesions have clear boundaries with peeling of the epidermis along the edge of the foci in the form of a border. In the center of the foci against the background of erythema and edema, there is moderate wetting, point erosions, multiple serous-purulent crusts, while on the periphery – pustular elements. On the lower extremities, erythema in foci has a bluish tint. One cannot help but be alarmed by the fact that the course of dermatosis in recent years has become more severe with frequent relapses, significant generalization of the process on the skin, and resistance to treatment [4, 5]. The most frequent complication of the eczematous process is the addition of a secondary pyococcal and fungal infection, associated with a decrease in the antimicrobial resistance of the skin. The therapy of infectious lesions is complicated due to the growing resistance of the main causative agents of pyoderma - *Staphylococcus aureus* and *Staphylococcus epidermidis* - to widely used antibiotics. The main principle of eczematous manifestations therapy, considering the polyetiological nature of this disease, is a complex effect on the body, considering the severity, nature, localization of the pathological process, the duration of the disease, previous treatment and its effectiveness,

the age of the patient and the available concomitant pathology [6].

The ability of staphylococci to migrate, survive in adverse conditions, and exchange genetic loci of acquired antibiotic resistance leads to the spread of "aggressive" strains, which causes the development of severe complications. Such strains, as a rule, are resistant to several groups of antibacterial drugs, which significantly complicates therapy [7]. In addition, staphylococci are characterized by the presence of a large number of pathogenicity factors that allow them to survive under various adverse environmental influences, one of which is the ability to form biofilms. We commonly understand biofilms as a living community of several types of bacteria, fixed on a substrate and surrounded by a protective matrix. The presence of a well-designed consortium of bacteria in the biofilm matrix ensures their survival. Treatment with antibiotics can destroy only the planktonic forms of this bacterium, while pathogens immobilized in a biofilm can survive and multiply after therapy discontinuation [8, 9].

AIM

The purpose of the study was to establish taxonomic composition, population level and microecological indicators of the «macroorganism-microbiota» ecosystem by determining the expression levels of individual pathogenicity factors of microorganisms isolated from the skin areas of patients with paratraumatic eczema developed as a result of combat injuries.

MATERIALS AND METHODS

The authors conducted a laboratory microbiological study of eczematous lesions content of 54 military men treated in the dermatological department of the State Institution «Institute of Dermatology and Venereology, National Academy of Medical Sciences of Ukraine». 77 strains of microorganisms, representatives of 6 taxonomic groups were isolated. All patients were male. The patients were from 19 to 57 years old, the average age was (34.3 ± 1.1) years. The comparison group consisted of 19 people of representative age and gender.

The authors collected and primarily inoculated biological material by generally accepted methods. Moreover, the material was microbiologically studied immediately after collection, no later than 2 hours. Morphological, tinctorial, cultural, and biochemical properties helped identify the selected taxa [10]. In addition, to characterize the population-species and microecological indicators, we determined index of constancy, frequency of occurrence according to the value of Margalef's species richness index, the Whittaker's species diversity index, as well as Simpson's and Berger-Parter's indices of species dominance [11].

The study also determined separate factors of pathogenicity (resistance to antibiotics and the ability to form biofilms). Using the method of adhesion to polystyrene in flat-bottomed plastic tablets, we confirmed the ability to form a biofilm. The optical density (OD) was measured on a microplate reader at 540 nm, comparing the OD 540 of the samples with that of the negative control. Absence of biofilm was recorded when the OD of the sample ≤ OD of the control, a weak degree of biofilm production - when the OD of the control < OD of the sample ≤ 2 OD of the control, the average degree of biofilm production - at 2 OD of the control < OD of the sample ≤ 4 OD of the control, a high level of biofilm production - at 4 OD of the control < OD of the sample, in accordance with the recommendations of Rodrigues et al., 2010. All experiments were performed in six replicates [12, 13]. Statistical data analysis was carried out using MS Excel packages.

RESULTS

54 patients took part in bacteriological examination with the aim to isolate and identify aerobic bacteria in eczematous contents obtained from patients with paratraumatic eczema. In total, we isolated 77 strains of opportunistic bacteria belonging to 6 different taxonomic groups. During the examination of the comparison group (19 people),we isolated a total of 21 strains of microorganisms belonging to the genus *Staphylococcus.* Table 1 shows the taxonomic composition of the «macroorganism-microbiota» ecosystem of the aerobic lesions microflora in patients with paratraumatic eczema and practically healthy individuals.

54 patients with paratraumatic eczema resulting from combat injuries (wounds) had 77 strains of conditionally pathogenic aerobic bacteria isolated and identified. This confirms that associations of these bacteria are involved in the eczematous process in some patients. In comparison, in the control group, only 2 people showed the presence of two-component associations including types of staphylococci. Fig. 1 shows quantitative characteristics of opportunistic aerobic microbiota associations in the eczematous process.

Qualitative characterization of associations is important for the eczematous process. Table 2 shows qualitative characteristics of opportunistic bacteria association contaminating eczematization foci, as compared to the skin of practically healthy individuals.

To identify the leading causative agent of the inflammatory process caused by associations, it is

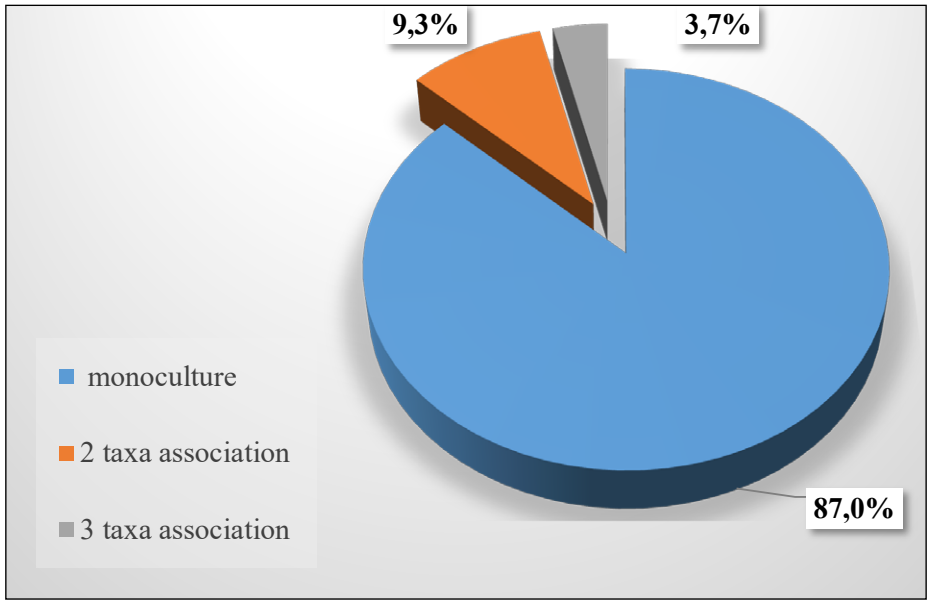

Fig. 1. Quantitative characteristics of opportunistic aerobic microbiota associations in lesions of patients with paratraumatic eczema.

Table 1. Taxonomic composition of the aerobic lesions microflora in patients with paratraumatic eczema and practically healthy individuals

Microorganisms	Typology of dominant microorganisms		Indices of species dominance				Typology of dominant microorganisms		Indices of species dominance				P*
	Affected skin (n=54)						Healthy skin (n=19)						
	Determined and identificated strains	Constancy index (%)	Index of species dominance	Whittaker's species diversity index	Simpson's species dominance indices	Berger-Parter's species dominance indices	Determined and identificated strains	Constancy index (%)	Index of species dominance	Whittaker's species diversity index	Simpson's species dominance indices	Berger-Parter's species dominance indices	
S.aureus	41	53,2	0,761	0,878	0,282	0,53	2	9,5	2,88	9,5	0,009	0,991	≤ 0,05
S.epidermidis	16	20,8	2,267	3,812	0,044	0,21	17	80,9	0,71	0,23	0,65	0,35	≤ 0,05
S.haemolyticus	9	11,7	3,09	7,555	0,014	0,12	2	9,5	2,88	9,5	0,009	0,991	
S.pyogenes	7	9,1	3,46	10,0	<0,001	0,09	-	-	-	-	-	-	
E.faecalis	2	2,6	5,267	37,5	<0,001	0,03	-	-	-	-	-	-	
K.pneumoniae	2	2,6	5,267	37,5	<0,001	0,03	-	-	-	-	-	-	

* p given in comparison with a control group of practically healthy individuals.

necessary to determine the population level of each component of the microbial community. Table 3 shows the population level and microecological indicators of opportunistic bacteria contaminating foci of eczematization.

The next stage of the study relates to sensitivity determination of clinical strains of *S.aureus* isolated from affected areas of the skin of military personnel with paratraumatic eczema to antibacterial drugs, as the most represented and pathogenic representative of the genus *Staphylococcus*. Fig. 2 shows the obtained data.

The study of various formation (or destruction) aspects of bacterial biofilms is a relevant and promising direction. It will enable scientists to optimize approaches to the diagnosis and treatment of a number of infections, including purulent-inflammatory complications of paratraumatic eczema [8]. Table 4 presents the summarized adhesion degree data to polystyrene of the extracted clinical strains of staphylococci as dominant representatives in the structure of eczematous lesions microbiocenosis.

DISCUSSION

Using the species constancy index and Whittaker's species diversity index, the authors analyzed micro-

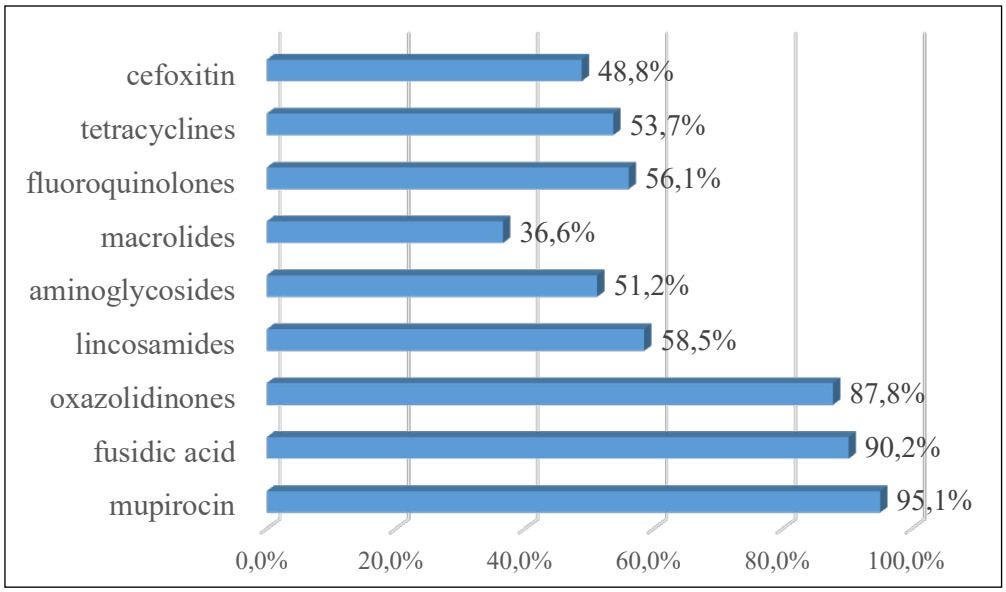

Fig. 2. Sensitivity of *S. aureus* clinical strains isolated from the affected skin areas in military personnel with paratraumatic eczema, (n = 41).

Table 2. Qualitative characteristics of opportunistic bacteria association, contaminating foci of eczematization compared to the skin of practically healthy individuals

Affected skin (n=54)				Healthy skin (n=19)			
Association composition	Association forming bacteria	abs.		Association composition	Association forming bacteria	abs.	
monoculture	*S.aureus*	37	48,1	monoculture	*S.aureus*	1	5
	S.epidermidis	13	16,9		*S.epidermidis*	15	71,4
	S.haemolyticus	5	6,5		*S.haemolyticus*	1	5
	S.pyogenes	6	7,8				
2- taxa associations	*S.aureus+ S.epidermidis*	2	2,6	2- taxa associations	*S.aureus+ S.epidermidis*	1	5
	S.aureus+ S.haemolyticus	2	2,6		*S.haemolyticus+ S.epidermidis*	1	5
	S.pyogenes+ E.faecalis	1	1,3				
3- taxa associations	*K.pneumoniae+ E.faecalis+ S.haemolyticus*	1	1,3	3- taxa associations			
	K.pneumoniae+ S.haemolyticus+ S.epidermidis	1	1,3				

ecologically the selected associations of microorganisms. Depending on the value of the constancy index, microorganisms were divided into permanent (with a constancy index of 50% or more), additional (with a constancy index of 25% - 50%) and random (with a constancy index of 25% and below). A low index means the homogeneity of the system, that is, the predominance of one group of microorganisms.

The combination of a low diversity index with a high constancy index of one species indicates a decompensated dysbiosis or a disease most likely caused by this species. The study of the taxonomic composition of the «macroorganism-microbiota» ecosystem of the aerobic lesions microflora in patients with paratraumatic eczema (Table 1) shows that the absolute majority of eczematized areas are contaminated with *S.aureus* (53.2% of patients). However, *S.epidermidis, S.haemolyticus, S.pyogenes* are less often isolated and identified – 20.8%, 11.7% and 9.1%, respectively.

Gram-negative bacillus *(K.pneumoniae*) and gram-positive cocci *(E.faecalis)* occurred in 2.6% of the examined. According to the constancy index, the frequency of occurrence, the value of Margalef's species richness index, Whittaker's species diversity index, and Simpson's and Berger-Parter's species dominance indices, the main part of the microbiocenosis of eczematization centers contained

Table 3. Population level and microecological indicators of opportunistic bacteria contaminating foci of eczematization (n=54)

Microorganisms	Population level in lg CFU/ml	Coefficient of quantitative dominance	Significance coefficient
S.aureus	5,4 ± 0,3	53,25	0,024
S.epidermidis	4,87 ± 0,35	20,78	0,063
S.haemolyticus	3,67 ± 0,36	11,69	0,11
S.pyogenes	4,27 ± 0,21	9,09	0,14
E.faecalis	4,52 ± 0,05	2,59	0,5
K.pneumoniae	4,39 ± 0,21	2,59	0,5

Table 4. Intensity assessment of biofilm formation by clinical strains of staphylococci

Strains of microorganisms/ quantity	Index of film formation (OD_{540})					
	low		average		high	
	Quantity of strains	OD_{540}	Quantity of strains	OD_{540}	Quantity of strains	OD_{540}
S. haemolyticus / 9			4	0,31±0,01	5	0,41±0,04
S epidermidis / 16	2	0,179±0,02	10	0,29±0,01	4	0,44±0,03
S. aureus/ 41			5	0,33±0,01	36	0,369±0,01
Total	2		19		45	

S.aureus. *S. epidermidis* occupied the second position, considering the tendency to its frequent extraction within the limits of the constancy index values, as an additional microorganism. Other bacteria (*S.haemolyticus, S.pyogenes, E.faecalis, K.pneumoniae)* were rare and accidental**,** according to the data of the study. The data we obtained were compared with similar studies conducted with the participation of civilian patients with the study of the microbiological status of venous ulcers of the lower extremities. There were, that alert pathogens are defined as drug-resistant, life-threatening microorganisms that constitute a significant epidemiologic problem. Among other microorganisms, the group of alert pathogens includes *S.aureus, Pseudomonas aeruginosa, Enterococcus* spp., *Streptococcus pneumoniae, Acinetobacter* spp., *and Enterobacteriaceae*, which were isolated from 28.6%of culture-positive ulcers. Alert pathogens, especially *P.aeruginosa* and *S.aureus*, are commonly isolated from venous leg ulcers [14]. It should be noted that the microflora of eczematous ulcers that developed as a result of combat injuries had higher degrees of colonization and population level. Due to the lack of data on the determination of statistical microbiological parameters in the study of the severity of combat injuries, a comparative analysis was not conducted

77 strains of conditionally pathogenic aerobic bacteria were isolated and identified in 54 patients with paratraumatic eczema, resulting from combat injuries (wounds).This is evidence that associations of these bacteria take part in the eczematous process in some patients. The quantitative characteristics of aerobic bacteria associations isolated from lesions (Fig. 1) show that a monoculture contaminated 87.0% of patients with paratraumatic eczema; 9.3% had associations of aerobic bacteria consisting of two different taxa; in 3.7% - contamination consisted of three different taxa. The study also determines qualitative characteristics of aerobic bacteria associations extracted from areas of eczematization in patients with paratraumatic eczema. (Table 2). The research has established that the inflammatory process in most patients is due to monoculture of *S.aureus* (48.1%). Other microorganisms in monoculture were rare: *S.epidermidis* in 16.9% of the examined, *S.haemolyticus* in 6.5% and *S.pyogenes* in 7.8%. In 9.3%, an association consisting of 2 different taxa: *S.aureus*+ *S.epidermidis, S.aureus*+ *S.haemolyticus* and *S.pyogenes*+ *E.faecalis* (2.6%, 2.5% and 1.3%, respectively) supported the inflammatory process. Associations consisting of three different taxa contained *K.pneumoniae* + *E.faecalis* + *S.haemolyticus* and *K.pneumoniae* + *S.haemolyticus* + *S.epidermidis*. Thus, *S.aureus* is the main cause of the inflammatory bacterial process that develops in paratraumatic eczema.

The study of the population level and microecological indicators of aerobic bacteria in the microbiota of eczematous lesions (Table 3) shows that the highest population level among them is in *S.aureus.* The values of the rating indicators (Margalef's and Whittaker's indices) were the highest among aerobic bacteria and characterized the spatial and nutritional resources of the biotope as well as the habitat conditions of

S.aureus. All other anaerobic bacteria have a lower population level - *S.epidermidis* by 23.9%, *S.haemolyticus* by 42.7%, *S.pyogenes* by 33.3%, *E.faecalis* and *K.pneumoniae* by 42.3% . The revealed value of the population level determines different dominance levels of these bacteria. Thus, the highest level of quantitative dominance was in *S.aureus.* The quantitative dominance of other species was much smaller: *S.epidermidis* - 2.56 times, *S.haemolyticus* - 4.56 times, *S.pyogenes* - 5.86 times, *E.faecalis* and *K.pneumoniae* - 20.56 times.

In order to predict the epidemic situation and develop anti-epidemic measures, in particular, empirical antibiotic therapy in each specific hospital and even department, it is important to constantly monitor not only the species composition and levels of resistance to antibiotics of the causative agents of purulent-inflammatory diseases of the wounded, but also the genes encoding this resistance. In addition, a comparative analysis data of the phenotypic signs of microorganisms resistance to antibiotics (antibiotic resistance profiles) with the presence of certain antibiotic resistance genes in their genome will help predict possible expression of genes that are in an inactive state. Such studies are especially relevant in countries with military conflicts. Therefore, the number of patients with wounds of various severity infected with microorganisms, in particular, resistant to antibiotics, is increasing. When determining the sensitivity of clinical strains of *S. aureus* isolated from patients with paratraumatic eczema to ABP, we established high levels of sensitivity to mupirocin, fusidic acid and oxazolidinones - 95.1%, 90.2% and 87.8%, respectively (Fig. 2) . At the same time, the number of polyresistant strains was 54.2%, with extensive resistance - 4.7%. Analysis of the sensitivity of pathogens to individual groups of drugs, obtained in another study, showed that K. pneumoniae is sensitive mainly to tetracyclines. fluoroquinolones, moderately sensitive to cephalosporins. Among other pathogens (*A.lwoffii, K.oxytoca, P.mirabilis, S. aureus, S. epidermidis, S. haemolyticus, S.hominis, S. maltophilia*), the general pattern of sensitivity was the following: most of them were resistant to penicillins, almost half - to carbapenems and fluoroquinolones, and the distribution of sensitivity did not depend on tinctorial properties [1].

Bacteria are able to adapt to changes in nutrition, the presence of stresses caused by external conditions, presence of inhibitory compounds, as well as to immune protection. One of the particularly important examples of bacterial adaptation mediated by the systematic action of genes is the ability to reproduce in immobile polymicrobial communities known as biofilms. Significant changes in gene expression and synthesis of additional proteins, manifested by resistance to antimicrobial agents and immune defense factors, accompany existence in the form of biofloods. The data in Table 4 show that the ability to form biofilms of varying degrees of density characterizes clinical strains of microorganisms. Such strains accounted for 68.2% (45 strains), among which *S.aureus* dominated (three quarters of the total number of these strains). 28.8% of strains had average ability to form biofilms, and dominated in *S. epidermidis* - 52.6%. Due to the lack of data on the determination biofilm parameters in another study of combat injuries microorganisms, a comparative analysis was not conducted.

CONCLUSIONS

1. *S.aureus* is the leading pathogen in the infectious-allergic process with paratraumatic eczema according to the constancy index of each taxon, frequency of manifestation, Margalef's species richness index, Whittaker's species diversity, the value of Simpson's and Berger-Parter's species dominance indices, as well as the population level of each taxon, the coefficient of quantitative dominance and the coefficient of significance. This microorganism causes the inflammatory process in 48.1% of patients in monoculture. Therefore, the therapeutic tactics of treating paratraumatic eczema should take into account sensitivity of *S.aureus* to antibacterial drugs.
2. When determining the sensitivity of *S. aureus* clinical strains isolated from patients with paratraumatic eczema, the study established high levels of sensitivity to mupirocin, fusidic acid, and oxazolidinones - 95.1%, 90.2%, and 87.8%, respectively. In case of necessity, it is possible to prescribe these drugs empirically before obtaining the results of a bacteriological study with the actual determination of the pathogens sensitivity to antibacterial drugs.
3. The study reveals that clinical strains of staphylococci have the ability to form biofilms of varying degrees of density. Strains with a high ability to form biofilms made up 68.2%, with *S.aureus* dominating (three quarters of the total number of these strains). Strains with an average ability to form biofilms accounted for 28.8% and contained *S. epidermidis* strains.

Prospects for further research the need for further research into the taxonomic composition of aerobic microorganisms with further study of the population level of autochthonous and allochthonous microbiota, their sensitivity to antibacterial agents for therapeutic and preventive purposes.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Effects of (EGFR-Her1) with continuous illumination on the immunohistochemical and histomorphometric changes of sublingual glands in male mice

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ABSTRACT

Aim: To evaluate the effects of constant light on the histological, histomorphometric and expression of EGFR of sublingual.

Materials and Methods: 20 male mice were applied and classified in to group A was kept in continuous light and control group B was kept in the normal daily variance for 45 days.

Results: Group A changes in behavior they become irritable, aggressive, loss of appetite, lethargic& lose weight. Histological examination with H&E showed degeneration of the sublingual tissue, loss architecture and necrosis (acini and ducts), the distraction of cell membrane and vacuolization, loss of nuclei in the necrotic tissue about 60%. Histomorphometric revealed significant differences in acini diameter between groups ($p \leq 0.001$). The mean of group A was 116.5960 ± 3.41668 compared with B group was 340.2720 ± 6.95821. Immunohistochemistry technique by used EGFR antibody in group A revealed expression evaluated as 0.8362 ± 0.00822 increase in values in comparison with group B 0.6988 ± 0.02489.

Conclusions: Light has an indirect oxidative stress effects that causes oxidative damage to glands, which affects mucin secretion. Therefore, there was a decrease in diameter of acini sublingual glands of light group than control group, however, there was increase in the expression EGFR of sublingual glands in light group compare with control group as compensatory mechanism for oxidative stress in glands by light.

KEY WORDS: salivary gland, circadian rhythms, EGFR

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INTRODUCTION

The smallest of the three salivary glands is a sublingual gland, which involves the sublingual, mandibular, and parotid glands. Salivary glands are fundamental organs that secrete and produce viscous saliva composed mainly of sulfated-glycoproteins and neuronal and epidermal growth factors, encouraging lubrication and protection of the oral mucous [1-3]. The sublingual glands receive their parasympathetic input from the chorda tympani nerve, which is a branch of the facial nerve, by the submandibular ganglion. The nerve functions in a secretomotor capacity [4]. Life on our planet is adapted to the 24-hour solar day. The prestable light/dark cycle has been internalized in the form of circadian rhythms. Circadian rhythms allow the concurrence of behavioral and biological processes with the external environment. Thus, the optimal timing of physiological events is harmonic by these internal timekeepers. Endogenous circadian rhythms have a time of ~24 hours and are reset daily to explicitly 24 hours via exposure to a dark-light cycle [5]. Despite that, the outline between night and day has been confused via the spread adoption of electric light at night. As a result, psychiatric consequences and behavioral health of circadian disruption by night light are becoming increasingly apparent [6, 7] furthermore, there is a relationship between mood disorders and the light/dark cycle. Mood disorders are often related to disrupted circadian clock-controlled responses, such as cortisol secretion and sleep, whereas disruption of circadian rhythms by jet lag or exposure to artificial light at night or night-shift work can precipitate or exacerbate affective signs in susceptible individuals. Evidence shows a strong link between mental health and circadian rhythms [8]. Often, all physiological processes show circadian rhythms, which are controlled via the circadian clock, permitting organisms to be familiar with the changes. In recent years, there has been increasing evidence that the circadian rhythm

system is involved in physiological processes in the maxillofacial region, as well as pathological processes, such as tooth and jaw development, oral carcinoma, salivary gland function, craniofacial malformations, and other diseases. Dialectically analyzes the importance of the circadian rhythm and circadian clock system to the activities of maxillofacial and oral tissues [9]. The epidermal growth factor receptor (EGFR; ErbB-1; HER1 in humans) is a protein transmembrane; it is a receptor for many proteins of the epidermal growth factor family (EGF family) ligands of extracellular protein. EGFR has been embroiled in the function of circadian and is expressed in the SCN suprachiasmatic nuclei. The response to EGFR activation is expected to count on circadian rhythms [10]. This research revealed the indirect effect of change in the light-dark cycle on sublingual glands. This is consistent with other research that have found effects of changing in the circadian rhythm on different organs, like the effect on the thyroid gland [11], ovary [12], and mammary gland [13].

AIM

The current study aims to evaluate the effects of constant light on the histological, histomorphometric and expression of EGFR of sublingual.

MATERIALS AND METHODS

Experimental Animals: twenty adult healthy male mice (albino mice) were obtained from College of pharmacy Al-Nahrain University at about 12-14 weeks of age and weighing 20-30 g, and were kept and fed under specified conditions. The animals were placed in a plastic cage, easy to clean with free access to water (fresh tap water) and food (standard pellet diet), and 10 animals for each cage. These were kept at room temperature (20±2 ºC) in a clean and well-ventilated room. Collections of samples from the animals were divided into two groups and were kept in the special illuminating-conditioned room according to daily illumination times (dark/light cycle). There were 20 male mice applied in the study and were assigned to groups A and B in accordance to the periods in which the animals were kept. The Group A include 10 animals that were kept with full-time light in 24 hrs. Light for forty-five days; the Group B - control group, include 10 animals, which were kept with a normal dark/light cycle (10 hrs. darkness/14 hrs. light) for forty-five days. Animals were sacrificed and a midline inverted T-shaped incision was made on the ventral side from the chin to the thoracic region of the animal to expose the salivary glands and obtain samples using a dissecting microscope. After dissection of the salivary glands from the cervical region, samples were chemically fixed by immersion in 10% neutral buffered formalin, then 70% ethanol. Then further histological procedures were done to obtain a paraffin block of specimens for histological and immunohistochemical assessment. Then we prepare the paraffin section. Glandular specimens were prepared for paraffin sectioning as follows: initial fixation, dehydration, clearing, impregnation and embedding in tissue blocks, sectioning on glass slides, then dewaxing, hydration, staining and finally embedding according to Kim SS et al [14]. Staining was used for histological examination for tissue preparation with Harris hematoxylin (Fisher) and eosin yellow (Fluke) (ready to use).

IMMUNOHISTOCHEMICAL STAINING

Dako company supplied the EGFR protein, the immunohistochemical staining of the EGFR-H11 antibody (Epidermal growth factor receptors) was used is Code M3563. Mouse monoclonal anti-epidermal Growth Factor Receptor (anti-EGFR) for use in laboratories for qualitative identification by light microscopy as epidermal growth factor receptor-positive in both experimental and normal tissues using immunohistochemical (IHC) test method. Epidermal growth factor receptor, EGFR is a 170 kD transmembrane glycoprotein bind thin activated by various ligands such as EGF, TGFα and some virally encoded growth factors. The histological tissue slides prepared from the tissue samples, stained with hematoxylin and eosin stain and IHC for EGFR-H11 were subjected to histological evaluation and estimation under the light microscope. Image J software was used for histomorphometric to measure the diameter of acini (a Java-based image processing program developed at the National Institutes of Health, USA).

STATISTICAL ANALYSIS OF DATA

For statistical analysis of the data we used the Statistical Package for Social Sciences (SPSS). software, version 23. The data are expressed as mean and standard error of the mean (SEM). The variance between the mean percentages of EGFR expression in sublingual glands was examined for statistical significance using an independent T-test to show the difference in the mean positively ± standard error between groups according to circadian rhythms [15].

IMMUNOHISTOCHEMICAL ASSESSMENT

The Aperio image inspection software with 12 scoring algorithms was used to measure the amount of a par-

Table 1. Diameter of the acini of the sublingual salivary glands

Group	Diameter	P-value
Control	340.2720 ± 6.95821	0.001
Light	116.5960± 3.41668	

ticular color in tissue sections, which depend on color quantification parameters in three intensity ranges (strong positive (brown), positive (orange), and weak positive (yellow) and negative (blue).

RESULTS

This study shows many morphometric and histological changes in sublingual salivary glands in the experimental animal when exposure male mice (albino mice) to the light period for 45 days were internalized in the form of circadian rhythms; these differences varied compared with the control group.

EXTERNAL FEATURES OF THE ANIMALS

During the present study, several changes in the animals' behavior were observed: they became irritable, quarrelsome and aggressive, lost their appetite, became lethargic, apathetic, became fearful, lost weight, lost hair, and became weaker compared to the control groups.

HISTOLOGICAL CHANGES OF GLANDS

The statistical analysis results showed that there were significant differences between groups exposed to the light and control groups ($p \leq 0.001$). The mean diameter of the acini of the sublingual salivary glands of animals exposed to light after 45 days was 116.5960±3.41668 compared to the control group, which was 340.2720±6.95821 (Table 1, Fig.1).

In the animals in the light group (Group A), the histological features appearance of necrosis and degeneration of the salivary gland tissue loss architecture and small acini (116.5960± 3.41668), distraction of cell membrane and vacuolization, loss of nuclei in the necrotic tissue compromised about 60%, degenerative acini show vacuolization of cell with enlarged hyperchromatic nuclei, ducts necrotic (Table 1, Fig.2).

In the present study, the results obtained by using H&E stains showed the histological appearance that the normal section of sublingual salivary glands of the control group (Group B) of albino mice showed the mucous glands with tubular an arrangement of poorly stained mucous cells with small interlobular duct are seen in connective tissue and normal size of acini (Table 1, Fig.2).

EFFECTS OF CIRCADIAN RHYTHMS ON THE MARKER EXPRESSION OF THE EGFR OF SALIVARY GLANDS

The samples were prepared for two groups to perform immunohistochemical method using epidermal growth factor receptor antibody in the mild group; they showed a pronounced expression, which was estimated as an increase in the acinar cell values in the salivary glands by 0.8362±0.00822 compared with the control group, was recorded as 0.6988±0.02489 (Table 2, Fig.3, Fig.4).

DISCUSSION

In this study the effect of options of illumination on animal behavior we revealed that as a result of exposing experimental animals to continuous lighting for 45 days, many changes occurred in the animals, including behavioral changes, as it was observed that the animals suffer from symptoms of stress and anxiety represented by loss of appetite, lack of movement and laziness. This agrees with the author's finding that excessive exposure to light wreaks havoc on circadian rhythms in different ways. It decreases the production of melatonin, the hormone that promotes sleep., another agent regulated by circadian rhythms, disrupting not just sleep but other biological operations, as well as finding a clear correlation between nighttime light, including shift work, and psychiatric disorders such as depression, insomnia, and anxiety that were most influenced by minim influential factors in light exposure. Excessive exposure to night light in particular contributed to a 20% increase in mental health symptoms [16-19]. Moreover, those who work shifts at night expose their bodies to constant light, they are forced to become depressed more often compared to those who sleep at night; this brings back the attention of teenagers who spend most of their time at night on their gadgets, such as computer, smartphone, etc., which has been shown to interfere with the synthesis of melatonin, which affects their sleep patterns, sleep and mood, especially anxiety. [20]. Furthermore, researches revealed that through several retina-brain pathways light influences our mood. They proved that mice subjected to acute bright light exposure exhibited anxiety-related phenotype in chronic condition. Since mice are active during the night, confrontation with light activity of some threat, for example, fire and the loss of a shelter [21, 22]. We have revealed that exposure of a group of exper-

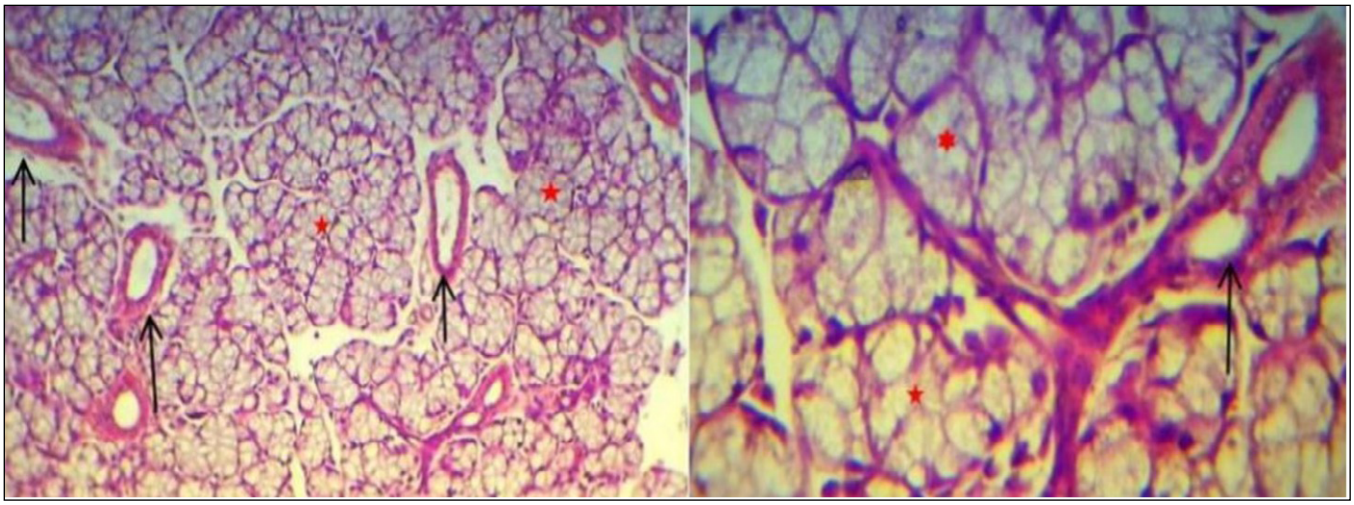

Fig. 1. Cross-section of the sublingual salivary glands in the control groups showed the presence of predominantly mucous glands with interlobular ducts (arrows) with normal sizes and shapes of acini. (Asterisk) (H&E) X10, X40.

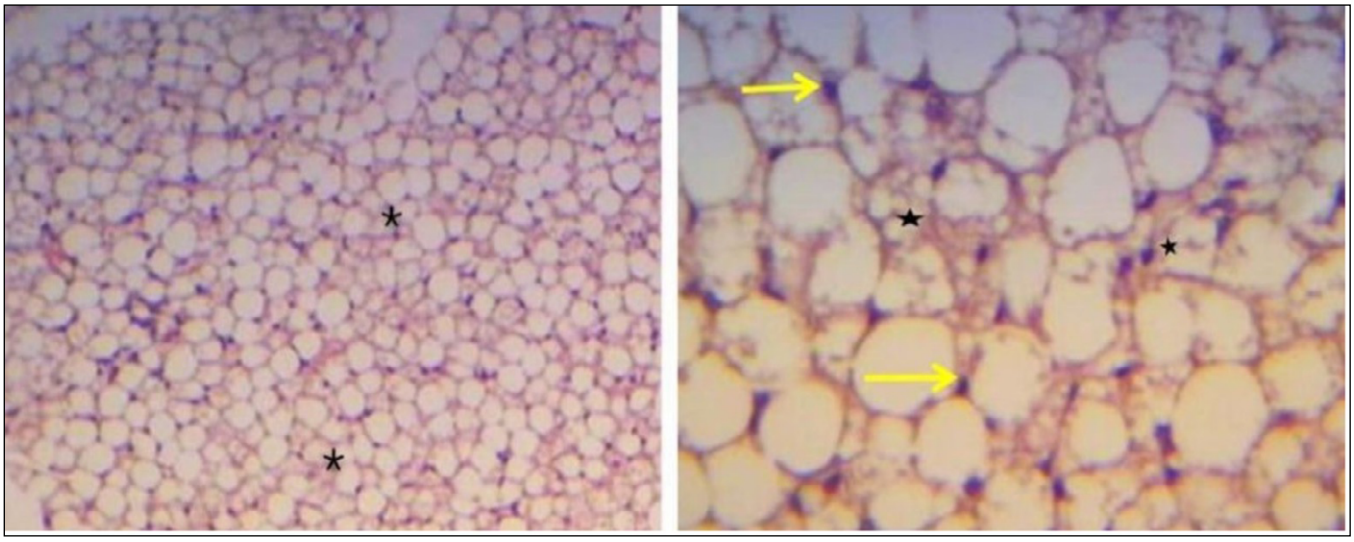

Fig. 2. Cross-section of sublingual salivary glands in light groups showed loss of architecture, necrotic ducts, necrosis of acini (asterisk), enlarged hyperchromatic nuclei (arrows) (H&E) X10, X40.

imental animals to continuous lighting led to behavioral changes in the animals, including anxiety and stress. Thus, lighting directly affects the nervous ssystem, and it is consistent with studies, which appears that exposure to night light can disrupt circadian clock mechanisms in the peripheral and CNS tissues [23]. The primary role of the salivary glands are to liberate saliva, which is essential for coated tongue, immunity, and oral digestion, as well homeostasis in the body. Sympathetic and parasympathetic division of the autonomic nervous therefore controls salivary secretion [24, 25]; moreover, the light in humans activates the sympathetic nervous system, as explained by a transient increase in sympathetic nerve activity [26]. The role of illumination on histological changes of sublingual glands: as a result of continuous illumination for 45 days in routine stain (H&E), histological examination with the usual dye showed that there was necrosis and degeneration of the sublingual gland tissue, loss of architecture and acini, distraction of cell membrane and vacuolization, loss of nuclei in the necrotic tissue compromised about 60% t as shown in Figure 2, and the size of the acini decreased to 116.5960±3.41668 compared to the control group 340.2720 ± 6.95821, as shown in Table 1. Since the necrosis in our cells occurs as a result of a lack of access to blood to the tissue, this is in accord with the generally held opinion that the nerve fibers supplying the salivary gland secretory elements are parasympathetic (cholinergic). At the same time sympathetic action stimulates secretion of a large amount of saliva in the mouth. However, stimulation of the sympathetic nervous system results in either little or no protein-rich blood flow [26, 27]. Furthermore, salivary production is controlled by sympathetic permanently oversees the production of saliva of through the superior cervical ganglion. These changes lead to; (a) decrease in saliva secretion by the acinar cells; (b) increase in protein

Table 2. Expression of EGFR in the sublingual salivary glands

Group	Mean ±Std Error	P-value
Control	0.6988 ± 0.02489	0.001
Light	0.8362±0.00822	

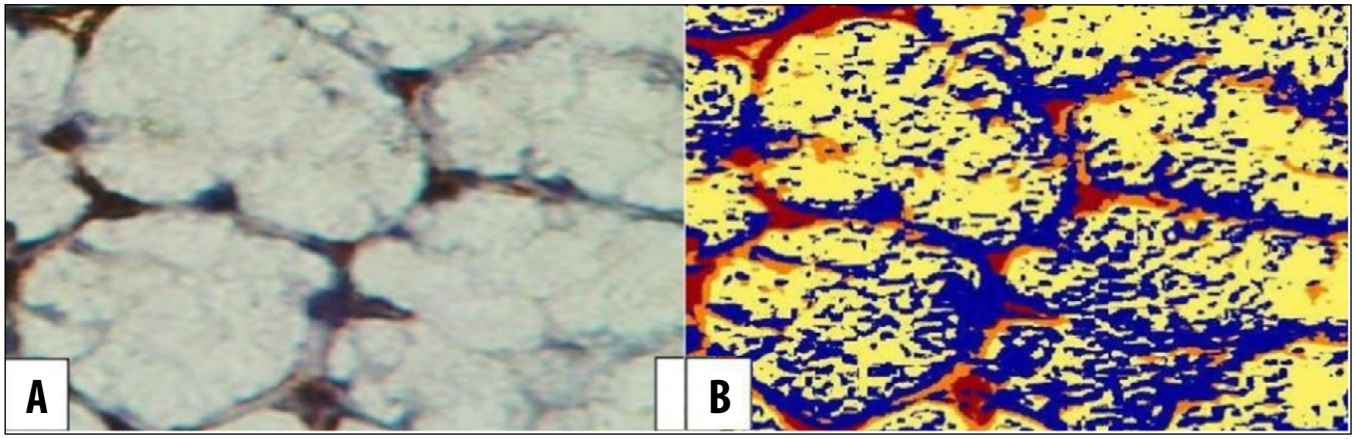

Fig. 3. A - EGFR expression in the sublingual salivary gland of the control group, B - EGFR expression in the sublingual salivary gland of the control group in analytical representation.

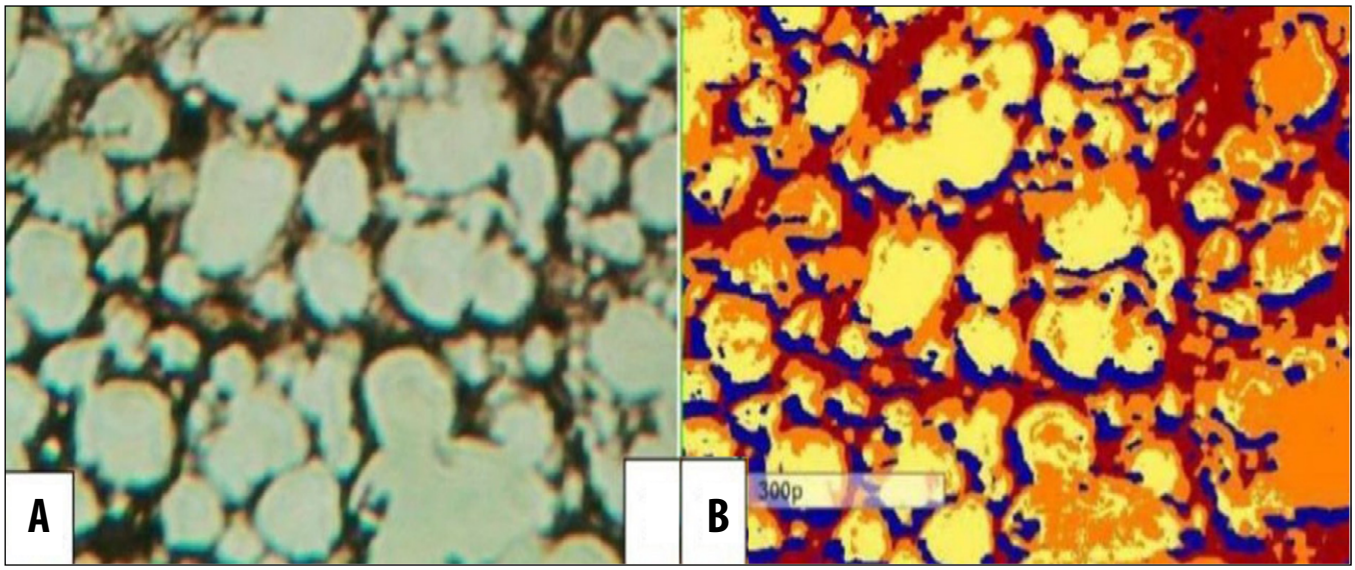

Fig. 4. A- EGFR expression of the sublingual salivary gland of the light group, B- EGFR expression of the sublingual salivary gland of the light group in analytical representation.

secretion; and (c) decrease in blood flow to the glands [28]. Moreover, sympathetic flow outcomes cause the release of acetylcholine to M3 muscarinic receptors. This leads to (a) stimulated acinar cells secretion of saliva; (b) HCO3 secretion rise by duct cells; (c) an increased blood flow to the submandibular gland via co-transmitters; and (d) an increased rate of saliva evacuation as a result of myoepithelium contraction [29-31]. Oxidative stress effect of circadian rhythm on EGFR expression of sublingual salivary gland present in this study reveals an increase in the expression of EGFR in light group (0.8362±.00822) mice than in control groups (0.6988±.02489) (Table 2, Fig.3&4). So that the present study concluded that stress effects of light on stimulation of the supraoptic nucleus in the hypothalamus will activate its sympathetic nucleus which affects the blood supply of the salivary glands consequently leading to hypoxic oxidative stress, therefore it will interfere with mucin protein production in the salivary gland acini and its excretion via their ducts, this agrees with researchers found the light plays an important role in oxidative stress. As a grant to study mechanisms of stress related to cellular signaling passage, light permits temporal and spatial control of ROS output in the heterogeneous environment of living cells [32]. In this study, it has been spotted that the antioxidant and oxidant systems of the salivary gland were affected by

any foreign oral application. These showed that the salivary glands were affected by alteration in the mouth so quickly and gave them suitable biochemical and physiological responses [33]. Moreover, other researchers have used the same pointing out that the epidermal growth factor (EGF) detected in the salivary glands promotes tissue damage, gastric wounds and healing there by increasing saliva EGF synthesis [34, 35]. In addition, the researchers reported that changes were recorded in the presence of the EGF receptor and oxidative events in the submandibular salivary glands, which localize the source of EGF [36, 37]. In another research, Garcia-Ojalvo et al., found the EGF effective in antioxidant markers and oxidative stress and synthesis of a significant oxidative. Also, they showed that locally penetrated EGF had a systemic influence on the recuperation of a pro-physiological redox balance and was effective in the level of stress [38]. Discovered that the use of diagnostic salivary biomarkers of oxidized condition in saliva gland of children is due to elevated oxidative degradations of saliva proteins and lipids linked with chronic kidney disease that affect the enzymes of antioxidant and non-antioxidant systems. They also found out that the salivary parameters of redox homeostasis can be potential diagnostic biomarker of the patients suffering from chronic kidney disease in children. Which is responsible for alterations in salivary antioxidant systems and for oxidative changes in salivary proteins secretion [39, 40].

CONCLUSIONS

Light has strenuous effects on various body organs, like salivary glands. These animals of the light group reveal irritability, quarreling aggressive behavior, loss of appetite, and lethargy. The acini in the sublingual salivary glands of the animal's light group reveal a marked decrease in the mean diameter and low mucin protein excretion than that of the control groups. There was an increase in the expression of EGFR of acini cells in the salivary glands of the light group mice than the control group, probably due to a compensatory mechanism to the oxidative stress in salivary glands.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Gender dimorphism of neurodynamic and cognitive functions of sub-elite and elite athletes (on the example of sports acrobatics)

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ABSTRACT

Aim: To evaluate the impact of a specific acrobatic load on the neurodynamic and cognitive functions of acrobats, as well as to study how these functions change depending on the gender, age and level of sports qualification of athletes (sub-elite and elite athletes).

Materials and Methods: Athletes specializing in pair and group acrobatics, of two age categories: 1. Sub-elite - athletes of puberty age, which included: girls (n=32), average age 13.96±1.02 years; boys (n=36), average age 14.88±2.32 years. 2. Youth acrobats (elite athletes): girls (n=30), average age 19.36±2.86 years; young men (n=34), average age 20.38±2.89. The computer program "Diagnost-1" was used in researches.

Results: In female acrobats, the latency period of simple visual-motor information, the latent period of the visual-motor reaction to choosing two out of three stimuli are statistically different from those of young acrobats. Boys acrobats are faster in terms of functional mobility of nervous processes than girls in both age categories (at $p<0.05$). A greater amount of processed information was found in acrobat boys than in adolescent girls. According to the indicators of cognitive characteristics features of sexual dimorphism between acrobats of different age categories were revealed due to different information processing strategies taking into account sexual characteristics.

Conclusions: Boys-acrobats of puberty and adolescence showed more effective processing of simple and complex visual-motor information compared to girls-acrobats of the same age categories. The indicators of neurodynamic and cognitive functions of young acrobats are better than those of pubescent athletes and different by gender ($p<0.05$).

KEY WORDS: cognitive functions, sport, sexual dimorphism, technical readiness, acrobatics, neurodynamic indicators

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INTRODUCTION

The problems of sexual dimorphism in sports have received considerable attention in recent years. And currently, this direction in the sport of higher achievements continues to be relevant. Every year there is an increase in the training exertion of female athletes, and in some cases they practically don't differ from those of men [1-3].

The analysis of a number of scientific works on the diagnosis of sensorimotor qualities of athletes of various sports and the prediction of effective sports activity showed that some experts cite examples of studies that showed the leveling of gender differences in female athletes when moving to the stage of higher sports skill [4].

Korobeinikov H., Prystupa Y, Korobeinikova L., Briskin Y. studied the peculiarities of the formation of the psychophysiological organization of the information processing system in highly qualified athletes in martial arts, game sports and gymnasts [1].

Chernozub A., Kochyna M., Chaban I. et al. note that when comparing the psychophysiological indicators of hand-to-hand fighting athletes in the gender aspect, no reliable differences were found in the indicators of male and female athletes [5].

Other experts claim that changes in both morpho-functional indicators and psychophysiological functions are observed during sports in female athletes [6].

Fedorchuk S., Ivasevych D., Borysova O. et al. investigated the psychophysiological characteristics of handball athletes with different levels of motivation for sports results [7].

Other domestic scientists (Fedorchuk S, Lysenko O, Shynkaruk O.) considered the issues of constructive and non-constructive coping strategies and psychophysiological properties of elite athletes [8]. Vovkanych

L., Dunaets-Lesko A., Penchuk A., Karchmar P. studied the features of sensorimotor reactions of athletes of various sports specializations [9]. Features of psychophysiological status in combination with a number of other factors, such as physical development, technical, general and special physical fitness, features of the training process depending on the stage of multi-year improvement, determine the possibility of forecasting and achieving high sports results.

Studies of psychophysiological indicators allow scientists to qualitatively assess the functional state of the central nervous system of athletes and its changes in different periods of the annual training cycle and stages of multi-year improvement [10].

A high level of speed of sensory information analysis, the strength of nervous processes, lability are important for an accurate assessment of the psychophysiological status of athletes for a scientifically based analysis of their functional readiness and, if necessary, making corrections in the educational and training process [11]. High indicators of efficiency, accuracy, speed of visual signal perception are the basis for achieving high sports results. Sports acrobatics is a complex coordination sport, which is characterized by the speed of thinking and specific technical actions, the ability of acrobats to display their best psychological and physical qualities in the conditions of a stressful situation. In educational and competitive activities, athletes have various mental reactions in case of changes in psychophysiological functions [6, 12].

Therefore, the study of the structure and content of the manifestation of psychophysiological features of acrobats at important stages of sports improvement is significant and provides additional information about the functional state of athletes. The analysis of special scientific and methodical literature and Internet resources showed that the study of psychophysiological indicators for highly qualified athletes in pair-group acrobatics, taking into account sexual dimorphism, is lighting is not enough . This became the goal of our research.

The results of our research of the neurodynamic and cognitive functions of acrobats of different sexes, ages and qualifications when performing a specific load can make a significant contribution to the medical field, in particular in several important aspects:

1. Understanding the physiological and cognitive aspects of sports activities.

Studies of neurodynamic functions, obtained results, help to understand how acrobats of different genders, ages and qualifications (elite and sub-elite) react to complex specific loads. This can help design training methods to improve performance and reduce the risk of injury. Assessment of cognitive aspects of acrobats in the important pre-competition period for athletes, such as attention, memory and the ability to make quick decisions, allows us to understand how athletes cope with competitive loads (a versatile mixed exercise is a multifunctional acrobatic action) that require a high level of concentration and quick decision-making.

2. Development of individual training programs for acrobats. The results of the neurodynamic and cognitive characteristics of acrobats of different ages, gender and qualification obtained during the study allow for the development of personalized training programs that take into account the individual characteristics of athletes to optimize their training and improve results in competitions.
3. Optimization of sports strategies. The results obtained during the experiment will be able to expand the range of knowledge about neurodynamic and cognitive aspects, which will provide an opportunity for the improved, necessary correction of the technique of performing acrobatic elements, in particular through the understanding of how reaction speed and cognitive load affect the performance of complex elements and acrobatic connections.

Thus, the results of studies of neurodynamic and cognitive functions of acrobats of different sexes in the age categories 12-16 and 17-23 years (sub-elite and elite) can significantly expand knowledge in the field of sports medicine, neuropsychology and cognitive sciences, which will help in further research and development of new training programs, improving safety, and promoting a general understanding of the physiological and cognitive aspects of sports activities.

The essence of the experiment in the study of neurodynamic and cognitive functions of acrobats of different genders, ages and sports qualifications consisted in the following aspects.

Thus, the scientific novelty of our work consists in the study from the standpoint of sexual dimorphism of changes in neurodynamic and cognitive functions of athletes of different sexes and age categories (12-16 and 17-23 years old)of different qualifications (sub-elite and elite athletes) under the influence of a specific load (universal acrobatic force-balance and tempo exercise), which is inherent only to pair-group acrobatics.

An important aspect follows from the above, that the results obtained in other sports cannot be used in the form of automatic data transfer in connection with the specifics of acrobatics. As you know, pair-group acrobatics is characterized by the educational and training process of athletes with a number of personal factors that can individually affect the personal performance of acrobats of different genders and ages. One of the most important factors is the delay in puberty (explained by

Table 1. Comparative characteristics of sensorimotor and neurodynamic indicators of acrobats of different gender before and after a specific exertion (pre-competition period)

Statistical indicators	Sub-elite athletes of puberty age				Elite athletes of youth age			
	To a specific exertion		After exertion		To a specific exertion		After exertion	
	Girls (n=32)	Boys (n=36)	Girls (n=32)	Boys (n=36)	Girls (n=30)	Young men (n=34)	Girls (n=30)	Young men (n=34)
	1	2	3	4	5	6	7	8
Indicators of the latent period of simple visual-motor information, milliseconds								
X±SD	255,70± 3,97	242,57± 6,05	244,02± 3,34	253,44± 6,19	245,00± 3,90	231,60± 6,26	252,45± 3,99	241,54± 8,36
Confidence level (p)	$p_{1,2} < 0,05$; $p_{2,4} < 0,05$; $p_{3,4} < 0,05$				$p_{5,6} < 0,05$; $p_{6,7} < 0,05$; $p_{7,8} < 0,05$			
Indicators of the latency period of the visual-motor reaction of choosing two out of three stimuli, milliseconds								
X±SD	384,70± 3,39	360,28± 6,21	398,61± 3,34	378,75± 4,85	367,38± 3,21	352,84± 5,84	379,16± 3,08	368,98± 5,12
Confidence level (p)	$p_{1,2} < 0,05$; $p_{2,4} < 0,05$; $p_{3,4} < 0,05$				$p_{5,6} < 0,05$; $p_{5,7} < 0,05$; $p_{6,7} < 0,05$; $p_{7,8} < 0,05$			
Indicators of functional mobility of nervous processes (imposed rhythm mode), seconds								
X±SD	98,81± 7,68	90,00± 2,23	88,87± 5,21	80,51± 2,17	89,44± 6,78	81,87± 2,30	84,07± 4,47	76,35± 2,57
Confidence level (p)	$p_{1,3} < 0,05$; $p_{2,4} < 0,05$				$p_{5,8} < 0,05$			
Indicators of the strength of nervous processes (imposed rhythm, number mistakes)								
X±SD	13,91± 2,55	10,29± 1,62	18,27± 2,82	14,89± 1,98	11,87± 2,28	9,50± 1,31	15,60± 1,84	13,92± 1,74
Confidence level (p)	$p_{1,3} < 0,05$; $p_{2,3} < 0,05$				$p_{6,7} < 0,05$; $p_{6,8} < 0,05$			
Indicators of the balance of nervous processes, milliseconds								
X±SD	18,03± 2,55	16,8± 1,62	22,93± 2,82	26,36± 1,98	16,24± 2,28	15,62± 1,31	20,83± 1,84	22,42± 1,74
Confidence level (p)	$p_{1,4} < 0,05$				$p_{5,8} < 0,05$			

Notes: the differences in the values of the indicators before and after the specific exertion, as well as between girls and boys are reliable according to the Mann-Whitney test ($p<0.05$).

the specificity of the selection and loads in the multi-year structure of acrobats' training).

AIM

The purpose of this work is to evaluate the impact of a specific acrobatic load on the neurodynamic and cognitive functions of acrobats, as well as to study how these functions change depending on the gender, age and level of sports qualification of athletes (sub-elite and elite athletes).

TASKS

1. To determine the main neurodynamic indicators of acrobats of different sexes and ages.
2. Assess cognitive functions (attention, memory, ability to make quick decisions).
3. Compare the results among acrobats of different genders, ages and sports qualifications.
4. Evaluation of how a specific load affects various neurodynamic and cognitive aspects in acrobats of different genders, ages and sports qualifications.
5. To identify the relationship between neurodynamic and cognitive functions and specific load (mixed universal acrobatic exercise).

RESEARCH METHODOLOGY

1. Preparatory stage of the experiment: conducting basic tests to determine the level of neurodynamic and cognitive functions before carrying out a specific load.
2. Evaluation of changes after exercise (re-evaluation) of neurodynamic and cognitive indicators to identify changes compared to initial data.
3. Comparative analysis of results – analysis of changes in neurodynamic and cognitive functions in different groups before and after performing a specific load.

On the basis of the received data, it is planned to develop, on a scientific basis, recommendations for opti-

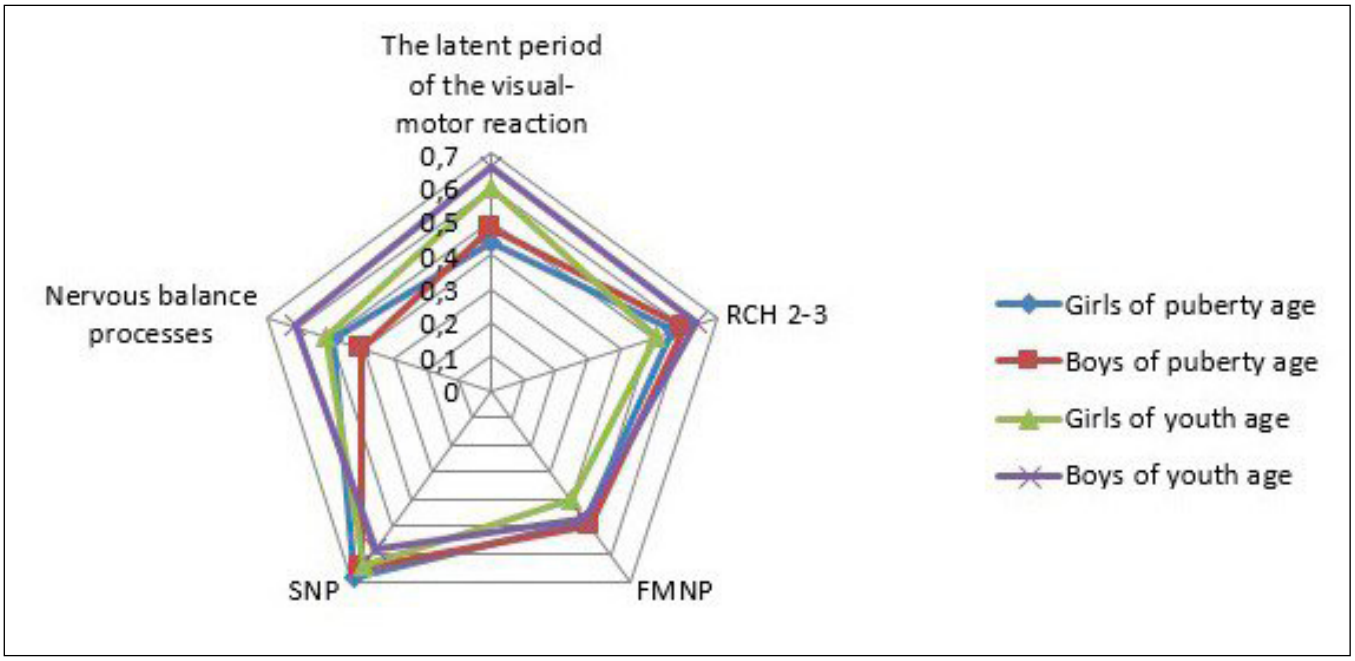

Fig. 1. Reliable correlations between parameters of psychophysiological characteristics and technical readiness of acrobats of different genders and age categories (pre-competition period).

mizing training programs, improving the technique of performing acrobatic exercises.

Thus, the experiment allows for a comprehensive study of the impact of acrobatic loads on neurodynamic and cognitive functions, which contributes to the improvement of understanding and optimization of training programs for acrobats of different genders, ages and qualifications.

MATERIALS AND METHODS

Athletes specializing in pair and group acrobatics, of two age categories: puberty and youth ages , participated in the experiment. The first age category (sub-elite) - athletes of puberty age, which included: girls (n=32), average age 13.96±1.02 years; boys (n=36), average age 14.88±2.32 years. Youth acrobats (elite athletes): girls (n=30), average age 19.36±2.86 years; young men (n=34), average age 20.38±2.89.

For the reliability and security of the results of our study, the method of stratified sampling (homogeneous groups) was chosen, where the participants of the experiment were divided into groups by age, gender, and level of sports skill. Each of the groups included athletes of the same (from a statistical point of view) level of physical development and sports qualification, which increases the overall reliability, accuracy and use of data in practice, the results can be applied to a larger number of subjects and different situations. In this way, it reduces the bias of the result and the possibility of systematic error. The homogeneity of the sample was revealed, it is representative of the general population.

The research was conducted in the pre-competition period with the study of individual typological properties of the higher nervous activity of acrobats with the help of the computer program «Diagnost-1» [1, 6, 10, 12]. Sensorimotor reactions of varying degrees of complexity were studied, namely: latent periods of simple and complex reactions, selection of two (RV2-3) from three signals, as well as individual-typological properties of the VND: functional mobility, strength and balance of nervous processes. A comparison of indicators before and after a specific exertion (mixed universal acrobatic exercise) was carried out.

Statistical processing was performed using the STATISTICA 10.0 computer program and MS Excel XP software packages. The main indicators of mathematical statistics were: arithmetic mean (X), standard deviation (SD), standard error of the arithmetic mean (m), Pearson-Brave correlation analysis (r). To study the differences between the indicators used, the non-parametric U-Mann-Whitney statistical test was applied, the level of significance was taken as $p<0.05$.

ETHICAL STATEMENTS AND PARTICIPANTS

The studies were performed in accordance with all relevant national norms and rules of institutional policy and the National Health Council, according to the Declaration of Helsinki. All athletes and parents of minors gave written consent for the study and were informed of the purpose and procedures of testing and

Table 2. Results of evaluation of cognitive functions of acrobats depending on age, gender and age categories in the pre-competition mesocycle

Indicators	Acrobats of puberty age		Acrobats of youth age	
	Gender			
	Female	Male	Female	Male
	Group 1 (n=32)	Group 2 (n=36)	Group 3 (n=30)	Group 4 (n=34)
Time perception error (s)	9,54±3,74	9,32±4,02	7,25±3,24	7,15±3,56
Confidence level (p)	$P_{1,2}$>0,05		$P_{3,4}$>0,05	
Volume of voluntary attention (%)	72,37±7,89	70,98,98±6,28	79,45±6,78	74,39±6,18
Confidence level (p)	$P_{1,2}$>0,05		$P_{3,4}$<0,05	
Volume of short-term memory (%)	57,24±5,83	50,36±4,98*	64,87±6,12	56,84±5,78*
Confidence level p)	$P_{1,2}$<0,05		$P_{3,4}$<0,05	
Coefficient of operational thinking (conventional unit)	1,76±0,45	1,23±0,54*	2,35±0,38	1,68±0,67*
Confidence level (p)	$P_{1,2}$<0,05		$P_{3,4}$<0,05	

Note: * – the differences compared to the Coefficient of operational thinking (conventional unit)group of female athletes are statistically significant according to the Mann-Whitney test (p<0.05).

of the possibility of withdrawing consent at any time for any reason.

RESULTS

To effectively predict the reliability and success of sports activities, it is important to study the functional state of the central nervous system of athletes. In order to predict undesirable phenomena of emotional overstrain, to assess the emotional stability or, on the contrary, the instability of athletes, the characteristics of the behavior of acrobats in various conditions of training and competitive activities, an assessment of the typological characteristics of athletes was carried out [1, 6].

Studies of psychophysiological indicators were conducted in the pre-competition period after performing a specific exertion- a mixed (universal) acrobatic exercise, which combines force-balance and tempo elements to assess the level of adaptation of the functional systems of the body of acrobats of different sexes to conditions that cause an increase in the intensity of physical and psycho-emotional exertion.

Due to the fact that fatigue develops primarily in the central nervous system, in the process of researching the typological properties of the nervous system, the deterioration of indicators can determine the level of overtraining of athletes. In this case, timely correction of training exertion is extremely important in order to optimize the working capacity of those who are engaged.

Testing of simple and complex speed reaction is important, which allows to reveal the psychological readiness of athletes, which is important for monitoring and predicting sports results at the same level as technical and physical training. According to the data we obtained, the speed of simple visual-motor information in young men is statistically significantly higher (p<0.05) than the similar indicator in female acrobats (Table 1). This confirms the qualitative uniqueness of the neurofunctional organization of the male and female brain.

Age and sports experience are also important in the study of psychophysiological functions. This is confirmed by our data, where the comparative characteristics of athletes of puberty and youth ages showed statistically significant differences in most indicators of neurodynamic functions (p<0.05).

Analyzing the obtained data, it can be seen that the indicators of the latent period of simple visual-motor information (lmilliseconds), the latent period of the visual-motor reaction of choosing two out of three stimuli (milliseconds) in girls are statistically different from those of young acrobats.

In the group of elite acrobats of youth age, statistically better indicators were found in terms of latent period indicators than in the group of athletes of puberty age (p<0.05), including gender characteristics, which indicates their higher adaptive and compensatory capabilities of the body.

As is known, indicators of the functional mobility of nervous processes are used to characterize rapid changes in the processes of excitation and inhibition and the strength of nervous processes. The strength of nervous processes characterizes the endurance of the nervous system, working capacity, based on the obtained data, it is possible to draw conclusions about

the ability to tolerate both short-term and long-term excitation and inhibition.

Boys were faster according to the indicator of functional mobility of nervous processes than girls in both age categories (the difference is also significant at $p<0.05$). A greater amount of processed information was found in acrobat boys than in adolescent girls, which corresponds to a higher level of strength of neural processes.

The strength of nervous processes (imposed rhythm, number of mistakes) tends to change this indicator in boys, but in comparison with girls, no statistically significant differences were found ($p>0.5$).

However, after the specific exertion, the number of mistakes increased compared to the background indicators for both girls and boys (reliable differences were detected with a probability of 95%), which indicates the activation of adaptation processes as a result of performing a mixed exercise as a specific exertion (Table 1).

According to the indicators of the accuracy of reactions to a moving object, boys of puberty and youth have a statistically significant smaller amount of reaction deviations than girls acrobats, i.e. boys have a higher balance of nervous processes (95% probability level).

The next task was to perform a Pearson -Brave correlation analysis between the studied indicators (Fig. 1). A significant number of correlational relationships of psychophysiological characteristics and specific exertion in the form of a universal mixed exercise (points) was revealed.

The correlation analysis showed the informativeness of the values studied between the data characterizing the technical readiness of acrobats (scores) in the form of a universal mixed exercise (performance of a composition with force-balance and tempo exercises) of puberty and youth and psychophysiological indicators. The highest correlations were found between the specific exertion in the form of a mixed exercise and neurodynamic indicators in girls of pubertal age ($r=0.435 – 0.689$; $p<0.05$), in boys of pubertal age ($r=0.405 – 652$; $p<0, 05$), in adolescent girls (puberty age) ($r=0.402 – 643$; $p<0.05$) and in adolescent boys ($r=0.468 – 0.658$; $p<0.05$).

According to the indicators of cognitive characteristics, during the research, the features of sexual dimorphism between acrobats of different age categories were revealed, namely: the coefficient of operational thinking (conventional unit) the volume of voluntary attention (%) and short-term memory (%) are statistically significantly better in the group of girls - acrobats of puberty and youth compared to boys and young men of the same age categories ($p<0.05$) (Table 2).

DISCUSSION

A number of specialists were involved in the research of neurodynamic and cognitive functions of athletes in various sports. In the framework of our work, we will focus on individual publications of scientists, where specialists expressed different views, which were made in research, regarding the issues being studied.

Scientists studied the manifestation of sexual dimorphism of highly qualified judokas: Korobeinikova L., Korobeinikov H., Mishchenko V. et al. [13]. In the course of the experiment of the aforementioned experts, the indicator of the latent reaction time in athletes of different genders didn't have a statistically significant difference. However, it was found that there was a significant difference in terms of bandwidth and impulsivity between groups of judo athletes of different genders.

It was also revealed the presence of a reliable difference in terms of accuracy and stability between judo athletes of different genders. The best indicator of accuracy was found in female judokas, within the framework of these studies, better abilities to perform cognitive tasks that require concentration of attention and the involvement of the thinking function were found.

The stability indicator is significantly better in women, who have higher stability in the implementation of neurodynamic functions. According to the features of the manifestation of the balance of nervous processes, no significant difference between women and men engaged in judo was found. The study of cognitive functions in the above-mentioned groups showed significantly better indicators of short-term memory volume and operational thinking coefficient in women compared to men practicing judo.

Thus, on the example of studies in judo, the authors found that the functional organization of psychophysiological functions in highly qualified judo athletes has different information processing strategies depending on gender. The analysis of neurodynamic indicators in qualified athletes specializing in sports games didn't reveal any significant gender differences.

Research results obtained by specialists Korobeinikova L.,Korobeinikov H.,Berezhna A., Danko [14] established that the endurance of the nervous system in men is higher than in women practicing judo. At the same time, it was found that the speed of visual perception is better in women, and the efficiency is better in men, which indicates, from the authors' point of view, also the presence of faster perception of non-verbal stimuli in women (this indicates the predominance of non-verbal intelligence).

The data obtained by Korobeinikov H. , Prystypa Y.,Korobeinikova L., Briskin Y. [1] in athletes of complex coordination sports indicate a probably reduced time of the

motor component of the complex sensorimotor reaction in men compared to female athletes. Also, within the framework of the experiment of the above-mentioned authors, it was found that the values of the complex visual-motor reaction are significantly reduced in qualified sportswomen who are engaged in all-around sports compared to sportswomen who are engaged in complex coordination sports and sports games.

The specialists note that within the limits of the experiment conducted by them, the study of neurodynamic functions testifies to the excellent significance of the time indicator of both simple and complex sensorimotor reactions depending on the type of sport in men and women.

Interesting studies were conducted by Fedorchuk S.,-Kutsenko T.,Yakovenko O., Lysenko O.(2023) [15]. These scientists determined gender differences in the state of psychophysiological functions based on indicators of the reaction to a moving object of qualified rowers with different levels of stress. the authors presented data that male athletes compared to female athletes demonstrated higher response accuracy to a moving object in total and mean deviation time, total delay time, and mean lead time when testing with the subdominant hand, and fewer delay responses during testing dominant hand.

Also, male and female subjects engaged in rowing sports differed in the ratio of the number of anticipatory reactions and the number of delayed reactions during testing with the dominant hand: in men, anticipatory reactions prevailed to a greater extent. The authors explain this by the predominance of visual-spatial coordination functions associated with the right parietal associative cortex in male rowers.

Research by Vilianskyi V., Bachynska N. (2019) [12] on the example of athletes engaged in karate and acrobatics, they showed the following: it was found that between girls and boys in both acrobatics and karate, a reliable difference was found with indicators of the latent periods of simple and complex visual-motor reactions and the strength of nervous processes. Better indicators of the strength of nervous processes and latent periods were found in young men of both sports ($p \leq 0.05$, probability level 90 and 95%).

Psychophysiological indicators of acrobat athletes and those who specialize in karate show better indicators against the background of a certain decrease in the level of neurodynamic characteristics in girls compared to boys.

Compared to acrobats, in karate athletes, the functional mobility of nervous processes is observed by higher values of the strength of nervous processes (at $p \leq 0.05$), that is, lower values of the percentage of false reactions, which may indicate a higher strength of nervous processes, for example, more significant dynamics of specificity fights The revealed difference in the speed of reactions between young acrobats and karate players, as well as female acrobats and karate girls, can be explained, for example, by the features of sexual dimorphism, the length of training in these sports, the specifics of training and competition (the difference is significant at $p \leq 0, 05; 0.01$).

Sports activity has such an important component as the need for quick decision-making in connection with the specifics of the sport. Therefore, we used the evaluation of the current state of psychophysiological indicators and the control of the dynamics of sensory analyzers and the motor apparatus in research for an objective integral criterion of work and the state of the nervous system of acrobats [16, 17].

Our study showed that the latent period of simple visual-motor information (milliseconds), the latent period of the visual-motor reaction to choosing two out of three stimuli (milliseconds)in female acrobats statistically different from the similar ones in young men (95% probability level), which we deal with a higher level of psycho-emotional stress. This, in turn, can contribute to the development of inhibitory processes and a decrease in the efficiency of the nervous system.

According to the indicators of the accuracy of reactions to a moving object in boys and young men of puberty and youth ages, a statistically significant (95% probability level) smaller amount of reaction deviations was found than in female acrobats, i.e. boys and young men have a higher balance of nervous processes.

According to the obtained data, after performing a specific exertion, the latent period of the visual-motor reaction of choosing two out of three stimuli exceeds the simple visual-motor reaction in adolescent acrobat girls by 38.78%, in boys by 33.00%.

Among young athletes, this difference was 33.41% for girls, 34.53% for boys. We consider the change in sensorimotor reactions in elite acrobats after performing a control exercise within the limits of up to 35% as a marker of mobilization of functional reserves and restructuring of the regulatory mechanisms of the central nervous system in relation to adaptation to a specific load: a control mixed competitive exercise in which force-balance and tempo elements are balanced.

During the individual analysis of the obtained results in three female acrobats and two male acrobats of puberty age, it was found that the indicators of individual psychophysiological indicators decreased by more than 50% after a specific exertion compared to the background ones. This indicates a lack of optimal adaptation to those training exercises that were proposed and performed within the plans.

Exceeding the 35 percent decrease in the number

of accurate actions in spatio-temporal tests towards the deterioration of indicators after a specific exertion may be associated with a decrease in the adaptation capabilities of acrobats to competitive and training activities. Therefore, as methodical recommendations, we proposed the individualization of exertion, regular monitoring of the current condition of acrobats and timely correction of training process plans (can be used as an indicator of detecting low exertion tolerance).

Analyzing the data obtained during the study, it was found that female acrobats need more time to process visual information than male acrobats. In boys, these indicators indicate better possibilities of neurodynamic functions compared to acrobat girls. The detected increase in the number of mistakes in the interpretation of the results of simple and complex visual information after a specific exertion can be explained by an increase in the tone of the sympathetic nervous system.

Gender differences in the cognitive functions of acrobat athletes may be due to differences in the brain physiology of women and men, for example, the distribution of neurotransmitters or the volume of certain brain regions. But in practical activities, it is necessary to take into account the individual performance of each athlete, regardless of gender, taking into account strengths and weaknesses.

Thus, pair-group types of acrobatics contribute to positive adaptation in the vestibular and visual systems. It is necessary to regularly monitor neurodynamic and psychophysiological characteristics both in the preparatory and competitive periods at all stages of multi-year improvement, for timely adjustment of exertions in accordance with changes (indicators) of the functional state of athletes.

Thus, analyzing the data presented by a number of authors in various sports, as well as based on the results obtained by us in this work, it was found that in the majority of studies by scientists, there are gender differences in the neurodynamic and cognitive components of athletes. The absence of gender differences in individual indicators (tests) of neurodynamic functions indicates that the issues under study require additional scientific research and the minimization of factors that may affect the reliability of the results. This can serve as a strong basis for future research and the interest of scientists in a wider study of this aspect.

CONCLUSIONS

1. As a result of the experiment, the influence of specific acrobatic loads on the neurodynamic and cognitive functions of elite and sub-elite acrobatic athletes of different genders was assessed.
2. When determining the main neurodynamic indicators, it was found that in female acrobats, the latent period of simple visual-motor information and the latent period of the visual-motor reaction to the choice of two out of three stimuli were statistically different from those of male athletes (95% confidence level), due to a higher level of psycho-emotional stress. For example, after performing the specific load, in the group of sub-elite athletes of pubertal age, the latent period of simple visual-motor information for girls was 244.02±3.34, while for boys it was 253.44±6.19 milliseconds (the difference is significant at $p<0.05$).
3. According to cognitive characteristics during the study, features of sexual dimorphism between acrobats of different age categories were identified ($p<0.05$) related to gender differences in cognitive functions in brain physiology between women and men (due to different information processing strategies considering gender characteristics): the operational thinking coefficient (conditional units), the volume of voluntary attention (%), and short-term memory (%) were statistically significantly better in the group of female acrobats of pubertal and youth age compared to boys and young men of the same age categories ($p<0.05$). For example, the volume of short-term memory in female acrobats of pubertal age was 57.24±5.83%, while in male acrobats it was 50.36±4.98% (the difference is significant at $p<0.05$); the operational thinking coefficient for female acrobats was 1.76±0.45, while for male acrobats it was 1.23±0.54 conditional units ($p<0.05$).
4. In the comparative analysis of the results among acrobats of different genders, ages, and sports qualifications, it was found that in the group of elite youth athletes, the indicators of latent periods were statistically better than in the group of acrobats of pubertal age ($p<0.05$), including by gender characteristics, indicating their higher adaptive-compensatory capabilities: boys were faster in terms of functional mobility of nervous processes than girls in both age categories (for example, in the group of elite youth athletes, this indicator for girls was 84.07±4.47 milliseconds, while for boys it was 76.35±2.57 milliseconds, the difference is significant at $p<0.05$). Additionally, male acrobats showed a greater amount of processed information than female acrobats of youth age, corresponding to a higher level of strength of nervous processes. The strength of nervous processes (imposed rhythm, number of errors) showed a tendency to change in boys, but no statistically significant differences were found compared to girls ($p>0.5$): after the spe-

cific acrobatic load, the number of errors increased compared to baseline indicators in both girls – from 13.91±2.55 to 18.27±2.8 – and in boys of pubertal age – from 10.29±1.62 to 14.89±1.98 (significant differences were found with 95% probability), indicating the activation of adaptive processes as a result of performing mixed exercises as a specific load.

5. The highest correlation relationships were found between specific acrobatic loads in the form of mixed exercises and neurodynamic indicators in girls of pubertal age ($r=0.435 – 0.689$; $p<0.05$), in boys of pubertal age ($r=0.405 – 652$; $p<0.05$), in girls of youth age ($r=0.402 – 643$; $p<0.05$), and in boys of youth age ($r=0.468 – 0.658$; $p<0.05$).

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Impact of INSR (rs2229429) G>A genetic polymorphism on response to exogenous insulin in type 1 diabetic Iraqi patients

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ABSTRACT

Aim: To examine prevalence of genotypic distribution, particularly assessing how genetic polymorphisms in Insulin Receptor gene influence effectiveness of insulin therapy in a sample of Iraqi population.

Materials and Methods: Effect of Single Nucleotide Polymorphisms rs2229429 G>A have been investigated in 99 T1DM individuals, with a mean age of 12.3 years. These patients were managed with exogenous insulin through a basal-bolus monotherapy regimen. Genotyping was performed using an allele-specific polymerase chain reaction technique, and the data were statistically analyzed.

Results: The prevalence of the minor allele frequency is 12% in a sample of Iraqi population. Homozygous mutant carriers of rs2229429 G>A were 10.479 times at higher risk for developing poor glycemic control (HbA1c >86 mmol/mol) compared to wild genotype in type 1 diabetes mellitus, p=0.008. Ultimately poor responders to exogenous insulin, demonstrating significantly higher plasma insulin receptors levels p<0.001.

Conclusions: The investigated Single Nucleotide Polymorphisms is significantly associated with hyperglycemia in type 1 diabetes mellitus and contributes to the development of double diabetes.

KEY WORDS: pharmacogenetics, SNPs, insulin receptor, hyperglycemia

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INTRODUCTION

Pharmacogenomic studies in drug discovery offers significant advantages for individuals with genetic predispositions, introducing novel approaches for personalized medicine and genetic diagnostics [1]. Thereby the efficacy of pharmacotherapy is enhanced, and the risk of drug-induced toxicity is minimized through this approach [2]. Notably, approximately 25-60% of patients exhibit interindividual variability, leading to divergent pharmacological responses to the same medication. The present pharmacogenetic research has the potential to elucidate the underlying factors contributing to individual differences in drug responses among patients. It underscores the importance of employing advanced methodologies for rapid, high-throughput DNA testing, which enables the prompt identification of numerous genetic variations [1, 2]. This study particularly focuses on variations in a specific gene sequence located within the insulin receptor (INSR) gene on the short arm of chromosome 19 (19p13.2). It provides a detailed illustration of the impact of these variations on exogenous insulin monotherapy in type 1 diabetes mellitus (T1DM). According to a previous study, lifelong administration of exogenous insulin is crucial for T1DM individuals. It has been proven to be highly effective in achieving glycemic control and increasing life expectancy [3]. Nonetheless, despite strict adherence to exogenous insulin protocols, a significant number of T1DM individuals exhibit insufficient responsiveness to insulin therapy. As a result, they experience persistent difficulty in attaining glycemic control, leading to chronic hyperglycemia [4]. As a result, uncontrolled T1DM can lead to serious complications, such as nephropathy, retinopathy, neuropathy, diabetic foot ulcers, diabetic ketoacidosis, infections, and serve as risk factors for cardiovascular diseases. Furthermore, environments conducive to the proliferation of cancer cells may be generated. Genetic variations in pharmacognosy can affect the interaction between insulin and its receptor in T1DM, significantly influencing glycemic control [5, 6]. Approximately 99.9% of nucleotide bases are identical across all individuals; however, the remaining 0.1% accounts for roughly 1.4 million unique variations among humans. These dispersed SNPs arise approximately every 300 to 2000 base pairs throughout the genome. Occurring in at least 1% of the population, they contribute significantly to human diversity [7]. Previous studies have

documented that various homozygous and heterozygous mutations in the INSR gene indicate a potential link to altered insulin sensitivity. These alterations result from receptor dysfunction in either the extracellular ligand-binding domain (alpha-domain). Substantial evidence supports the association between insulin resistance and single nucleotide polymorphisms (SNP) induced dysfunction in the INSR alpha-domain [8, 9]. Different forms of insulin resistance resulting from mutations in the extracellular binding domain have been described in numerous studies. These mutations reduce or completely inhibit insulin binding affinity, leading to impaired intracellular signal transduction, contributing to the development of insulin resistance [10, 11]. Insulin resistance type A, Rabson-Mendenhall syndrome, Leprechaunism syndrome, Donohue syndrome, and Polycystic Ovary Syndrome are well-documented examples of genetic forms of extreme insulin resistance. Additionally, patients with double diabetes and reduced responsiveness to platinum-based chemotherapy in epithelial ovarian cancer demonstrate insulin resistance, which are all linked to dysfunction in the alpha-domain of the INSR gene [12, 13]. Additionally, many polymorphisms in the coding region of in the INSR gene had shown correlation with insulin resistance and type 2 diabetes mellitus (T2DM) [14]. Insulin resistance is a condition, mainly prevalent in T2DM, and develops when tissues demonstrate a decreased response to insulin, resulting in hyperglycemia. Studies of insulin resistance and T2DM have suggested that insulin-signaling abnormalities may occur due to decreased cellular INSR content and/or reduced tyrosine kinase activity [15]. Causes of insulin resistance can be divided into pre-receptor, insulin receptor, and post-receptor defects. Given the crucial role of the INSR in initiating insulin action, a mutation in the INSR gene is a likely candidate for explaining genetic forms of insulin resistance [16]. The molecular mechanism of insulin resistance includes defects in receptor maturation and folding, improper transport from the nucleus to the cell surface, or impaired receptor accelerated degradation. As a result, a decreased number of INSR are produced of target cells surfaces or reduced INSR function [10]. A defective INSR function leads to decreased insulin binding affinity and reduced tyrosine kinase activity [17]. Consistent with this hypothesis, T1DM individuals enrolled in the current study have been proposed to exhibit insulin resistance due to receptor defects. Individuals with T1DM who are overweight and have a family history of type 2 diabetes or exhibit clinical signs of insulin resistance are considered to have "double" diabetes [18]. A specific SNP rs2229429 G>A exist in the coding region of exon 8 in the alpha-subunit in the INSR gene and have been connected with insulin resistance. Therefore, we look to explore the prevalence of genotypes of INSR gene rs2229429 G>A among T1DM individuals. Additionally, to investigate predictive role of this polymorphism in a sample of T1DM Iraqi patients treated with exogenous insulin monotherapy. Furthermore, to analyze if there is any association between different genotypes of INSR gene rs2229429 and hyperglycemia in insulin-treated patients with T1DM. This could assist in the diagnosis of double diabetes, thereby allowing for the concurrent consideration of alternative therapeutic options. The SNP rs2229429 G>A has been characterized in the alpha-subunit (FnIII-1) domain of the INSR gene. It is thought to be a benign synonymous mutation. Although the protein sequence is unchanged, the synonymous variant (rs2229429) G>A may influence gene expression, mRNA stability, translation efficiency, and protein folding [19]. The synonymous variant (rs2229429) G>A impact the abundance of insulin receptors on cell surfaces through multiple mechanisms. The secondary structure of mRNA might be altered, and may result in the production of fewer receptors. Furthermore, it affects the speed and efficiency with which ribosomes translate mRNA into mature INSR. Thereby, potentially affecting post-translational modifications and eventually produce misfolded proteins. Moreover, the transport of receptors to the cell surface may be disrupted, or the rate of degradation of existing receptors may be increased [20]. Previous evidence suggests that misfolded proteins may impede insulin binding by altering the three-dimensional structure of the insulin-binding pockets within the receptor's ectodomain, a consequence of defective cleavage into subunits. Since the investigated SNP (rs2229429) G>A is located within the (FnIII-1) alpha-subunit domain, it is expected to distort the docking sites. The (FnIII-1) structural domains are essential for receptor dimerization and high-affinity Insulin binding. Therefore, insulin binds with low affinity to the deformed (FnIII-1) alpha-subunit [21, 22]. Almost any deformation to an amino acid inside the binding interface, alters the receptor's affinity for exogenous insulin. Consequently, preventing the receptor from going through the required conformational changes to activate its intracellular signaling machinery. This in turn reduces receptor activation, which disrupts the signal transduction pathway [21-23]. The underlying cause of this phenomenon is a geometrical constraint, the introduction of a bulky side chain into the narrow binding pocket obstructs access to the active site. This affects the stability and functionality of the INSR, which in turn causes insulin molecules to have difficulty in binding, and even when they do, the connection is weaker and more prone

Table 1. Primers sequences of rs2229429 G>A genetic polymorphism

Primers	Primer sequence (5 '->3 ')	Primer size (bp)	Product size (bp)	Reference
Forward Primer	AACCTCACTGCATCAGCCT	19	319	Current study
Reverse Primer Allele G	CAGAATGTGACGGAGTTCGAC	21		
Reverse Primer Allele A	CAGAATGTGACGGAGTTCGAT	21		

Table 2. Distribution of Genetic Variants in INSR rs2229429 G>A among T1DM individuals

Gene	Genotype	patients	Frequency %	Allele		p-value
rs2229429	GG wild	83	83.8	G	A	<0.0001
	GA heterozygous mutant	8	8.1	(174) 0.88	(24) 0.12	
	AA homozygous mutant	8	8.1			
	Total	99	100			

Table 3. Hardy-Weinberg equilibrium for the INSR rs2229429 G>A genotypes among T1DM individuals

Genotype Observed % N=99		HWE Expected %			Fisher exact test	P-value
G	A	G	A	GA/AG	GG/observed vs GG/expected	<0.0001
88	12	77.23	1.47	21.3	GA/observed vs GA/expected	<0.0001
					AA/observed vs AA/expected	<0.0001

HWE: Hardy-Weinberg Equilibrium, numbers are presented as numbers and percentages, p-value<0.05 is indicated as significant.

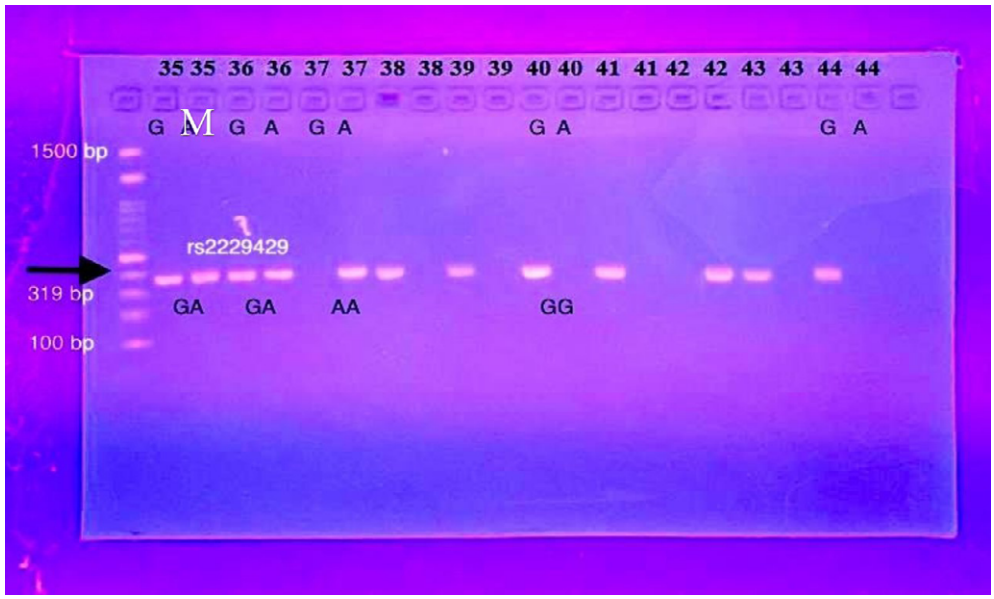

Fig. 1. Genotyping of rs2229429 genetic polymorphism G>A by Allele-Specific PCR Technique.

to breaking. The intended metabolic effects of exogenous insulin are lessened because of this weak or unstable binding. Thus, chronic hyperglycemia in Type 1 diabetic patients in the current study with the above-mentioned SNPs is further explained by the weak INSR activation due impairment in the binding site, as well as reduced protein amount and increased rate of degradation [24]. A decreased insulin binding affinity hinders tyrosine kinase activity to a certain extent. Therefore, impairs the ability of the receptor to transmit signals across the membranes. This suggests that T1DM individuals possessing a SNP in the (FnIII-1) domain may develop insulin resistance, due homozygosity for INSR gene mutations [25]. We hypothesized that the SNP rs2229429 G>A affects glycemic control.

To investigate this, we genotyped patients with T1DM for the SNP rs2229429 G>A. Subsequently, an evaluation of this SNP has been performed to determine whether they are linked to the circulating levels of HbA1c or soluble insulin receptor (sIR) levels in patients who are on insulin basal-bolus monotherapy.

AIM

The primary objective of this study is to examine the prevalence of genotypic distribution, particularly as-

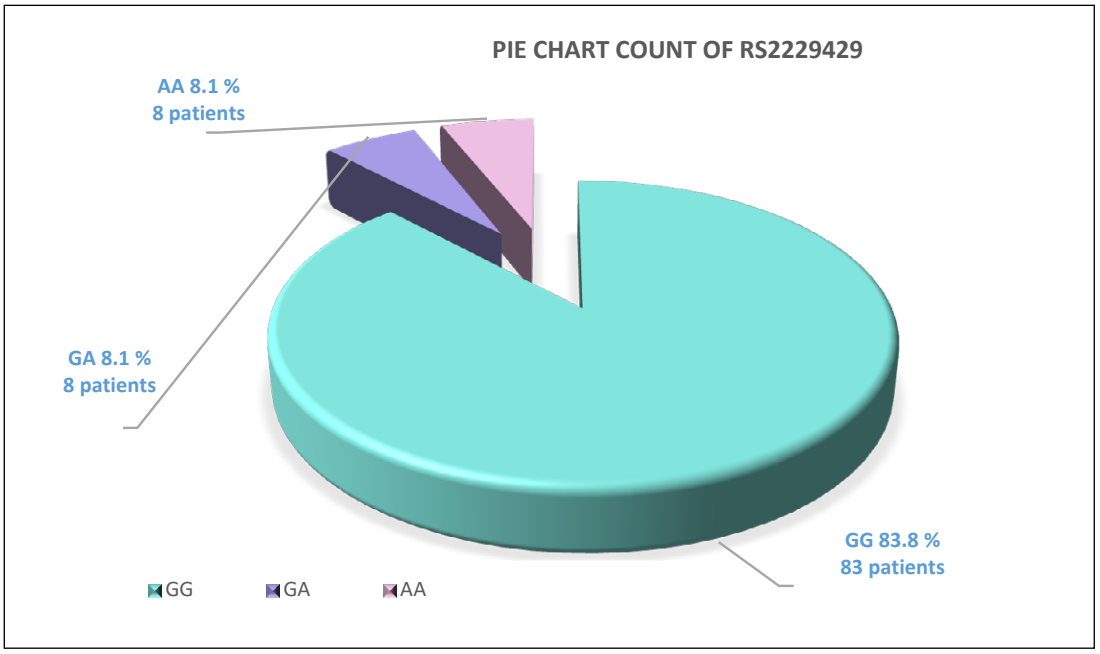

Fig. 2. Distribution of genetic variants of rs2229429 across three genotypes among T1DM individuals.

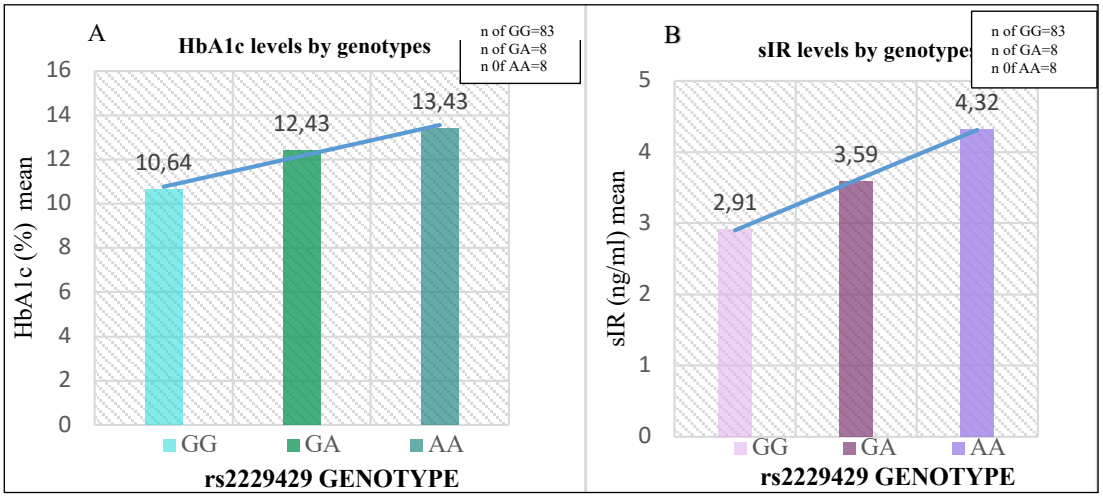

Fig. 3. Comparison of HbA1c levels (A) and sIR Levels (B) by rs2229429 genotypic distribution among T1DM individuals.

sessing how genetic polymorphisms in the INSR gene influence the effectiveness of insulin therapy in a sample of the Iraqi population.

MATERIALS AND METHODS

PATIENTS SELECTION

This study was designed as a cross-sectional study. Ninety-nine T1DM individuals, ranging in age from 5 to 17 years old, were retained in this study during their visit to the Al-Hassan Metabolism and Diabetes Center, Kerbala, Imam Alhassan Almojtaba Teaching hospital/Kerbala, Almarjan Hospital/Babylon and Alzahraa Hospital, Al Najaf City between September 2023 and March 2024. Patients were previously diagnosed according to diagnostic criteria, they received a daily dose of the exogenous insulin basal-bolus monotherapy regimen according to their body weight for at least 1 year, and they followed both inclusive criteria exclusive criteria.

SOLUBLE INSULIN RECEPTOR MEASUREMENT

Shedding of soluble insulin receptors in human plasma may reflect biological response to hyperglycemia. Elevated sIR levels have been associated with diabetes [26]. The determination of human sIR by commercially available INSR-specific sandwich ELISA system, Sunlong Biotech Co (China). Following manufacturer's instructions, specifically designed to detect sIR concentrations in human serum samples, sandwiched by two antibodies.

Table 4. Distribution of genotypic variations of INSR rs2229429 G>A gene across socio-demographic variable in T1DM

Variable		INSR rs2229429 genotypes mean (±SD)						p-value		
		n	GG (n=83)	n	GA (n=8)	n	AA (n=8)	GG vs GA	GG vs AA	GA vs AA
Age (years)	5-12	38	9.58±2.29	2	10.50±2.12	5	10.20±1.79	0.48	0.56	0.86
	13-17	45	15.02±1.44	6	15.00±1.41	3	15.00±2.00	0.97	0.98	>0.99
BMI (kg/m$^{2)}$	Underweight	39	14.89±1.23	1	14.92±0.0	2	17.05±4.03	0.98	0.03	0.74
	Normal	34	21.60±1.96	2	22.40±1.69	2	21.90±0.99	0.57	0.83	0.75
	Overweight	7	28.80±0.89	2	26.60±0.71	3	27.90±0.98	0.01	0.19	0.21
	Obese	3	30.27±0.46	3	30.27±0.37	1	30.01±0.0	0.98	0.67	0.59
DM duration (years)	1 -5	83	4.40±2.96	8	4.00±2.62	8	3.94±1.94	0.71	0.67	0.96
Duration of Rx (years)	1-3	83	2.60±1.39	8	2.25±1.04	8	2.56±1.04	0.49	0.94	0.51

BMI: body mass index, DM duration: diabetes mellitus duration, Duration of Rx Duration of insulin therapy, n=number of patients.

Table 5. Impact of exogenous insulin on glycemic control in respect to INSR rs2229429 G>A gene variations in T1DM

Variable	INSR rs2229429 genotypes			p- value	Reference
	GG (n=83) Mean (± SD)	GA (n=8) mean (± SD)	AA (n=8) mean (± SD)		
FBS (mg/dL)	188.76±52.99	222.38±90.32	233.1±25.78	0.03	Current study
RBS (mg/dL)	317.27±73.79	375.63±72.19	344.00±112.20	0.09	
HbA1c %	10.64±1.44	12.43±1.03	13.43±1.39	< 0.001	
sIR (ng/ml)	2.91±0.87	3.59±0.92	4.32±0.89	< 0.001	

FBS: fasting blood sugar, RBS: random blood sugar, HbA1c: glycated hemoglobin presented in percentage, sIR: soluble insulin receptor, n=numbers of patients

GENETIC ANALYSIS

The genomic DNA was extracted from frozen blood of T1DM patients employing the Favorprep TM Blood gDNA extraction mini kit favorgen. Molecular studies of the INSR gene were performed using Allele specific Polymerase chain reactions (AS-PCR). PCR was performed using specific primer pairs designed for (rs2229429) G>A (Macrogen, Korea) [27]. The primers were designed using Primer Blast Software [28], primers sequences utilized for amplification analysis of *INSR* gene for SNP identification is shown in Table 1.

STATISTICAL ANALYSIS

Statistical analysis of data was performed using Statistical Package for Social Sciences (SPSS), version 26 software (IBM, USA). Independent t test was performed to assess significant differences between means. Chi-square was used to assess significant differences among percentages. To test for statistically significant differences in basal characteristics among the genotypes, a one-way Analysis of Variance (ANOVA) was utilized. For each test, a p-value of <0.05 was considered statistically significant. Haldane-Anscombe correction and Hardy-Weinberg Equilibrium (HWE) (MedCalc Software for HWE) were performed as well. Odds ratio was also estimated.

RESULTS

RESULTS OF AMPLIFICATION REACTION OF THE EXTRACTED HUMAN DNA

The gene polymorphism rs2229429 produced a clear band with a molecular size of 319 bps amplicon using a UV-transilluminator, performed at the Research Center for Genetic Testing (Fig.1). The size of the amplicon was estimated by comparing it to a 100-1500 bp DNA ladder, indicated by lane M. Lane 40 represented individuals with the GG genotype (wild type), lane 37 represented individuals with the AA genotype (mutant type), and lanes 35 and 36 represented individuals with the GA genotype (heterozygous). The gel electrophoresis was conducted at 45 volts. This analysis successfully distinguished between different genotypes of the rs2229429 G>A polymorphism, providing valuable genetic information that may have clinical implications, such as drug response.

Figure 1 illustrates the results by using UV-transilluminator after having performed the Agarose gel electrophoresis by using 1.5% agarose per TBE buffer.

Table 6. Association between INSR rs2229429 G>A genotypes and poor glycemic control in T1DM

SNP allele		HbA1c ≤ 86 mmol/mol				HbA1c > 86 mmol/mol				OR (95% CI)	p-value
		rs2229429 G>A Genotypes				rs2229429 G>A Genotypes					
		n	GG	GA	AA	n	GG	GA	AA		
rs2229429 G>A	G	33	33 (100%)	0	0	50	50 (75.8%)	0	0	10.479 (1.964-55.916)	0.008
	A	0	0	0	0	16	0	8 (12.1%)	8 (12.1%)		
Total patients (99)		33 (100 %)				66 (100%)					

SNP: single nucleotide polymorphism, OR: Odds ratio, 95 % CI: 95 % confidence interval, Haldane-Anscombe correction used for OR, HbA1c: glycated hemoglobin, n=numbers of patients are shown as numbers and frequencies.

Table 7. Relationship between HbA1c levels according to INSR rs2229429 G>A genotypes in T1DM

Variable	Genotype	HbA1c ≤ 10% Mean (±SD)	HbA1c > 10 % Mean (±SD)	p-value	Reference
rs2229429 G>A	GG	9.34±0.57	11.51±1.16	<0.001	Current study
	GA	0±0	12.43±1.03	<0.001	
	AA	0±0	13.43±1.39	<0.001	

HbA1c: glycated hemoglobin presented in percentage, independent sample t-test, p-value (zero-inflated model)

Table 8. Comparison between two age groups according to according to INSR rs2229429 G>A genotypes in T1DM

Variable	Genotype	Age (5-12 years) HbA1c %, mean (±SD)	Age (13-17 years) HbA1c %, mean (±SD)	p-value	Reference
rs2229429 G>A	GG	10.74±1.59	10.57±1.3	0.60	Current study
	GA	11.95±0.07	12.59±1.17	0.49	
	AA	13.1±1.63	13.99±0.87	0.43	
	Total	11.05±1.73	10.98±1.58		

HbA1c: glycated hemoglobin presented in percentage; independent sample t-test used

GENOTYPE AND ALLELE FREQUENCIES ANALYSIS FOR INSR rs2229429 G>A GENOTYPES IN T1DM INDIVIDUALS

The data presented in Table 2 and Figure 2 show the different genotypes distribution among the 99 enrolled patients. Results of allele frequency revealed that patients with the allele A are significantly at a high risk for developing insulin resistance in T1DM patients as compared with patients with the allele G as indicated by a P-value of <0.0001.

HARDY-WEINBERG EQUILIBRIUM ANALYSES FOR INSR rs2229429 G>A GENOTYPES

The following findings underscore the presence of genetic deviations from the Hardy-Weinberg equilibrium (HWE) within this sample across all three genotypes among the enrolled 99 patients. Table 3 revealed the observed and expected genotype counts and Hardy-Weinberg frequencies. The observed frequencies of genotypes (GG: 83.8, GA: 8.1, AA: 8.1) significantly differ from the expected frequencies (GG: 77.23, GA: 21.3, AA: 1.47). The P-value of <0.0001 indicates that the observed genotype frequencies significantly differ from the expected frequencies under HWE, implying that the data supports a conclusion of departure from HWE. We reject or the null hypothesis that the population is at H-W equilibrium

GENOTYPIC VARIATIONS OF INSR rs2229429 G>A GENE ACROSS SOCIO-DEMOGRAPHIC VARIABLE IN T1DM

Table 4 compares basal characteristics, including age, BMI, duration of diabetes and therapy across three genotypes of the rs2229429 gene in 99 T1DM patients. Mean basal bolus daily Insulin dose for the GG genotype was 1.06±0.25 IU, for GA genotype 1.27±0.17 IU and for AA genotype 1.16±0.14 IU. Age, the BMI across genotype groups shows statistical significance only for GA genotype of overweight (p=0.01) patients and AA genotype of underweight (p=0.03) patients compared to the wild type.

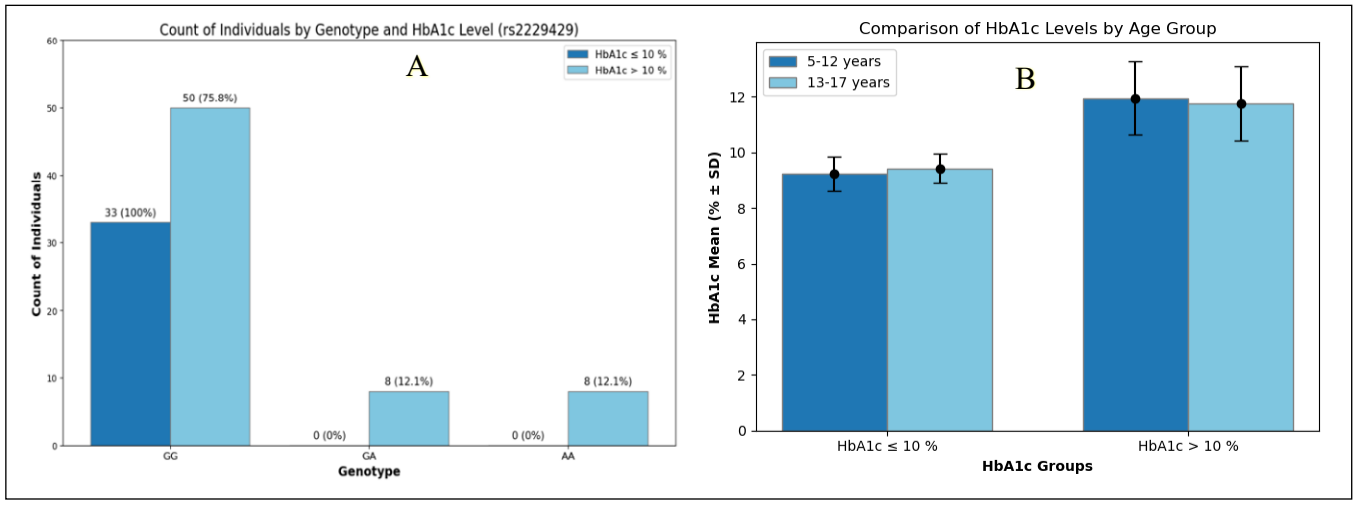

Fig. 4. The distribution of rs2229429 G>A genotypes and HbA1c level groups (A) and age groups (B) among T1DM individuals.

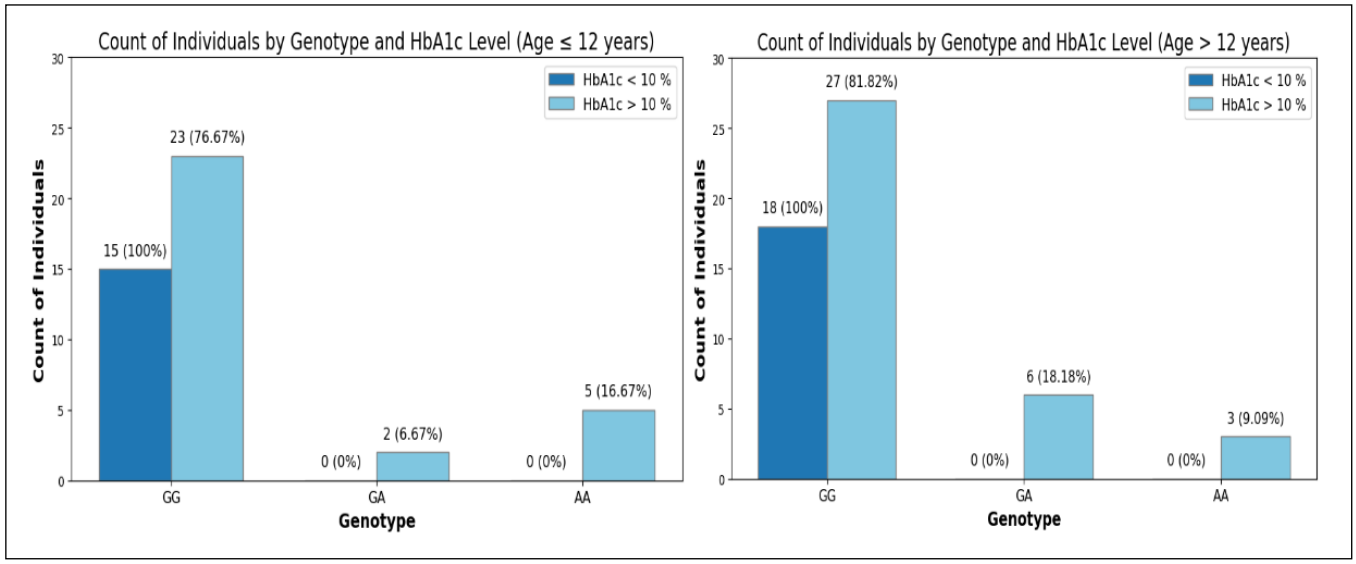

Fig. 5. Counts of T1DM individuals by two age groups and HbA1c levels according to INSR rs2229429 G>A genotypes in T1DM.

Table 9. Relationship between serum sIR levels and HbA1c groups according INSR rs2229429 G>A genotypes in T1DM patients

Variable sIR mean (±SD)		HbA1c ≤ 10 % group	HbA1c > 10 % group	p-value	Reference
		sIR mean (±SD)			
rs2229429	GG	2.74±0.56	3.02±1.01	0.16	Current study
	GA	0±0	3.59±0.92	<0.001	
	AA	0±0	4.32±0.89	<0.001	

sIR: soluble insulin receptor, independent sample t-test used (zero-inflated model)

THE IMPACT OF EXOGENOUS INSULIN ON LABORATORY PROFILES IN RESPECT TO INSR rs2229429 G>A GENETIC VARIATIONS IN T1DM

Patients were on a mean daily dose of 1.16±0.08 IU according to their body weight. None of the patients enrolled in the study received a drug dosage of 2 or more IU kg/day. In Table 5 mean FBS and HbA1c levels show a statistically significant difference of p=0.03 and p < 0.001, with the AA genotype group having the highest mean HbA1c. The GA group exhibits the highest mean RBS; however, it shows no significant association between the different genotypes. Plasma sIR levels (p<0.001) vary noticeably, with the GA and AA genotypes having higher mean values than the GG genotype.

It is shown are serum HbA1C levels of patients with T1DM (n=99) in relation to (Fig.3A) INSR rs2229429 (p <0.001), significant difference is shown in GA heterozygous mutant allele (jade green bar) compared

to homozygous GG (turquoise bars). Elevated HbA1C levels were observed in patients with the mutant AA (teal bar) genotypes. Differences in sIR levels among groups based on rs2229429 genotypes ($p<0.001$) elevated soluble insulin receptor levels were observed in patients with the mutant GA (lilac bar) and AA (amethyst purple bar) genotypes, compared to GG genotypes (lavender bar) (Fig.3B).

DISTRIBUTION OF GENOTYPES INSR rs2229429 G>A IN RELATION TO HBA1C IN T1DM INDIVIDUALS

Table 6 presents the association between rs2229429 genotypes and HbA1c levels (≤ 86 mmol/mol and >86 mmol/mol). Genotype distribution of GA and AA genotypes is highly prevalent in HbA1c > 86 mmol/mol group, with identical distributions for GA (8 patients) and AA (8 patients) genotypes. The data suggest that carriers of the homozygous mutant type are more likely to develop insulin resistance than the homozygous wild type and are 10.479 at higher risk developing insulin resistance.

Figure 4A illustrates the distribution of the different genotypes among HbA1c level groups. Genotype GA and AA is highly prevalent in the HbA1c >10 % level group. Figure 4B shows near equal HbA1c levels among both age groups. A significant association between rs2229429 genotypes and HbA1c mean levels is illustrated in Table 7. HbA1c mean levels are presented in percentage. GA (12.43±1.03) and AA (13.43±1.39) genotypes is higher than GG (11.51±1.16) genotypes in HbA1c > 10% group. Additionally, GG genotypes of the HbA1c >10% groups are higher than the GG genotype of the HbA1c ≤ 10% group.

The HbA1c levels among different genotypes showed no statistical significance among the two age groups (Table 8).

Patients with the heterozygote and homozygote genotype demonstrating HBA1c levels > 10% are distributed over two age groups (Fig. 5).

RELATIONSHIP BETWEEN SIR AND HBA1C GROUPS ACCORDING TO INSR rs2229429 G>A GENETIC DISTRIBUTION IN T1DM INDIVIDUALS

Serum soluble insulin receptor levels were analyzed according to genotypic distribution and HbA1c groups are illustrated in Table 9. sIR values according to manufacturer's protocol for normal individuals was identified by a range from 1 to 3 ng/ml. HbA1c >10 % group shows higher mean values for the minor allele of rs2229429 compared to the major allele and is of statistical significance.

DISCUSSION

SNP selection of rs2229429 G>A was based on a literature search of previously reported associations with glycemic control and reduced insulin sensitivity in T1DM and was proposed to be associated with insulin resistance. The synonymous SNP exists in the coding region at exon 8, and is located in the FnIII-1 domain on the in the alpha-subunit of the INSR gene, on chromosome 19 (locus chr19:7166377). This SNP is quite common in European ethnicity with a heterozygote frequency of at least 8 % [19]. A minor allele frequency for the A allele in Qatari population was of 29 % and 0.05 % in Korean and Japanese ethnicity, however the minor allele frequency in the Caucasian ethnicity was reported to be 19 % for the A allele. Our results are consistent with earlier studies in populations according to 1000 Genomes Project Consortium. The results of the DNA amplification reaction of the investigated polymorphism in this study revealed three genotypes. GG, GA, and AA, with a minor allele frequency of 12 % for the A allele in a sample of Iraqi population. Our data reveals that the most common genotype prevalence was for the homozygous mutant GG (83.8%), followed by an equal distribution for the heterozygous GA (8.1%) and homozygous AA (8.1%). Recent excellent researches identified that SNPs within the INSR gene ectodomain (alpha-chain), are associated with hyperglycemia [14]. This hypothesis meets our results. We examined if the SNP in INSR gene rs2229429 G>A is associated with high levels of HbA1c in T1DM. The results showed that the enrolled T1DM patients had poor glycemic control with a HbA1c total mean of 11.01±1.64. We observed that patients who were heterozygous for the minor allele A in rs2229429 tended to have higher HbA1c levels and consequently, indicating poor glycemic control than patients who were homozygous for the wild type G allele. From hypotheses from many studies, we assume according to evidence that the synonymous SNP rs2229429 might have led to reduced insulin sensitivity. Consequently, leading to an unstable signaling cascade. As a results, we propose that insulin resistance could be associated to the enrolled patients carrying mutant alleles in the *INSR* gene possessing over a high BMI [29, 30]. The results suggest that the Odds Ratio 10.479 for the rs2229429 SNP A genotype is of statistical significance (p-value = 0.008), indicating difference of having > 86 mmol/mol with the G allele as reference. These results demonstrated that patients carrying the A allele are significantly at high risk of 10.479 times for

developing chronic hyperglycemia associated with insulin resistance as compared to the G allele carriers in the same HbA1c group. Furthermore, the current study demonstrated a significant association between rs2229429 G>A genotypes and HbA1c mean levels in T1DM patients, along with the p-value (<0.001). HbA1c mean±SD levels of GA (12.43±1.03) and AA (13.43±1.39) genotypes is higher than GG (11.51±1.16) genotypes in HbA1c > 10% group. This suggests a possible association between genotype and insulin sensitivity in T1DM patients. Similarly, the results of the mean HbA1c were compared in two age groups, despite that the adolescents experiencing pubertal and hormonal changes. Their mean HbA1c levels were lower than the school-aged group, however results showed no statistical association between both age groups and the mean HbA1c levels of those with the wild type genotype. This analysis suggests that patients carrying the "wild type" who experiences hyperglycemia, might be due to poor compliance, dehydration, stress, physical inactivity, hormonal changes and sedentary lifestyle. T1DM individuals carrying the minor allele of rs2229429 showed an increased prevalence of chronic hyperglycemia, regardless of the age. There is a direct correlation between HbA1c and insulin resistance, where HbA1c has been shown to be more strongly associated with the insulin sensitivity in healthy subjects with normal glucose tolerance. As a result, HbA1c is a reliable biomarker and an excellent indicator of insulin resistance for testing individuals for diabetes [31]. A family history of diabetes has been reported in 39 % of patients in the current study. The two patients who were overweight and were heterozygote for the minor allele (p=0.01) have reported to have parental history of diabetes. This may suggest the possibility that these patients exhibit characteristics of double diabetes. Further investigation for lipid profile, metabolic biomarkers, blood pressure, waist circumference is fundamental to the 9 patients with a high BMI possessing over the investigated SNP are essential to exclude double diabetes. Based on the hypothesis that soluble sIR may exist in human plasma, we attempted to detect sIR in human plasma in T1DM individuals [31, 32]. Previous research has proposed that the increased soluble IR ectodomain level appears to be a more rapid glycemic marker than A1C or glycoalbumin. This hypothesis is consistent with the current study, sIR levels have been analyzed and have shown significant differences (p<0.001) across genotypes. This indicates a possible association between genotype and sIR levels. The half-life of the insulin receptor was discovered to be 7–12 hours, however a previous study estimated a half-life of 6 hours in diabetic patients [32]. This is explained by the fact that shedding of the insulin receptor appeared to be parallel to the blood glucose levels, which is consistent with our results. Healthy normoglycemic individuals' results for sIR have shown normal values (data not shown).

CONCLUSIONS

The prevalence of minor allele frequency of the INSR rs2229429 G>A gene polymorphism is 12 % in a sample of T1DM Iraqi individuals. The investigated SNP is identified as a biomarker for predicting poor glycemic control. Results indicate that patients carrying the minor allele are 10.479 times (p=0.008) at a higher risk as compared with patients with the G allele for developing poor glycemic control (HbA1c >10 %). Ultimately, we suggest that elevated levels of the soluble insulin receptor may serve as a rapid glycemic biomarker for hyperglycemia.

RECOMMENDATIONS FOR FUTURE PERSPECTIVES

This research prompts further inquiries that require additional investigation, including the clinical, metabolic, and immunological profiles of affected individuals, which would be highly valuable. Extensive, long-term clinical trials are also essential to evaluate the safety and effectiveness of non-insulin antidiabetic medications for patients with insulin-resistant T1DM or double diabetes. Future research should focus on therapies targeting insulin resistance, such as metformin and pramlintide, as well as investigate various drug classes, including thiazolidinediones, GLP-1 receptor agonists, SGLT2 inhibitors, and DPP-4 inhibitors. Further, in-depth exploration of the novel once-weekly basal insulin formulation, insulin Efsitora alpha, is crucial. Hypoglycemia concerns were among the reasons why the U.S. Food and Drug Administration recently voted against its approval for type 1 diabetes, though it has been approved in several other countries. Consequently, conducting comprehensive studies on patients with double diabetes would be highly beneficial. Researchers have been exploring the potential of CRISPR-Cas9, an innovative and powerful gene-editing technology, for correcting genetic mutations in stem cells, with the goal of transforming them into healthy cells. Currently, CRISPR-Cas9 research is in early stages, primarily focused on preclinical models. Although this technology shows great promise, it may take several years before it is ready for cww linical application. Therefore, further research is essential to establish comprehensive ethical guidelines, regulatory frameworks, and public policies to create globally accepted standards for CRISPR use in human genetic modification. While preclinical trials have shown the potential of CRISPR-Cas9, the long-term

effects of gene editing in humans remain largely unknown, emphasizing the need for studies that prioritize long-term safety assessments to evaluate the stability of edited genes and possible immune responses. Additionally, developing computational models to simulate soluble insulin receptor interactions and predict the impact of modulating its levels or activity could provide insights into insulin sensitivity and glucose regulation.

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This study was carried out in accordance with ethical guidelines to ensure the protection of participants. Informed consent was obtained from all participants, who were made fully aware of the study's objectives and procedures. Participation was voluntary, and individuals were free to withdraw from the study at any time without any restrictions or sanctions. All participant data were anonymized and securely stored in password-protected files, accessible solely to the research team. The study received approval from the Research Committee of Kerbala Health Directorate (2023167/Kerbala) in 8/09/2023.

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Socio-psychological causes and consequences of combat stress in armed conflict participants

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ABSTRACT

Aim: The aim is to determine the causes and consequences of combat stress in armed conflict participants.

Materials and Methods: The research involved 109 service members (under 30) who participated in the russian-Ukrainian war in 2022-2024 on a rotational basis for different durations (from 4 to 18 months). Research methods included theoretical analysis and generalization of literary sources, interviews, questionnaires, and statistical methods.

Results: The causes of combat stress in service members participating in military operations have been identified. It has been established that during the first rotation, service members most often suffer from being in conditions of constant threats (71.6 %), experience fear of death and injury (70.6 %), fear of killing a person (67.9 %), loud sounds (63.3 %) and lack of sleep (52.3 %). With the acquisition of combat experience, the frequency of these factors decreases. The research revealed the consequences of combat stress, which worsen depending on the duration of stay in a combat situation: increased aggressiveness (71.1 %), anxiety, fear (68.4 %), sleep problems (31.6 %), cognitive impairment (28.9 %), and psychosomatic disorders (26.3 %).

Conclusions: Ensuring military personnel's access to qualified psychological assistance, creating a support and rehabilitation program, and raising public awareness of the problems faced by service members participating in combat operations will reduce the negative effects of combat stress and help improve the quality of life of military personnel and their families.

KEY WORDS: health, stress, combat stress, military personnel, armed conflict participants, war

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INTRODUCTION

The realities of the current Russian-Ukrainian war have forced many scientists to turn to stress as one of the main factors in the battle of attrition [1, 2]. Modern psychological science has not stayed away from this issue. Researchers note that stress is the main factor that affects the quality of people's lives and is closely related to both mental health and many physical health problems [3, 4]. A study on the causes and consequences of combat stress due to the full-scale invasion of Ukraine by the Russian federation is particularly relevant. However, despite the breadth of interest in the stress problem, insufficient research substantiates the relevant theories.

The analysis of scientific sources on various aspects of combat stress showed that much research has been conducted to address this problem. This is because an increase in local and regional armed conflicts around the globe has characterized recent decades [5]. These conflicts have diverse localizations and durations and differences in strategic goals, intensity of hostilities, nature, and specifics of military operations. Unfortunately, Ukraine has faced such large-scale armed aggression, which has been going on for three years in a row. Hundreds of thousands of service members from both sides are involved in the fighting. Armed conflict participants experience the effects of combat stress for a long time [6]. The circumstances of service members' stay in conditions of significant psychological and physical stress with the need to make optimal decisions in a limited time make high demands on the mental health of service members, values, and motivation, as well as on their psychological and physical qualities and adequacy of behavior in a combat situation [7]. Despite the high motivation to protect the territorial integrity and sovereignty of Ukraine, to improve military professional and psychological training, some service members have mental health problems, which are manifested by the development of acute stress reactions, combat

and chronic stress and post-traumatic stress disorder (PTSD), and, as a result, an aggravation of a complex of problems at the interpersonal and social levels, and a deterioration in physical health.

Studies conducted over the past thirty years [8, 9, 10] have revealed the significance of the impact of combat stress on the psyche of a serviceman. Such factors as combat situation can influence the development of the consequences of combat stress, individual psychological characteristics of a person, the level of professional and psychological preparation of a combatant for combat, the nature, and level of motivation of a service member prediction of the impact of combat stress factors on the future life of servicemen in a "peaceful environment," etc. [11]. However, this list of factors and their content, taking into account the realities of today, need to be clarified and supplemented in light of the experience of countering russian armed aggression by the security and defense forces of Ukraine, which has led to the relevance of the research of this problem.

AIM

The aim is to determine the causes and consequences of combat stress in armed conflict participants.

MATERIALS AND METHODS

The research involved 109 male service members of Bohdan Khmelnytskyi National Academy of the State Border Guard Service of Ukraine (Khmelnytskyi, Ukraine) of the first and second age groups (under 25 and 30, respectively) who participated in the russian war in 2022-2024 on a rotational basis for various periods (from 4 to 18 months). Among the service members interviewed, 42 were privates and non-commissioned officers, and 67 were officers.

To achieve the research aim, we used the following scientific methods: theoretical analysis and generalization of literary sources, interviews, questionnaires, and statistical methods. The method of theoretical analysis and generalization of literary sources was used to conduct an analytical review of scientific sources on the outlined range of issues (18 sources from PubMed, Scopus, Web of Science, Index Copernicus and other databases were analyzed). Interviews with armed conflict participants were conducted orally to determine the main factors, in their opinion, that cause combat stress in service members and the consequences of combat stress that they experience after returning from rotations. The questionnaire method involved surveying service members using a questionnaire developed by the author's team. The questionnaire contains 20 questions, that are aimed at studying the causes and consequences of combat stress in armed conflict participants.

The results of the survey revealed the frequency of combat stress symptoms experienced by service members during participation in military operations, depending on combat experience (during the first and second or subsequent rotations), as well as the consequences of combat stress depending on the duration of stay in a combat situation (up to 6 months or 12 months or more). The survey also asked service members to identify the factors that most influence the occurrence of combat stress, in their opinion. The factor that they considered the most traumatic was assigned the 8th rank, slightly less traumatic – the 7th rank, and so on, down to the least traumatic, which was assigned the 1st rank. Based on the results of the survey, we calculated the standardized ranks of combat stress factors and built their rating depending on the combat experience of the respondents (number of rotations and their duration) and depending on the category of service members (private, non-commissioned officer or officer) and, accordingly, the combat tasks performed during the rotations. The questionnaire was anonymous, which helped to increase the accuracy of the formulated conclusions. The questionnaire was assessed by the experts in this field (1 professor and 3 associate professors) and was approved by the Academic Council of Bohdan Khmelnytskyi National Academy of the State Border Guard Service of Ukraine (Khmelnytskyi, Ukraine) (Protocol No. 16 dated 19.08.2022). Consent to voluntary participation in the survey was obtained from all respondents involved in the study. Data collection, processing, and analysis were performed on a personal computer using MS Excel, a software package that can use statistical data processing methods. The procedure for organizing the study and the topic of the article were previously agreed with the committee on compliance with Academic Integrity and Ethics of the Bohdan Khmelnytskyi National Academy of the State Border Guard Service of Ukraine. Also this study followed the regulations of the World Medical Association Declaration of Helsinki. Informed consent was received from all participants who took part in this study.

RESULTS

Interviews with service members who participated in combat operations revealed that the psyche of a serviceman in combat conditions is affected by many factors that can lead to a pre-pathological destabilizing state of combat stress, including fear of death or injury, fear of killing a person (even if it is the enemy), being

Table 1. The frequency of combat stress factors experienced by service members during combat operations depending on their combat experience (n=109, %)

Combat stress factors	Frequency of factors manifestation			
	Often	Sometimes	Very rarely	Never
During the 1st rotation				
Fear of death and injury	70.6	21.1	8.3	0
Fear of killing a human enemy	67.9	17.4	12.8	1.9
Lack of support from commanders	12.8	24.8	25.7	36.7
Insufficient amount of sleep	52.3	33.0	13.8	0.9
Insufficient resources	10.1	14.7	30.3	44.9
Unfavorable climatic and geographical conditions	31.2	31.2	23.9	13.7
Living under constant threats	71.6	14.7	11.0	2.7
Loud sounds	63.3	25.7	11.0	0
During the 2nd and subsequent rotations				
Fear of death and injury	59.6	17.4	15.6	7.4
Fear of killing a human enemy	26.7	16.5	31.2	15.6
Lack of support from commanders	35.8	29.4	14.7	20.1
Insufficient amount of sleep	43.1	28.4	23.9	4.6
Insufficient resources	13.8	19.3	29.4	37.5
Unfavorable climatic and geographical conditions	33.0	29.4	26.6	11.0
Living under constant threats	56.9	17.4	16.5	9.2
Loud sounds	54.1	32.1	10.1	3.7

Table 2. Rating of combat stress factors of armed conflict participants (n = 109), depending on the category of service members and their combat experience, points

Combat stress factors	Privates and non-commissioned officers (n = 42)		Officers (n = 67)	
	During the 1st rotation	During the 2nd rotation	During the 1st rotation	During the 2nd rotation
Fear of death and injury	8	5	8	8
Fear of killing a human enemy	7	2	6	7
Lack of support from commanders	3	8	1	2
Insufficient amount of sleep	5	7	5	6
Insufficient resources	1	6	2	1
Unfavorable climatic and geographical conditions	2	4	3	4
Living under constant threats	6	3	7	5
Loud sounds	4	1	4	3

in conditions of the constant threat to life and health, unfavorable climatic and geographical conditions, lack of rest (sleep), insufficient resources, constant exposure to loud sounds, inadequate support from commanders. Let's look at each factor identified by armed conflict participants. *Fear of dying or being injured*, in other words, the fear of losing bodily integrity or seeing others seriously injured or killed. The human brain has centers for assessing the types of threats, so the stress level will be much higher if another person poses a threat. Thus, a service member may be most afraid of being killed or seeing torture, killing of prisoners or civilians, or being injured by the actions of another person. Also, by default, every human being has an evolutionarily necessary socially oriented attitude of *"thou shalt not kill thy fellow man*," which is intended for the survival of the population of the human species in intraspecific forms of aggression. Given this, most people without serious military moral and psychological training are almost incapable of killing an "enemy person." This kind of act creates very strong stress. At the same time, psychological pressure in terms of the complexity of moral and

ethical dilemmas: between the obligation to execute a combat order, which may contradict personal moral principles, and the need to make quick decisions in conditions of uncertainty, most of which can have lethal consequences for the enemy, accelerates the mental exhaustion of a fighter. In addition, the complexity and danger of combat missions, limited by the amount of intelligence information, require a combatant to concentrate a lot of mental, emotional, purposeful, and physical effort to solve them.

It has also been established that *loud sounds* are a factor in combat stress. It is well known that humans, as a biological species, have historically evolved in an environment where loud sound was a priori perceived as dangerous. It was a signal to run away, to save your life as quickly as possible. The modern type of general military combat is full of loud sounds, ranging from unmanned systems, drones, airplanes, helicopters, tank shots, artillery systems, explosions of shells, mines, and small arms to the screams of wounded soldiers. *Long periods spent in an environment of constant threats* to the life and health of a serviceman due to the time required to move out, deploy, wait at combat positions, and regroup within the unit to other positions close to the enemy. This leads to the development of mental deprivation states caused by the inability to satisfy important life needs for a long time (change from a civilian to a military lifestyle, separation from loved ones, etc.). In addition, a state of frustration can arise when faced with the realization of objective insurmountable difficulties (inability to defeat the enemy for objective reasons of its better technical equipment and training) or subjective experiences of internal psychological conflict (prohibitions to defeat the enemy with actual capabilities, but due to the lack of such orders from the command). Peculiarities of *unfavorable climatic and geographical conditions* such as extreme heat, cold, wind, snow, or other precipitation. Unusual geographical conditions and a different mentality of the local population can significantly complicate the process of performing combat missions and increase the level of stress. *Insufficient rest and sleep time*, even if available, leads to physical and psycho-emotional exhaustion. Armed conflict participants are often forced to perform combat missions for long periods without the possibility of adequate rest, and the nervous system is in a prolonged state of excitement. *Lack of basic resources to maintain vital forces*, such as water and food, following the specifics of the combatant's physical or mental activity. We have also identified another group of factors contributing to combat stress: the *lack of proper support from the command*. The directive, dry style of communication between commanders and subordinates, the remoteness of headquarters and command posts from the line of contact in the context of the combatant's actual location in the combat zone, and insufficient moral support from the leadership, or sometimes miscalculations in the planning of operations, can increase the level of helplessness and isolation of an armed conflict participant in the performance of their duties. Also, the nature of combat missions to repel an enemy offensive, rather than conducting active offensive operations, often causes frustration due to the mismatch between reality and the combatant's expectations.

The study results of the frequency of armed conflict participants' experience of the above factors of combat stress, depending on combat experience, are presented in Table 1.

The results presented in Table I show that during the first rotation, service members most often experience the following combat stress factors: being under constant threat – 71.6 %, fear of death and injury – 70.6 %, fear of killing a person – 67.9 %, loud sounds – 63.3 %, and lack of sleep – 52.3 %. With the acquisition of combat experience, during the second and subsequent rotations to the combat zone, the frequency of combat stress factors most often experienced by service members decreased significantly: fear of death and injury – by 11.0 %, being under constant threat – by 14.7 %, fear of killing a person – by 41.2 %, loud sounds – by 9.2 %, and lack of sleep – by 9.2 %. Instead, the frequency of such a factor as lack of support from commanders increased by 23.0 %.

Studying service members' opinions about the factors that most influence the occurrence of combat stress, we calculated the standardized ranks of combat stress factors and built their ratings depending on the combat experience of the respondents (number of rotations) and the category of service members (private, non-commissioned officer, or officer) (Table 2).

It has been found that the rating of combat stress factors has significant differences between service members with the rank of privates and non-commissioned officers and officers, especially after the second rotation. Thus, during the 1st rotation, the first place (8 rating points) for privates and non-commissioned officers was taken by the factor referred to as "fear of death and injury," the second – "fear of killing a person," the third – "being in conditions of constant threats"; among officers – the first place – "fear of death and injury", the second – "being in conditions of constant threats", the third – "fear of killing a person". During the 2nd rotation, the first place for privates and non-commissioned officers was taken by the factor "lack of support from commanders," the second – "lack of sleep," the third – was "lack of resources"; for officers – the first place was

Table 3. The frequency of manifestation of combat stress effects in service members depending on the duration of participation in combat operations (n = 109)

Consequences of combat stress	Frequency of factors manifestation			
	Often	Sometimes	Very rarely	Never
Rotation duration – up to 6 months (n = 71)				
Increased anxiety, fear	19.7	39.4	28.2	12.7
Depression	8.5	11.3	45.1	35.1
Suicidal thoughts	0	11.3	12.7	76.0
The onset of alcohol dependence	0	5.6	16.9	77.5
Increased aggressiveness	32.4	42.3	18.3	7.0
Violation of relations in the team	9.8	25.4	19.7	45.1
Violation of family relationships	2.8	8.4	18.3	70.5
Problems with sleep	12.7	21.1	39.4	26.8
Psychosomatic disorders	7.1	15.5	29.6	47.8
Cognitive impairment	4.2	8.4	18.3	69.1
Rotation duration – 12 months or more (n = 38)				
Increased anxiety, fear	68.4	21.1	10.5	0
Depression	28.9	39.5	21.1	10.5
Suicidal thoughts	7.9	15.7	18.4	58.0
The onset of alcohol dependence	18.4	42.1	26.3	13.2
Increased aggressiveness	71.1	15.7	7.9	5.3
Violation of relations in the team	23.7	39.5	26.3	10.5
Violation of family relationships	7.9	18.4	21.1	52.6
Problems with sleep	31.6	50.0	15.8	2.6
Psychosomatic disorders	26.3	39.5	28.9	5.3
Cognitive impairment	28.9	34.2	26.3	10.6

taken by "fear of death and injury," the second – "fear of killing a person", the third – "lack of sleep". It is important to add that for privates and non-commissioned officers during the second rotation, such factors as "being under constant threat," "fear of killing a person" and "loud sounds" received the lowest number of rating points and, accordingly, are the least significant in the formation of combat stress. Among officers, the lowest number of points corresponds to such factors as "loud sounds,""lack of support from commanders," and "insufficient resources." Thus, with the acquisition of combat experience and depending on the combat tasks that servicemen have to perform in a combat environment, the strength of the impact of a particular combat stress factor changes.

The survey also revealed the consequences of combat stress on service members, depending on the duration of their stay in a combat situation (Table 3).

It has been found that after a rotation lasting up to 6 months, service members most often show such consequences of combat stress as increased aggressiveness (32.4 %), anxiety and fear (19.7 %), and sleep problems (12.7 %). However, after rotations lasting 12 months or more, the effects of combat stress are more pronounced: increased aggressiveness (71.1 %), increased anxiety, fear (68.4 %), sleep problems (31.6 %), cognitive disorders (memory problems, decreased concentration – 28.9 %), depression (28.9 %), psychosomatic disorders (cardiovascular diseases, digestive system problems, headaches, fatigue, etc. – 26.3 %). It should be noted that the number of armed conflict participants with frequent signs of alcohol dependence (18.4 %), disruption of team and family relationships (23.7 %), and suicidal thoughts (7.9 %) has increased.

DISCUSSION

Significant physical and mental health problems occurred among U.S. and coalition forces service members during and after combat operations in Iraq and Afghanistan [12, 13]. Mental disorders in combatants were manifested in persistent performance disorders, fatigue, sleep problems (depth, quality, and duration), headaches, convulsive disorders, vomiting, tachycardia,

panic attacks, and other reactions associated with the impact of various stressors in the combat environment, as well as the emergence of more complex and severe mental disorders such as PTSD and depression [14].

The results of the analysis of theoretical and methodological approaches to the study of the socio-psychological causes and consequences of combat stress on service members are highlighted in the works of many scientists [3, 5, 8, 15], but these studies were conducted before the russian aggressor's full-scale invasion of Ukraine. After the outbreak of the Great War, the scale of this problem has increased significantly, and both the factors of combat stress and their consequences need to be clarified. Thus, we generally agree with the conclusions of scientists [7, 16] regarding the lack of research on combat stress and its impact on the personality of a serviceman. Firstly, there are many different points of view on the nature of this phenomenon, which makes it difficult to coordinate approaches to its study. Secondly, the complexity of conducting research in combat and clinical settings limits the possibility of obtaining a complete picture of what is happening and the reliability of the data since most of the material is a reproduction of the memories of armed conflict participants. Thirdly, the lack of specialized tools for psychodiagnosis of combat stress in real combat environments significantly complicates its study and diagnosis [17]. Given these limitations, we analyzed the objective and subjective groups of factors of the combat environment in which a service member may experience the effects of combat stress. It has been found that the frequency of combat stress factors significantly depends on the combat experience of service members.

In general, understanding the essence of the term "combat stress," which we interpret as a short-term, multi-level process of intensive attempts of adaptive activity of the serviceman's body in response to extreme conditions of the combat environment, accompanied by a significant strain on biological and psychological mechanisms of self-regulation, allows us to correct the consequences and find ways to avoid negative consequences.

It has been found that combat stress's effects on service members vary in intensity from mild to severe. As the stressors increase, the manifestations of combat stress can become more pronounced and lead to serious social and psychological consequences. Service members may face problems in interpersonal relationships, including in the family, which often leads to conflicts and divorces. The lack of adequate social support and understanding of society can also exacerbate these problems, causing isolation and alienation.

In its more severe stages, combat stress can lead to serious mental disorders, such as post-traumatic stress disorder, depression, and suicidal tendencies. The loss of comrades-in-arms and the experience of personal loss can cause deep emotional trauma that affects the ability of service members to perform their duties and adapt to civilian life [14, 15, 18].

Particular attention should be paid to combat stress's social and psychological aspects, such as changes in public opinion and perceptions of the military's role in society. Public support and positive attitudes toward veterans can be important in their rehabilitation and integration into civilian life. At the same time, negative attitudes or indifference can increase feelings of isolation and hopelessness.

CONCLUSIONS

The article has identified the social and psychological causes of combat stress in armed conflict participants. It has been found that during the first rotation, service members most often suffer from being in conditions of constant threats (71.6 %), experience fear of death and injury (70.6 %), fear of killing a person (67.9 %), loud sounds (63.3 %), and lack of sleep (52.3 %). With the acquisition of combat experience, the frequency of combat stress factors significantly decreases. Based on the study of combat stress factors, their rating depends on combat experience and the service members' category.

The consequences of combat stress in service members, depending on the duration of their stay in a combat situation, have been revealed: after a rotation of up to 6 months, service members most often have increased aggression (32.4 %), anxiety and fear (19.7 %), and sleep problems (12.7 %); after rotations of 12 months or more, they have increased aggression (71.1 %), anxiety, fear (68.4 %), sleep problems (31.6 %), cognitive impairment (28.9 %), depression (28.9 %), and psychosomatic disorders (26.3 %).

Combat stress effects are multifaceted and require a comprehensive approach to studying and overcoming them. Service members must be provided with access to qualified psychological assistance, support, and rehabilitation programs must be created, and public awareness of the problems faced by armed conflict participants must be raised. This will help reduce the negative effects of combat stress and improve service members' and their families' quality of life.

PROSPECTS FOR FURTHER RESEARCH

It is planned to substantiate a program of rehabilitation measures to reduce the effects of combat stress in armed conflict participants.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

The pharmacogenomic biomarkers and clinical effect of FSHR gene variants on female infertility

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ABSTRACT

Aim: The aims of this study are to detect the genetic polymorphisms of FSHR rs6166 (C> T) and rs6165 (C> T) gene particularly that associated with the response to FSH treatment and their effects on the pathogenesis of infertility in Iraqi women.

Materials and Methods: 210 Iraqi women, aged 20 to 34, who had just been diagnosed with infertility were included in this prospective case control research, whereas the control group consisted of 50 clinically healthy women who were free of any disorders. Following the guidelines for inclusion and exclusion in the study, each of the participating women saw a gynecologist to confirm. The time frame for this From November 2021 to June 2022, the investigation was carried out.

Results: The findings of this study in infertile women, clearly indicates that multiple genotypes of FSHR gene particularly (rs6166) (C>T) and (rs6165) (C>T), that include the homozygous wild genotype (CC), homozygous mutant (TT) and heterozygous (CT) genotype. The T allele was significantly increased ($P<0.05$) in poor responder infertile women for both rs6166 and rs6165 in FSHR which associated significantly with poor response to FSH in Iraqi infertile women.

Conclusions: Polymorphisms in FSHR gene may be associated with decrease in response to FSH treatment and it was associated with pathogenesis of infertility in Iraqi women/ Kerbala province

KEY WORDS: motivators, barriers, plans and factors

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INTRODUCTION

The World Health Organization (WHO) defines infertility as the failure to produce a clinical pregnancy after engaging in continuous, unprotected sexual activity for at least a year [1]. Infertility is a public health problem affecting 8% to 12% of couples worldwide [2]. In addition to its impact on reproductive health, female infertility also has psychological, economic, and physical repercussions it can lead to stress and trauma, particularly in cultures and countries where having children is highly valued [3]. There are two types of infertility in women: "Is unable to bear a child" refers to a woman's incapacity stress and trauma can result from it, especially in societies and nations where having children is highly regarded. This condition is known as primary infertility as well as secondary infertility, which happens when a woman has already given birth. Just 1.9 percent of couples experienced primary infertility, whereas 10.5% experienced subsequent infertility [2-4]. Every gender experiences infertility for the same reasons, despite the stereotype that women are more affected than males. Thirty percent of infertile couples have no known explanation for their infertility, a condition known as unexplained infertility. Forty percent of infertile couples attribute their infertility to the male spouse, forty percent to the female partner, and forty percent to neither of the two [5]. Recombinant DNA technology creates new genetic combinations that are used in industry, science, medicine, and agriculture by joining DNA molecules from two different species. By creating novel therapy strategies, monitoring tools, and diagnostic instruments, it also significantly contributes to the improvement of medical diseases. Two of the most prevalent applications of genetic engineering in health care are the creation of novel kinds of genetically modified bacteria to create synthetic human insulin, erythropoietin, and FSH as well as experimental mutant mice for research [6]. The treatment of infertility greatly benefits from the use of exogenous follicle stimulating hormone (FSH). Exogenous hFSH novel kinds of genetically modified bacteria to create synthetic human insulin, erythropoietin, and FSH as well as experimental mutant mice for research [7, 8]. There are several ways to

obtain follicle stimulating hormone. One technique is to extract various urinary proteins and FSH: LH activity at a 1:1 ratio from the urine of menopausal women [9-11]. In terms of amino acid sequence, glycosylation location, receptor binding capacity, and in vitro biologic activity. Comparable to urinary or pituitary FSH is recombinant FSH. Furthermore, the structures of recombinant and native carbohydrates are the same [12]. At the moment, follitropin α, β, and δ are the three r-hFSH medicines available on the market [13]. Despite having the identical amino acid sequence, follitropin α, β, and δ differ in terms of glycosylation, silica acid residue composition, and isoelectric coefficients: follitropin α has slightly altered biological activity, half-life, and metabolic clearance due to its higher acidity compared to follitropin β [14, 15]. The synthesis of FSHR is succeeded by suitable folding, post-translational modifications (PTM), and the ER and Golgi apparatus creation of highly-ordered di/oligomers. These events are followed by binding and anchoring to the cell surface, which is necessary for FSHR signaling events to function. After FSH binds, the Gas protein separates from the receptor and initiates processes that lead to Gas activation. These include protein kinase A (PKA) and extracellular signal-regulated kinase (ERK), which are triggered by phosphorylating cAMP [16, 17]we compared signaling triggered by human pituitary FSH preparations (FSH(18/21, which regulate the aromatase of downstream effectors of the mitogen-activated protein kinase pathway (MAPK) and the transcription of CREB cAMP response element-binding proteins, including phosphorylation, which are required for the processes of luteinization and ovulation. G protein-related kinases (GRKs) phosphorylated the desensitized receptor in the ILs and its C-terminal tail (Fig.1). Following phosphorylation, beta arrestin proteins interacted with the receptor to enable endocytosis coupling with clathrin-coated pits [18-19].

AIM

The aims of this study are to detect the genetic polymorphisms of FSHR rs6166 (C> T) and rs6165 (C> T) gene particularly that associated with the response to FSH treatment and their effects on the pathogenesis of infertility in Iraqi women.

MATERIALS AND METHODS

This prospective case-control study included 210 Iraqi women aged 20 to 34 years who were newly diagnosed with infertility, while the control group consisted of 50 clinically healthy women with no abnormalities. Following the guidelines for inclusion and exclusion in the study, each of the participating women saw a gynecologist to confirm. The time frame for this research is from November 2021 to June 2022, the investigation was carried out. The private clinic in the city of Kerbala provided samples. The practical part was conducted in the pharmacology and toxicology labs of the College of Pharmacy at the University of Kerbala. The Scientific and Ethical Committee approved this study, and each participant was asked to sign an informed consent form after being informed about it. A distinct patient card was made for every patient. All women were diagnosed with infertility by a consultant gynecologist and underwent laboratory tests (measurements of FSH, LH, Prolactin, TSH, E2, and AMH) as well as a physical examination and vaginal ultrasonography on the cycle's second day. Each infertile woman received subcutaneous injections of 75 IU follitropin-α on the third day of her menstrual cycle. After six days of stimulation, women who were infertile underwent vaginal ultrasound and had their E2 levels measured. Follicular growth was monitored every other day using transvaginal sonography until the minimum size of a single follicle reached eighteen millimeters. Then, to start ovulation, a single intraperitoneal injection A dose of 10,000 IU of HCG was given.

Exclusion criteria: Women are excluded if any of the following apply to them: previous ovarian surgery:

1. Systemic (diabetes mellitus, hepatic, renal, or cardiovascular illnesses) and endocrine problems
2. Infertility due to male factors
3. Ovarian polycystic syndrome
4. Ovarian Endometriosis

In this experiment, the concentration and purity of DNA were measured using a Nano-spectrophotometer, or Nano Drop.

Using the absorbance approach, the Nano drop device was utilized to assess the concentration and purity of isolated DNA. At 260 and 280 nm, absorbance readings were recorded [21]. Light is greatly absorbed by DNA at 260 nm, although it is most powerfully absorbed by protein at 280 nanometers. The A260/A280 ratio was used to determine the DNA's purity. A260/A280 ratios in the range of 1.8 to 2.0 are commonly mentioned as trustworthy indicators of DNA samples of superior quality [22]. Extremely sensitive Nano drop micro detector used as a blank. After cleaning the micro detector from blank High-quality DNA samples are often identified by their A260/A280 ratios, which are typically reported to be between 1.8 and 2.0 [23] (Fig.2).

STATISTICAL ANALYSIS

A260/A280 ratios between 1.8 and 2.0 are frequently cited as reliable markers of high-quality DNA samples.

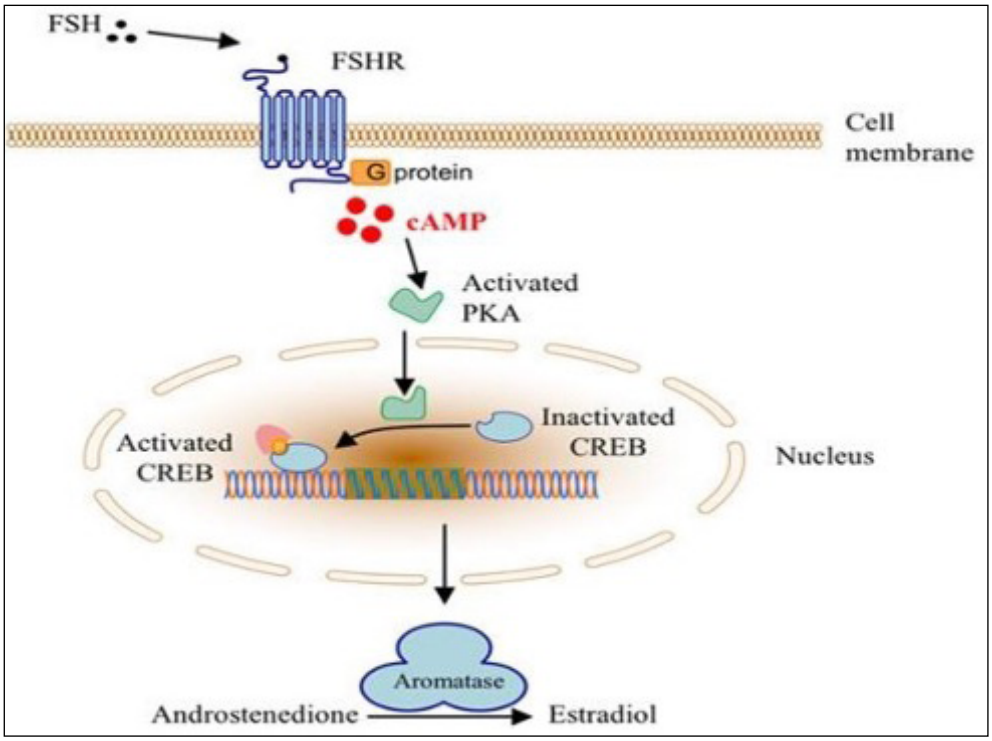

Fig. 1. Signaling mechanism (FSH and FSHR cellular activity) via the standard cyclic AMP/protein kinase An [20].

The results were examined using Pearson's correlation coefficients and the Statistical Package for the Social Sciences (SPSS) version 21.0. A P-value of less than 0.05 was considered statistically significant. Allele frequency for each genotype in the current study was estimated using the Hardy-Weinberg equilibrium online calculator; a P-value of ≤ 0.05 was deemed significant. In addition to clinical and biochemical markers, the study used the 95% confidence interval (CI 95%) and odds ratio (OR) to assess the relationship between these genotypes and the start of infertility.

RESULTS

A FEW CLINICAL AND BIOCHEMICAL CHARACTERISTICS IN THE PATIENT AND CONTROL GROUPS PRIOR TO THERAPY

Table 1 shows the biochemical marker results for this investigation. The blood readings of prolactin, AMH, TSH, LH, E2, and antral follicle count prior to treatment (for both high responder infertile women and others) were reported as mean ± standard deviation (SD). For the control group as well as the patient groups (moderate, poor) the pre-FSH, LH, TSH, prolactin, AMH, E2, and antral follicle count (for both high responder infertile women and others) therapy blood values were represented by the standard deviation ± mean (SD). The pre-treatment serum values of FSH, LH, TSH, prolactin, AMH, E2, and antral follicle count (for both high responder infertile women and others) were shown by the mean ± standard deviation (SD). It occurs in the infertile women's group with high response rates compared to the control group; among the infertile women's groups (moderate and high responder), the high responder group's mean serum FSH levels are statistically significantly lower than the poor responder group's ($P < 0.01$). For the three patient groups (poor, moderate, and high responder infertile women), the mean ± standard error of serum LH levels was 5.85 ± 0.72 mIU/mL, 8.09 ± 1.14 mIU/mL, 5.48 ± 1.52 mIU/mL, and 7.42 ± 0.66 mIU/mL, respectively. An ANOVA revealed no statistically significant difference in mean blood levels of LH between the moderate responder group and the control group ($P >0.05$). When compared to the moderate responder and control groups, the mean LH levels in the poor responder group were considerably lower ($P ^ 0.001$), whereas the mean LH levels in the high responder group were significantly higher ($P ^ 0.001$) than those in the poor responder groups. The patient groups (none of whom differed substantially from the others; $P > 0.05$ and the control group (poor, moderate, and high responder infertile women) did not exhibit significant differences in the means of their serum levels of prolactin and TSH. There were no statistically significant differences in the means of the patient groups (poor, moderate, and high responder infertile women) or in the serum levels of TSH and prolactin between the control group and the patient groups ($P > 0.05$). The results for infertile women are displayed in Table 1. Together with the serum AMH values for the patient and control groups (low, moderate, and high responder in that order, 3.24±0.96 ng/mL,

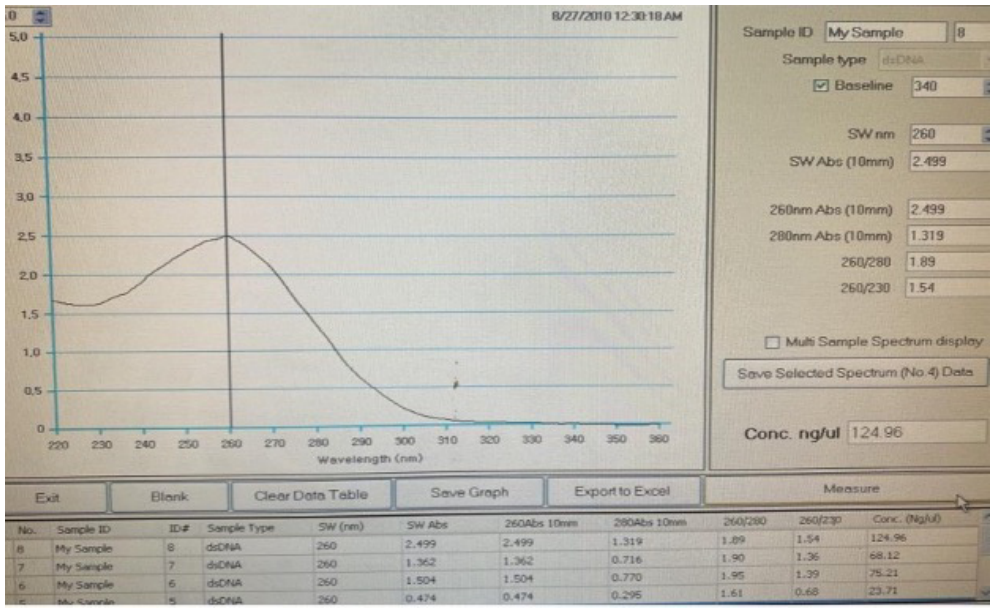

Fig. 2. DNA Nano drop results (concentration and purity).

0.63±0.12 ng/mL, 2.89±0.66 ng/mL, and 7.98±1.46 ng/mL). There was no significant difference between the moderate responder group and the control group ($P > 0.05$). The mean serum AMH level increased significantly ($P \wedge 0.001$) between the high responder group and the control group. This was seen in the subset of respondents who did not do well. Comparing the average of AMH for the high and moderate responder groups to the poor responder group and the high responder group to the moderate responder group revealed a significant increase ($P < 0.001$). Prior to therapy, the control and patient groups (poor, moderate, and high responder infertile women) had blood levels of E2 at mean±SD of 31.17±6.35 pg/mL, 38.96±6.52 pg/mL, 33.22±5.62 pg/mL, and 30.23±5.24 pg/mL. Although there was no statistically significant difference ($P>0.05$) between the high and moderate responder groups and the control group, an ANOVA comparison of the E2 before treatment results revealed that the poor responder group had very high significant increases ($P \wedge 0.001$) in the mean of E2 before treatment compared to the control group. The mean E2 before treatment for the moderate responder group exhibited very substantial significant decreases ($P \wedge 0.001$) compared to the poor responder group. Prior to treatment, the high responder group's mean E2 level in their sera was considerably lower than that of the moderate and poor responder groups ($P < 0.001$). The control and patient groups consist of infertile women who are poor, moderate, and high responders. Their respective mean ±SD values for the antral follicle counts were 11.7±1.23, 4.14±0.72, 11.16±1.01, and 16.59±2.16, respectively. The results of an ANOVA showed that the mean antral follicle count of the moderate responder group and the control group did not differ statistically significantly ($P > 0.05$). On the other hand, the average number of antral follicles was considerably lower ($P \wedge 0.001$) in the poor responder groups and significantly larger ($P \wedge 0.001$) in the high responder group compared to the control group. The mean antral follicle count for the high and moderate responder groups was significantly higher ($P \wedge 0.001$) than for the poor person group. This increase was also observed when comparing the moderate responder group to the high responder group.

The present study discovered that, in contrast to the control group, the mean serum FSH greatly increased in the poor responder group, but it sharply decreased in the infertile women groups with moderate and high responders. In contrast to the poor responder group, table 1 shows how the pituitary gland raises the quantity of FSH to maintain normal follicular growth. Therefore, the body's reaction to ovarian stimulation therapy is predicted by the basal FSH level [24]. Women with high blood FSH levels who were infertile were more likely to have a negative response to ovarian stimulation therapy, according to research by Jaiswar *et al.* These outcomes were consistent with their research, which showed that basal serum FSH levels significantly predicted ovarian reserve and response [25]. The current study's findings were in line with previous research's findings showing patients with greater serum FSH levels were less fertile, even if its discovery was distinct [26]. The results showed no significant differences in FSH levels across the several groups of ovarian stimulation treatment responders. According to table 1, the basal level of E2 in the current study revealed that the moderate and high responder infertile women experienced a very high significant reduction, while the poor responder group experienced a very high significant increase when compared to the control group. How-

Table 1. Evaluation of biochemical parameters of the control group and patient groups (women with low, medium and high response to therapy, suffering from infertility), (ANOVA test; all data were expressed as mean ±SD)

Hormonal and clinical parameters	Control	Poor responder	Moderate responder	High responder
Number	50	70	61	79
FSH (mIU/mL)	6.27 ±1.43	9.64 ±0.45 $^{a▲***}$	6.64±1.48 $^{aNS,b▼***}$	5.67 ±0.26$^{a▼**,b▼***,c▼***}$
LH (mIU/mL)	5.85 ±0.72	8.09±1.14 $^{a▲***}$	5.48±1.52 $^{aNS,b▼***}$	7.42. ±0.66 $^{a▲***,b▼***,c▲***}$
TSH (mIU/mL)	1.9 ±0.39	2.06 ±0.57 ^{NS}	2.04 ±0.41 ^{NS}	2.06 ±0.44^{NS}
Prolactin (ng/mL)	19.55±2.26	19.91 ±2.38 ^{NS}	20.08 ±2.51 ^{NS}	19.23 ±3.22 ^{NS}
AMH (ng/mL)	3.24±0.96	0.63±0.12 $^{a▼***}$	2.89±0.66 $^{aNS,b▲***}$	7.98±1.46 $^{a▲***,b▲***,c▲***}$
E2 before treatment (pg/mL)	31.17±6.35	38.96±6.52 $^{a▲***}$	33.22±5.62 $^{aNS,b▼***}$	.30.23±5.24$^{aNS,b▼***,c▼***}$
Antral follicle count	11.7±1.23	4.14±0.72$^{a▼***}$	.11.16±1.01 $^{a NS, b▲***}$	.16.59±2.16 $^{a▲*** b▲***,c▲***}$

a – ANOVA test between poor, moderate, high responder groups versus control group. b – ANOVA test between moderate, high responder groups versus poor group. c – ANOVA test between high and moderate responder groups. ▼*** – very high significant decrease (P<0.001); ▼** – high significant decrease (P<0.01); ▲*** – very high significant increase (P<0.001); NS – non-significant difference.

Table 2. The mean ±SD of E2 before and after therapy, as well as the size and quantity of Graafian follicles in the patient groups (poor, moderate, and high responder infertile women)

Clinical and hormonal parameters		Poor responder	Moderate responder	High responder
Number		70	61	79
E2 (pg/mL)	Before treatment	38.96±6.52	33.22±5.62	30.23±5.24
	After treatment	80.26±4.67 $^{c▲***}$	311.02±34.61 $^{a▲***,c▲***}$	704.78±138.85$^{a▲***,b▲***,c▲***}$
Graafian follicle size (mm)		9.76±2.23	18.31±1.16 $^{a▲***}$	21.91 ±0.9 $^{a▲***,b▲***}$
Graafian follicle count		1.04±0.2	2.07±0.25 $^{a▲***}$	3.05±0.22 $^{a▲***,b▲***}$

a – ANOVA test between moderate, high responder infertile women groups versus poor responder group. b – ANOVA test between moderate and high infertile women groups. c – Paired t-test between basal E2 and after 6 days of stimulation for poor, moderate and high responder infertile women groups. ▲*** – very high significant increase.

ever, the groups of moderate and high responders did not differ significantly from the control group. Women's baseline E2 serum levels as part of an ovarian reserve test were [27], according to studies by Carvalho et al., determining baseline FSH and E2 together may reduce the possibility of false-negative tests that just look for FSH. The ovarian response may not be sufficient if both indicators are raised too soon [28]. Prasad *et al*. found that the number of graafian follicles and the pregnancy rate increase in tandem with baseline E2 levels. Patients with high basal E2 levels should also be assessed for other stimulation options [29]. AFC measures on the second day of the menstrual cycle should show a significant increase in high responders and no appreciable changes between the moderate and control groups, as predicted by this study. The primordial follicle pool from which antral follicles are drawn determines how many antral follicles are detected by vaginal ultrasonography; the more primordial follicles available, the more follicles will proliferate. The reducing primordial follicle pool is reflected in the declining antral follicle count, which may help explain why the AFC is regarded as a predictor of ovarian response [30]. Lower AFC levels are associated with decreased ovarian reserve and response to ovarian stimulating drugs [31]. The findings showed that while the ovarian reserve of the poor responder group was considerably lower than that of the groups with moderate, high, and control responses, the ovarian reserve of the high and moderate responder groups was determined to be adequate. These results were consistent with the study's conclusions [32], Barbakadze *et al*. argue that blood AMH and AFC have a significant correlation and that combining AMH and AFC could improve the estimate of ovarian reserve, even though AFC assessment by ultrasonography is a useful way for predicting ovarian response. Groups with low AFC may be more likely to have reduced ovarian reserve [33].

SEVERAL CLINICAL AND BIOCHEMICAL MARKERS IN PATIENT GROUPS FOLLOWING THERAPY

Significant correlations have been observed between serum AMH and AFC, and combining AMH and AFC may improve the assessment of ovarian reserve. According to Barbakadze et al., low AFC and ovarian reserve can be re-

Table 3. Comparison of the mean ± SD of the studied parameters (AMH, E2 prior to therapy, and antral follicle count) in patient groups (poor, moderate, and high responder) with varying genotypes of the FSHR gene (rs6165) SNP

Biochemical parameters	Genotype	Poor responder	Moderate responder	High responder
Number		70	61	79
AMH (ng/mL)	CC	0.78±0.08	3.19±0.65$^{a▲***}$	8.51±0.32$^{a▲***,b▲***}$
	CT	0.65±0.07$^{c\ NS}$	3.1±0.57$^{a▲***,\ c\ NS}$	8.56±1.16 $^{a▲***,b▲***,\ c\ NS}$
	TT	0.46±0.18$^{c\ ▼*,\ d\ ▼*}$	2.47±0.49$^{a▲***,\ c\ NS,\ d\ NS}$	7.61±0.91 $^{a▲***,b▲***,c\ NS,\ d\ NS}$
E2 before treatment (pg/mL)	CC	36.36±10.17	33.53±5.96$^{a▼***}$	30.76±4.1$^{a▼***,\ b▼***}$
	CT	37.29±3.19$^{c\ NS}$	33.25±2.19 $^{a\ ▼***,\ c\ NS}$	30.25±5.38 $^{a\ ▼***,\ b\ ▼***,\ c\ NS}$
	TT	41.77±4.2$^{c\ ▲***,\ d\ ▲***}$	34.26±0.03 $^{a\ ▼***,\ c\ NS,\ d\ NS}$	31.56±4.31 $^{a\ ▼***,\ b\ ▼***,\ c\ NS,\ d\ NS}$
Antral follicle count	CC	5.41±0.61	11.7±1.13 $^{a\ ▲***}$	17.84±1.28 $^{a▲***\ b***}$
	CT	4.19±0.79$^{c\ NS}$	10.75±0.73 $^{a\ ▲***,\ c\ NS}$	16.44±1.52 $^{a\ ▲***,\ b\ ▲***,\ c\ NS}$
	TT	2.9±0.75$^{c\ ▼***,\ d\ ▼***}$	10.7±0.48 $^{a\ ▲***,\ c\ NS,\ d\ NS}$	16.44±1.52 $^{a\ ▲***,\ b\ ▲***,\ c\ NS,\ d\ NS}$

c – ANOVA test between (CT, TT) and CC genotype. d – ANOVA test between TT and CT genotype. c NS, d NS ▼* – significant decrease (P<0.05); ▼*** – very high significant decrease (P<0.001); ▲*** – very high significant increase (P<0.001); NS – nonsignificant difference.

Table 4. Analyzing the mean ± SD of the investigated parameters (Graafian follicle size, Graafian follicle number, and E2 after treatment) in the patient groups (poor, moderate, and high responder) after FSH treatment according to different genotypes of the FSHR gene (rs6165)

Biochemical and clinical parameters	Genotype	Poor responder	Moderate responder	High responder
Number		70	61	79
E2 after treatment (pg/mL)	CC	85.74±5.55	319.62± 1.78$^{a▲***}$	763.89± 86.65 $^{a▲***,b▲***}$
	CT	84.59±4.33$^{c\ NS}$	329.76± 18.57$^{a▲***,\ c\ NS}$	734.26±98.75$^{a▲***,b▲***,\ c\ NS}$
	TT	71.79±4.5$^{c\ ▼***8,\ d\ ▼***}$	303.66± 1.78$^{a▲***,\ c\ NS,\ d\ NS}$	695.84± 21.43$^{a▲***\ b▲***,\ c\ NS,\ d\ NS}$
Size of graafian follicle (mm)	CC	11.96±1.61	18.11±1.29$^{a▲***}$	21.75±0.94$^{a▲***,b▲**}$
	CT	11.04±2.33$^{c\ NS}$	19.01±0.93 $^{a\ ▲***,\ c\ NS}$	21.97±0.86 $^{a\ ▲***,\ b\ ▲**,\ c\ NS}$
	TT	5.84±2.44$^{c\ ▼***,\ d\ ▼***}$	18.6±0.41 $^{a\ ▲***,\ c\ NS,\ d\ NS}$	22.29±0.79 $^{a\ ▲***,\ b\ ▲***,\ c\ NS,\ d\ NS}$
Number of graafian follicle	CC	1.23±0.43	2.09±1.12 $^{a\ ▲*}$	3.17±0.53$^{a▲***,\ b▲*}$
	CT	1.27±0.72$^{c\ NS}$	2.06±0.92 $^{a\ ▲*,\ c\ NS}$	3.11±1.13 $^{a\ ▲***,\ b\ ▲*,\ c\ NS}$
	TT	0.65±0.49$^{c\ ▼**,\ d\ ▼**}$	2.01±0.44 $^{a\ ▲**,\ c\ NS,\ d\ NS}$	3.01±0.48 $^{a\ ▲***,\ b\ ▲*,\ c\ NS,\ d\ NS}$

c – ANOVA test between (CT, TT) and CC genotype. d – ANOVA test between TT and CT genotype. significant c NS, d NS ▼* – significant decrease (P<0.05); ▼** – high significant decrease (P<0.01); ▼*** – very high decrease (P<0.001); NS – non-significant difference.

lated. E2 results for medium, high, and poor respondents. The equivalent FSH levels for infertile women following treatment are displayed in Table 2: 311.02±34.61 pg/mL, 704.78±138.85 pg/mL, and 80.26±4.67 pg/mL comparing the moderate and high responder groups to the poor responder group. Table 2 shows that the E2 outcomes for the poor after receiving FSH treatment were 80.26±4.67 pg/mL, 311.02±34.61 pg/mL, and 704.78±138.85 pg/mL, respectively. The paired t-test showed very substantial significant increases (P ^ 0.001) in the E2 mean values when comparing the pre-treatment E2 mean values of the poor, high, and moderate responder groups with the post-treatment E2 mean values of the same groups employing FSH. Following FSH treatment, the mean ± SD Graafian follicle diameters for the three patient groups (poor, moderate, and high responder infertile women) were 9.76±2.23 mm, 18.31±1.16 mm, and (21.91±0.9) mm, respectively. The moderate and high responder groups' mean Graafian follicle size increased significantly (P ^ 0.001) in comparison to the poor responder group, according to the ANOVA test results. The mean ± SD values of Graafian follicles in infertile women in the moderate, high responder, and poor groups were 2.07±0.25, 3.05±0.22, and 1.04±0.2, respectively, after FSH treatment. When comparing the moderate responder group to the poor responder group, an ANOVA test showed a significant increase (P < 0.001) in the mean number of high responder groups and Graafian follicles.

Following six days of exogenous FSH injection, the serum levels of estradiol in the patient groups (poor, moderate, and high responder) are displayed in table 3 of the current study. It demonstrates that, in contrast to the groups of moderate and poor responders, the high

responder group experienced a very high and significant rise. Measurement of the E2 level after ovarian stimulation treatment may be useful to assess follicle maturation and predict the ovarian response to treatment. The steroid hormone E2 is released by granulose cells secreted by developing ovarian follicles. Because the primary functions of FSH are follicular development and stimulation of estradiol synthesis, a low level of estradiol indicates a reduction in the capacity of ovarian follicles to proliferate and produce estradiol in response to FSH [34]. This stimulation may be uncoupled or include distinct downstream pathways of the FSH receptor. The outcomes of this inquiry were consistent with those of study [35]. Researchers found that follicular maturation and a significantly lower pregnancy rate were associated with lower levels of estradiol in the poor responder group. Given their significant association with the size and quantity of graafian follicles after ovarian stimulation therapy, Malathi et al. claim that estradiol levels are a useful therapeutic tool in predicting maturity [36]. This stimulation may be uncoupled or include distinct downstream pathways of the FSH receptor. The results of this study were also consistent with the results of the study [35]. Researchers found that follicular maturation and a significantly lower pregnancy rate were associated with lower levels of estradiol in the poor responder group. Given their significant association with the size and quantity of graafian follicles after ovarian stimulation therapy, Malathi *et al.* claim that estradiol levels are a useful therapeutic tool in predicting maturity [36, 37]. This treatment slows down the ovaries' maturation and growth. Based on these findings, the poor responder group's poor follicle development was primarily caused by decreased granulose cell stimulation and ovarian follicle sensitivity to FSH. After this procedure, the ovaries grow and mature more slowly. Lower granulose cell stimulation and decreased ovarian follicle sensitivity to FSH were the primary causes of the poor follicle development in the poor responder group, according to the data [38].

EFFECT OF THE C>T GENETIC POLYMORPHISM IN THE FSHR GENE (rs6165) ON BIOCHEMICAL MARKERS WAS EXAMINED IN THREE PATIENT GROUPS: POOR, MODERATE, AND HIGH RESPONDERS

Table 3 presents the results of the current study, where one-way ANOVA was used to demonstrate the biochemical characteristics of each FSHR (C> T) (rs6165) genotype. To study differences between patients, it was necessary to establish a relationship between genotypes and levels of hormonal, clinical and biochemical markers, including prolactin, AMH, FSH, LH, TSH, E2 before treatment and the number of antral follicles.

In comparison to the groups with the CC and CT genotypes, the poor responder group with the TT genotype in this study showed a considerably lower AFC value for both the rs6166 and rs6165 tables 3.

THE POOR RESPONDER GROUP WITH THE TT GENOTYPE IN THIS STUDY HAD SIGNIFICANTLY LOWER AFC VALUES FOR THE rs6166 AND rs6165 TABLES THAN THE GROUP WITH THE CC AND CT GENOTYPES

Table 3 shows the E2 results after treatment in (pg/mL), the size of Graafian follicle in (mm), and the number of graafian follicles for patient groups (poor, moderate, and high responder) following an analysis of the impact of an SNP in the FSHR gene (6165) on the response to FSH through treatment with FSH. When comparing the genotypes CC, CT, and TT in the high responder group to the corresponding genotypes in the poor responder group, the averages of E2 after treatment (pg/mL) show a very high significant increase (P ^ 0.001).Additionally, the data show a highly significant increase ($p<0.001$) in the means of E2 after treatment (pg/mL) for the genotypes CC, CT, and TT in the high responder group as compared to the same genotypes in the moderate responder group. The mean ± standard deviation (SD) of serum E2 levels for the CC, CT, and TT genotypes in the poor responder group were 85.74±5.55 (pg/mL), 84.59±4.33 (pg/mL), and 71.79±4.5 (pg/mL) following the initiation of FSH treatment. The mean blood E2 levels for the (CC) and (CT) genotypes in the poor responder group do not differ significantly ($P > 0.05$), according to the results of the ANOVA test. Comparing the TT genotype to the CC and CT genotypes in the same group, however, revealed a substantial decrease ($P < 0.001$). There is no significant difference ($P > 0.05$) between the moderate and high responder groups' average E2 levels after therapy for any genotype (CC, CT, and TT). When comparing the mean size of graafian follicle (mm) findings for all patient groups with the analogues genotypes in the poor responder group, the ANOVA test showed a highly significant increase (P value < 0.001) in the (CC, CT, and TT) genotypes for the moderate and high responder groups. A highly significant increase (P value < 0.001) was observed in the (TT) genotype in the high responder group, which was linked to a similar genotype in the poor responder group. The results demonstrated a significant increase (P value < 0.01) in the case of the genotypes (CC and CT) in the Graafian follicle size when comparing the genotypes in the high responder group and the moderate responder group. The poor responder group with the CC, CT, and TT genotypes had mean ± (SD) Graafian follicle sizes in millimeters (mm) of 11.96±1.61, 11.04±2.33, and 5.84±2.44 after taking FSH medication. After FSH treatment, the poor

responder group with the CC, CT, and TT genotypes had mean ± (SD) Graafian follicle sizes in millimeters (mm) of 11.96±1.61, 11.04±2.33, and 5.84±2.44, respectively. The means of graafian follicle levels following treatment for the two groups exhibiting moderate or strong response do not differ statistically significantly ($P > 0.05$) among the genotypes (CC, CT, and TT).There are no statistically significant differences ($P > 0.05$) in the means of graafian follicle levels after treatment between the genotypes (CC, CT, and TT) for the two groups that exhibit moderate or strong response. When comparing the genotypes, the number of Graafian follicle levels increased significantly ($P < 0.001$). Compare the poor responder group with the high responder group (CC, CT, and TT). Furthermore, the genotypes in the high responder group had considerably ($P < 0.05$) more graafian follicles than the corresponding genotypes in the moderate responder group. Examine and contrast the responses of the poor responder group with those of the high responder group (CC, CT, and TT). Additionally, the TT genotype displayed a significant decrease ($P < 0.01$) in comparison to the CC and CT genotypes, while the number of graafian follicle levels for the genotypes in the high responder group showed a significant increase ($P < 0.05$) in comparison to the equivalent genotypes in the moderate responder group. Following medication, there is only a minor difference ($P>0.05$) in the average number of graafian follicles between the moderate and high responder groups across genotypes (CC, CT, and TT).

DISCUSSION

Infertile women with the TT genotype in the current study's poor responder group had somewhat lower serum levels of estradiol than women with the CC and CT genotypes after receiving the same dosage of FSH for ovarian stimulation. The special FSHR that FSH binds to in the ovary's granulose cells, which is essential in determining female reproduction. Connection between FSHR and FSH triggers an intracellular signaling mechanism that regulates granulose cell proliferation and differentiation. Granulose cells are activated by FSH to generate E2. Therefore, rs6166 and rs6165's altered FSHR activity result in lower E2 production and poor granulose cell differentiation and proliferation [38, 39]. These findings demonstrated that the T allele might be in charge of the FSH receptor's lower sensitivity to FSH, and that the FSHR polymorphisms (rs6166 and rs6165) were connected to a poor response to FSH. Both for rs6166 and rs6165, The CC, CT, and TT genotypes did not significantly differ between the moderate and high responder groups, suggesting that these genotypes and blood estradiol levels are unrelated. The current study's findings differed from those of Trevisan et al., who showed no correlation between the FSHR polymorphisms (rs6166 and rs6165) and blood levels of FSH and estradiol; nevertheless, the findings were consistent with a number of other research, such, It found When compared to other genotypes, infertile women with homozygous mutant genotypes for the two research SNPs (rs6166 and rs6165) displayed less mature oocytes and lower E2 levels [40]. In the current study, graafian follicles were considerably smaller in size and quantity in the poor responder group of FSHR (rs6166 and rs6165) TT carriers as opposed to CC and CT carriers. Estradiol synthesis, ongoing follicular development, and permanent accumulation in granulose cells, estradiol and FSH must predominate in follicular fluid. Thus, it was postulated that the FSHR's sensitivity to FSH may have been decreased by the genetic variation connected to the FSHR SNPs (rs6166 and rs6165), Due to this, Graafian follicle size and quantity have decreased, suggesting that the effect of FSH may have been diminished. Nonetheless, no appreciable variation was observed among the groups of moderate and strong responders who had CC, CT, and TT, suggesting that there was no correlation between the FSHR polymorphisms and the genotypes of these groups [41].

CONCLUSIONS

According to the study's findings, for both SNPs, the heterozygous genotype CT was more prevalent than the other genotypes, CC and TT. Additionally, FSHR polymorphisms, rs6166 and rs6165, may contribute to the genetic diversity in Iraqi infertile women's FSH responsiveness. The presence of both SNPs in the FSHR gene was linked to an increased risk of infertility in Iraqi women, according to the odd ratio. Strong ties exist between the TT genotypes of rs6166 and rs6165 and the clinical and hormonal markers of a poor ovarian response to FSH treatment. This is shown by a significant decrease in the mean of AFC, AMH, and E2, in addition to a notable rise in baseline FSH levels.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Patients 'satisfaction and views about pharmacists in community pharmacies as healthcare providers in Iraq: Najaf province

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ABSTRACT

Aim: To assess the level of patient satisfaction and their opinions about the services provided by community pharmacists and their changes depending on the socio-demographic characteristics that influence the level of satisfaction and expectations of patients served in the city of Al-Najaf Al-Ashraf city of Iraq.

Materials and Methods: A structured questionnaire was allocated among patients who were staying pharmacies regarding aspects like accessibility, communication, and quality of services. A descriptive cross-sectional survey was executed utilizing a questionnaire that encompassed various dimensions of pharmaceutical services. The sample population comprised 449 patients who frequented a range of pharmacies with varying regularity within the province.

Results: A significant majority of individuals exhibit a favorable perception regarding community pharmacists in Al-Najaf Al-Ashraf, with patients articulating substantial satisfaction, recognition, and esteem for the contributions that pharmacists make within the healthcare team.

Conclusions: Pharmacists play a vital role in providing pharmaceutical care, and patients generally express satisfaction with their services. However, there is a significant need for additional initiatives to enhance the clinical skills of community pharmacists, identify factors affecting patient satisfaction, and conduct comparative research on pharmacy services in different regions of Iraq.

KEY WORDS: community pharmacists, patient satisfaction, pharmaceutical care

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INTRODUCTION

Of the health professionals, community pharmacists are the most accessible. For one thing, the community pharmacy has longer hours of operation than other healthcare facilities, and a consultation with a pharmacist does not require an appointment [1, 2]. Many patients consider community pharmacists as their first point of contact with healthcare because advice regarding medication is provided more quickly and cheaply than from general practitioners' surgeries. This is especially evident in cities with less established medical services [3]. They develop strong relationships with their patients and are consequently able to promote trust and open communication. This ease of access allows them also to be very involved with major public health initiatives, including vaccinations and screenings, which further solidifies their value as healthcare providers. Their personalized advice and medication management further enhance patient outcomes, thereby making them indispensable in the chain of healthcare. The International Pharmaceutical Federation, known as FIP, and WHO define GPP as the provision of optimum, evidence-based care for the benefit of patients with their needs being given due consideration. GPP is thus aligned with the services of pharmacists to meet the expectations of the public with respect to safety, efficacy, and access. GPP underpins the delivery of quality, patient-centered services that improve health outcomes and engender public trust [4]. The joint International Pharmaceutical Federation (FIP)/World Health Organization (WHO) guidelines defined good pharmacy preparation as the practice of pharmacy that responds to the needs of the people who use the pharmacists' services by providing optimal, evidence-based care, this definition helps to ensure that the pharmacist's practice is in line with what the people who use the pharmacists' services for [5].

PHARMACIST ROLE

The primary responsibility of the community pharmacist is still to prepare medication; however, community pharmacists have a bigger part in ensuring patients' well-being than just that. When it comes to proper drug administration, dosage, side effects, storage, and drug–drug and drug–food interactions, they assist patients in getting the most out of their medications [6, 7]. Community pharmacists also help patients adhere to their medication regimens better, recognize, treat, and avoid drug therapy issues in conjunction with other healthcare providers and prescribe and dispense medications in a reasonable and cost-effective manner [8, 9]. In addition, they lessen the financial and medical load of the patients while also educating them about their conditions and offering advice on minor illnesses [10]. Stronger relationships between pharmacists and healthcare providers can facilitate coordinated care for better chronic condition management and optimize medication therapy [2]. The preparation, procurement, storage, security, distribution, administration, dispensing, and disposal of medical supplies are all part of a pharmacist's job description [11]. Furthermore, pharmacists play a crucial role in monitoring patient outcomes and ensuring that therapeutic goals are met, which can lead to improved health results and reduced hospital readmissions [12]. They also contribute to public health initiatives by providing immunizations and health screenings, thereby enhancing community wellness and preventing disease outbreaks [13].

PERCEPTION OF COMMUNITY PHARMACISTS IN THE WORLD

Various international studies are conducted to assess the perception, degree of satisfaction, opinions, and observations of the public about the community pharmacy services. The empirical results in the United States and Canada showed that the patients were extremely satisfied with the service level of community pharmacies and had positive opinions about the community pharmacists [14] albeit in a retail environment, Canadian consumers viewed pharmacists as health professionals. European countries' patients generally had good perceptions of the role of a community pharmacist and appreciated their important contribution to the healthcare system [15-18]. In contrast, studies conducted in the Middle East, specifically in Saudi Arabia, the UAE, Iraq, Palestine, Jordan, and Qatar, have shown that there is mainly a negative perception of the current role of community pharmacists, with marked ignorance about their role [19-25].

PATIENT SATISFACTION AND ITS USE AS INDICATOR

Patient satisfaction is a measure of the quality of the treatment received by the patient and services provided, so it might be used to the assessment of healthcare services as a whole [26]. Since patient satisfaction reflects the actual quality of the treatment or service received, it has become a prominent metric for evaluating the quality of health care and pharmaceutical services play a critical role in this [27]. Information on patient satisfaction may be used to pinpoint particular areas of the service that require improvement in order to deliver high-quality pharmacy services. It is the duty of pharmacists to satisfy patients while acting in a polite and professional manner [28]. Patients adherence, compliance, and seeking medical assistance, are influenced by their level of pleasure, patients who are happy with their overall treatment are more likely to take their prescriptions as prescribed and are also less inclined to switch to a new healthcare provider and high levels of patient satisfaction are anticipated if Good Pharmacy Practice is implemented in the pharmacy and pharmacists fulfill the tasks that patients and society demand of them [29, 30].

AIM

The aim of this project is to assess the level of patient satisfaction and their opinions about the services provided by community pharmacists and their changes depending on the socio-demographic characteristics that influence the level of satisfaction and expectations of patients served in the city of Al-Najaf Al-Ashraf city of Iraq.

MATERIALS AND METHODS

STUDY DESIGN

This was through the employment of a cross-sectional descriptive study between November 2023 and March 2024 by interviewing patients visiting different pharmacies across various districts in Al-Najaf city, Iraq, using a pre-piloted questionnaire adapted from previous research work in Riyadh [19]. A total of 449 respondents were sampled. The survey instrument was divided into two parts in a systematic manner. The first part intended to produce relevant demographic information: age, gender, educational attainment, occupation, and frequency of visit to the pharmacy. The second part aimed to elicit attitudes towards community pharmacists by assessing patients' perceptions, opinions, and overall satisfaction with the role of the pharmacists in community pharmacy services. This survey employed a nominal scale because it encourages a response format that was simplified with three responses, "Yes," "No," and "I don't know." These response alternatives allow the ones responding to the survey to account for uncertainty.

Inclusion criteria stated that participants in the study included healthy adults of both genders, 18 years and

Table 1. The Survey's Collection of Questions

Q1	Is the pharmacist available during the labelled hours?
Q2	Is the pharmacist primarily acting as a vendor or dispenser of treatment drugs?
Q3	Does the pharmacist offer counselling without needing to be asked? (Consider rephrasing to: "Does the pharmacist proactively offer counselling on medication use, side effects, or health conditions without being asked?")
Q4	Does the pharmacist request about accordance with formerly dispensed prescriptions?
Q5	Does the pharmacist ask about other health issues or medications the patient has used previously before dispensing a new prescription? (This clarifies that the pharmacist should assess overall health conditions before providing new medications)
Q6	Does he inform the patients/consumers about the ongoing health camps and campaigns in his vicinity? e.g.: Polio eradication, cataract removal and family planning etc.
Q7	How important do you consider the role of a pharmacist in the healthcare system? (Avoid leading language and provide response options like: "Not important," "Somewhat important," "Important," "Very important," "Indispensable.")
Q8	Does the pharmacist provide clear instructions on the timing and method of drug administration?

Table 2. Sociodemographic data of the respondents

	Parameter	Frequency	Percent
Age	18 – 29	209	46.5
	30 – 45	103	22.9
	46 – 60	83	18.5
	> 60	54	12.0
Gender	Male	202	45.0
	Female	247	55.0
Education	Uneducated	11	2.4
	Primary, secondary or high school level	122	27.2
	Collage or beyond bachelor degree	316	70.4
Employment status	Unemployed	189	42.1
	Government sector	152	33.9
	Private sector	80	17.8
	Retired	28	6.2
Number of visits to the pharmacy last year	1	33	7.3
	3 – 2	90	20.0
	5 – 4	82	18.3
	10 – 6	65	14.5
	>10	179	39.9

above, who can read and write to ensure that information derived from them is reliable.

Exclusion criteria comprised of exclusion of individuals below 18 years and for those with severe cognitive impairment, disabling them to understand the questionnaire properly, as well as those people who have severe health conditions that may hamper the successful running of the research (Table 1).

STATISTICAL ANALYSIS

For discrete variables, frequencies and percentages were calculated, and variables were cross-tabulated to derive the relationship between the demographic factors and responses. Furthermore, the thematic analyses of the qualitative data provided through open-ended questions give a clear glimpse of in-depth experiences and perceptions of participants regarding their experiences with respect to the study's focus. Comparisons across demographic groups highlighted differential responses significantly that would provide a basis for making decisions on further directions of research and practical application of findings. The major trend emergences out of the data pointed out not only similarities among participants but also identified unique challenges for particular groups.

Table 3. Distribution of patients' responses on the role of the pharmacist

Question	Frequency	Percent
Q1 Is the pharmacist available at the designated hours?		
Yes	309	68.8
No	44	9.8
Don't know	96	21.4
Q2 Is the pharmacist a mere vendor/dispenser of prescription drugs?		
Yes	85	18.9
No	346	77.1
Don't know	18	4.0
Q3 Does he offer counselling without asking?		
Yes	261	58.1
No	137	30.5
Don't know	51	11.4
Q4 Does he extract information about the compliance to the previously dispensed prescription?		
Yes	179	39.9
No	195	43.4
Don't know	75	16.7
Q5 Does he enquire about the related health problems and any other medication used in the past?		
Yes	268	59.7
No	135	30.1
Don't know	46	10.2
Q6 Does he inform the patients/consumers about the ongoing health camps and campaigns in his vicinity? e.g.: Polio eradication, cataract removal and family planning etc.		
Yes	73	16.3
No	271	60.4
Don't know	105	23.4
Q7 Do you perceive a pharmacist as an indispensable and effective part of the health care system?		
Yes	403	89.8
No	36	8.0
Don't know	10	2.2
Q8 Does the Pharmacist instruct about timings of drug administration?		
Yes	430	95.8
No	14	3.1
Don't know	5	1.1

RESULTS

AGE DISTRIBUTION

The age group of 18-29 years is the highest percentage among total respondents, at 46.5%, and this means that young adults are the frequenters of pharmacies, possibly due to augmented responsiveness about health or medication essentials on explanation of lifestyle circumstances. In the age group of 30-45 years there were 22.9%, in the groups of 46-60 years - 18.5% and over 60 years - 12%. Female respondents make up 55%, male - 45%.This might be indicative of the relative more active role of women in maintaining health for themselves and their families, or an increased willingness on the part of females to participate in surveys about health services. A total of 70.4% of the respondents reported having a college education or higher, indicating a high level of educational attainment among participants. The highest percentage being 27.2%, have completed their primary, secondary, or high school. A small number of 2.4% represent uneducated. The rather high percentage of educated respondents could mean that people with a higher education level may be more likely to go to pharmacies or answer health-related surveys. The data indicates that 42.1

Table 4. Distribution of patients' responses by age

	Age group											
	18 - 29			30 – 45			46 - 60			> 60		
	Yes	No	Don't know	Yes	No	Don't know	Yes	No	Don't know	Yes	No	Don't know
Q1	138 (66.0%)	19 (9.1%)	52 (24.9%)	66 (64.1%)	15 (14.6%)	22 (21.4%)	62 (74.7%)	8 (9.6%)	13 (15.7%)	43 (79.6%)	2 (3.7%)	9 (16.7%)
Q2	41 (19.6%)	158 (75.6%)	10 (4.8%)	26 (25.2%)	76 (73.8%)	1 (1.0%)	9 (10.8%)	71 (85.5%)	3 (3.6%)	9 (16.7%)	41 (75.9%)	4 (7.4%)
Q3	125 (59.8%)	69 (33.0%)	15 (7.2%)	53 (51.5%)	31 (30.1%)	19 (18.4%)	55 (66.3%)	21 (25.3%)	7 (8.4%)	28 (51.9%)	16 (29.6%)	10 (18.5%)
Q4	82 (39.2%)	85 (40.7%)	42 (20.1%)	39 (37.9%)	56 (54.4%)	8 (7.8%)	35 (42.2%)	33 (39.8%)	15 (18.1%)	23 (42.6%)	21 (38.9%)	10 (18.5%)
Q5	114 (54.5%)	67 (32.1%)	28 (13.4%)	64 (62.1%)	34 (33.0%)	5 (4.9%)	60 (72.3%)	18 (21.7%)	5 (6.0%)	30 (55.6%)	16 (29.6%)	8 (14.8%)
Q6	30 (14.4%)	124 (59.3%)	55 (26.3%)	17 (16.5%)	62 (60.2%)	24 (23.3%)	13 (15.7%)	53 (63.9%)	17 (20.5%)	13 (24.1%)	32 (59.3%)	9 (16.7%)
Q7	184 (88.0%)	19 (9.1%)	6 (2.9%)	90 (87.4%)	13 (12.6%)	0 (0.0%)	80 (96.4%)	2 (2.4%)	1 (1.2%)	49 (90.7%)	2 (3.7%)	3 (5.6%)
Q8	197 (94.3%)	8 (3.8%)	4 (1.9%)	98 (95.1%)	5 (4.9%)	0 (0.0%)	82 (98.8%)	0 (0.0%)	1 (1.2%)	53 (98.1%)	1 (1.9%)	0 (0.0%)

are unemployed; hence, this is the biggest single category. This may comprise of students, homemakers, or people who, at the time of inquiry, are not working. It follows that 33.9% are employed in government, and 17.8% work in the private sector. The relatively high part of government organizations could be connected to the employ assistances related with such jobs, like healthcare coverage, entailing regular visits to pharmacies. Another 6.2% of the respondents were retired; this means that smaller groups could have different health needs or routines compared to other segments. Employment status shows that 42.1% are unemployed, 33.9% work in the government sector, 17.8% are employed privately, and 6.2% are retired. In terms of pharmacy visits, 39.9% visited 10 times or more in the last year, 20.0% made 2-3 visits, 18.3% visited 4-5 times, 14.5% visited 6-10 times, and 7.3% had a single visit (Table 2). A considerable proportion 68.8% affirm that pharmacists are accessible during specified hours, while 89.8% regard them as a crucial component of the healthcare framework (Table 3). This suggests a robust conviction regarding the pharmacist's function extending beyond the mere distribution of pharmaceuticals.

Descriptive statistics of the responses to the patient questionnaires are presented as frequencies and percentages (Table 3). Pharmacist availability: most patients thought that pharmacists were available at particular times, which suggests that access was good. Indeed, 68.8% of patients thought that pharmacists were available at particular times, which suggests that they had good access, and negatively (9.8%), uncertain (21.4%). Although a significant portion of respondents answered yes 18.9% as opposed to "no" 77.1% when asked about pharmacists' roles in healthcare, the majority of consumers do not see them as solely sellers, suggesting that they are aware of their wider responsibilities beyond simply delivering prescription drugs. Don't know were answered by 4.0% respondents. Very few people are unsure suggesting that the role of the pharmacist has been clearly taken into account. The results generally show that patients have a positive attitude towards pharmacists and their role, but at the same time indicate areas of concern in proactive counseling, compliance checks, and the communication of health campaigns. This will no doubt be helpful for the pharmacists in order to improve in their services and further strengthen their integral role within the healthcare system.

Descriptive statistics of the responses to the patient questionnaires by age are presented as frequencies and percentages (Table 4). The data underlines the fact that, in different age groups, there is considerable variation in response patterns, with the older ones tending to build stronger consensus while their younger counterparts reflect more uncertainty. These may be useful in framing appropriate communication strategies in order to bridge the knowledge gaps and enhance understanding across demographics.

Descriptive statistics of the responses to the patient questionnaires by gender are presented as frequencies and percentages (Table 5). The data shows strong agreement between the genders on some topics, like Q7 and Q8, but also points out the difference in uncertainty and disagreement, with females generally showing higher uncertainty and varied opinion. These gender-based insights can usefully inform more tailored communication and engagement strategies. The following are trends in the responses from male and female patients based on the eight questions (Q1

Table 5. Distribution of patients' responses by gender

	Gender					
	Male			Female		
	Yes	No	Don't know	Yes	No	Don't know
Q1	145 (71.8%)	27 (13.4%)	30 (14.9%)	164 (66.4%)	17 (6.9%)	66 (26.7%)
Q2	49 (24.3%)	142 (70.3%)	11 (5.4%)	36 (14.6%)	204 (82.6%)	7 (2.8%)
Q3	116 (57.4%)	62 (30.7%)	24 (11.9%)	145 (58.7%)	75 (30.4%)	27 (10.9%)
Q4	71 (35.1%)	93 (46.0%)	38 (18.8%)	108 (43.7%)	102 (41.3%)	37 (15.0%)
Q5	116 (57.4%)	69 (34.2%)	17 (8.4%)	152 (61.5%)	66 (26.7%)	29 (11.7%)
Q6	27 (13.4%)	135 (66.8%)	40 (19.8%)	46 (18.6%)	136 (55.1%)	65 (26.3%)
Q7	178 (88.1%)	20 (9.9%)	4 (2.0%)	225 (91.1%)	16 (6.5%)	6 (2.4%)
Q8	193 (95.5%)	8 (4.0%)	1 (0.5%)	237 (96.0%)	6 (2.4%)	4 (1.6%)

Table 6. Distribution of patients' responses by education level

	Uneducated			Primary, secondary or high school level			College or beyond bachelor degree		
	Yes	No	Don't know	Yes	No	Don't know	Yes	No	Don't know
Q1	9 (81.8%)	0 (0.0%)	2 (18.2%)	88 (72.1%)	5 (4.1%)	29 (23.8%)	212 (67.1%)	39 (12.3%)	65 (20.6%)
Q2	2 (18.2%)	8 (72.7%)	1 (9.1%)	25 (20.5%)	90 (73.8%)	7 (5.7%)	58 (18.4%)	248 (78.5%)	10 (3.2%)
Q3	4 (36.4%)	6 (54.5%)	1 (9.1%)	66 (54.1%)	42 (34.4%)	14 (11.5%)	191 (60.4%)	89 (28.2%)	36 (11.4%)
Q4	8 (72.7%)	3 (27.3%)	0 (0.0%)	38 (31.1%)	63 (51.6%)	21 (17.2%)	133 (42.1%)	129 (40.8%)	54 (17.1%)
Q5	7 (63.6%)	4 (36.4%)	0 (0.0%)	63 (51.6%)	37 (30.3%)	22 (18.0%)	198 (62.7%)	94 (29.7%)	24 (7.6%)
Q6	3 (27.3%)	7 (63.6%)	1 (9.1%)	18 (14.8%)	74 (60.7%)	30 (24.6%)	52 (16.5%)	190 (60.1%)	74 (23.4%)
Q7	11 (100.0%)	0 (0.0%)	0 (0.0%)	103 (84.4%)	15 (12.3%)	4 (3.3%)	289 (91.5%)	21 (6.6%)	6 (1.9%)
Q8	11 (100.0%)	0 (0.0%)	0 (0.0%)	114 (93.4%)	6 (4.9%)	2 (1.6%)	305 (96.5%)	8 (2.5%)	3 (0.9%)

through Q8). More than 88% of the male patients and more than 91% of the female patients strongly agreed "Yes" with Q7 and Q8. In table 5 there is clear agreement between the two groups in this trend.

Descriptive statistics of the responses to the patient questionnaires by education level (Table 6), employment status (Table 7) and number of visits to the pharmacy last year (Table 8) are presented as frequencies and percentages. Moreover, patients who visited the pharmacy six to ten times in the last year reported a higher availability rate of 84.6%, while patients who visited the pharmacy once a year reported the least availability 54.5%; males reported higher availability compared to females 71.8% and 66.4% respectively. The study showed that about 77 % of the respondents anticipate the pharmacist to do more than just dispensing of medicine, and did not consider him as a mere vender. Only 18.9% respondents perceived him as mere vendor (Table 3). Respondents aged 30-45, males and government sector workers reported more negatively 25.2%, 24.3% and 22.4% respectively (Tables 4, 7). People with education level of primary, secondary and high school reported more negatively in that regard with 51.6% (Table 6). The data showed that respondents were more positive in relation to the respondents aged 46-60, employees in the private sector and patients with single pharmacy visit annually 72.3%, 70.0% and 69.7% respectively (Tables 4, 7-8). The survey indicates that 89.9% of respondents felt that pharmacist is indispensable and an effective part of the health care system while 8% gave negative response. Respondents aged 46-60 and that which are uneducated reported more positively 96.4% and 100% respectively (Tables 4, 6). In response to the question about whether the pharmacist gives instructions about timing of the drug administration, the survey shows

Table 7. Distribution of patients' responses by employment status.

	Unemployed			Government sector			Private sector			Retired		
	Yes	No	Don't know	Yes	No	Don't know	Yes	No	Don't know	Yes	No	Don't know
Q1	129 (68.3%)	11 (5.8%)	49 (25.9%)	106 (69.7%)	17 (11.2%)	29 (19.1%)	52 (65.0%)	15 (18.8%)	13 (16.3%)	22 (78.6%)	1 (3.6%)	5 (17.9%)
Q2	37 (19.6%)	144 (76.2%)	8 (4.2%)	34 (22.4%)	114 (75.0%)	4 (2.6%)	11 (13.8%)	65 (81.3%)	4 (5.0%)	3 (10.7%)	23 (82.1%)	2 (7.1%)
Q3	113 (59.8%)	61 (32.3%)	15 (7.9%)	78 (51.3%)	46 (30.3%)	28 (18.4%)	52 (65.0%)	23 (28.8%)	5 (6.3%)	18 (64.3%)	7 (25.0%)	3 (10.7%)
Q4	75 (39.7%)	70 (37.0%)	44 (23.3%)	52 (34.2%)	81 (53.3%)	19 (12.5%)	39 (48.8%)	32 (40.0%)	9 (11.3%)	13 (46.4%)	12 (42.9%)	3 (10.7%)
Q5	98 (51.9%)	61 (32.3%)	30 (15.9%)	101 (66.4%)	42 (27.6%)	9 (5.9%)	56 (70.0%)	21 (26.3%)	3 (3.8%)	13 (46.4%)	11 (39.3%)	4 (14.3%)
Q6	31 (16.4%)	112 (59.3%)	46 (24.3%)	15 (9.9%)	99 (65.1%)	38 (25.0%)	22 (27.5%)	40 (50.0%)	18 (22.5%)	5 (17.9%)	20 (71.4%)	3 (10.7%)
Q7	166 (87.8%)	19 (10.1%)	4 (2.1%)	140 (92.1%)	11 (7.2%)	1 (0.7%)	73 (91.3%)	4 (5.0%)	3 (3.8%)	24 (85.7%)	2 (7.1%)	2 (7.1%)
Q8	179 (94.7%)	7 (3.7%)	3 (1.6%)	144 (94.7%)	6 (3.9%)	2 (1.3%)	79 (98.8%)	1 (1.3%)	0 (0.0%)	28 (100.0%)	0 (0.0%)	0 (0.0%)

Table 8. Distribution of patients' responses by number of visits to the pharmacy last year

	Number of visits to the pharmacy last year														
	1			2 - 3			5 - 4			10 – 6			>10		
	Yes	No	Don't know	Yes	No	Don't know	Yes	No	Don't know	Yes	No	Don't know	Yes	No	Don't know
Q1	18 (54.5%)	2 (6.1%)	13 (39.4%)	55 (61.1%)	6 (6.7%)	29 (32.2%)	62 (75.6%)	2 (2.4%)	18 (22.0%)	55 (84.6%)	3 (4.6%)	7 (10.8%)	119 (66.5%)	31 (17.3%)	29 (16.2%)
Q2	7 (21.2%)	24 (72.7%)	2 (6.1%)	16 (17.8%)	69 (76.7%)	5 (5.6%)	13 (15.9%)	65 (79.3%)	4 (4.9%)	14 (21.5%)	48 (73.8%)	3 (4.6%)	35 (19.6%)	140 (78.2%)	4 (2.2%)
Q3	22 (66.7%)	9 (27.3%)	2 (6.1%)	55 (61.1%)	29 (32.2%)	6 (6.7%)	42 (51.2%)	23 (28.0%)	17 (20.7%)	40 (61.5%)	16 (24.6%)	9 (13.8%)	102 (57.0%)	60 (33.5%)	17 (9.5%)
Q4	13 (39.4%)	14 (42.4%)	6 (18.2%)	31 (34.4%)	40 (44.4%)	19 (21.1%)	28 (34.1%)	31 (37.8%)	23 (28.0%)	28 (43.1%)	30 (46.2%)	7 (10.8%)	79 (44.1%)	80 (44.7%)	20 (11.2%)
Q5	23 (69.7%)	7 (21.2%)	3 (9.1%)	45 (50.0%)	29 (32.2%)	16 (17.8%)	51 (62.2%)	17 (20.7%)	14 (17.1%)	43 (66.2%)	18 (27.7%)	4 (6.2%)	106 (59.2%)	64 (35.8%)	9 (5.0%)
Q6	4 (12.1%)	17 (51.5%)	12 (36.4%)	10 (11.1%)	55 (61.1%)	25 (27.8%)	9 (11.0%)	45 (54.9%)	28 (34.1%)	8 (12.3%)	43 (66.2%)	14 (21.5%)	42 (23.5%)	111 (62.0%)	26 (14.5%)
Q7	28 (84.8%)	4 (12.1%)	1 (3.0%)	77 (85.6%)	9 (10.0%)	4 (4.4%)	76 (92.7%)	5 (6.1%)	1 (1.2%)	62 (95.4%)	2 (3.1%)	1 (1.5%)	160 (89.4%)	16 (8.9%)	3 (1.7%)
Q8	31 (93.9%)	2 (6.1%)	0 (0.0%)	86 (95.6%)	4 (4.4%)	0 (0.0%)	76 (92.7%)	3 (3.7%)	3 (3.7%)	63 (96.9%)	0 (0.0%)	2 (3.1%)	174 (97.2%)	5 (2.8%)	0 (0.0%)

that the majority of the patients 95.8% reported that the pharmacist gives.

DISCUSSION

A basic expectation of any profession that is designed to serve the public directly is that the profession should be accessible to the public. Most of the available positions should be filled. Whereas the absolute percentage of 68.8% is considerably less than the ideal of 100%, some leeway can be given because patients did not know which of the individuals the technician was and which was the pharmacist. Despite this, in a 2012 study conducted by Al-Arifi in Riyadh, Saudi Arabia, as many as 71.4% of respondents said community pharmacists are accessible in their places of employment [19], indicating a positive perception of availability. This would tend to suggest that although confusion regarding function differentiation may exist, the community stays last to opinion pharmacists as a key basis for health-related questions and sustenance. Increasing the awareness among the public with

respect to the different roles of pharmacists and technicians would also quite adequately enable service utilization and, consequently, improve patient outcomes. Furthermore, it is stated that the primary factor preventing the patient from seeking guidance and, as a result, leading to inadequate therapy is unavailability [29]. Patients have high expectations from pharmacists; 77.1% of patients believe that pharmacists should assume more responsibilities and Bawazir (2004) found in the Kingdom of Saudi Arabia that 56.1% of the respondents believed that pharmacists were more interested in the business of dispensing of drugs than in other aspects of the profession. The poll puts into perspective the importance attached to pharmacist-driven counseling regarding drug use as stated by 58.1% of the respondents. According to Singhal et al., (2002), higher magnitudes of pharmacist-driven support are associated with increased patient satisfaction, reinforcing the need for personalized counseling at the level of pharmacy practice. In contrast, an Indian study showed that only 3 percent of their respondents replied that they received counseling spontaneously. This disparity in response suggests that more must be done in terms of communication and proactive involvement between the pharmacist and the patient for the complete pharmaceutical care package to be achieved. Moreover, initiated education and training programs targeting the enhancement of communication skills are ways through which the pharmacist can be better prepared with the means of handling questions from patients and furthering a collaborative healthcare setting. With 43.4% responding negatively, the pharmacist's contribution to increasing patient compliance is only viewed as adequate. This is greater than the 34.9% recorded by Al-Arifi in Saudi Arabia. Regarding the pharmacist's information-gathering role, the poll reveals that around 60% of respondents find the pharmacist asks about the patient's health state. Ibrahim et al., (2013) discovered that 60% of Baghdad residents do not think the pharmacist is attentive to that element [22]. A sizable fraction of respondents disagree that pharmacists ought to promote health campaigns more actively. This might be due to a number of things, such as a poor healthcare system, a lack of resources, and a failure to make the most of the pharmacist's abilities in this role. Numerous people die each year from diseases that can be avoided with immunizations [31]. Though pharmacies can be accommodating in this regard. Pharmacists are able to advise vaccinations and enlighten the public about immunizations. By doing this, the number of fatalities will be reduced and society's health will improve. In a 2016 research, Merks et al. discovered that many individuals in rural UK locations were unaware of the vaccination programs that were offered, which prevented them from taking use of them [32]. An effective profession should be seen as having important or essential individuals. If it were not the case, that profession's future would be in doubt [9]. Positive views of the role of pharmacists in society are evident from the 89.9% of people who believe they are necessary. The pharmacist is the last healthcare provider to see a patient, so they have a crucial role to play in educating them about drug use. They should provide patients with clear instructions regarding medication forms and administration timings, as well as additional information if they need it. 95.8% of patients agree that the pharmacist has a responsibility to give clear instructions. Providing advice on when to administer drugs. Both doctors and pharmacists play a role in patient education. However, according to Alkhawajah et al., 20% of patients believe that pharmacists are more explicit in their instructions than doctors [33].

Descriptive statistics of the responses to the patient questionnaires are presented as frequencies and percentages (Table 3). Pharmacist availability: most patients thought that pharmacists were available at particular times, which suggests that access was good. Indeed, 68.8% of patients thought that pharmacists were available at particular times, which suggests that they had good access, and negatively (9.8%), uncertain (21.4%). Although a significant portion of respondents answered yes 18.9% as opposed to "no" 77.1% when asked about pharmacists' roles in healthcare, the majority of consumers do not see them as solely sellers, suggesting that they are aware of their wider responsibilities beyond simply delivering prescription drugs. Don't know were answered by 4.0% respondents. Very few people are unsure suggesting that the role of the pharmacist has been clearly taken into account. The results generally show that patients have a positive attitude towards pharmacists and their role, but at the same time indicate areas of concern in proactive counseling, compliance checks, and the communication of health campaigns. This will no doubt be helpful for the pharmacists in order to improve in their services and further strengthen their integral role within the healthcare system.

Descriptive statistics of the responses to the patient questionnaires by age are presented as frequencies and percentages (Table 4). The data underlines the fact that, in different age groups, there is considerable variation in response patterns, with the older ones tending to build stronger consensus while their younger counterparts reflect more uncertainty. These may be useful in framing appropriate communication strategies in order to bridge the knowledge gaps and enhance understanding across demographics.

CONCLUSIONS

Pharmacists play a vital role in providing pharmaceutical care, and patients generally express satisfaction with their services. However, there is a significant need for additional initiatives to enhance the clinical skills of community pharmacists, identify factors affecting patient satisfaction, and conduct comparative research on pharmacy services in different regions of Iraq.

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ORIGINAL ARTICLE

CONTENTS

The study of the base resin impact on planktonic growth of microorganisms of oral origin

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ABSTRACT

Aim: To study the main differences in the intensity of growth of microbial strains of oral origin when they are cultivated in the presence of base resins that are widely used in the clinic of prosthetic dentistry.

Materials and Methods: 3 types of base resin samples were used to evaluate the impact on the growth of microorganisms. Strains of opportunistic pathogens representing the facultative anaerobic transient microflora of the oral cavity were used for the research. Microbial cultures were isolated from the oral mucosa of the patients with removable dentures. The optical density of the culture was determined using a multi-mode photometer. Control growth of the cultures was evaluated under similar cultivation conditions in test tubes with a nutrient medium without resin samples.

Results: All resins reduced the intensity of polyantibiotic-resistant *S. aureus* MRSA growth compared to control samples. The impact on *S. epedermidis* growth was different. Thus, Vertex and Breflex reduced the growth intensity insignificantly, and Villacryl increased it to the level of 1.104 ±0.026 CU in comparison with glass constituting 1.178 ±0.033 CU. All studied resins inhibited the growth of polyantibiotic-resistant S. aureus MRSA. Villacryl resin had the least impact on the growth of S. epedermidis. Villacryl resin inhibited *C. albicans* growth only by 13.70%, and the other two resins inhibited it by 25% on average.

Conclusions: The conducted microbiological studies showed the diverse influence of base materials on such important indicators as the optical density of cultures grown in the presence of base resins and the growth inhibition index of cultures.

KEY WORDS: removable dentures, base resin, microbial cultures

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INTRODUCTION

The prevalence and intensity of dental diseases remains at a high level despite the implementation of a significant number of treatment and prevention programs aimed at reducing their level. This leads to edentulousness and the occurrence of a significant percentage of pathological and structural-functional changes in the maxillofacial system. According to the WHO, partial edentulousness is observed in 75% of the world's population [1]. Ukrainian adult population's need for the prosthodontic treatment in case of partial edentulousness constitutes 57.5 people per 1000 examined [2].

The prosthodontic treatment of patients with partial edentulousness should prevent and remove the consequences of the edentulousness, namely an atrophy of the maxillofacial skeleton and muscles, and is aimed at a person's general improvement and prolongation of the active period of their life [3, 4].

The prosthodontic measures during the treatment of patients with edentulousness consist in the justification of the choice of the optimal dental prosthesis, the material of the dental prosthesis and the patient's treatment approach [5].

Most often, an orthopedic surgeon chooses any standard dental prostheses and materials that are constantly used in modern practice [6]. Until this point, we have mostly mentioned removable partial dentures made of acrylic, that is, made of plastics, but nowadays dentures made of thermoplastic are very popular [7]. Thermoplastics are a type of plastic based on thermoplastic polymers. Thermoplastics soften under the influence of high temperatures and become viscous fluid, and return to a solid form upon cooling [8]. Despite the positive results of these materials, most patients try to avoid the use of removable dentures [9].

AIM

To study the main differences in the intensity of growth of microbial strains of oral origin when they are cultivat-

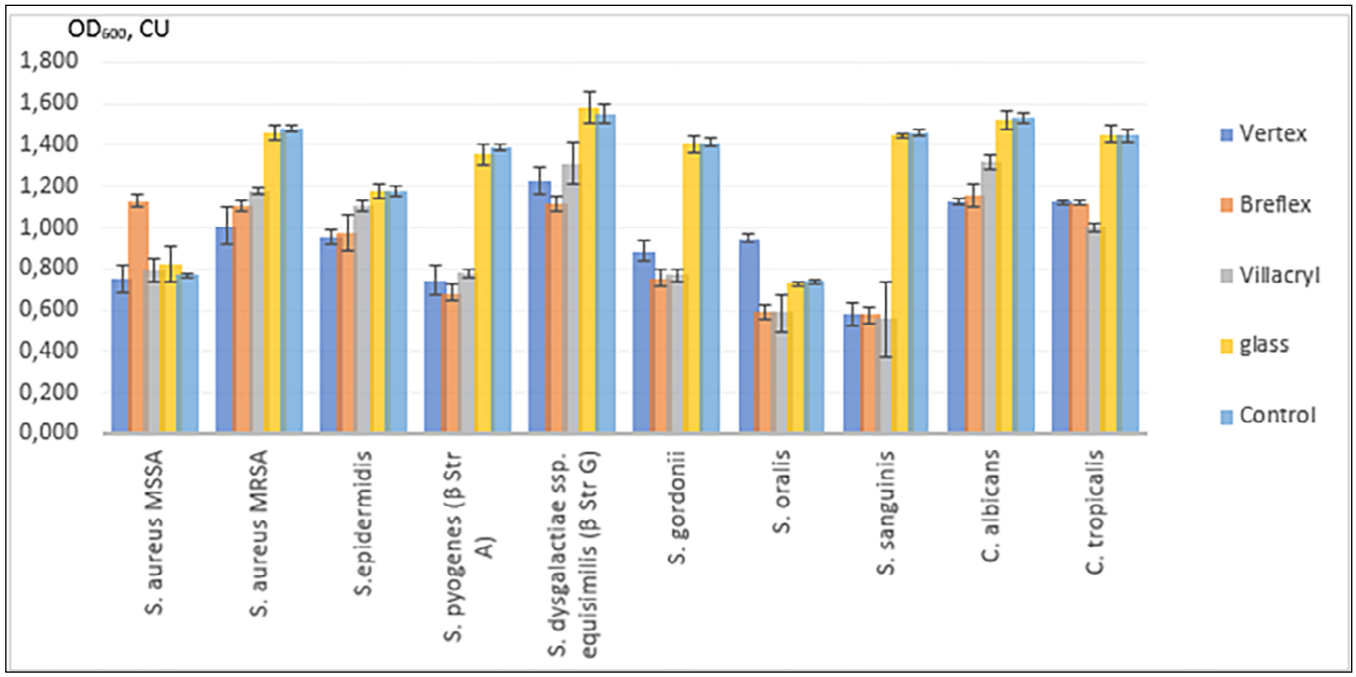

Fig.1. Optical density of cultures (CU) grown in the presence of resins.

Table 1. Optical density of cultures (CU) grown in the presence of resins

	S. aureus MSSA	*S. aureus* MRSA	*S.epidermidis*	*S. pyogenes* (β Str A)	*S. dysgalactiae ssp. equisimilis* (β Str G)	*S. gordonii*	*S. oralis*	*S. sanguinis*	*C. albicans*	*C. tropicalis*
Vertex	0.751±0.066	1.010±0.087*	0.953±0.035*	0.744±0.070*	1.224±0.067*	0.886±0.050*	0.947±0.021*	0.579±0.055*	1.130±0.015*	1.124±0.012*
Breflex	1.130±0.027*	1.107±0.026*	0.975±0.089*	0.686±0.038*	1.116±0.036*	0.757±0.039*	0.592±0.032*	0.577±0.038*	1.154±0.055*	1.120±0.011*
Villacryl	0.794±0.053	1.176±0.018*	1.104±0.026	0.778±0.020*	1.310±0.102*	0.769±0.030*	0.588±0.093*	0.557±0.180*	1.316±0.033*	1.002±0.023*
Glass	0.821±0.086	1.461±0.035	1.178±0.033	1.356±0.049	1.580±0.076	1.408±0.041	0.731±0.010	1.445±0.014	1.525±0.046	1.454±0.042
Control	0.770±0.012	1.479±0.017	1.179±0.028	1.390±0.014	1.553±0.046	1.412±0.021	0.740±0.011	1.461±0.011	1.530±0.030	1.448±0.030

* significant differences at $p < 0.05$.

ed in the presence of base resins that are widely used in the clinic of prosthetic dentistry.

MATERIALS AND METHODS

This research was conducted according to the WMA Declaration of Helsinki – "Ethical Principles for Medical Research Involving Human Subjects" and approved by the Ethics Committee of the Ivano-Frankivsk National Medical University (protocol No. 131/22 dated November 24, 2022).

3 types of base resin samples such as Vertex poly methyl methacrylate, Breflex polyamide and Villacryl polymethacrylate were used to evaluate the impact on the growth of microorganisms.

In total, the results of microbiological studies of prostheses of 55 patients were studied.

3 groups of patients were formed. The group with prostheses, where the base resin was Vertex poly methyl methacrylate, consisted of 18 patients; Breflex polyamide – 16 patients; Villacryl polymethacrylate – 21 patients.

Ready resin samples for the experiment had the form of plates with a thickness of 2 mm and an area of 1 cm^2. Glass plates of similar size were used as controls. The study and control samples were placed in a sealed cellophane package and sterilized by X-ray irradiation at a dose of 0.44 mGy for 1.540 s.

Strains of opportunistic pathogens representing the facultative anaerobic transient microflora of the oral cavity were used for the research: *Streptococcus pyogenes* (Group A β-hemolytic streptococcus), *Streptococcus dysgalactiae ssp. equisimilis* (Group G β-hemolytic streptococcus), methicillin-susceptible *Staphylococcus*

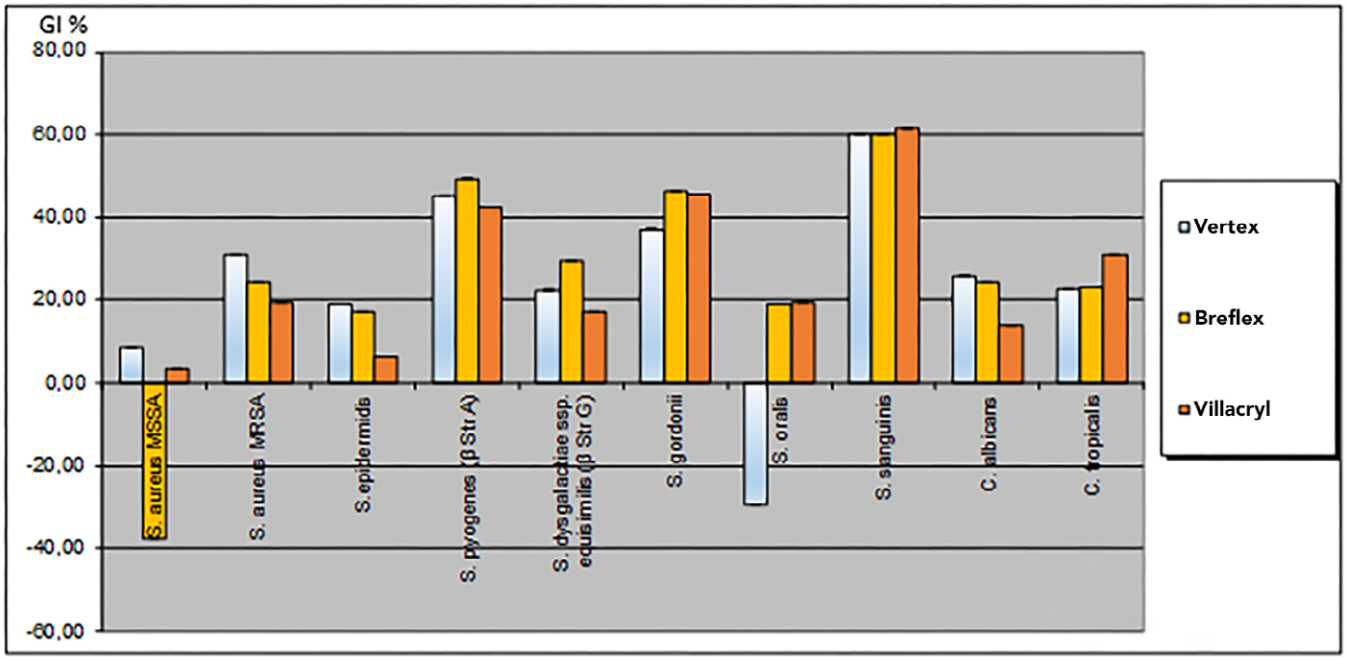

Fig.2. Growth inhibition index (%) in the presence of resins (in relation to glass).

Table 2. Growth inhibition index (%) in the presence of resins (in relation to glass)

	S. aureus MSSA	*S. aureus* MRSA	*S.epidermidis*	*S. pyogenes (β Str A)*	*S. dysgalactiae ssp. equisimilis (β Str G)*	*S. gordonii*	*S. oralis*	*S. sanguinis*	*C. albicans*	*C. tropicalis*
Vertex	8.53	30.87*	19.10*	45.13*	22.53*	37.07*	-29.55*	59.93*	25.90*	22.70*
Breflex	-37.64*	24.23*	17.23*	49.41*	29.37*	46.24*	19.02*	60.07*	24.33*	22.97*
Villacryl	3.29	19.51	6.28	42.63*	17.09*	45.38*	19.56*	61.45*	13.70*	31.09*

* significant differences at $p < 0.05$.

aureus (MSSA), methicillin-resistant *S. aureus* (MRSA) with associated resistance to fluoroquinolones, macrolides, tetracyclines and aminoglycosides, methicillin-susceptible *Staphylococcus epidermidis*; yeast-like fungi *Candida albicans* and *Candida tropicalis* as well as the mitis group α-hemolytic streptococci (*Streptococcus oralis*, *Streptococcus sanguinis*, *Streptococcus gordonii*) as the main representatives of the resident microflora of this biotope. Microbial cultures were isolated from the oral mucosa (prosthetic bed, gingival pockets) of the patients with removable dentures with manifestations of prosthetic stomatitis and identified on the basis of morphological, cultural properties and biochemical microtests "STAPHYtest 16", "STREPTOtest 16" (Lachema, Czech Republic) and VITEK 2 GP and VITEK 2 YST test systems (Biomerieux, France) using the VITEK 2 Compact analyzer.

The study sample was placed in a test tube with 2.0 ml of nutrient Brain Heart Infusion Broth (HiMedia Laboratories Pvt. Ltd., India) with the addition of 1% glucose, previously freshly inoculated with test strains of microorganisms at a final concentration of 1×10^4CFU/ml. Cultures were cultivated for 24 hours at a temperature of 37°C with constant stirring by means of an MR-1 shaker (SIA BIOSAN, Latvia) with a stirring frequency of 20 times/min. 5 portions of planktonic culture with a volume of 200 µl were taken into the cells of a polystyrene tablet after the cultivation. The optical density (OD) of the culture was determined using a multi-mode photometer for Synergy™ NTX S1LFTA microplates (BioTek Instruments, Inc., USA) at a wavelength of 495 nm using Gen5™ Data Analysis Software. Control growth of the cultures was evaluated under similar cultivation conditions in test tubes with a nutrient medium without resin samples.

Based on the results of the experiment, the growth inhibition indices of test cultures of microorganisms were calculated for each sample (1):

$$GI(\%) = 100 - \frac{OD_{experiment} \times 100}{OD_{control}} \quad (1)$$

Statistical processing of the obtained data was presented in the form of mean values of measurements ± standard deviation for three independent experiments. Results were processed using a two-sample t-test, differences were considered statistically significant at $p<0.05$.

RESULTS

We analyzed the differences in the growth intensity of microbial strains of the oral origin in the course of their cultivation in the presence of the resins widely used in a clinic of prosthetic dentistry (Table 1 and Fig. 1).

All resins reduced the intensity of polyantibiotic-resistant *S. aureus MRSA* growth compared to control samples. The impact on *S. epedermidis* growth was different. Thus, Vertex and Breflex reduced the growth intensity insignificantly, and Villacryl increased it to the level of 1.104 ±0.026 CU in comparison with glass constituting 1.178 ±0.033 CU ($p < 0.05$). All samples reduced the growth intensity of Group A β-hemolytic streptococci *S. Pyogenes* and Group G *S. dysgalactiae ssp. Equisimilis,* moreover a sample of Breflex resin did it to the greatest extent ($p < 0.05$). When studying the representatives of the oral cavity microflora, namely α-hemolytic streptococci, the most significant growth inhibition influenced by the samples of all three basic resins was observed in the cultures of *S. gordonii* and *S. sanguinis*. The growth rate of *S. gordonii* culture constituted 53.7% in the presence of Breflex and Villacryl resins and 62.9% in the presence of Vertex resin in relation to the growth intensity in test tubes with glass as the control material ($p < 0.05$). The growth rate of *S. sanguinis* culture constituted 40% in the presence of Vertex and Breflex base resins and 38.6% in relation to glass ($p < 0.05$).

Somewhat different picture was observed when *S. oralis* cultures were studied. Thus, Vertex stimulated its growth up to 0.947 ±0.021 CU relative to glass (0.731 ±0.010 CU) ($p < 0.05$), and the other two resins reduced it. Vertex and Breflex resins equally reduced the growth intensity of yeast-like fungi *C. albicans*, *C. tropicalis*. Villacryl base resin caused a more significant growth inhibition of *C. tropicalis* than *C. albicans*.

The growth inhibition index is a more informative criterion characterizing the impact of base resins on microbial cultures growth. It was calculated in relation to the control material, namely glass (Table 2 and Fig. 2). All studied resins inhibited the growth of polyantibiotic-resistant *S. aureus MRSA*: Vertex – by 30.87%, Breflex by 24.23% and Villacryl by 19.51% compared to glass ($p < 0.05$). Villacryl resin had the least impact on the growth of *S. epedermidis* (inhibition by 6.28% compared to glass) ($p < 0.05$). The growth rates of *S. pyogenes (β Str A)* in the studied resins were approximately at the same level and ranged from 49.41% to 42.63% ($p < 0.05$). Villacryl base resin inhibited *S. dysgalactiae ssp. equisimilis (β Str G)* growth by 17.09% ($p < 0.05$). *S. gordonii* growth was inhibited by Vertex base resin most of all. S. *oralis* culture growth was stimulated by Vertex by 29.59% ($p < 0.05$), however it was inhibited by other resins, on the contrary (by 19% compared to glass). Resins did not show any significant difference on the growth inhibition index of *S. sanguines* ($p < 0.05$).

Important results were obtained regarding the impact of resins on *yeast-like fungi cultures*. Thus, Villacryl resin inhibited *C. albicans* growth only by 13.70% ($p < 0.05$), and the other two resins inhibited it by 25% on average ($p < 0.05$). Villacryl resin inhibited the growth intensity of *C. tropicalis* most of all, namely by 31.09% ($p < 0.05$).

DISCUSSION

Dental implantation has become widespread at the current stage of the dentistry development. This treatment is aimed at restoring a patient's chewing efficiency and aesthetic comfort [10]. Unfortunately, dental implantation is not always possible immediately after tooth extraction and requires some time and appropriate preparation of the oral cavity [11].

In order to prevent bone tissue atrophy and preserve the supporting teeth, we offer temporary removable dentures for patients with edentulousness who are scheduled for dental implantation. This problem is quite relevant and has become the objective of our research, namely, the determination of the material that should be used for temporary removable dentures manufacturing in order to prevent the development of dysbacteriological complications and the development of the maxillofacial skeleton diseases, which will eventually become contraindications to implantation surgeries.

As is well known, the wrong choice of structural material is the cause of microbial imbalance, which leads to the accumulation of pathogenic microflora, which leads to a shortening of the period of use of prostheses, and in many cases to the refusal of their use [12, 13].

Scientists have proven that as the period of use of partial removable lamellar prostheses increases, the negative impact of the bases of the prostheses on the microflora of the oral cavity increases, regardless of the construction material. This is an excellent environment for increased reproduction of microorganisms, primarily for *C. albicans* and *E. Coli* [14].

Colonization by fungi of the genus *C. albicans* was observed in 22.3%-30% of the examined patients in

patients who use removable prostheses and steadily increased to 41.5% with increasing duration of use [15].

A number of scientists claim that along with the massive insemination of the mucous membrane of the oral cavity in elderly patients who use removable orthopedic structures, the number of microflora increases, among which dominate staphylococci in 90.5%, lactobacilli in 76.2% and yeast-like fungi in 57.6% of cases [16].

The obtained data on the optical density of microbial cultures characterized the intensity of their planktonic growth.

Comparing the base resins impact on the growth of *S. aureus MSSA*, the most intense growth was observed in the presence of Breflex resin (it constituted 1.130±0.027 CU). Growth indicators of Vertex and Villacryl resins were at the same level, even slightly lower than the corresponding indicators compared to the control samples made of glass constituting 0.82±0.086 CU and the control amounting 0.77±0.012 CU.

The conducted microbiological studies showed different impact of base resin samples on the growth inhibition of antibiotic-sensitive *S. aureus MSSA*. Vertex inhibited growth by 8.53% in relation to glass, and Breflex, on the contrary, sharply stimulated the culture growth by 37.64%. The impact of Villacryl resin was the closest to the control indices in comparison with glass.

The period of adaptation is a very difficult period for patients and dentists, so the obtained research results contribute to the improvement of the selection of structural elements of partial removable lamellar prostheses in the treatment of partial edentulousness.

CONCLUSIONS

The conducted microbiological studies showed the diverse influence of base materials on such important indicators as the optical density of cultures grown in the presence of base resins and the growth inhibition index of cultures.

According to the obtained results, such basic resins as Breflex, Vertex increased the optical density of microbial cultures and could not be recommended for the manufacture of temporary removable laminar dentures.

The index of cultures growth inhibition and the impact on the optical density provided an opportunity to recommend Villacryl base resin as a base material for the manufacture of temporary removable laminar dentures.

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CONFLICT OF INTEREST

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ORIGINAL ARTICLE

CONTENTS

Synthesis and evaluation of Sulfonamide-Thiazolidinone conjugates as promising anticancer agents via carbonic anhydrase inhibition

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ABSTRACT

Aim: This study focuses on developing and evaluating five newly created compounds targeting carbonic anhydrase isoforms found in solid tumors. Our primary goal is to create exceptionally potent anti-cancer drugs.

Materials and Methods: Sulfanilamide, chloroacetyl chloride, Gamma-aminobutyric acid, thionyl chloride, methanol, hydrazine hydrate, aromatic aldehyde derivatives, glacial acetic acid, and thioglycolic acid were used in the chemical synthesis. We performed docking studies using the Molecular Operating Environment software program version 2015.10, and used MTT assay to predict cytotoxic activity.

Results: The compounds we developed demonstrated impressive antineoplastic action in both in silico experiments as well as cell line experiments. Their toxicity to normal cells varied significantly, but their efficacy against cancer cells differed significantly from cisplatin. When compared to acetazolamide, each of the produced compounds exhibited significant differences in their effects on MCF7 cells. Based on these findings, synthetic compounds may serve as antineoplastic medications. Including the thiazolidinone ring in these compounds enhanced their affinity for the receptor by binding to multiple crucial amino acids that play a significant role in our target's enzymatic activity and substrate binding.

Conclusions: Our synthetic compounds revealed cytotoxicity and inhibitory potencies against carbonic anhydrase. Moreover, they exhibited cytotoxicity.

KEY WORDS: Carbonic Anhydrase Inhibitors, In Silico, Thiazolidin-4-one, Sulfonamide

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INTRODUCTION

Cancer is a complex group of disorders distinguished by the unregulated proliferation and spreading of irregular cells, and these abnormal cells can develop into tumors, infiltrate neighboring tissues, and spread to other areas of the body through a process known as metastasis [1]. Cancer is a significant worldwide public health issue, with millions of new cases diagnosed each year. It is a prominent factor contributing to mortality on a global scale [2]. According to the World Health Organization (WHO), in 2020, over 19.3 million individuals globally received a cancer diagnosis, and 10 million of them died from the disease [3]. With an expected 2.3 million new cases (11.7% of the total), female breast cancer has surpassed lung cancer as the most often diagnosed cancer. Afterward, colorectal 10.0%, prostate 7.3%, and stomach 5.6% cancers were listed in that order of frequency [4]. Although there have been significant improvements in cancer treatment, cancer still poses a considerable challenge [5]. Current research efforts are mainly focused on understanding the fundamental principles of cancer development and discovering new targets for therapy [6]. Nowadays, numerous researchers are attempting to gain a more comprehensive understanding of the tumor microenvironment, which substantially impacts the development of tumors [7]. The tumor microenvironment consists of various elements that create a complex network, including nutrition, oxygen levels, metabolites, pH, and growth factors, so they contribute to therapeutic resistance by providing a protective niche for cancer cells against chemotherapy, radiation therapy, and targeted therapies [8]. Hypoxia is a significant carcinogenic factor present in all solid tumors, contributing to enhanced malignancy and associated with resistance to ionizing radiation and chemotherapy [9]. One of the proteins induced by hypoxia via the hypoxia-inducible factor 1α (HIF-1α) is carbonic anhydrase IX (CA IX), a membrane-associated enzyme. CA IX expression is markedly elevated in human cancer cells while remaining minimal in normal tissues [10]. Human carbonic anhydrases (hCAs) are essential me-

talloenzymes capable of converting CO_2 and H_2O into HCO_3^- and H^+. There are about 16 isoforms, and we will focus on CA IX as it is the most active CA isoform for the CO^2 hydration reaction, playing a significant role in regulating the tumor acid-base balance [11-12]. Targeting CAIX could be a beneficial strategy for delivering cytotoxic medications directly to the desired place, minimizing adverse effects on normal tissues [13]. All currently utilized CA inhibitors are either sulfonamide or a structurally similar sulfamido or sulfamato group. This group acts as a zinc-binding group (ZBG) and mimics the transition state analogue, resembling the tetrahedrally bound zinc to bicarbonate [14-15]. Since 2005, sulfonamides have attracted considerable attention because of their capacity to inhibit carbonic anhydrase, potentially making them suitable for use as anticancer therapies. Additionally, sulfonamide has an essential functionality with various pharmacological applications, such as antihypertensive and antibacterial, antiprotozoal, antifungal, and anti-inflammatory medications [16-17]. The sulfonamides interact with the enzyme in the following manner: Initially, the nitrogen atom in the R-SO2-NH- molecule must establish an ionic bond with the Zn^{2+} ion present in the main site of the carbonic anhydrase. Establishing two hydrogen bonds with the THR-199 amino acid is also necessary [18]. In medicinal chemistry, five-membered heterocyclic molecules, especially those with several heteroatoms, have shown various biological activities [19]. Thiazolidinone is a compound characterized by a sulfur atom at the 1-position, a nitrogen atom at the 3-position, and a carbonyl group at the 2-, 4-, or 5-positions [20]. Compounds that include a Thiazolidinone group have shown diverse pharmacological effects, such as anti-HIV, anti-malarial, anti-inflammatory, and hypoglycemic activity [20]. Incorporating Thiazolidinone into a molecule can enhance its interaction with biological targets. Thiazolidinone fragments are frequently chosen to modify lead compounds in developing anti-tumor medications [22]. These derivatives have the potential to combat cancer through various mechanisms, including initiating apoptosis, preventing the cell cycle, generating reactive oxygen species (ROS), and inhibiting a diverse range of enzymes that are directly associated with the survival of cancer cells, including our target enzyme, CA [23].

AIM

This study focuses on developing and evaluating five newly created compounds targeting carbonic anhydrase isoforms found in solid tumors. Our primary goal is to create exceptionally potent anti-cancer drugs.

MATERIALS AND METHODS

EXPERIMENTAL SECTION

All reagents and anhydrous solvents were supplied by Sigma-Aldrich, Germany, Riedel de Haën, Germany, Hangzhou Hyper Chemicals, and Merck, Germany. We used the Thomas Hover apparatus and the capillary tube method to determine the melting points. Ascending thin-layer chromatography confirmed the reaction steps, purified the synthesized compounds, and measured their retention factor (Rf) values. This was accomplished by the use of a mobile phase that was composed of methanol and acetone at a ratio of one to one [24]. The scanning FT-IR and spectra estimation were carried out utilizing KBr discs by means of a Japanese Shimadzu spectrophotometer at the University of Kufa. Recordings of H^1NMR were made using the Bruker 300 MHz instrument at the University of Mashhad, with DMSO as the solvent.

TYPICAL PROCEDURE FOR THE REACTIONS

We first dissolved the sulfanilamide in a benzene and dimethylformamide solvent; we then added Triethylamine to the solution. The mixture was agitated in a chilled container. Chloroacetyl chloride (CAC) dissolved in benzene is then added in small amounts to the sulfonamide mixture to form acetyl sulfonamide. GABA carboxyl (COOH) esterified using thionyl chloride in cold methanol. The GABA's methyl ester participated in the reaction with acetyl sulfonamide, which resulted in the formation of a secondary amine bond. The compound that was formed as a result of this reaction possessed 99% of hydrazine hydrazide, and this resulted in the creation of hydrazide. The hydrazide underwent a reaction with aromatic aldehyde derivatives in the presence of glacial acetic acid as a catalyst, leading to the synthesis of imines. The imines underwent a reaction with thioglycolic acid to yield sulfonamide derivatives with a heterocyclic ring known as thiazolidin-4-one. The production procedure for synthesizing molecules (IVa–IVe) derived from sulfanilamide is described in Fig. 1.

2-CHLORO-N-(4-SULFAMOYLPHENYL) ACETAMIDE (A) IS SYNTHESIZED ACCORDING TO MINA ET. AL., [25]

Two grams of sulfanilamide, which equals 11.6 mmol, were dissolved in 40 ml of a solution that included 1:3 ratio of dimethylformamide to benzene. Subsequently, we added Triethylamine (1.6 mL, 11.6 mmol). The reaction solution was stirred continually in an ice bath throughout the experiment. Gradually, CAC (0.92 mL, 11.6 mmol in 10 mL benzene) was added while

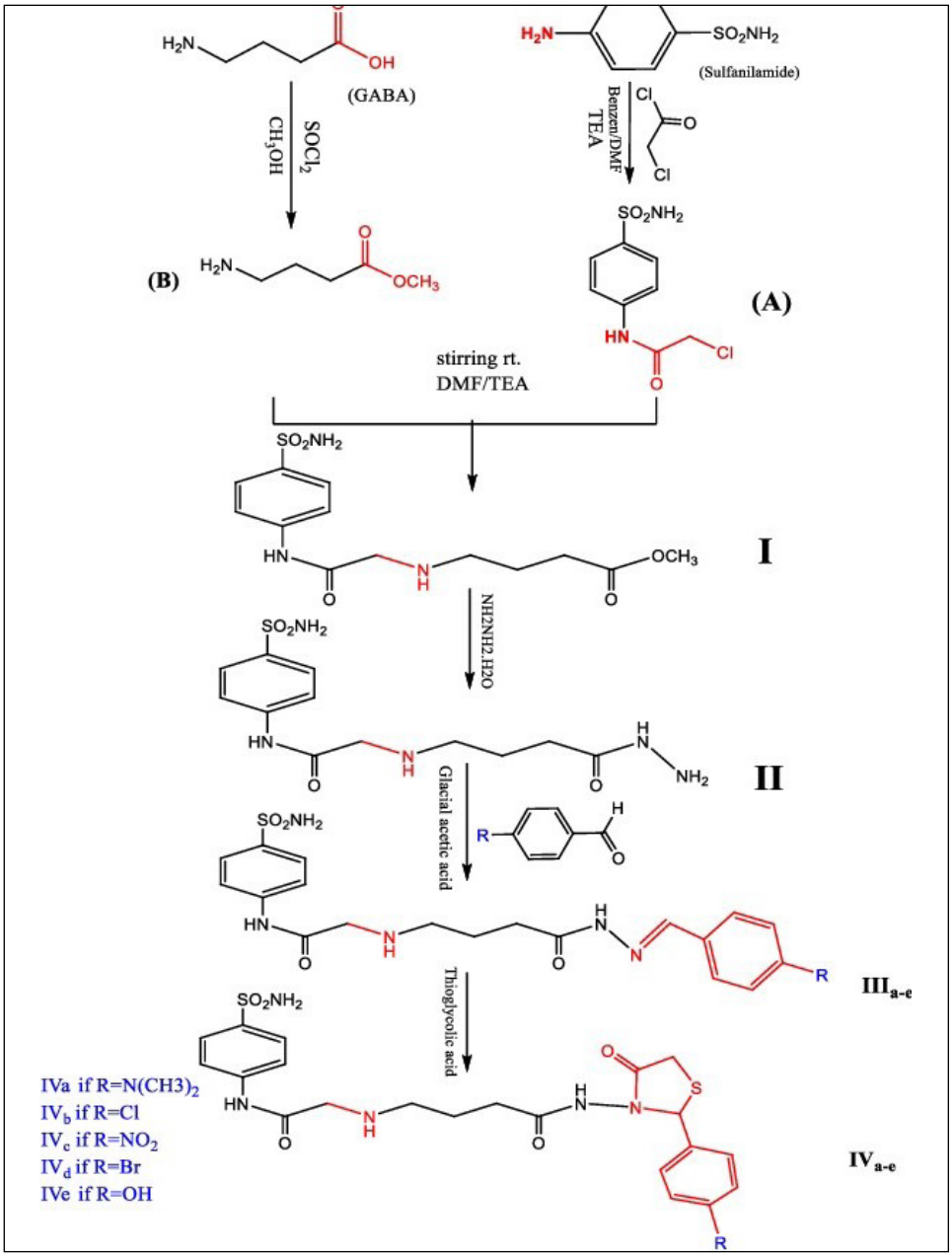

Fig. 1. Synthesis of target compounds.

continuously stirring for one hour. The mixture was then heated to 76°C and subjected to reflux for three hours. We then introduced additional crushed ice water, which led to the formation of a solid substance. Filtering separated this solid, and ethanol-based recrystallization purified it, producing compound A. Table 1 provides %yield, physical data, and Rf values.

METHYL-4-AMINOBUTYRATE HYDROCHLORIDE (B) IS SYNTHESIZED ACCORDING TO LI, J. AND Y. SHA., [26]

Gamma-aminobutyric acid (1g, 9.7 mmol) was dissolved in methanol and then cooled to a temperature of 0°C. We gradually added 0.7 mL (9.7 mmol) of thionyl chloride. The solution was left at room temperature for 45 minutes and then refluxed. After the reaction was finished, the solvent was completely evaporated. Then, the remaining substance was subjected to multiple washes with diethyl ether and purified by recrystallization using a combination of methanol and diethyl ether. This process resulted in compound B. Table 1 provides % yield, physical data, and Rf values.

METHYL 4-((2-OXO-2-((4-SULFAMOYLPHENYL)AMINO)ETHYL) AMINO) BUTANOATE (I) IS SYNTHESIZED ACCORDING TO PARK, H.-S., ET AL., [27]

We used a round-bottom flask to dissolve 0.6 of compound B, which equals 4 mmol in 20 ml of DMF. Before adding compound A (1g, 4 mmol), 0.75 mL of Triethylamine was slowly added to the reaction solution. We stirred the mixture throughout the night. Subsequently, the solvent evaporated, and the resul-

Table 1. Type of aldehyde compounds and their amounts used in the above reaction

Aldehyde compounds	Number of grams
4-Bromobenzaldehyde	0.276
4-Chlorobenzaldehyde	0.213
4-Dimethylaminobenzaldehyde	0.226
4-Hydroxybenzaldehyde	0.185
4-Nitrobenzaldehyde	0.229

tant residue was gathered and subjected to numerous washes with diethyl ether, creating a viscous oil compound I. Table 1 provides % yield, physical data, and Rf values.

2-((4-HYDRAZINYL-4-OXOBUTYL)AMINO)-N-(4-SULFAMOYLPHENYL) ACETAMIDE (II) IS SYNTHESIZED ACCORDING TO JUBIE ET. AL., [28]

The compound (I) (1.5g, 4.5 mmol) was dissolved in absolute ethanol in a flask. We introduced a 0.63 mL solution of 99% hydrazine hydrate, equivalent to 13 mmol, into the flask. The resulting mixture was refluxed for 10 hours at a temperature of 75°C. After finishing the reaction, we evaporated the solvent until it reached half of its initial volume. A solid substance was acquired upon cooling, which underwent filtration, drying, and further purification via recrystallization utilizing 100% ethanol, leading to compound II. Table 1 provides %yield, physical data, and Rf values.

2-((4-(2-BENZYLIDENEHYDRAZINEYL)-4-OXOBUTYL) AMINO)-N-(4-SULFAMOYLPHENYL) ACETAMIDE (IIIA-E) IS SYNTHESIZED ACCORDING TO XAVIER A ET AL., [29]

We carefully added five droplets of glacial acetic acid to a reaction mixture with compound II (1.52 mmol, 0.5 g) and the right aldehydes (1.52 mmol), both dissolved in 25 mL of methanol:

1. Sulfanilamide ($C_6H_8N_2O_2S$) M. WT = 172.20, off white crystals, Melting point in °C = 165. Rf value= 0.90.
2. GABA ($C_4H_9NO_2$) = M. WT = 103.12, white crystals, melting point in °C= 203 and Rf value = 0.35.
3. Compound A ($C_8H_9ClN_2O_3S$) M. WT = is 248.69, yield=76.9%, grey powder, melting point in °C = 203-205 and Rf value is 0.74.
4. Compound B ($C_5H_{12}ClNO_2$) M. WT = is 153.06, yield =96%, white crystal, melting point in °C = 120-122 and Rf value is 0.62.
5. Compound I ($C_{13}H_{19}N_3O_5S_2$) M. WT = 329.37, Brown, yield = 80% and Rf value = 0.81.
6. Compound II ($C_{12}H_{19}N_5O_4S$) M. WT = 329.38, yellowish white powder, yield = 82%, melting point in °C = 50-52 and Rf value = 0.72.
7. Compound IIIa ($C_{21}H_{28}N_6O_4S$) M. WT = 460.55, red crystals, yield = 72%, melting point in °C = 256-258 and Rf value = 0.90.
8. Compound IIIb ($C_{19}H_{22}ClN_5O_4S$) M. WT = 451.93, yellow crystals, yield = 74%, melting point in °C = 209-211 and Rf value = 0.77.
9. Compound IIIc ($C_{19}H_{22}N_6O_6S$) M. WT = 462.84, yellow crystals, yield = 75%, melting point in °C = 307-309 and Rf value = 0.75.
10. Compound IIId ($C_{19}H_{22}BrN_5O_4S$) M. WT = 496.38, yellow crystals, yield = 75%, melting point in °C = 226-228 and Rf value is 0.69.
11. Compound IIIe ($C_{19}H_{23}N_5O_5S$) M. WT = 433.48, white crystals, yield = 60%, melting point in °C = 208-210 and Rf value = 0.78.
12. Compound IVa ($C_{23}H_{30}N_6O_5S_2$) M. WT = 534.65, orange oil, yield = 68% and Rf value = 0.68.
13. Compound IVb ($C_{21}H_{24}ClN_5O_5S_2$) M. WT=526.02, White oil, yield= 70% and RF= 0.66.
14. Compound IVc ($C_{21}H_{24}N_6O_7S_2$) M. WT=536.58, yellow oil, yield=66% and RF=0.71.
15. Compound IVd ($C_{21}H_{24}BrN_5O_5S_2$) M. WT=570.48, White oil, yield=72% and RF=0.69.
16. Compound IVe ($C_{21}H_{25}N_5O_6S_2$) M. WT=507.58, yellow oil, yield=71% and RF=0.73.

We then refluxed the reaction mixture for 2–3 hours. Next, we separated the formed precipitate using filtration and purified it by recrystallization using ethanol. This process resulted in the formation of compounds IIIa–e, table 1 presents the percentage yield, physical data, and Rf values for the compounds.

4-((2-OXO-2-((4-SULFAMOYLPHENYL) AMINO)ETHYL)AMINO)-N-(4-OXO-2-PHENYLTHIAZOLIDIN-3-YL)BUTANAMIDE (IVA-E) IS SYNTHESIZED ACCORDING TO NEUENFELDT PD ET AL., [30]

The procedure involved heating a mixture consisting of 3 mL of thioglycolic acid and 1 mmol of one of the compounds IIIa-e to a temperature of 60°C. The mixture was continuously stirred until the reaction was fully completed, normally taking around 3 hours. Next, we introduced 5 mL of ethyl acetate into the reaction mixture. Next, we rinsed the organic layer three times with 20 mL of a saturated sodium bicarbonate solution and once with 10 mL of water. After removing any remaining water using anhydrous magnesium sulfate, the mixture was concentrated to produce an oily substance. We rinsed the oil with diethyl ether to get the compounds IVa-e. Table 1 provides the % yield, physical data, and Rf.

Table 2. Cytotoxic activity (IC50, μM) of sulfonamide derivatives and the reference drugs against MCF7 and MCF10a

Compound	MCF7 Results		MCF10a Results	
	IC_{50}(μM) ± SD	p-value	IC_{50}(μM) ± SD	p-value
Acetazolamide	67.53 ± 1.80	Standard	53.91± 5.636	Standard
Cisplatin	15.09 ±2.25	Standard	13.55 ±3.45	Standard
IVa	33.57 ± 1.438	0.0024 a	567.24± 5.072	0.0001 a
		0.0081 b		0.0001 b
IVb	34.60 ±1.42	0.0028 a	511.04±2.422	0.0001 a
		0.0076 b		0.0001 b
IVc	41.29 ± 1.29	0.0067 a	452.75± 1.194	0.0001 a
		0.0039 b		0.0001 b
IVd	28.55±1.342	0.0001 a	240.51±3.068	0.0001 a
		0.0096 b		0.0001 b
IVe	30.20 ±2.014	0.0019 a	490.38 ±3.97	0.0001 a
		0.0087 b		0.0001 b

Letters a and b: There is a considerable variation compared to cisplatin and acetazolamide, respectively. Not significant (Ns).

Table 3. Results of the investigated compounds' S scores and rmsd values

Compound Name	R group	Docking-Scores	RMSD	Total affinity sites	Molecules that are involved in binding
Acetazolamide	----------	-6.5	1.66	5	Zn301, Thr199, Thr200, His96, His119
IVa	N(CH3)2	-8.8	1.44	7	Zn301, Thr199, Leu198, Gln92, Asn62, His96, His119
IVb	Cl	-9.1	1.9	6	Zn301, Thr199, Leu198, Trp5, His96, His119
IVc	NO2	-8.55	1.7	6	Zn301, Thr199, Leu198, Trp5, His96, His119
IVd	Br	-9.16	1.9	10	Zn301, Thr199, Leu198, Gln67, Gln92, Trp5, His64, His94, His96, His119
IVe	OH	-8.68	1.7	8	Zn301, Thr199, Leu198, Gln92, His64, His94, His96, His119.

SPECTROSCOPIC ANALYSIS [30]

Compound A ($C_8H_9ClN_2O_3S$) FT-IR (cm^{-1}) 3327-3213 (N-H_2 of sulfonamide), 3134 (N-H of amide), 1689 (C=O amide), 1602-1548 (C=C of aromatic ring) and 684 (C-Cl stretching). Compound B ($C_5H_{12}ClNO_2$) FT-IR (cm^{-1}) 3018 (N-H of primary amine salt), 1735 (C=O of ester), 1132 (C-N stretching). Compound I ($C_{16}H_{14}N_4O_3S_2$) FT-IR (cm^{-1}) 3433-3390 (N-H_2 of sulfonamide), 3178 (N-H of amide), 1695 (C=O of ester), 1666 (C=O of amide), 1595-1496 (aromatic C=C). Compound II ($C_{12}H_{19}N_5O_4S$) FT-IR (cm^{-1}) 3294-3190 (N-H_2 of sulfonamide overlap with NH2 of amine), 1668 (C=O of amide). Compound IIIa ($C_{21}H_{28}N_6O_4S$) FT-IR (cm^{-1}) 3442-3375 (N-H_2 of sulfonamide), 3196 (N-H of amide), 2947-2816 (C-H of N-$(CH_3)_2$), 1658 (C=O of amide overlap with C=N), 1365 (N-$(CH_3)_2$ stretching). Compound IIIb ($C_{19}H_{22}ClN_5O_4S$) FT-IR (cm^{-1}) 3520 and 3446 (N-H_2 of sulfonamide), 3229 (N-H of amide), 1660 (stretching of C=N), 1624 (C=O of amide), 817 (stretching of C-Cl). Compound IIIc ($C_{19}H_{22}N_6O_6S$) FT-IR (cm^{-1}) 3444-3292 (N-H_2 of sulfonamide), 3186 (N-H of amide), 1668 (stretching of C=N), 1620 (C=O of amide), 1546-1334 (asymmetric and symmetric of NO_2). Compound IIId ($C_{19}H_{22}BrN_5O_4S$) FT-IR (cm^{-1}) 3477-3415 (N-H_2 of sulfonamide), 3145 (N-H of amide), 1624 (C=O of amide overlap with C=N), 636 (stretching of C-Br). Compound IIIe ($C_{19}H_{23}N_5O_5S$) FT-IR (cm^{-1}) Broad 3396 Overlap of O-H and of NH2 of sulfonamide, 1658 (C=O of amide overlapping with C=N), 1018 (alcoholic C-O). Compound IVa ($C_{23}H_{30}N_6O_5S_2$) FT-IR (cm^{-1}) 3435 (N-H_2 of sulfonamide), 3170 (N-H of amide), 2933 (C-H of alkane), 1718 (C=O of thiazolidinone overlap with C=O of amide), 1294 (C-S stretching). ^{1}H NMR (ppm): 1.09(2H, M, CH_2 of GABA); 2.89 (6H, S, CH_3 of N$(CH_3)_2$); 3.32-3.39 (4H, M, CH_2 of GABA); 3.61 (1H, S, NH of amine); 3.67(2H, S, CH_2-C=O); 3.99-4.06 (2H, Doublet of doublet, CH_2 of thiazolidinone); 5.17 (1H, S, CH of thiazolidinone); 6.68 (2H, S, NH_2 of sulfonamide); 7.19-7.82 (8H, M, Aromatic CH); 10.18 (1H, S, NH of amide); 10.52 (1H, S, NH of amide). Compound IVb ($C_{21}H_{24}ClN_5O_5S_2$) FT-IR (cm^{-1}) 3441-3348 (N-H_2 of sulfonamide), 3182 (N-H of amide), 1718 (C=O of thiazolidinone), 1645 (C=O of amide), 1294 (C-S stretching), 669 (stretching of C-Cl). ^{1}H NMR (ppm): 1.22(2H, M, CH_2 of GABA); 3.20 (2H, S, CH_2-C=O); 3.34(1H, S, NH of amine); 3.62-3.65 (4H, M,

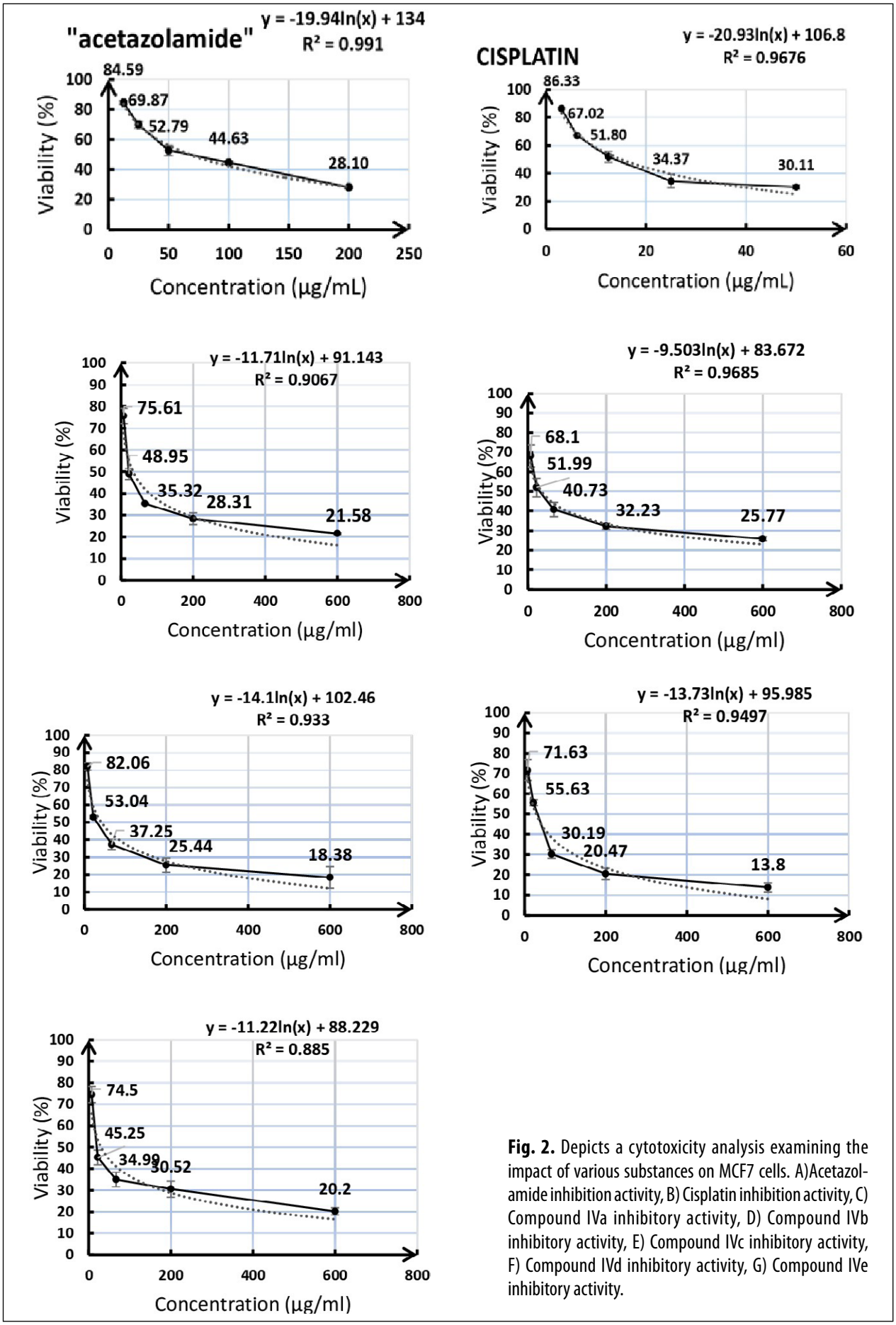

Fig. 2. Depicts a cytotoxicity analysis examining the impact of various substances on MCF7 cells. A)Acetazolamide inhibition activity, B) Cisplatin inhibition activity, C) Compound IVa inhibitory activity, D) Compound IVb inhibitory activity, E) Compound IVc inhibitory activity, F) Compound IVd inhibitory activity, G) Compound IVe inhibitory activity.

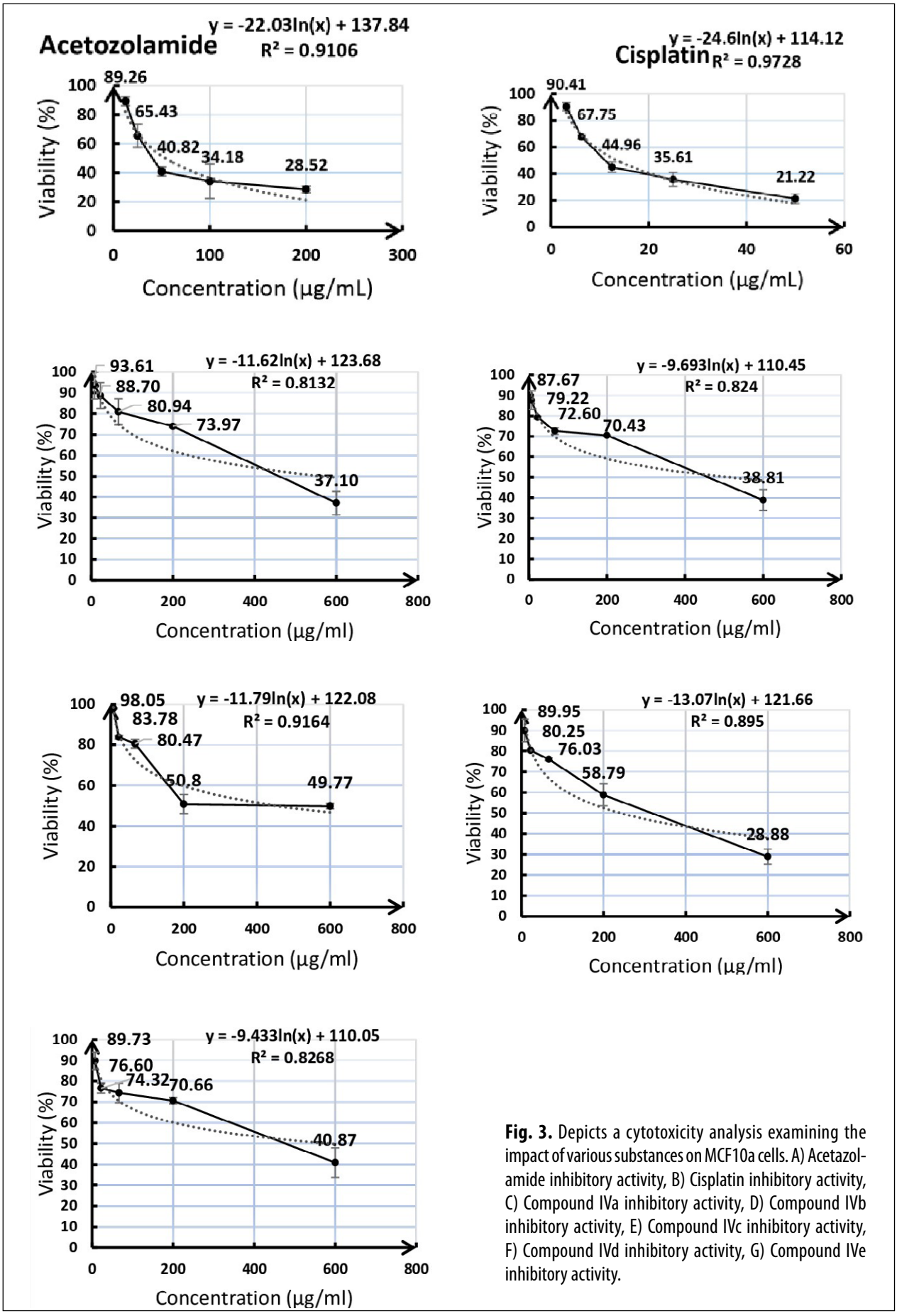

Fig. 3. Depicts a cytotoxicity analysis examining the impact of various substances on MCF10a cells. A) Acetazolamide inhibitory activity, B) Cisplatin inhibitory activity, C) Compound IVa inhibitory activity, D) Compound IVb inhibitory activity, E) Compound IVc inhibitory activity, F) Compound IVd inhibitory activity, G) Compound IVe inhibitory activity.

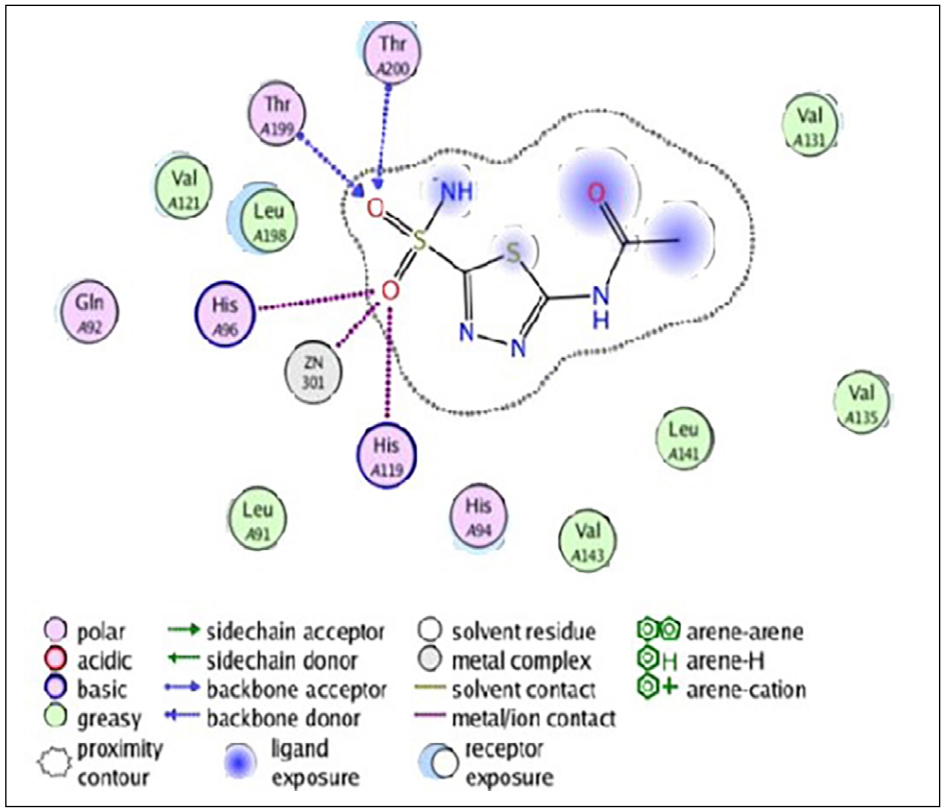

Fig. 4. Acetazolamide in combination with HCA IX (PDB code: 4M2V) (2D).

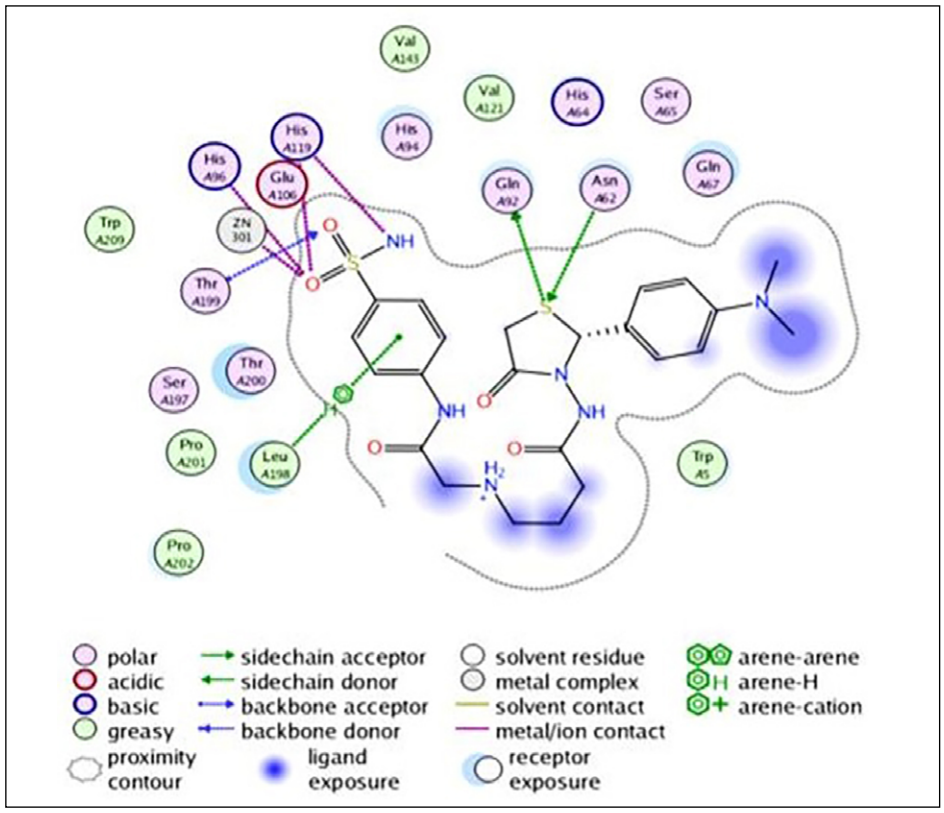

Fig. 5. IVa in combination with HCA IX (PDB code: 4M2V) (2D).

CH_2 of GABA); 3.88-3.98 (2H, Doublet of doublet, CH_2 of thiazolidinone); 5.24 (1H, S, CH of thiazolidinone); 6.53 (2H, S, NH_2 of sulfonamide); 7.34-7.41 (8H, M, Aromatic CH); 9.29 (1H, S, NH of amide); 10.21 (1H, S, NH of amide). Compound IVc ($C_{21}H_{24}N_6O_7S_2$) FT-IR (cm^{-1}) 3471 and 3435 (N-H_2 of sulfonamide), 3265 (N-H of amide), 1714 (C=O of thiazolidinone), 1676 (C=O of amide), 1519-1390 (asymmetric and symmetric of NO2), 1207 (C-S stretching). ^{1}H NMR (ppm): 1.04(2H, M, CH_2 of GABA); 3.22 (2H, S, CH_2-C=O); 3.29(1H, S, NH of amine);

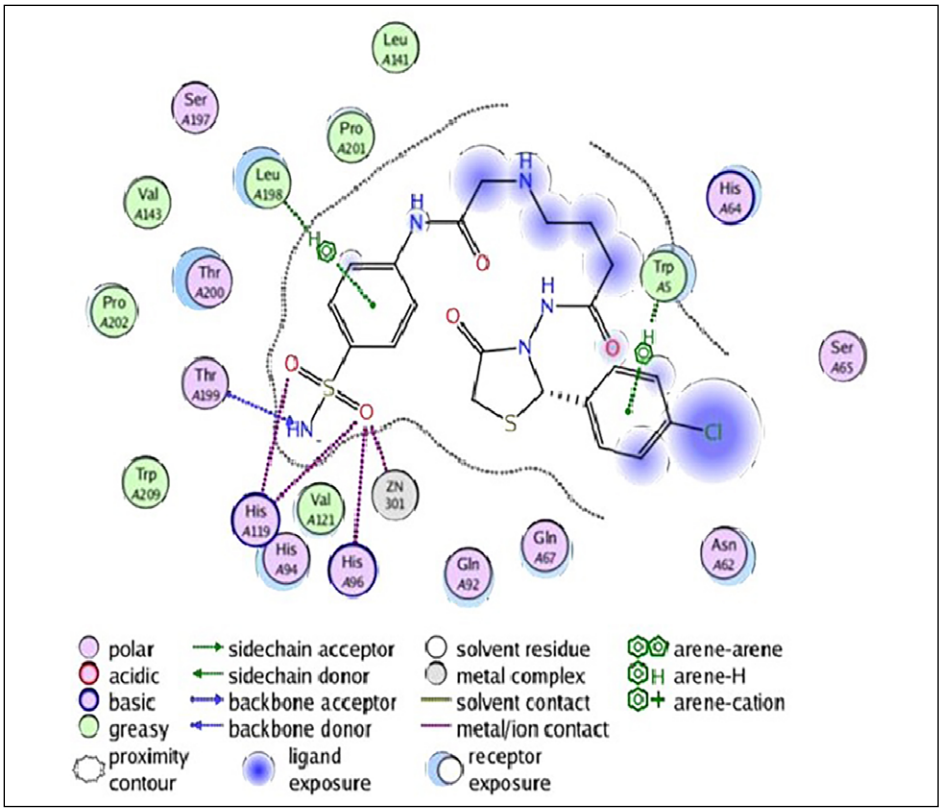

Fig. 6. IVb in combination with HCA IX (PDB code: 4M2V) (2D).

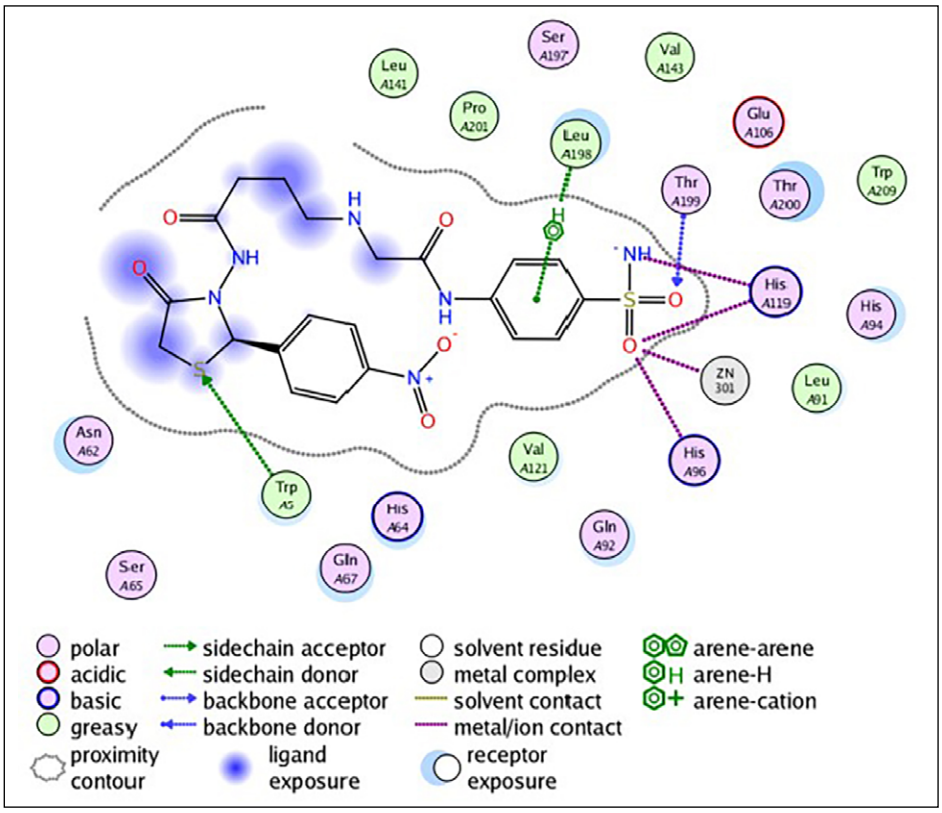

Fig. 7. IVc in combination with HCA IX (PDB code: 4M2V) (2D).

3.61-3.64 (4H, M, CH_2 of GABA); 3.67-3.95 (2H, Doublet of doublet, CH_2 of thiazolidinone); 5.41 (1H, S, CH of thiazolidinone); 6.60 (2H, S, NH_2 of sulfonamide); 7.64-7.28 (8H, M, Aromatic CH); 10.37 (1H, S, NH of amide); 10.51 (1H, S, NH of amide). Compound IVd ($C_{21}H_{24}BrN_5O_5S_2$) FT-IR (cm^{-1}) 3439-3392 (N-H_2 of sulfonamide), 3201 (N-H of amide), 1730 (C=O of thiazolidinone), 1668 (C=O of amide), 1286 (C-S stretching), 644 (stretching of C-Br). ^{1}H NMR (ppm): 1.28 (2H, M, CH_2 of GABA); 3.24 (2H, S, CH_2-C=O); 3.42 (1H, S, NH of amine); 3.64-3.73 (4H, M,

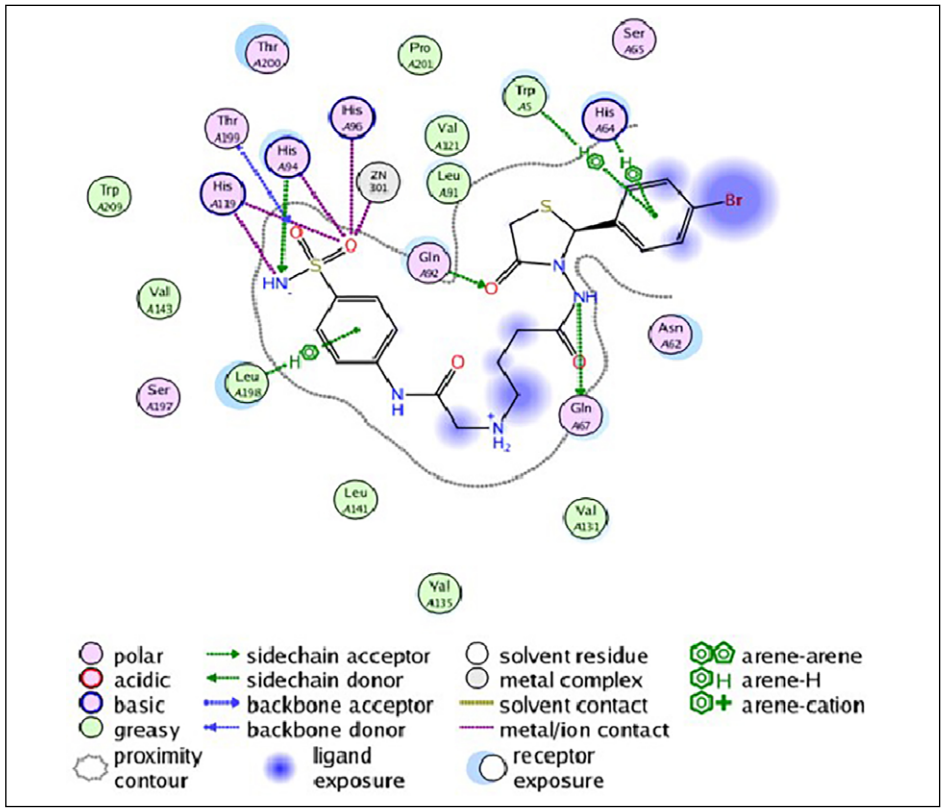

Fig. 8. IVd in combination with HCA IX (PDB code: 4M2V) (2D).

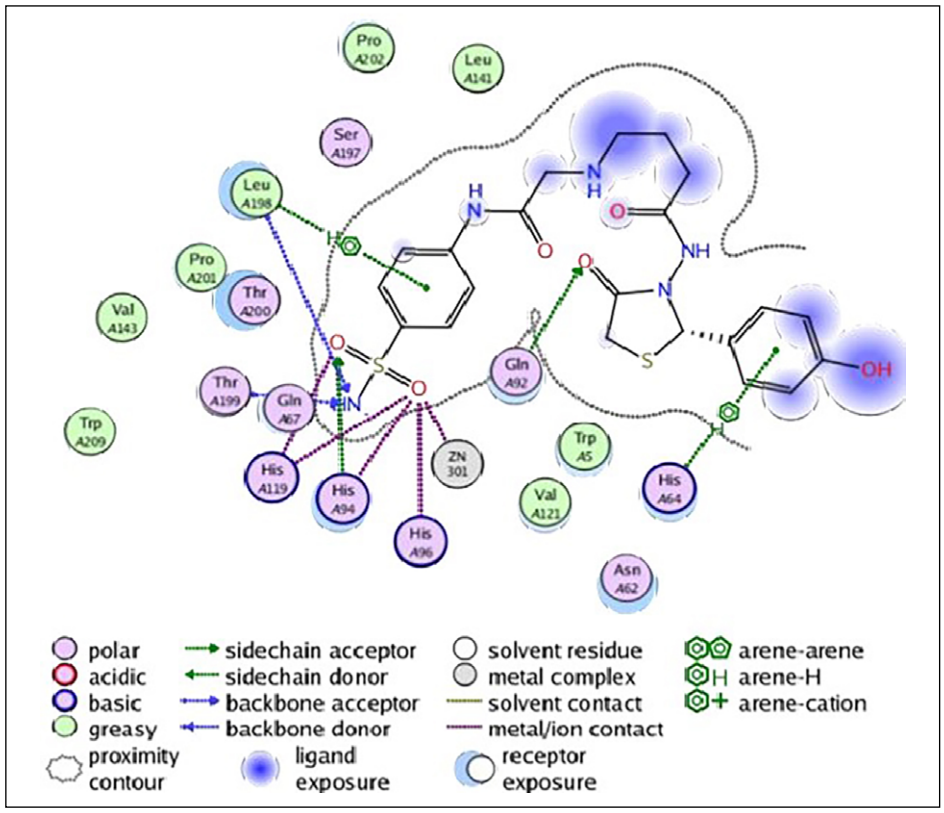

Fig. 9. IVc in combination with HCA IX (PDB code: 4M2V) (2D).

CH_2 of GABA); 3.90-4.03 (2H, Doublet of doublet, CH_2 of thiazolidinone); 5.29 (1H, S, CH of thiazolidinone); 6.68 (2H, S, NH_2 of sulfonamide); 7.35-7.59 (8H, M, Aromatic CH); 10.31 (1H, S, NH of amide). Compound IVe ($C_{21}H_{25}N_5O_6S_2$) FT-IR (cm^{-1}) 3433 (O-H stretching), 3342-3217 (N-H_2 of sulfonamide), 3142 (N-H of amide), 1718 (C=O of Thiazolidinone), 1641 (C=O of amide), 1292 (C-S stretching), 1156 (alcoholic C-O). ^{1}H NMR (ppm): 1.22 (2H, M, CH_2 of GABA); 3.21 (2H, S, CH_2-C=O); 3.31 (1H, S, NH of amine); 3.58-3.65 (4H, M, CH_2 of GABA); 3.95-4.02

(2H, Doublet of doublet, CH_2 of thiazolidinone); 5.16 (1H, S, CH of thiazolidinone); 6.63 (2H, S, NH_2 of sulfonamide); 7.17-7.79 (8H, M, Aromatic CH); 9.67 (1H, S, alcoholic OH); 10.37 (1H, S, NH of amide).

DOCKING STUDY

Molecular docking analysis, including protein and ligand structure preparation, was carried out by the study using the MOE (Molecular Operating Environment) software version 2015.10. We used ChemDraw Professional 12.0 to draw the ligand structures precisely. Afterward, we protonated the ligands in their 3D structure in MOE and applied partial charges. Then, we performed energy minimization. The structure of carbonic anhydrase IX (PDB code: 4M2V) was obtained from the Protein Data Bank database and imported into MOE for analysis. Isolating the target protein included identifying and eliminating non-functional chain sequences and small molecules. Hydrogen bonds were introduced, and the atomic potentials of the protein were modified. After identifying the active site, the next step is to import the already prepared ligands into MOE, and the docking process is carried out.

CYTOTOXIC STUDY

In the cytotoxicity study, we used two types of breast cells: MCF7 (malignant) and MCF10a (benign), both sourced from the National Cell Bank of Iran. Cell cultures were maintained in RPMI-1640 and DMEM media, each supplemented with 10% fetal bovine serum (FBS) and antibiotics (penicillin at 100 U/mL and streptomycin at 100µg/mL). The cells were incubated at 37°C in a controlled atmosphere with 5% CO2 and appropriate humidity. Trypsin/EDTA and phosphate-buffered saline (PBS) were used for cell passage. The same culture conditions and media were used for 3D colony formation and regular monolayer cell cultures. The MTT assay was employed to assess cell growth and viability. For the monolayer culture, cells were trypsinized, counted, and seeded into 96-well plates at a density of 1.4×10^4 cells per well in 200 µL of fresh media. After forming a monolayer, the cells were exposed to various concentrations of compounds (ranging from 600µg/mL to 7.4µg/mL) for 24 hours at 37°C with 5% CO2. Post-treatment, the media were removed, and cells were incubated with MTT solution (0.5 mg/mL in PBS) for an additional 4 hours at 37°C. Subsequently, the MTT solution was replaced with 100 µL of dimethyl sulfoxide (DMSO) per well, and the cells were agitated at 37°C until complete dissolution of the formazan crystals. Cell viability was measured using an ELISA reader at an absorbance of 570 nm. The IC50 values, representing the concentration of compounds causing 50% cell death, were estimated by analyzing the dose-response curves.

RESULT AND DISCUSSION

CHEMISTRY

The appearance of a sharp band at 1689 cm^{-1} for the amide group signifies a nucleophilic attack by the electron pairs on the nitrogen atom of the primary amine in sulfonamide. This interaction with CAC leads to the formation of the 2-chloroacetamide derivative. Using methanol and thionyl chloride, Gamma-aminobutyric acid was esterified to produce its methyl ester. The presence of a 1730 cm^{-1} carbonyl band (for ester) indicates that GABA has been converted to methyl 4-aminobutanoate and the disappearance of the broad OH group of the carboxylic acid. The coupling of GABA ester with compound A in which the CABA ester amino group undergoes nucleophilic attack on the electrophilic carbon atom of chloroacetamide to give compound I, and it is formation confirmed by the absence of a 690 cm^{-1} peak corresponding to the stretching vibration of C-Cl, and the emergence of a 3292 cm^{-1} peak indicating the presence of a secondary amine. Hydrazide was obtained by reacting the resulting ester with 99% hydrated hydrazine. The ester's carbonyl group disappeared, while the amide band at 1668 cm^{-1} was unaffected. Additionally, at 3294-3190 cm^{-1}, the NH2 sulfonamide group overlapped with the primary amine group. The compounds (IIIa-e) were synthesized through Schiff base formation by reacting compound (II) with appropriate aldehydes in the presence of an acid catalyst. The formation of the imine bond was confirmed by FT-IR spectroscopy, indicated by absorption in the range of 1600-1660 cm^{-1}. Additionally, the absence of the aldehyde carbonyl bond and the disappearance of the characteristic aldehydic C-H stretching in the 1750-1850 cm^{-1} range further verified the reaction. The final cyclization and formation of the 4-thiazolidine ring, as depicted in Scheme 1, were achieved through a solvent-free synthesis by reacting compounds (IIIa-e) with thioglycolic acid, resulting in the formation of compounds (IVa-e). The structures of these compounds were confirmed by the appearance of new characteristic peaks corresponding to the carbonyl group of thiazolidinone in the range of 1718-1730 cm^{-1} and the disappearance of the thioglycolic acid broad OH peak.

CYTOTOXIC EVALUATION

We evaluated the cytotoxicity of substances (IVa, IVb,

IVc, IVd, and IVe) using MTT assays on cell lines. We employed two cell types, cancer cells (MCF7) and normal cells (MCF10), to assess the effects of our synthesized compounds. We determined the IC50 for each compound. The inhibitory action of each compound mentioned in this study was evaluated compared to acetazolamide, and their cytotoxic activity was compared to cisplatin, as indicated in Table 2.

The results indicate that each compound synthesized displayed inhibitory activity and possesses the potential to be developed into potent anticancer medications. The compounds showed varying levels of IC50 against the MCF7 and MCF10a cell lines, ranging from 28.55 to 41.29 μM and from 240.51 to 567.24 μM, respectively. All the compounds exhibited notable cytotoxicity variations compared to cisplatin, suggesting their lower efficacy against cancer cells. On the other hand, as compared to cisplatin, these compounds have a far lower toxicity to normal cells, suggesting that they may be more selective and have fewer side effects. Fig. 2 illustrates the correlation between the concentration of each compound and its impact on the viability of MCF7 cells, in which the inhibitory activity of acetazolamide is shown in Fig.2 A, while that of cisplatin is shown in Fig. 2B. Figures 2C, 2D, 2E, 2F, and 2G show the inhibitory actions of compounds IVa-e. Fig. 3 shows the relationship between the concentration of each compound and its effect on the viability of MCF10a cells. It depicts the inhibitory effects of various substances, including acetazolamide, in Fig. 3A, while that of cisplatin is shown in Fig. 3B. Figures 3C, 3D, 3E, 3F, and 3G show the inhibitory actions of compounds IVa-e.

DOCKING STUDY

The docking simulations identified multiple binding forms for the ligands within the 4M2V protein's binding pocket. The binding modes were identified based on specific residues in ligand-protein interactions, such as hydrogen bonding and electrostatic interactions. The results demonstrated varying binding affinities across the synthesized compounds, with greater interactions observed with the target protein than with acetazolamide. We observed a degree of similarity in the inhibition action of the produced compounds. All compounds bind to zinc, essential for inhibition activity and establishing hydrogen bonds with Th199 in the enzyme's active region. Furthermore, thiazolidinone moiety plays a vital role in binding to the enzyme's catalytic region by linking to important amino acids in substrate binding. Table 3 shows the result of the docking study.

Their S. score values were -8.8, -9.1, -8.55, -9.16, and -8.68, respectively, while their rmsd values were 1.6, 1.4, 1.9, 1.9, and 1.7, respectively.

CONCLUSIONS

This study aimed to explore the potential of newly developed sulfonamide-thiazolidinone hybrids as effective anticancer drugs, specifically targeting breast cancer. Compounds IVa-e were designed, thoroughly examined using computer simulations, chemically synthesized, and subjected to biological testing to evaluate their effectiveness against cancer. The compounds were examined for their cytotoxic effects against a cell line, resulting in IC50 values ranging from 28.55 to 41.29 μM. Among these compounds, the parabromo-derivative exhibited the highest cytotoxic activity against the tested breast cancer cell lines, with an IC50 of 28.55 μM. Furthermore, the same compound exhibited the highest s.score of 9.1 in relation to the in silico investigation, making it an excellent candidate for use as an anti-cancer drug. Furthermore, the four other compounds generated also displayed significant changes in cytotoxicity when compared to cisplatin. These compounds demonstrated promising efficacy against the enzyme carbonic anhydrase IX, as indicated by the docking results in MOE. However, these compounds demonstrate a notable variation in toxicity compared to cisplatin in normal cells, suggesting that they cause less harm to these cells. In summary, these results emphasize the capability of the produced compounds to inhibit tumor cells while minimizing harm to normal cells specifically. The data indicate that all five generated compounds, classified as sulfonamide-thiazolidinone, can potentially be employed as therapeutic agents for breast cancer. Further research is required to determine the in vivo anticancer efficacy more precisely.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Assessing the psychological well-being among adolescent and children

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ABSTRACT

Aim: To assess the psychological well-being of children and adolescents, socio demographic distribution data of parents and children and to find out the relationship between psychological well-being of children and adolescents and their socio demographic characteristics.

Materials and Methods: A descriptive cross- sectional study to assess the psychological well-being of children and adolescents in Al-Nasiriyah schools. The study period was extended (from December 1, 2023 to April 1, 2024). A non- probability (purposive sample) consist of (200) children and adolescents.

Results: Results revealed That children and adolescent in current study have moderate levels of Psychological Well-Being, our study revealed that is significant correlation (between participants' age and Psychological Well-being, difference in psychological wellbeing between history of school fail, difference in psychological Well-being among between mother educational groups and difference in Psychological well-being among father job groups) and no statistically Significant (between difference psychological well-being and school history, difference in psychological well- being Among socioeconomic status groups, difference in psychological well-being among father level of education Groups and difference in psychological wellbeing among father job groups).

Conclusions: Study provides valuable insights into factors influencing the psychological well- being of children and adolescents, highlighting the complex interaction between age, social status, parental education, and parental occupation. Academic performance and school experiences play a significant role in psychological well-being, especially in cases of academic failure.

KEY WORDS: Assessing, Psychological, Well-Being, Children

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INTRODUCTION

Creating a supportive school environment, empowering students, and addressing social and environmental factors are key to enhancing adolescent emotional well-being and resilience. Factors like social media impact, cyber bullying, and school pressures contribute to rising mental health issues among adolescents. A great deal of research has focused on the psychological well-being of children and adolescents, and therefore it is incredibly important that assessments are available to measure this trait [1]. Psychological well-being is a key facet of mental health, which is becoming of increasing concern, as demonstrated by the growing number of children and adolescents being referred to psychologists. The impact of these referrals is not only obvious in the short term but these emotional problems have the potential to continue into adulthood. A significant majority of people experiencing anxiety had first done so during childhood [2]. In order to discuss psychological well- being, it is important to start with a definition of this construct. Psychological well-being is a broad term, and there is no consensus as to what exactly this term denotes; this is partly because the discipline of psychology has focused almost entirely on psychological ill-being [3]. Despite no initial consensus as to the nature of psychological well-being, some basic themes do tend to emerge. Indeed, the term 'psychological well-being' is used to refer to other terms which, when combined, provide an overall understanding or definition of the phenomenon. Such sub-themes include life satisfaction, positive affect, autonomy, self-acceptance, environmental mastery, and positive relations [4]. Mentally healthy children and adolescents tend to have high scores on one or all of these sub-themes, as well as on additional characteristics and outcomes that may be increased by one or more of these factors, such as scholastic competence and social skills [5]. The focus of research based on positive psychology has been the

examination of increasing the presence of well- being using the theoretical perspective and ideas related to authenticity, happiness, and positive affect. Indeed, positive psychology is concerned with the scientific study of what makes life most worth living. In contrast, there has been a recent increase in resilience-based interventions in schools, due to the finding that resilient children are not only better adjusted but also have more optimal well-being than their non-resilient peers [6].

AIM

To assess the psychological well-being of children and adolescents, socio demographic distribution data of parents and children and to find out the relationship between psychological well-being of children and adolescents and their socio demographic characteristics.

MATERIALS AND METHODS

A descriptive cross-sectional study to assess the psychological well-being of children and adolescents in AI-Nasirihay schools, the study period was extended from (December 1, 2023 to April 1, 2024).

STUDY INSTRUMENT

A questionnaire was developed by the researcher through review of related literature. Thequestionnaire consists of four main parts for includes the following:

Part (I): Questionnaire Related to the Demographic Characteristics of the child

This part is concerned with the collection of basic demographic Data was obtained from the Child'sparents, consisting of the following items; Age, gender, residence, child's failure in school and typeof study.

Part (II): Questionnaire Related to the Demographic Characteristics of the parents this part is concerned with the collection of basic demographic data for parents, consisting of the following items; Educational level, occupation, economic status, Kinship link parents.

Part (III): Questionnaire Related to The psychological state of the child This part was constructed to assess Children's psychological well-being, It consisted of (25) a question.

Part (IV): Questionnaire Related to The difficulties the child faces, this part was constructed toassess the difficulties the child faces, It consisted of (4) questions.

STATISTICAL ANALYSIS

The statistical package of social sciences (SPSS) version (26), which included the functions for frequency, percent, arithmetic mean, standard deviation, mean of the score (MS), P-values, df, andx^2, was used to analyze the data.

RESULTS

The result indicate that the mean age is 1.84 ± .702; more than two-fifth age 10-13-years (n = 97;48.5%). Concerning gender, most are males (n = 149; 74.5%) compared to females (n = 51; 25.5%). As per monthly income, most have mild income (n = 145; 72.5%), followed

Table 1. Distribution of Children according to their Sociodemographic Characteristics

No.	Characteristics		f	%
1	Age (year) M ± SD = 1.84 ± .702	6-9 years	67	33.5
		10-13 years	97	48.5
		14-16 years	36	18.0
		Total	200	100.0
2	Sex	Male	149	74.5
		Female	51	25.5
		Total	200	100
3	Socioeconomic status	Good	19	9.5
		Mild	145	72.5
		Poor	36	18.0
		Total	200	100.0
4		Yes	155	77.5
		No	45	22.5
		Total	200	100.0

No= Number, f= Frequency, %= Percentage, M= Mean, SD=Standard Deviation

Table 2. Assessing the Psychological Well-Being of Children and Adolescents

No	Questions	False		Some times		True		M	S.D.	Ass.
		F	%	F	%	F	%			
1	Interested in the feelings of others	48	24.0	83	41.5	69	34.5	2.10	.66	M
2	Unquiet, overactive, cannot sit for a long time	59	29.5	106	53.0	35	17.5	1.88	.76	L
3	Often complains of headache, stomach pain, or nausea	40	20.0	109	54.5	50	25.0	2.05	.75	L
4	Easily shared with other children (candy, toys, pens, etc.)	20	10.0	106	53.0	74	37.0	2.27	.79	H
5	Often screams angrily or is angry	54	27.0	99	49.5	47	23.5	1.96	.72	L
6	Be somewhat isolated and tend to play alone	61	30.5	87	43.5	52	26.0	1.95	.73	L
7	General obedience, usually does what adults ask	24	12.0	99	49.5	77	38.5	2.26	.72	L
8	Many anxiety, often seem anxious	50	25.0	81	40.5	69	34.5	2.09	.68	L
9	Useful if someone is injured, troubled or feeling sick	50	25.0	87	43.5	63	31.5	2.06	.69	H
10	Stirring or swaying continuously	62	31.0	74	37.0	64	32.0	2.01	.72	L
11	Has at least one good friend	31	15.5	73	36.5	96	48.0	2.32	.70	H
12	Often quarrels with or bullies other children	71	35.5	88	44.0	40	20.0	1.84	.76	L
13	Often unhappy, upset, or crying	59	29.5	95	47.5	46	23.0	1.93	.73	L
14	Generally loved by other children	24	12.0	81	40.5	95	47.5	2.35	.66	H
15	Disperses easily, concentrates tense	44	22.0	103	51.5	53	26.5	2.04	.75	L
16	Nervous or attached to others in new situations,easily losing confidence	56	28.0	95	47.5	49	24.5	1.96	.56	M
17	Cute with younger children	26	13.0	72	36.0	102	51.0	2.38	.75	H
18	Often lying or cheating	106	53.0	58	29.0	36	18.0	1.65	.66	L
19	Targeted or bullied by other children	63	31.5	92	46.0	45	22.5	1.91	.65	L
20	Often volunteer to help others (parents, dependents)	26	13.0	99	49.5	75	37.5	2.24	.44	M
21	Think about things before acting	56	28.0	87	43.5	57	28.5	2.00	.66	M
22	Steals from home, school or other places	152	76.0	37	18.5	11	5.5	1.29	.76	L
23	Deals better with adults than with other children	45	22.5	84	42.0	71	35.5	2.13	.75	L
24	Many fear, easily terrified	40	20.0	113	56.5	47	23.5	2.03	.79	L
25	Gets things done to the end, good attention	19	9.5	90	45.0	90	45.0	2.36	.72	H

by those whoare poor income (n = 36; 18%), those who are good income (n = 19; 9.5%) (Table 1).

The study results reveals that most question answered with low levels (Table 2).

The study results reveals there is statistically no significant relationship between participants' ageand Psychological Well-being in Children and Adolescents at (*r*=-.075) (Table 3).

The study results reveal that there is no statistically significant difference between psychological well-being and their Gender at (p=.045) (Table 4).

The study results reveal that there is statistically significant difference in psychological well-being between history of school fail at (p =.000) when participant with history of fail most affected main=(52%) (Table 5).

The study results display that there is no statistically significant difference in psychological well-being among socioeconomic status groups (p-value = .188) (Table 6).

DISCUSSION

This finding suggests that, on average, the participants reported lower levels of psychological well- being. For children and adolescents, low psychological well-being can have a number of effects, including a higher chance of mental health conditions including anxiety and depression [7]. It's important to consider the factors contributing to these low levels, such as family dynamics, socioeconomic status, and school experiences, as they can provide insights into potential interventions to improve psychological well-being in this population. Age may not be a reliable indicator of psychological well-being in this population, as evidenced by the lack of a significant correlation between age and psychological well-being (r = -0.075). This finding contradicts some previous research indicating that there is a strong relationship between psychological well-being and age [8]. However, it's important to note that other factors, such as individ-

Table 3. Correlations among participants' age and Psychological Well-being

Correlations			
		Age	**Overall**
Age	Pearson Correlation	1	-.075
	Sig. (2-tailed)		.290
	N	200	200

Table 4. Gender differences in Psychological Well-being

Independent Samples Test										
		Levene's Test for Equality of Variances		**t-test for Equality of Means**						
		F	**Sig.**	**t**	**df**	**Sig. (2-tailed)**	**Mean Difference**	**Std. Error Difference**	**95% Confidence Interval of the Difference**	
									Lower	**Upper**
Psychological Well- being	Equal variances assumed	3.245	.073	2.021	198	.045	3.245	.073	2.021	198
	Equal variances not assumed			2.195	101.600	.030			2.195	101.600

df: Degree of freedom; F: F-Statistics; Sig.: Significance; Std. Error Difference: Standard Error Difference.

Table 5. School history differences in Psychological Well-being

Independent Samples Test										
		Levene's Test for Equality of Variances		**t-test for Equality of Means**						
		F	**Sig.**	**t**	**Df**	**Sig. (2-tailed)**	**Mean Difference**	**Std. Error Difference**	**95% Confidence Interval of the Difference**	
									Lower	**Upper**
Dose fail in school	Equal variances assumed	.071	.790	3.726	198	.000	2.87240	.77087	.071	.790
	Equal variances not assumed			3.618	68.628	.001	2.87240	.79389		

df: Degree of freedom; F: F-Statistics; Sig.: Significance; Std. Error Difference: Standard Error Difference.

ual differences and environmental influences, may play a more significant role in shaping psychological well-being in children and adolescents. History of School Failure and Psychological Well-being: The statistically significant difference in psychological well-being between participants with a history of school failure ($p = 0.000$) suggests that this factor may strongly influence psychological well-being in children and adolescents. These results are consistent with study by [9] that found there is a negative association between the psychological well-being and academic achievement finding that participants with a history of school failure are most affected 52% underscores the importance of addressing academic struggles and providing support to improve psychological well-being in this group. The fact that there was no statistically significant difference in psychological well-being between the socioeconomic status groups ($p = 0.188$) implies that psychological well-being may not be strongly predicted by socioeconomic position. This result is at odds with some other research that revealed that children and adolescents with lower socioeconomic status have worse psychological outcomes [10] (Reiss, 2013).

Table 6. Differences in Psychological Well-being among socioeconomic status groups

		ANOVA				
		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Psychological Well-being	Between Groups	105.643	3	35.214	1.611	.188
	Within Groups	4285.512	196	21.865		
	Total	4391.155	199			

df: Degree of freedom; F: F-Statistics; Sig.: Significance

CONCLUSIONS

The study provides valuable insights into the factors influencing the psychological well-being of children and adolescents. While age, socioeconomic status, parental education, and parental employment are important considerations, the findings suggest that their impact on psychological well-being can vary. Factors such as school history and academic experiences, particularly school failure, appear to significantly affect psychological well-being. Additionally, mother's education level and father's job type were found to be associated with psychological well-being, highlighting the complex interplay of various factors in shaping children's mental health.

RECOMMENDATIONS

Provide educational support for parents, especially mothers with lower education, offer support for low-income families to access mental health services and Schools should offer mental health support services for students.

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CONFLICT OF INTEREST

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ORIGINAL ARTICLE

CONTENTS

Association of program cell death ligand-1 (rs4143815 G>C) with some clinical symptoms and oral ulcer types in systemic lupus erythematosus

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ABSTRACT

Aim: The study aims to detect Association of Program Cell Death Ligand-1(PD-L1) rs4143815 G>C with some Clinical Symptoms and Oral Ulcer Types in Systemic Lupus Erythematosus (SLE).

Materials and Methods: A case control design was suggested in the study enrolled about 60 SLE patients and 20 healthy individuals as a control group, PD-L1 variation implemented using allele specific PCR.

Results: The findings showed significant changes in age ($p<0.011$), sex ($p<0.000$), and non-sig in BMI ($p<0.654$). in SLE group about 23.3% of patients have a family history, 93.7% suffered from fever, 51.66% have a Butterfly-shaped rash, 73.3% suffered from oral ulcer, single oral ulcer was observed 65.90% and multiple ulcer observed 34.09%. Oral mouth was more frequent (55%) while pharynx ulcer was low frequent (3.3%). The rs4143815 G>C showed three genotypes (GC, CC and GG), Significant association of CC with SLE patients (OR 67.8874, $p <0.003$), A non-sig association was observed in GG with SLE (OR 5.8571, $p <0.395$), significant association of C allele with SLE group (OR 1.3529, $p< 0.0008$). The PD-L1 rs4143815 (G>C) genotyping distribution according to some clinical symptoms showed a non-significant association with all symptoms. The PD-L1 rs4143815 (G>C) genotyping distribution according to oral ulcer sites showed a non-significant association also.

Conclusions: The present results concluded closed correlation between rs4143815 G>C and SLE patients, but no association was observed with some clinical symptoms and oral ulcer types.

KEY WORDS: Program Cell Death Ligand-1, (rs4143815 G>C), Clinical Symptoms, Oral Ulcer Types, SLE

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INTRODUCTION

One of the most diseases which increased in the last years in Iraq is SLE, this disease is an autoimmune disorders induced by a complex etiology, the interplay between genetic and environmental factors lead to clinically heterogeneous presentation [1]. The Immune regulatory molecules like programmed death receptor (PD-1) and ligands (PD-L1, PD-L2) are contributed in SLE development [2]. The Antibodies Targeting PD-1 receptors that block its stimulation is used to treat some cancers, Perhaps lead to the development of immune-associated inverse events that clinically appeared with symptoms same of autoimmune disorders such as SLE [1,3]. The PD-1 axis complexities are presented by the PD-1and PD-L1 expression of on lymphoid and myeloid subsets on the microenvironment of non- immune and immune cells. The role of PD-1 and its ligands in the immune response is well documented, it has been found that produced negative signals from them lead to attenuate and terminate immune response [4]. in an antigen presenting cell, the most interaction explained contributed the CD4 T cell PD-1 with both ligands 1 or 2, the Ligation of these receptors stimulates immunoreceptor tyrosine-based inhibitory motif (ITIM) activation in the PD-1 cytoplasmic tail that prevent stimulation sequences contained in the immunological synapse [5], moreover, PD-1 is detected on B cells and myeloid cells [6-8], while the ligands are detected on some cells such as lymphocytes, neutrophils tumors, epithelial, endothelial and stromal cells [9-14]. PD-L1 binds CD80 via antigen presenting cell and linked to CD4 T to reduce cell activation [4, 10]. Therefore PD-1 or PD-L1 signal regulates the adaptive immune response, the cell signals Dysregulated in SLE may discriminate

mechanisms contributed in the controlling of PD-1 response. Oral ulcer in SLE is one of the 4 criteria for the SLE diagnosis, many types of mouth ulcer were elucidated, ranging between common aphthous and injury, it may be a side impacts of SLE medications or rheumatoid arthritis [15-16]. Several ulcers are erupt on the hard palate and painless. In spite of rare, discoid lupus may develop in a child and appeared with ulcers on the soft palate, tongue and on the lining of the lips and cheeks (buccal mucosa), SLE often presents oral alteration in early stages of development [17]. The present study was aims to Association of program cell death ligand-1 with some clinical symptoms and oral ulcer types of SLE.

AIM

The study aims to detect Association of Program Cell Death Ligand-1(PD-L1) rs4143815 G>C with some Clinical Symptoms and Oral Ulcer Types in Systemic Lupus Erythematosus (SLE).

MATERIALS AND METHODS

STUDY SUBJECTS

A case control study was conducted to identify program cell death ligand-1 (PD-L1) rs4143815 G>C Association with some clinical symptoms and oral ulcer types of SLE A 60 SLE patients were enrolled in the present study which attended the chronic disease clinic of the Marjan hospital city; all cases were diagnosed by prof. Dr. Ali Al-kazaz /college of medicine, university of Babylon, and 20 healthy individuals were enrolled as a control group.

SAMPLE COLLECTION

Blood and data sample were collected from study subjects according to ethical approval of environment and health ministry in Iraq, and written consents from each contributor. Blood sample were collected then transferred to DNA Lab, College of Science, University of Babylon, for DNA extraction and (PD-L1) rs4143815 G>C genotyping identification, using the following primers:

FO: CTGTGACAGGGAGAAAGGATACTTCTG;
RO: AGCAAGTTTAGTTTGGCGACAAAATTGT;
FI: TTTGCCTCCACTCAATGCCTCAATATC;
RI: AACACTGAGACTCTCAGTCATGCAGAATAC [18] to produce Allele G=176 bp Allele C=203 bp, Control band = 322 bp, via 30 cycles 95, 58, and 72^{o}C for 30, 40 and 40 sec respectively, with final extension 72 ^{o}C for 10 min.

DATA VISUALIZATION AND ANALYSIS

The PCR products were visualized by electrophoresis pattern (1% agaros, 0.5 TBE, 100 V for 1 hour), 100-1000bp DNA ladder, data represented as mean ±SE for age and BMI, percentage% for genotyping, statistical analysis was used independent t test, and odd ratio (CI%) at p value < 0.05.

RESULTS

The present work was elucidated to evaluate association of PD-L1 (rs4143815 G>C) genotyping with some clinical symptoms and oral ulcer types in SLE patients, the study characteristics distribution exhibited significant differences between SLE and control group in age ($p<0.011$) and sex that showed the female percentage was higher than male significantly ($p<0.000$), non-sig in BMI differences between both groups ($p>0.654$). About 23.3% of patients have a family history, 93.7% suffered from fever, 51.66% have a Butterfly-shaped rash, 73.3% suffered from oral ulcer, single oral ulcer was observed in 65.90% and multiple ulcer observed in 34.09%, Table 1.

The oral ulcers were classified according to the ulcer sites, five types were detected (mouth, lips, tongue, gum and pharynx) oral mouth was more frequent 55% while pharynx ulcer was low frequent 3.3%, Fig. 1.

The DNA was extracted from whole blood and PD-L1 (rs4143815 G>C) was detected using allele specific PCR, the result was clarified in electrophoresis pattern Fig. 2, three genotypes were found (GC, CC and GG). A Significant association of CC with SLE patients (OR 67.8874, $p<0.003$), a non-sig association was observed in GG with SLE (OR 5.8571, $p>0.395$), significant association of C allele with SLE group (OR 1.3529, $p<0.0008$), Table 2.

The PD-L1 rs4143815 (G>C) genotyping distribution according to some clinical symptoms were elucidated in SLE patients, non-significant association were observed in all symptoms, Table 3.

The PD-L1 rs4143815 (G>C) genotyping distribution according to oral ulcer sites showed non-significant association also, GC more frequent in all types ($p>0.725$) (Table 4).

DISCUSSION

The current output proved significant role of PD-L1 rs4143815 G>C in SLE patients but non association with clinical symptoms, the SLE prevalence is increased in last years in Iraq, particularly among women which have higher percentage than men in present study, this agree with a study in United States, found the SLE incidence and prevalence were reached to 5.5 and 73 per

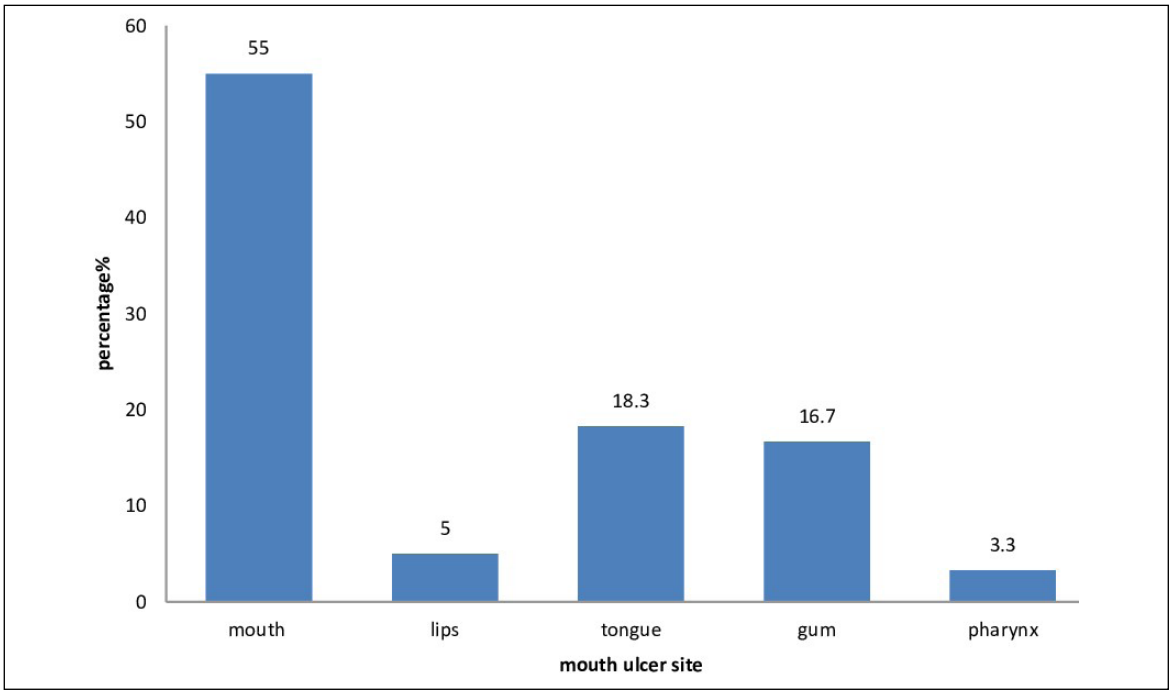

Fig. 1. The percentage of oral ulcer types of SLE patients.

Table 1. Characteristics distribution and some clinical symptoms of SLE group

Study variables	SLE group	Control group	p
Age (year)	35.80±1.453	28.97±1.43	0.011
BMI(kg\m2)	28.00±0.968	27.30±0.86	0.654
Duration (year)	7.00±0.841	-	-
Sex Male female	 5% 95%	 57.89% 42.10%	0.000
Married Yes no	 75% 25%	 47.36% 52.63%	0.000
Family history Yes no	 23 % 77%	-	
Fever Yes no	 93.7% 6.7%	-	
Butterfly-shaped rash Yes no	 52% 48%	-	
Oral ulcer Yes no	 73.3% 26.7%	-	
Number of oral ulcer Single Multiple	 65.90% 34.09%	-	

100,000 people respectively [19]. SLE predominantly impacts women of child-bearing age, with high percentage in African ancestry individuals [19-20]. The role of the PD1 and PDL-1in SLE has complex mechanisms, Regarding to PD-L1 that significant association in the present study, the PD-1^{+}Tfh cells number is increased with severity and progressing of SLE which regulated by PD-L1 ligation on B cell [21-23]. That may be prevented tyrosine phosphorylation of effector molecules as well as SYK, SHP-2, beneficially stopped signaling of B cell

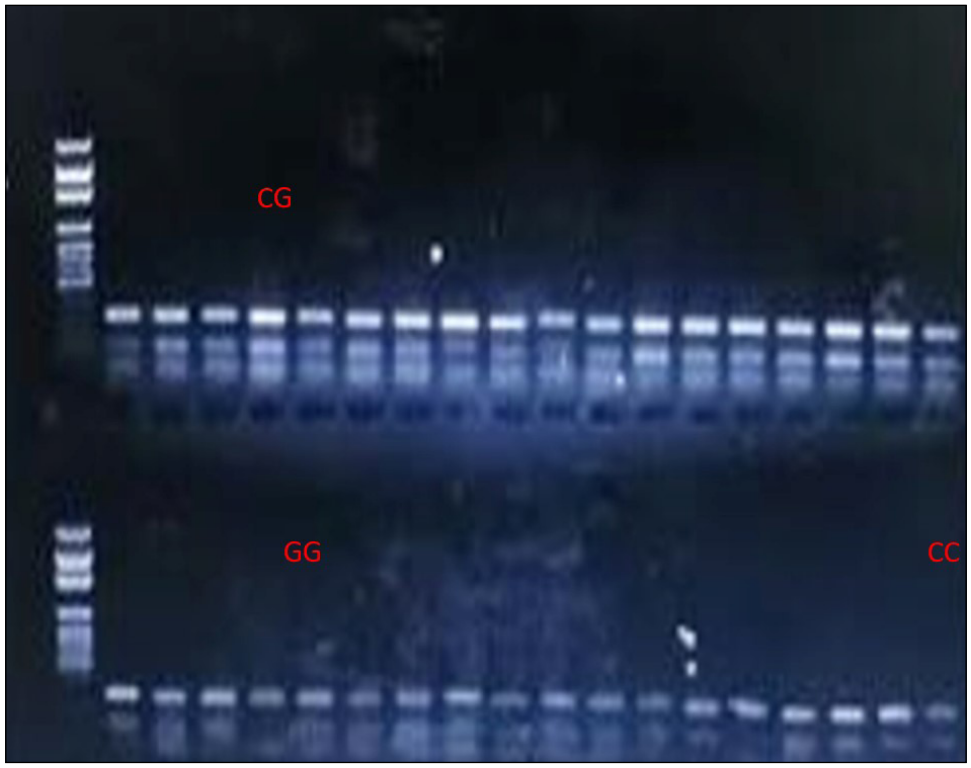

Fig. 2. Electrophoresis patterns of PD-L1 rs4143815 (G>C) genotyping in study groups (CC, GC and GG), DNA ladder 100 bp.

Table 2. PD-L1 rs4143815 (G>C) genotyping and allele frequency distribution in study groups (p<0.05)

rs4143815 (G>C) genotyping	SLE patients	Control group	Odd ratio	p
GC	(75%)	(100%)	67.8874 4.0677 to 1133.0016	0.0033*
CC	(20%)	0	5.8571 0.0990 to 346.6356	0.3959
GG	(5%)	0		
C	0.575	0.5	1.3529 1.1342 to 1.6138	0.0008*
G	0.425	0.5		

Table 3. The PD-L1 rs4143815 (G>C) genotyping distribution according to some clinical symptoms (p less than 0.05)

SEL symptoms	GC(%)	CC(%)	GG(%)	X^2	p
Fever					
Yes	42(70)	11(18.33)	3(5)	0.267	0.874
no	3(5)	1(1.66)	0		
Butterfly-shaped rash					
Yes	22(36.66)	8(13.33)	1(1.66)		
no	23(38.33)	4(6.66)	2(3.33)	1.624	0.444
Sex					
Male	3(5)	0	0	1.052	0.5908
female	42(70)	12(20)	3(5)		
Family history					
Yes	9(15)	4(6.66)	1(1.66)	1.118	0.5718
no	36(60)	8(13.33)	2(3.33)		
Oral ulcer					
Yes	31(51.66)	9(15)	3(5)	1.422	0.491
no	14(23.33)	3(5)	0		
Number of oral ulcer					
Single	24(40)	5(8.33)	2(3.33)	0.8008	0.670
Multiple	21(35)	7(11.66)	1(1.66)		

Table 4. PD-L1 rs4143815 (G>C) genotyping distribution according to oral ulcer sites

Oral ulcer sites	GC(%)	CC(%)	GG(%)	X^2	p
Mouth	22(44)	8(16)	3(6)	3.6363	0.7257
lips	3(6)	0	0		
tongue	8(16)	1(2)	2(4)		
Pharynx	2(4)	1(2)	0		

receptor [6], The B cells expansion in SLE, perhaps supposed that PD-1 in B cell is not beneficially produced or ligated in SLE in spite of elevation in transcript levels [24-25], The significant PD-1 production lacking on SLE patient CD8 T cells is detected depletion in regulatory cell signals for this cell type [26]. The macrophage in cancer, the expression of PD-1 is a negative association with their phagocytic potency, while the macrophages in SLE also produced PD-1 as a biomarker of reduction capability to clear apoptotic cells [7]. The PD-L1 gene (ID: 29,126) encoded type 1 transmembrane protein 40 kDa, which carried on at the chromosome 9p24.2, the 3'-UTR of mRNA is most vital regions in post-transcriptional gene expression regulation concerning to microRNAs targeting, that mediates repression of gene translational. The PD-L1 3'-UTR Structural variations, as well as duplications, deletions and translocations, result to PD-L1 production disturbing in numerous disease like tumors and autoimmune disease [27-29]. The rs4143815 G>C is a common SNP in PD-L1 gene which binding site of miR-570 and causes up-regulation production of PD-L1 by attenuated the miRNA mediated mRNA degeneration [30]. Oral ulcers in SLE have been reported in almost studies, in present study the high percentage of oral ulcer was reported but there was no association with PD-L1 in addition to types of oral ulcer, Zakeri et al., [31] found an oral ulcer in 61.4% of the study cases, and the most reported type was oral aphthous ulcers, erosion, hyperkeratosis and pigmentation, that observed on the hard palate, soft palate and the lower lip vermilion. However, in spite of the importance of the oral manifestations perception in the SLE prognosis, there are still not enough reports about them.

CONCLUSIONS

The present results concluded closed correlation between rs4143815 G>C and SLE patients, but no association was observed with some clinical symptoms and oral ulcer types. Further investigation should be implemented to evaluate the role of PDL-1 in the SLE symptoms.

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CONFLICT OF INTEREST

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

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ORIGINAL ARTICLE

CONTENTS

Clinical characteristics of temporomandibular disorders in a sample of Iraqi patients

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ABSTRACT

Aim: To evaluate the symptoms and indicators that people with TMD experience and encourage them to seek treatment at an academic oral medicine clinic.
Materials and Methods: The study includes temporomandibular joint patients who visited the oral medicine clinic at the University of Baghdad's College of Dentistry between October 2021 and May 2022. The information was gathered from temporomandibular joint patients' medical records.
Results: In the research herein, 323 patients in total were included, females 234(72.4%) and 89(27.6%) males, the patient's mean age was (31.38 ± 14.085), and there was no discernible gender difference in mean age. In this study, the most prevalent temporomandibular joint signs and symptoms were temporomandibular joint clicking 73.6% with no significant difference between females and males. In comparison, dislocation is the least prevalent and the most prevalent muscle spasm was the masseter muscle with a percentage of 50.4%) while the sternocleidomastoid muscle is the least one.
Conclusions: The research results demonstrated a significant prevalence of temporomandibular joint disorder and the necessity of preventing it to improve the overall well-being of people in general.

KEY WORDS: Temporomandibular joint pain, muscle spasm, clicking

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INTRODUCTION

The term "temporomandibular joint" (TMJ) originates from the bilateral synovial dynamic articulation act between the condylar process of the mandible below and the squamous region of the temporal bone of the skull above. The articular disc is situated in the space between these two bones. This joint is distinct because of its form, structural features, and bilateral joint that works as a single unit [1]. Temporomandibular joint disorder (TMD) has been recognized as a common orofacial pain. In 1983, the American Dental Association (ADA) first used the abbreviation "TMD" to describe a group of conditions marked by symptoms such as discomfort in the mastication muscles, preauricular area, or temporomandibular joint (TMJ); the sound produced by the TMJ when the jaw is functioning (audible); Abnormal or limited range of jaw movement [2]. There are no international standards, there are disparities in the types and quality of evaluation techniques used to estimate and report TMD, and there have been several published epidemiological studies on the prevalence of TMD in young people from different demographic groups, the prevalence of TMD ranging from 9.8% to 80% [3]. TMD's multifaceted etiology is presently understood to include systemic, genetic, parafunctional habits, trauma, mental stress, and occlusal variables. However, none of these criteria has consistently shown to outweigh the others [4]. The most common musculoskeletal disorders that lead to physical discomfort and impairment are TMDs [5].

TMD seriously affects the patient's quality of life, affecting work activities, studies, sleep, appetite, and eating habits. Additionally, the condition may worsen over time [6]. To enable medical professionals to collaborate and get a better awareness of the disease's prevalence in the general community, a deeper comprehension of TMD's symptoms and indicators is required [7]. The purpose of this study was to find out exactly how prevalent TMD symptoms were among a sample of Iraqi patients who consulted the oral medicine clinic at the University of Baghdad's College of Dentistry.

AIM

To evaluate the symptoms and indicators that people with TMD experience and encourage them to seek treatment at an academic oral medicine clinic.

Table 1. Comparison of the patients' mean ages

	Female	Male	P-value
Mean age	30.848 ± 14.146	32.842 ± 13.212	P> o.o5

MATERIALS AND METHODS

This study included TMJ patients who visited the oral medicine clinic at the University of Baghdad's College of Dentistry between October 2021 and May 2022. The information was gathered from TMJ sufferers' medical documents which included: patients' demography, self-reported complaints, the period from the onset of TMD symptoms to the visit, clinical signs/symptoms (TMJ pain, clicking, trismus, bruxism, dislocation, deviation and headache) seen at first presentation of patients. The patient's history, significant tooth wear, the presence of discomfort or tenderness in the muscles upon awakening, the patient's claims of teeth grinding while they sleep, and the patient's perceptions of themselves of stiffness during the day are all used to make the diagnosis of bruxism. Muscles of mastication were examined and the affected muscle with spasm was recorded. Version 18.0 of the SPSS program (SPSS Ins, Chicago, IL) will be used for statistical analysis. The independent T-test and the Chi square test were applied to express the results, which are given as mean SD and percentage. Statistical significance was defined as a p-value of less than 0.05.

RESULTS

There were 323 patients in total in this retrospective analysis, and their mean age was (31.38 ± 14.085), (Table 1) illustrates that there was no statistically significant variation in the mean age by gender, with 234(72.4%) females and 89(27.6%) men.

Temporomandibular joint (TMJ) clicking was the most common indication and symptom of TMJ dysfunction 73.6%, followed by TMJ pain 71.8%, Headache 59.4%, Bruxism 51.3%, Deviation 29.1%, Trismus 22.6% and the least one is Dislocation 12.3% however, no significant difference between females and males, while there was significant difference with deviation and highly significant difference with headache between females and males as shown in (Table 2).

The most prevalence of muscles spasm was the masseter muscle with percentage 50.4% followed by both temporalis and medial pterygoid with percentage 35.6%, Lateral pterygoid with percentage 47.3% and the least one was Sternocleidomastoid muscle with percentage 12.6%. There was no significant difference between females and males, while there was a significant difference with only temporalis muscle as shown in (Table 3).

DISCUSSION

This study's results display that a larger percentage of patients were female is consistent with earlier researches [8-12]. The possibility that hormonal and social variables have a role in this phenomenon might support this. With regard to gender, female hormones would be crucial in the development of TMD in women, which could be responsible for the peak of dysfunctions at the fertile age. Another reason why women are more common in the current study is because women may seek treatment more frequently than men, which might account for their higher proportion [13-15]. The results indicated that there were no statistically significant variations between men and females concerning the higher prevalence of TMD affected people at mean age (31.38 ± 14.085), this agrees with another study that found no difference found in gender regarding the age [16]. The most impacted age range, taking into account both genders, may be connected to the person's reproductive phase, thus maybe the hormone aspect is important in this regard [17]. The most common TMD symptom in the current research was clicking, with no gender difference; this is consistent with previous studies [18] that discovered that when their temporomandibular joint is moving, patients either complain of discomfort or pops, clicks, or other sounds [19-20]. Many epidemiological investigations have been demonstrated that variety of subjective symptoms and physical indications of mandibular dysfunction are frequently present, in random populations, there has only been a very tiny sex difference documented. The headache and deviation is more in female this is agree with other studies [21-22], this study has been showed that the most prevalence muscle spasm involved was masseter followed by both temporalis and medial pterygoid muscles and this is consistent with recent research showing a strong link between oral parafunctions and discomfort in the masticatory muscles. The sensation of discomfort or pain in the masticatory muscles increased when parafunctional behaviors were present [23-24]. It has been reported that approximately 50% of all TMDs are myogenic in origin; the masticatory muscles' myofascial discomfort is more usually brought on by stress [25]. Stress, anger, and disappointment are examples of emotional factors that can lead to oral habits like bruxism, which increases the activity of the masticatory muscles, particularly the masseter and temporal

Table 2. TMJ signs and symptoms

TMJ symptoms	Female n (%)	Male n (%)	Total n (%)	P value
TMJ pain	182 77.7%	50 56.17%	232 71.8%	0.031*
Clicking	168 71.7%	70 78.6%	238 73.6%	0.73
Trismus	49 20.9%	24 26.9%	73 22.6%	0.829
Bruxism	116 49.5%	50 56.1%	166 51.3%	0.758
Dislocation	22 9.4%	18 20.2%	40 12.3%	0.979
Deviation	80 34.1%	14 15.7%	94 29.1%	0.00296*
Headache	170 72.6%	22 24.7%	192 59.4%	0.0003**

*P < 0.05
**P < 0.001

Table 3. Muscles spasm

Muscles spasm	Female n (%)	Male n (%)	Total n (%)	P-value
Lateral pterygoid	112 47.8%	41 46.06%	153 47.3%	0.455
Medial pterygoid	90 38.46%	25 28.08%	115 35.6%	0.108
Masseter	120 51.28%	43 48.31%	163 50.4%	0.407
Temporalis	95 40.5%	20 22.4%	115 35.6%	0.009*
Sternocleidomastoid	29 12.3%	12 13.4%	41 12.6%	0.636

*P < 0.05

muscles [26]. The muscle which controls the position of the head while chewing, the sternocleidomastoid, had the lowest prevalence. Patients with asymmetry in the occlusion may also have an imbalance in the muscle's activity [27].

CONCLUSIONS

In this study the most prevalence TMJ signs and symptoms was TMJ clicking 73.6% with no significant difference between females and males while dislocation is the least prevalence and the most prevalence of muscle spasm was the masseter muscle with percentage 50.4%, while sternocleidomastoid muscle is the least one. The results revealed a significant frequency of TMD among population and the significance of preventing it in order to improve the overall well-being of people in general.

ABBREVIATIONS

TMD: Temporomandibular joint disorder
ADA: American Dental Association
TMJ: Temporomandibular joint

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

The impact of estrogen receptor β gene polymorphisms on the atherogenic index and coronary artery disease in Iraqi population

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ABSTRACT

Aim: To study the impact of the estrogen receptor beta polymorphisms rs4986938 (AluI) and rs1256049 (RsaI) on ischemic heart disease as well as the atherogenic index in Iraqi population.

Materials and Methods: A case-control research study was conducted on a sample of Coronary artery disease patients in addition to a group of normal individuals. Serum lipid concentrations were measured in the participants, and the ER gene (rs1256049, rs4986938) was genotyped by PCR- RFLP.

Results: Atherogenic serum lipid concentrations increased significantly in Coronary artery disease patients relative to the control group. Genotyping of the estrogen receptor β gene forrs4986938 SNP revealed a significant (OR=2.79, P=0.000) elevation of the GA genotype carriers in Coronary artery disease versus the control groups. The genotype analysis of thers1256049 SNP failed to exhibit a significant variation. Atherogenic index and serum lipid concentration markedly elevated with these polymorphisms.

Conclusions: The polymorphisms of ESRβ rs4986938, (not for rs1256049) was associated with risk of Coronary artery disease and implicated in changes of serum lipid concentrations and atherogenic index in Iraqi population. Additional studies of ESRβ genetic variation and risk of CVD are warranted.

KEY WORDS: estrogen receptor beta, coronary artery disease, atherogenic index, ischemic heart disease, Polymorphisms

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INTRODUCTION

Coronary artery disease (CAD) is the main cause of morbidity and mortality in the developed world and developing cities [1-2]. It is responsible for about 1 in every 3 deaths in people over the age of 35. Iraq ranks 20 in the world at age-adjusted death rates [3]. Cardiovascular disease is the first cause of disease-related death in Iraq [4]. CHD rate in our country is alarming and is mostly linked to the grouping of cardiovascular risk factors [5]. The primary cause of CAD is atherosclerosis that starts with coronary arterial streaks of fatty tissue lesions that restrict the coronary arteries or its branches impeding myocardial perfusion [6-7]. Coronary artery disease is a disease that has multi-factorial features, exaggerated by both environmental and genetic factors. It corresponds with a polygenic; many genes with different alleles that may have mild to moderate effects [8]. Studies highlighted the association of numerous gene polymorphisms with CHD [9-10]. The ER βgene, which is present in the layers of the vessel wall in the vasculature, is one of the genes suspected of being implicated in the cause of CHD [11]. ERβ is a protein of 530 amino acids [12]. ERβ is known to be expressed in both endothelial and VSMC of arteries [13]. The gene of ERβ is located in the 14q23.2-q23.3. It comprises 17 exons and 16 introns [14]. There are two polymorphisms in ERβ the geners1256049, rs4986938; they are located in exon 5, 8 respectively [15]. The first, rs125649G>A, is a silent polymorphism that replaces guanine with adenine [16], the second, rs4986938 G>A (replaces guanine with adenine) in non-coding area of 3 untranslated region of exon8 in the ERβ gene. Little attention was focused on estrogen receptor β polymorphism in CAD. Little emphasis was paid to estrogen receptorβ polymorphism. Few studies have found that these SNPs are linked to CAD in particular populations [17-18]; however, the association of ERβ gene polymorphisms with CAD has not been examined in Iraq. The current investigation was carried out to confirm the effect of estrogen receptor polymorphisms in the gene on the atherogenic indexes and coronary arteries in the Iraqi population.

MATERIALS AND METHODS

STUDY INDIVIDUALS

A case-control research study was conducted on a group of CVD patients as well as a group of individuals who were healthy. Serum lipid concentrations were measured in the participants, and the ER gene (rs1256049, rs4986938) was genotyped. Participants completed an extensive questionnaire that included age, gender, past family medical conditions, and other pertinent information. BMI values were calculated using weight and height measurements. It is worth noting that the Al-Najaf Centre for vascular surgery and catheterization of the cardiovascular system is a national institution that receives patients from all across Iraq, including the northern region, middle, and south. As a result, the current demographic sample is representative of the Iraqi population. All subjects provided informed consent. From March to August 2022, the biochemical and genetic studies were carried out in the laboratory of the Department of Biochemistry, the College of Medicine in Kufa, Iraq. Ethical Committee from the Kufa faculty of medicine authorized the study.

PATIENTS GROUP

It included 150 CAD patients who had undergone coronary surgery and performed cardiac catheterization at the Al-Najaf center. The patient's age was 52.06 ± 8.88 (Mean ± SD) years. Specialist physicians diagnosed the patients. Patients with unstable angina or myocardial infarction (MI) were eligible if their angiography indicated 70% or more obstruction of the coronary arteries or its branches, and their ages ranged from 30 to 75 years. The criteria for being excluded were as follows: Use of contraceptive pills and hormone or replacement treatment in the month preceding the study, family history of hypercholesterolemia, cancer, connective tissue disease and Diabetes mellitus patient.

CONTROL GROUP

It was made up of 150 healthy people. They were chosen from the general community who came to the hospital for a routine check; the ages were 53.25±8.83 years. The following were the inclusion criteria:

- no past medical history of CHD,
- no history in the family of CHD,
- no past medical history of DM,
- matched to patients with regard to age, sex, and geographical distribution.

BIOCHEMICAL MEASUREMENTS

Fasting serum lipid (cholesterol, triglycerides, and HDL-C) concentrations were measured. They were measured in accordance with the manufacturer's specifications. VLDL-C and LDL-C were calculated in an indirect manner. The atherogenic index in plasma (AIP) and the triglyceride/HDL-C ratio were also determined.

GENOTYPIC MEASUREMENTS

For DNA analysis, blood samples from CAD patients and control groups were obtained in EDTA-anticoagulant tubes. A small kit (FAVORGENTM Total DNA Extraction) was used to extract the DNA. RFLP was used to genotype the ERβ gene for rs4986938, rs1256049 SNPs using particular restriction enzymes (AluI), (RsaI) respectively and then analyzed by gel electrophoresis.

The primers were chosen as previously reported [19], those in favor of SNP were:

For rs4986938 were:
Forward primer: 5-GTGTGTGGTGGGACACAGAG-3
Reverse primer: 5-AGGCCATTGAGTGTGGAAAC-3
Those for rs1256049 SNP were:
Forward primer: 5-TTCTGAGCCGAGGTCGTAGT-3
Reverse primer: 5-TGAATCCTTGGACCCAACTC-3

The amplification process included an overall volume of 25 µl and was made up of 12.50 µl GoTaq® G2 Green's Masters Mix [28], 0.8 µl each of the primer, enough nuclease-free water to fill the whole volume, and 5 µl of the genomic DNA template. For the rs4986938 G/A, the PCR reaction program was as follows: 5 minutes at 95°C, 35 cycles of 95°C for 50 s, 63°C for 50 s, 72°C for 50 s, and final elongation at 72°C for 5 min. For the rs1256049, the PCR reaction program was as follows: 5 minutes at 95°C, 35 cycles of 95°C for 50 s, 54.5°C for 50 s, 72°C for 50 s, and final elongation at 72°C for 5 min. The rs4986938 polymorphism (which results in a band size of 646 bp for the normal estrogen receptorβ sequences (GG), a total of three distinct bands of 646, 445, and 201bp in a heterozygous one (GA), and two distinct bands of 445 and 201 bp in the homozygous polymorphism (AA) were produced when the PCR products were digested using the AluI restriction enzyme (Fig. 1).

Bands of sizes 582 bp were produced in the normal ERβ sequence (GG) by restriction digestion of the rs1256049 polymorphism with the RSAI enzyme; three distinct bands of 582, 293, and 289 bp were produced in the heterozygous polymorphism (GA); and two distinct band of 293 and 289 bp, respectively, in the homozygous variants (AA) (Fig. 2).

The amplification results were subjected to 1.5% agarose gel electrophoresis analysis.

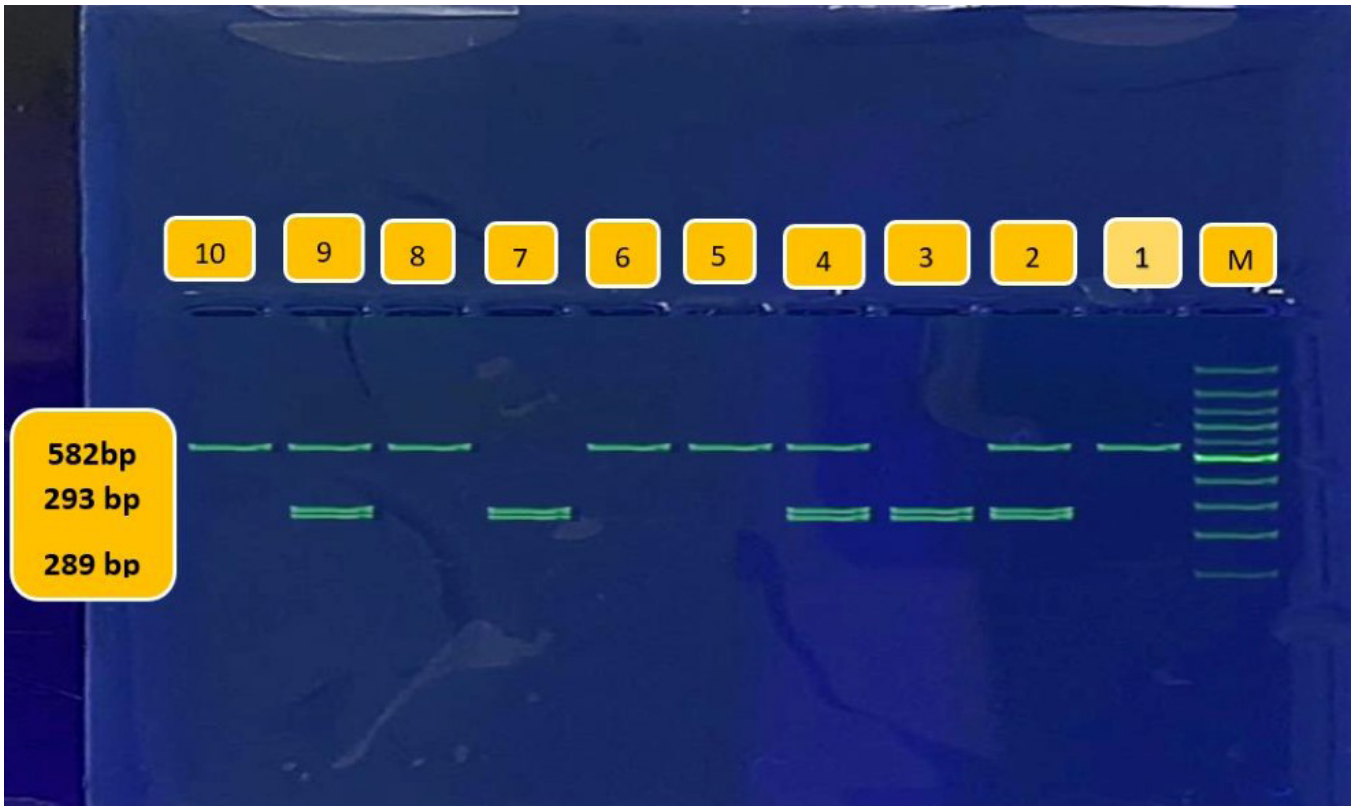

Fig. 1. Result of ERβ gene polymorphism (rs1256049) products on agarose gel electrophoresis following RSAI restriction enzyme digestion, visualization under UV light uses a green stars stain that indicated the presence of the allele. Lane M: ladder of 100 bp. Lanes 2, 4 and 9: (GA) genotype 582, 293 and 289 bp. Lanes 3 and 7: (AA) genotype 293 and 289 bp. Lanes 1, 5, 6, 8 and 10: (GG) genotype 582.

STATISTICAL ANALYSIS

ANOVA and the student t-test were utilized to compare patient's and control groups' continuous data. The Chi-square test was used to determine if there were any significant differences between the CAD and the control groups' genotype distributions and allele frequency.

RESULTS

The research groups' characteristics are listed in Table 1. Anthropometric parameters showed a difference between the CAD and control groups. When comparing the illness group to the control group, there was a substantial increase in atherogenic blood lipid concentrations and a decrease in HDL-c. Furthermore, the patient's group had significantly higher plasma atherogenic index or triglyceride/HDL-C ratios than the healthy ones. When comparing the GA carriers in the CAD group to the control group, the genotyping of thers4986938 G\ASNP in estrogen receptorβ revealed significant (OR =2.79, CI 95%=1.62 - 4.79, P =0.00) increases compared with the controls group (Table 2), for the GA carriers of the CAD group. The genotype analysis of thers1256049 SNP failed to exhibit a significant variation between patient and control group (Table 3). Atherogenic index and serum lipid concentration TC, TG, VLDL-C, LDL-C, HDL-C markedly elevated with the rs4986938 G\A polymorphism and TC, TG, VLDL-C, HDL-C (not for LDL-C) have been elevated with the rs1256049 G\A polymorphism (Tables 4-5).

DISCUSSION

Coronary artery disease (CHD) is a complicated medical condition that can be affected by hereditary and environmental factors [29]. Research on the role of many genes in the development of CHD is ongoing to understand its origins, alleviate treatment approaches, and understand its progression. This research focuses on the association between ERβ gene variation and cardiovascular disease in the Iraqi population, the first of its kind in humans. The study examined the association between variations in the ERβ gene (rs1256049 and rs4986938 SNPs) with CHD in the Iraqi population. These SNPs were chosen due to their known associations with cardiovascular characteristics and frequency-based confirmation of these associations [20-21]. The study found that the distribution of genotypes in the Iraqi population was stable from generation to generation, as the investigated SNPs were in obvious harmony with the HWE in the control group. Significant increases in atherogenic lipids were seen in the

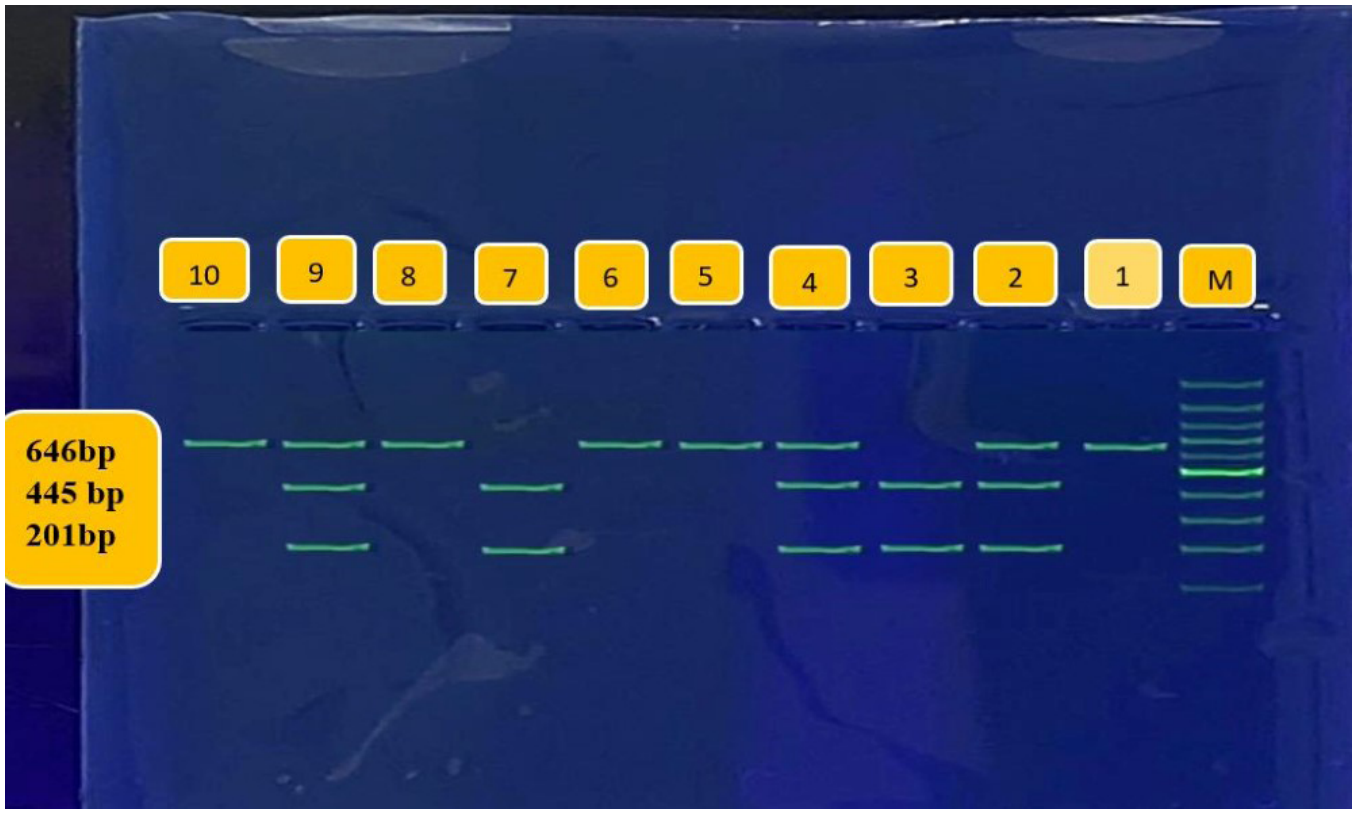

Fig. 2. ERβ polymorphism (rs4986938) results on electrophoresis of agarose gel following ALUI enzyme digestion. Direct detection under UV light using a green star stain that indicated a presence of alleles allowed for confirmation. The ladder of DNA 100 bp in Lane M. Lane1, 5, 6, 8&10: genotype (GG) 646 bp. Lane 2, 4 &9: (AG) genotype 646, 445 and 201bp.Lanes 3 and 7: (AA) genotype 445 and 201bp.

serum of both female and male CHD patients when compared to a control group. Atherogenic dyslipidemia is a major risk factor for the onset of atherosclerosis. LDL-c assessment alone, while looking for cardiovascular risk, would overlook the TG-rich lipoproteins. Our findings corroborate those of others [22-23] in showing that both the atherogenic index of plasma (AIP) and the atherogenic index (AI) are significantly higher in patients than in controls. The study reveals significant changes in remnant cholesterol (RC) and a high risk of coronary heart disease (CHD) in patients with advanced atherosclerosis (CAD). The atherogenic index in plasma (AIP) was higher in CAD patients than in the control group, indicating a greater CAD risk. ER signaling and ER gene polymorphism contribute to CAD etiology, with ERβ being more common in blood vessel tissue [24]. Recent research has shown that estrogen acting through ERβ may have anti-fibrotic effects on the heart [25-26]. Overexpression of the ERβ gene has been linked to improved cardiac function and survival in patients with a history of recent MI [27]. Vasodilation and the increase of eNOS expression are two further possible cardioprotective mechanisms of the ERβ gene in ischemia/reperfusion (I/R) damage, notably in women [28]. Coronary calcification is a hallmark of advanced atherosclerosis, and several studies have shown that ERβ is the most common estrogen receptors in human coronary arteries. Atherosclerosis and calcification progress in patients with elevated Erβexpression, hypertension and other cardiovascular illnesses [29-30], the link between ESR βpolymorphisms and LV structural variations [31-32], and so on. Genotypic analysis of the examined SNPs revealed that the rs1256049 G>A SNP is not associated with increased risk of coronary heart disease (CHD) in three different inheritance patterns: co-dominant, dominant, and recessive. These results suggest that rs1256049 in the ER βgene may have not a role in the onset of CHD in individuals from Iraq. Further investigation into the molecular basis is warranted. The single-nucleotide polymorphism rs 1256049 (or G1082A) are present in exon5, and it may alter ER2 gene expression and protein levels. Studies have linked rs 1256049 to elevated plasma lipoprotein concentrations and coronary artery disease in linkage disequilibrium with other loci [33]. In a case-control study of Chinese Han women, no evidence was found linking the genotypes or haplotypes of these two SNPs to an increased risk of coronary artery disease [34]. However, patients with the AG genotype of rs4986938 had a substantially decreased risk of CAD compared to those with the GG genotype, even among those younger than 40 years of age. The risk of coronary artery disease was greater

Table 1. Anthropometric and biochemical factors levels of CAD and control groups

Parameters	Control		CAD		*P*-Value
	Mean	SD	Mean	SD	
Age (y)	53.25	8.83	52.06	8.88	0.24
BMI (kg/m^2)	28.22	3.07	26.52	2.70	<0.0001
TC(mg/dl)	284.41	102.89	189.61	89.80	<0.0001
TG(mg/dl)	284.86	76.71	160.85	82.89	<0.0001
VLDL-C (mg/dl)	56.97	15.34	32.04	16.69	<0.0001
LDL-C(mg/dl)	193.17	99.14	115.59	88.52	<0.0001
HDL-C(mg/dl)	34.27	13.10	41.42	17.02	<0.0001
TG/HDL-C	0.93	0.27	0.58	0.36	<0.0001
LDL/HDL	6.98	4.80	4.26	4.98	<0.0001
CRI (TC/HDL)	9.95	5.64	6.38	5.94	<0.0001

Table 2. Differences in allele frequencies and genotype results of rs4986938 (AluI) SNP between patients and controls

	Patient (N=150)		Control (N=150)		OR (95%CI)	*P*-value
	No.	[%]	No.	[%]		
GG	90	60.0	121.0	80.7		
GA	54	36.0	26	17.3	2.79 (1.62 - 4.79)	0.000
AA	6	4.0	3	2.0	2.68 (0.65 – 11.04)	0.15

Table 3. Differences in genotype results of rs1256049 (RsaI) SNP between CAD and control groups

	Patient (N=150)		Control (N=150)		OR (95%CI)	*P*-value
	No.	[%]	No.	[%]		
GG	104	69.3	113	75.3		
GA	41	27.3	33	22.0	1.34 (0.79 - 2.29)	0.26
AA	5	3.3	4	2.7	0.86 (0.22 – 3.32)	0.65

Table 4. Results of phenotypic parameters of CAD patients analyzed in relevance to the rs4986938 (AluI) gene

	GG (N=90)		GA (N=54)		AA (N = 6)		*P*-value
	Mean	SD	Mean	SD	Mean	SD	
Age (y)	53.42	9.13	52.67	8.60	55.83	6.62	0.67
BMI (kg/m^2)	27.01	2.71	30.02	2.65	30.17	3.19	0.000
TC (mg/dl)	258.66	86.37	323.81	114.97	316.17	112.62	0.001
TG (mg/dl)	261.32	82.36	320.35	50.00	318.50	56.29	0.000
VLDL-C (mg/dl)	52.26	16.47	64.07	10.00	63.70	11.26	0.000
LDL-C (mg/dl)	167.98	83.87	231.82	109.45	223.30	109.83	0.001
HDL-C (mg/dl)	38.42	14.52	27.93	6.97	29.17	8.40	0.000
TG/HDL-C	0.84	0.30	1.07	0.15	1.05	0.17	0.000
LDL/HDL	5.66	4.42	8.96	4.69	8.65	5.36	0.000
CRI (TC/HDL)	8.33	5.29	12.42	5.27	12.03	6.12	0.000

among AA homozygotes. In another Iranian investigation, rs1256049 SNP showed no such associations, while rs1256049 genotypes were shown to be significantly associated with CAD [35]. Variation in starting conditions is mostly responsible for discrepancies in results. A study of phenotypic data stratified by genotype (GG, AG, and AA) for the rs 1256049 polymorphism found that total cholesterol, very low-density lipoprotein (VLDL), and triglyceride levels were considerably higher in the three genotypes. In this study, has been found that the ER rs4986938 SNP was found to elevate the risk of coronary heart disease in the GA genotype with (OR= 2.79, CI(1.62-4.79), P=0.000). The link between rs4986938 polymorphisms and the risk of cardiovascu-

Table 5. Results of phenotypic parameters of CAD patients analyzed in relevance to the rs1256049 (Rsal) gene

	GG (N=93)		GA (N=50)		AA (N = 7)		P-value
	Mean	SD	Mean	SD	Mean	SD	
Age (y)	53.69	8.91	52.07	8.67	53.60	9.40	0.61
BMI (kg/m2)	27.43	2.97	30.05	2.52	29.60	2.88	0.000
TC (mg/dl)	270.12	98.82	313.83	101.65	340.60	146.50	0.031
TG (mg/dl)	268.47	80.82	323.98	49.12	305.00	60.80	0.000
VLDL-C (mg/dl)	53.69	16.16	64.80	9.82	61.00	12.16	0.000
LDL-C (mg/dl)	179.74	95.80	220.55	97.29	248.00	137.94	0.36
HDL-C (mg/dl)	36.68	14.36	28.49	7.44	31.60	5.37	0.002
TG/HDL-C	0.87	0.29	1.07	0.15	0.98	0.14	0.000
LDL/HDL	6.34	4.90	8.40	4.21	8.39	5.42	0.53
CRI (TC/HDL)	9.13	5.78	11.86	4.80	11.39	6.04	0.26

lar disease (CVD) occurrence and severity is not without controversy, while one study conducted on the American population confirmed no association between the A allele of rs 4986938 SNP, another case-control study conducted on the Polish population suggests that the ESR2 rs4986938 (G1730A) variant may play a role in the risk factor of MI at a young age, albeit not as an independent but as a potential risk, ESR βgene polymorphism AluI (G1730A) was shown to be correlated with male body fat percentage in a study of 170 healthy Greeks (ages 22-59, mean 42 years) [36]. Researchers believe that ethnic variations among the studied populations account for the conflicting findings in the literature regarding the connection of certain ESRβ genotypes with BMI, body fat mass, hypertension, LDL, HDL, triglyceride, and glucose concentrations. The population we studied was composed entirely of Caucasian Poles. Despite the prevalence of young-age MI in males, there is currently little information available on the association between premature CAD and ESRβ genotypes in this cohort. Coronary artery intima from asymptomatic males who had atherosclerotic plaque on autopsy was shown to have elevated ER expression [37]. Plaque area associated favorably with ER expression, indicating a function for ER in both advanced atherosclerosis and early CAD. There was a correlation between the G1730A mutation and LDL level [38] in healthy young males. In conclusion, by studying the association between ER βgene variation and CHD, researchers can identify individuals at high risk for developing CHD and those who may be genetically protected against the illness. Further research is needed to fully understand the complex relationship between genetic predisposition and environmental factors in the development of CHD.

LIMITATIONS

1. Conduct a study with a large enough sample size to allow for separate men and women analyses.
2. Analysis of numerous SNPs of ERβ gene and analyzing their relation with other genes to discover which one is more prevalent in our community.
3. Assessment of gene-gene interaction of ERβ gene with other CHD relation genes.

CONCLUSION

The rs4986938 (not for rs1256049SNPs) are risk factors to develop CHD in Iraqi population and implicated in changes of serum lipid concentrations and atherogenic index.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

Use of honey in dentistry – literature review

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ABSTRACT

Aim: To assess the effects of honey and its derivatives on oral health.

Materials and methods: PubMed, Google Scholar, Scopus, Embase, Cochrane databases were searched using relevant keywords and Boolean operators.

Conclusions: Honey is a promising natural alternative for the treatment of various dental diseases due to its antimicrobial, anti-inflammatory and wound-healing properties. Scientific studies show that honey has numerous health properties, including antibacterial, antiviral, anti-inflammatory, wound-healing and anticancer effects. In the context of dentistry, honey shows potential in the treatment of various oral conditions, such as dental caries, gingivitis, halitosis, radiotherapy-induced mucositis, xerostomia or post-extraction stomatitis. Honey's safety and multifunctional benefits make it a valuable potential addition to dental treatments.

KEY WORDS: honey, mucositis, caries, halitosis, dry socket

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INTRODUCTION

Honey is a product naturally produced by honey bees. Chemically speaking, it consists of an average of 17% water, 38% fructose, 30% glucose, while the rest is made up of other disaccharides, gluconic acid, lactones and minerals and nitrogen [1]. Fructooligosaccharides, of which honey contains 4 to 5% in its composition, are a good source of prebiotics that support the intestinal bacterial flora. Honey also contains vitamins, including B2, B6 and C, as well as calcium, iron, zinc and potassium. Many of honey's pharmacological properties are derived from polyphenols and, as with the rest of the composition, the amount depends on the source of the nectar [2].The earliest known written reference to honey is on a clay tablet belonging to the Sumerians, who inhabited the Euphrates valley between 2100 and 2000 BC. This tablet mentions the use of honey as a drug and an ointment [3]. Propolis is a resinous product collected from plants by bees to cover holes and cracks in their hives. After collecting propolis, the bees mix it with wax flakes and saliva in the hive. Although bees use propolis to strengthen the walls of the hive and protect the hive from the onslaught of infection, humans use these products to strengthen their immune systems. Propolis has strong antibacterial, antiviral, anti-inflammatory, wound-healing and anti-cancer properties. It is similar to aspirin in its functions without side effects [4, 5].

AIM

The study aims to evaluate the medicinal properties of honey, particularly its applications in dentistry, by reviewing the existing literature. It seeks to explore the potential of honey as a treatment for various oral conditions, such as dental caries, gingivitis, halitosis, mucositis, xerostomia, and dry socket, and to determine its effectiveness as a natural adjunct in dental care.

MATERIALS AND METHODS

The PubMed, Scopus, Embase, Cochrane and Google Scholar databases were searched using syntaxes consisting of keywords and Boolean operators: (''honey'' OR ''propolis'' OR ''propolis farinosa'' OR ''propolis versicolor'' OR ''apitherapy'') AND (''oral

health" OR ''mouth diseases" OR ''periodontitis" OR ''ulceration" OR ''stomatitis" OR ''oral ulcer" OR ''mucositis" OR ''dental caries") adapted as appropriate for each database. Polish and English language search restrictions were used.

REVIEW AND DISCUSSION

CARIOUS CAVITIES

Caries is undoubtedly one of the biggest global problems of the present day [6]. A biofilm, comprising mucus, bacterial cells, saliva polymers and food particles, covers the tooth surface. If left unchecked, the biofilm can increase in thickness to a level of hundreds of cells on the tooth surface. This biofilm, known as plaque, provides an optimal surface for bacterial adhesion, colonization, and proliferation [7]. Laboratory studies have shown that different types of honey have significant antimicrobial activity against various periodontal pathogens, including *Streptococcus mutans*. Although the antibiofilmic effect of honey has been reported mainly against *S. mutans*, these results are limited to a few studies. In clinical settings, honey was found to significantly reduce plaque, but was not superior to conventional agents. Honey has shown positive in vitro results in the prevention of dental caries. Further robust clinical studies are needed to determine its efficacy in clinical settings [8]. Voidarou et al. showed that simultaneous preparations of artificial saliva into honeys, enhances their antimicrobial activity [9]. Patel et al. observed that the inhibitory effect on S. mutans had a honey concentration of no less than 60% [10]. Conversely, the interaction of honey with hard tissues remains uncertain. The low pH of the honey samples tested by Habluetzel et al. [11] and Grobler et al. [12] did not result in any erosive damage to enamel, as reported in their respective studies. Furthermore, one specific type of honey was demonstrated to alter the composition of the enamel membrane, thereby conferring a protective effect against demineralization caused by cariogenic factors [11]. Atwa et al. in their study showed that the pH decrease with honey application was greatest after 5 minutes, but quickly returned to its original value within 10-20 minutes, and additionally the pH decrease did not exceed the pH critical for demineralisation (pH = 5.5) [13].

GINGIVITIS

Gingivitis caused by plaque build-up is an inflammatory condition characterised by bleeding, redness and swelling of the gums. It is most often due to the presence of harmful bacteria in the gingival crevices, which triggers an inflammatory response in the tissues. It is one of the most common oral conditions, along with tooth decay. Although not all cases of gingivitis progress to an advanced state, i.e. periodontitis, treatment of gingivitis is a key strategy for the prevention of this condition [14]. Nayak et al. conducted a study comparing the efficacy of honey, chlorhexidine (0.2%) and xylitol gum rinses in reducing plaque and gingivitis. In the group utilising Manuka honey, participants were instructed to gently apply the honey to the gingival crevices of all teeth, wait for a period of five minutes, and then repeat the process on two further occasions. The application was conducted twice daily, following the consumption of meals. The use of both Manuka honey and chlorhexidine mouthwash was found to be significantly more effective in reducing plaque formation than the use of xylitol chewing gum [15]. Atwa et al. in a randomized trial considered honey chewing as a worthy alternative to traditional measures to prevent caries and gingivitis with orthodontic treatment. They found that bacterial counts were significantly reduced in the honey group compared to the other treatment groups, and honey significantly inhibited the growth of all strains tested compared to the inhibition observed with antibiotics [13]. A double-blind, randomised, study by Singhal et al. showed that manuka honey and raw honey were as effective as chlorhexidine as a mouthwash. However, chlorhexidine showed maximum reduction in average plaque and gums [16].

HALITOSIS

Halitosis is a common problem that manifests as an unpleasant and disgusting odour emanating from the mouth. The cause of the unpleasant odour is mainly due to the putrefactive action of microorganisms on endogenous or exogenous proteins and peptides. Bad breath is an embarrassing condition that affects a large percentage of the human population [17]. The antimicrobial action of honey effectively combats bacterial colonisation of healing wounds and provides nutrients to the bacteria so that they produce lactic acid during metabolism rather than odorous gases[18]. Drain and Fleming demonstrated that manuka honey is a safe, effective palliative treatment to reduce odour and inflammation in wounds secondary to oral squamous cell carcinoma in this patient. Makmuriana et al, on the other hand, demonstrated the superiority of honey over chlorhexidine in patients with post-stroke halitosis [19].

MUCOSITIS WITH RADIOTHERAPY

The mucosal epithelium's inflammatory response to the cytotoxic effects of chemotherapy and radiotherapy results in mucositis, a painful side effect of cancer treatment. Approximately 40% of patients undergoing chemotherapy will develop mucositis, with the incidence rising to approximately 90% among those with head and neck cancer who are treated with both chemotherapy and radiotherapy. Of these cases, 19% will require hospitalisation and result in delays to cancer treatment due to severe mucositis. This leads to a reduction in quality of life, an adverse prognosis and increased patient costs[20]. An important study by Biswal et al. first showed that honey was effective in preventing radiation mucositis [21]. Since then, many studies worldwide have shown that honey was beneficial in reducing the incidence of radiotherapy/chemotherapy-induced oral mucositis, reducing treatment interruptions, weight loss and delaying the onset of oral mucositis[22–25].

XEROSTOMIA

Saliva plays a key role in maintaining dental integrity, diluting food debris and bacteria, mechanical cleaning of the mouth and oral comfort. Saliva also provides an antimicrobial effect to prevent oral infections and plays an important role in upper gastrointestinal tract functions, including taste perception, food bite formation, facilitation of chewing, swallowing and speech, as well as oral and upper oesophageal mucosal orcristration. Accordingly, salivary gland hypofunction is associated with an increased risk of oral infections, candidiasis, scarring of teeth, dysgeusia, and oral mucosal discomfort [26]. A randomized study by Charalambous et al. showed that the use of honey by patients with radiotherapy-induced xerostomia resulted in significant improvements in both saliva secretion and quality of life for patients [27]. Similar results were obtained in another randomized study by Ibrahim et al. on a group of patients with end-stage renal failure treated for xerostomia[28]. Heydarirad et al. also obtained positive results in a randomized trial on patients with chemotherapy-induced xerostomia for breast cancer. They showed that the use of a honey and lemon spritz every 2 hours had a positive effect on saliva production[29]. It was also shown that in patients with xerostomia induced by radiation therapy, honey caused a reduction in S. mutans [30].

DRY SOCKET

Alveolar osteitis, or dry socket, represents a potential complication of tooth extraction, with a higher prevalence observed in cases involving mandibular molars. The condition is typified by acute discomfort two to three days following surgery, with or without halitosis, and an alveolus that may be partially or completely devoid of a blood clot, frequently necessitating more frequent postoperative visits [31]. Unlike eugenol used for dry alveolus, which can lead to bone necrosis, honey has no side effects, which is associated with its high biocompatibility [32]. To date, many studies have been produced that confirm the efficacy of honey in the treatment of post-extraction osteoarthritis by significantly reducing pain sensations, and reducing inflammation [32–34].Moreover, González-Serrano et al. showed that after using honey as a dressing, the infected wound showed sterility after 3-6 days [35].

ULCERATIONS

Mucosal cell turnover in the oral cavity depends on a balance between cell differentiation and exfoliation, which acts primarily as a defence mechanism against pathogens. However, this can be disrupted and lead to the development of several conditions, including hyperplasia and dysplasia and a reduced proliferation rate, which can lead to the development of ulcers [36]. A systematic review by Hunter et al. showed that honey is an effective treatment for various oral ulcer conditions. It accelerates healing time, reduces pain sensation in patients and reduces co-morbid inflammation [37].

As a natural product, honey is not only enriched with nutritional value, but also possesses a number of medicinal properties that make it a noteworthy agent in the field of dentistry. Its richness in fructooligosaccharides, vitamins, minerals and other components brings numerous health benefits, especially in the context of various oral disorders. In the treatment of tooth decay, honey has been shown to have antimicrobial effects, which can help to reduce plaque and inhibit the growth of harmful bacteria such as *Streptococcus mutans*. In addition, research suggests that honey may accelerate the healing process of wounds on the oral mucosa, which may be particularly beneficial in cases of ulcers and inflammation. In the context of gingivitis, honey shows potential in reducing plaque and reducing gingival inflammation. Its antimicrobial action can also help to combat harmful bacteria, which are the main cause of gingivitis. Halitosis, or bad breath, can also be alleviated by honey's antibacterial action, which helps to combat the bacteria responsible for putrefactive processes in

the mouth. In addition, honey can help to reduce inflammation and improve overall oral hygiene. For patients undergoing radiotherapy or chemotherapy, honey can be an effective agent in the prevention and treatment of radiation-induced oral mucositis. Its antimicrobial and wound-healing properties can provide relief to patients, reducing pain sensations and promoting tissue regeneration processes. The aforementioned benefits of honey in the context of various oral conditions suggest that honey may be a valuable addition to traditional dental therapies, offering patients natural and effective support in maintaining oral health. However, further clinical studies are needed to confirm these benefits and to determine the optimal doses and methods of using honey in dental practice.

CONCLUSIONS

Honey offers a promising natural alternative for managing various dental conditions due to its antimicrobial, anti-inflammatory, and wound-healing properties. While its efficacy has been supported by several studies, particularly in reducing plaque, gingivitis, and oral ulcers, more clinical research is necessary to validate its use in routine dental practice and establish appropriate application methods. Honey's safety and multifunctional benefits make it a valuable potential addition to dental treatments.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

Prospects of osteosynthesis with fixators based on magnesium alloys, mechanical and physiological properties. The state of the problem at the current stage

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ABSTRACT

Aim: The aim of this work is to analyze the available scientific information regarding to the prospects of metal-osteosynthesis with biodegradable fixators based on magnesium alloys.

Materials and Methods: A set of general and special methods of scientific knowledge are used in the article. Search and analysis of full-text articles and scientific publications - carried out in databases of systematic reviews of MEDLINE, PubMed, Web of Science, Google Scholar, Scopus.

Conclusions: Magnesium-based implants contribute to a tissue regeneration and healing during degradation and do not require removal. This allows you to avoid the second surgical intervention and reduces treatment costs. That is why the development and implementation of biodegradable fixators for osteosynthesis is of great importance.

KEY WORDS: magnesium alloy, bioresorbable fixators, magnesium fixators, bioresorbable alloy, biodegradable screws

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INTRODUCTION

Traditionally, metal implants are made of rust resistant steel, titanium (Ti) and cobalt-chromium (Co–Cr) alloys. Although these metals have demonstrated good biocompatibility, high wear resistance, and sufficient mechanical strength, but they have critical limitations [1].

Firstly, they are often incompatible with the physiological properties of natural bone, wear out mechanically and can cause inflammation and metallosis due to corrosion. Their use requires the second surgical intervention to remove fixators that have fulfilled their goal. In the place of implantation, tension is created due to the high Young's modulus, which is characteristic of traditional fixators for osteosynthesis. Reduction of peripheral bone density and loss of mechanical strength due to the presence of an implant increases the risk of secondary fracture. In order to overcome the large number of disadvantages of traditional implants at the present time, the leading countries of the world (USA, South Korea and Germany) are developing and clinically implementing implants made of bioresorbable materials, including metal implants based on magnesium alloys [2].

Magnesium-based implants are of particular interest because it is proved that they promote osteogenesis, angiogenesis, and neuroregeneration while oppressing osteoclast activity and inflammation [3]. Magnesium-based implants promote bone regeneration and remodeling. However, it is still unclear how magnesium affects metabolism and bone remodeling.

Magnesium is the fourth most popular cation in organism equal to approximately 1 mole (24 g) in an adult and more than 60% is accumulated in bones and teeth. Most of the magnesium that accumulates in bone tissue is concentrated on the hydrated surface layers of apatite crystals, which is promising in the development of fixators for osteosynthesis [4]. This ensures a rapid exchange of magnesium between the blood and the extracellular liquid, which leads to ion homeostasis. Magnesium has been found to be a cofactor for various enzymatic reactions related to energy metabolism, protein and nucleic acid synthesis, functional support of the parathyroid glands, and vitamin D metabolism, which are directly related to bone formation [5] . Several researchers who have studied the effects of a magnesium-depleted diet on rats have shown a decrease in

systemic bone density. Suppression of the growth of the proximal end of the tibia and even the development of osteoporosis in this group was noted [6]. In these studies, increased magnesium intake has been proven to prevent a decrease in bone mineral density (BMD) in patients with osteoporosis. It is not reported about symptoms of toxicity caused by excess magnesium, as magnesium concentration is strictly mediated by the kidneys through urinary excretion [7].

AIM

The aim of this work is to analyze the available scientific information regarding to the prospects of metal-osteosynthesis with biodegradable fixators based on magnesium alloys.

MATERIALS AND METHODS

A set of general and special methods of scientific knowledge are used in the article.Search and analysis of full-text articles and scientific publications - carried out in databases of systematic reviews of MEDLINE, PubMed, Web of Science, Google Scholar, Scopus.

REVIEW AND DISCUSSION

Studies [8] have shown that when the concentration of magnesium ions reaches the appropriate range (it means, 50–100 parts per million), it is able to enhance the viability of osteoblasts. It was found that the specific activity of alkaline phosphatase (AL) of osteoblasts cultivated on environments supplemented with Mg ions was significantly higher compared to the control. Real-time RT-PCR research also demonstrated higher expression levels of ALP and transcription factor - 2 (Runx2) after stimulation with appropriate amounts of Mg ions. The highest expression levels of collagen type I (Colla 1) and osteopontin (Opn) were detected on the third day in cells cultured in conditioned medium [9].

In other studies, magnesium was added to different types of materials, including hydroxyapatite and tricalcium phosphate, and then the biological activity of these materials was investigated and compared to a control. When apatite in collagen was completely replaced by magnesium, a toxic effect was observed in the form of inhibition of the extracellular matrix [10]. However, when the amount of magnesium fluctuated in the appropriate range, the formation of the osteogenic matrix, as well as the attachment to cells of osteoblasts, on the background of the synthesis of alkaline phosphatase, increased. An increase in osteogenic activity in vivo was also recorded [11].

Although the mechanism of influence of magnesium ions on fracture healing has not yet been fully investigated, the understanding of this mechanism has been significantly deepened in recent works. In particular, in the studies conducted by Yoshizawa etc. [12]it is suggested that the osteoregenerative effect of magnesium on undifferentiated stromal cells of human bone marrow (hBMSC) and osteogenic hBMSC is probably connected with the following organized reactions: 1) activation of hypoxia-inducible factor 2 a (HIF-2 a) and peroxisomes ; 2) gamma coactivation of the proliferator-activated receptor (PGC)-1a, accordingly.

Zhang et al. has found that rat bone marrow stem cells (BSCs) show significantly reinforced expression of integrin-a-5-b-1 when cultured with 5% calcium phosphate cement which contains magnesium (5MCFC), and accordingly promote osteogenic differentiation, whereas this effect was not observed when cultured with 10MCFC and 20MCFC. More recently, Zhang et al. demonstrated that magnesium ions can stimulate calcitonin-induced accumulation of neuronal polypeptide (CGRP) in both the peripheral cortex of the femur and the ipsilateral dorsal root ganglia (DRG), thus promoting fracture healing in a rat animal model [13].

This study revealed an uncertain role for Mg-2þ in CGRP-mediated osteogenic differentiation. In another study devoted to the long-term mechanism of magnesium alloy degradation in vivo, Makkar P. et al. found that the use of biodegradable fixators based on magnesium can promote the crystallization of calcium phosphate in a rabbit femoral condyle fracture model [14]. All these reports have emphasized one more time the importance of magnesium for fracture healing and indicate its therapeutic potential in bone fracture regeneration in orthopedic clinics.

Bone metabolism includes both the formation of bone tissue and its destruction. It is the dynamic balance of osteoblasts and osteoclasts that supports the stability of bone tissue regeneration. Currently, the factors that mainly affect osteoclast differentiation and osteoresorption are PI3K-Akt and OPG-RANK-RANKL signaling. Recent studies have shown that inhibition of PI3K-Akt signaling activity reduces osteoclast resorption [15]. While different concentrations of magnesium ions can regulate PI3K-Akt signaling in osteoblasts [16]. However, the OPG-RANKL-RANK signaling pathway promotes bone resorption. Gene transcription for osteoclast differentiation requires M-CSF and RANKL, which are released by osteoblasts. This indicates that a certain degree of differentiation and functional activity of osteoblasts contributes to the activity of osteoclasts [17].

Magnesium-based implants release its ions, which leads to an increase in local concentrations. When the appropriate concentration is reached, the PI3K-Akt signaling pathway is activated to promote osteogenic differentiation. Osteoblast differentiation leads to the secretion of RANK and M-CSF to induce osteoclast differentiation, which explains the coexistence of bone formation and bone resorption in the early stages after implantation of magnesium-based fixators. However, additional experiments are still needed to confirm this assumption. There is an opinion that simultaneous bone formation and bone resorption accelerates remodeling and promotes bone formation, which allows to increase the rate of callus formation [18].

Magnesium-based implants promote tissue recovery and regeneration during degradation and do not require removal. This allows to avoid the second surgical intervention and reduces treatment costs. It has been proven that magnesium can promote osteogenesis, angiogenesis, and neuroregeneration while inhibiting osteoclast activity and inflammation [19].

Most of the clinical researches are focused on the benefits of uses of magnesium, such as the stimulation of osteogenesis and angiogenesis. For example, Wang Z, et al. used magnesium-based implants in an experimental rat osteonecrosis model. The regenerative potential of implants based on magnesium alloys was proven [20]. Some investigators have attempted to use magnesium-based screws in anterior cruciate ligament reconstruction, but the strength of magnesium-based screws in such injuries remains questionable.

Lee J.W. et al. analyzed the voltage in the screw head and successfully improved its construction, which solved the key point of the clinical use of screws based on magnesium alloys [21]. Magnesium-based metal implants also show benefits in restoring bone fracture healing in elderly patients and in osteoporosis. Lin etc. added magnesium particles to clinically used polymethyl methacrylate (PMMA) bone cement to give it osseointegration, angiogenic, and anti-infective properties [22].

Bioactive magnesium metal powder has also been included into a composite of bioactive carcass that is made to repair large bone defects [23]. Recent studies have shown that, due to surface modification, corrosion properties decrease and biocompatibility of magnesium alloy implants increases [24]. One of the approaches is to significantly increase the corrosion resistance of magnesium alloys due to sol-helium and polyester-based synthetic coatings. For example, Zhang et al. developed a new double-crosslinked hydrogel with Mg ions by photocrosslinking gelatin methacryloyl (GelMA), thiolated chitosan (TCS) and modified polyhedral oligomeric silsesquioxane (POSS) nanoparticles with subsequent inclusion of magnesium ions through MgS coordination connections [25]. Researchers have used more than two approaches to successfully improve the corrosion properties and biocompatibility of magnesium-based implants, opening a new way to prepare biodegradable fixators [26].

Li et al. modified the inner layer of Mg(OH)2 with a nanocoating by introducing stearic acid (SA), which improved the adhesion strength between the outer and inner layers, avoiding the penetration of corrosive environment [27]. In a similar way, to improve the corrosion resistance and biocompatibility of magnesium alloys, Cheng et al. tested a Mg-Al layered double hydroxide (LDH) coating on the surface of pure magnesium and demonstrated not only greater corrosion resistance, but also improved osteogenic, angiogenic, and anti-inflammatory activity and better osseointegration in vivo than pure magnesium [28].

The use of other nanocoatings to improve the biocompatibility and degradation resistance of magnesium alloy was reported in another work [29]. Lin et al. used TiO2/Mg2TiO4 nanocoating on the outer layer of magnesium alloy to improve its biocompatibility and resistance to bacterial infections [30]. Also Liu et al. found that a lithium-bound nanoporous coating provides a magnesium alloy with increased corrosion resistance, and also promotes angiogenesis and bone formation [31].

Gao et al. used calcium phosphate to improve the biocompatibility and stability of the magnesium alloy [32]. Razavi et al. extended this approach using a new bioceramic nanocomposite coating that resulted to reducing corrosion rate and improved new bone formation while reducing inflammation at the border of implants and surrounding tissue [33]. Other authors achieved an increase of resistance to degradation and bactericidal activity by inputing graphene nanoparticles into a magnesium alloy [34]. Numerical studies also emphasize the improvement of corrosion characteristics of natural polymer coatings due to the inclusion of synthetic polymers. In addition, sol-gel and synthetic polyester coatings have demonstrated the ability to act as local drug delivery platforms [35]. Chen et al. produced a coating filled with zoledronate on the surface of an AZ31 magnesium alloy. The results showed that it has better corrosion resistance, and zoledronate may also play a role, confirming the possibility of its easy and effective use in the clinic [36]. It should be noted that researches in this field are very limited. In addition, there were attempts to make alloys in metallic glass to improve corrosion resistance [37]. With the development of materials science, more and more researchers used

nanocomposites to increase the corrosion resistance and biocompatibility of magnesium alloys [38]. The key problem, however, remains the dynamic balance between the rate of degradation and recovery of bone tissue.

The mechanical strength, corrosion resistance, and biocompatibility of magnesium alloys have also been shown to be improved by surface modification. However, cytotoxicity tests were not included in most known studies [39]. Based on the analysis of available sources, it was found that, unlike an inert metal, there is no unified standard for assessing metals for cytotoxicity in biodegradable fixators. Observation of tissue changes in the place of installation of implants requires further investigation.

In general, magnesium and its alloys have excellent biocompatibility with bone tissue. As a result, magnesium has become widely used in the field of biomaterials as a biodegradable and bioabsorbable material for medical usage. There are various methods of controlling the corrosion of magnesium, such as cleaning, anodizing, alloying (fusing) metals, and coating the surface with various materials. Studies have shown that magnesium purification significantly reduces corrosion [40]. But chemically pure magnesium has weak mechanical properties, it is very brittle and not strong in breaking. Magnesium alloys with different elements offer the opportunity to increase the mechanical strength of pure magnesium, but the alloying elements must be carefully selected to maintain biocompatibility and increase the corrosion resistance of magnesium.

CONCLUSIONS

The use of fixators made of magnesium alloys during osteosynthesis of bone fractures does not require the second surgical intervention with regard to remove the fixators, which have fulfilled their goal. Magnesium alloys are light and have one-third the density of titanium fasteners. Magnesium-based implants contribute to osteogenesis, angiogenesis, neuroregeneration, regeneration and remodeling of bones at the same time inhibiting osteoclast activity and inflammation. Magnesium and its alloys have excellent biocompatibility with bone tissue. Magnesium-based implants contribute to a tissue regeneration and healing during degradation and do not require removal. This allows you to avoid the second surgical intervention and reduces treatment costs. That is why the development and implementation of biodegradable fixators for osteosynthesis is of great importance.

Overall, several challenges remain to be resolved for the clinical usage of magnesium-based biodegradable osteosynthesis fixators.

Firstly, it is necessary to control the degradation rate of magnesium fixators and maintain the desired mechanical properties throughout the treatment period to support tissue healing.

Secondly, in vivo animal limb experiments still can not reproduce the assessment of workload required for interventions in patients. In the future, it is likely that experiments on primates will be necessary to obtain better evaluation data.

Thirdly, the question of reducing gas formation of magnesium-based implants in vivo still needs further researches.

Fourthly, there is still no appropriate system for biological assessment of in vitro and in vivo effects on cells for biodegradable alloys.

That is, the issue of efficiency and safety of magnesium alloys has been considered in a number of studies in recent years. The use of magnesium alloys in fixators for bone osteosynthesis provides an economical improvement and a possible solution for the consolidation of bone fractures.

When the therapeutic effect and mechanism of action of biodegradable fixators are clearly understood, then the development of clinical protocols will be possible. That is why the search for components and the study of the effect on the regeneration of bone tissue with the use of implants based on magnesium alloys is quite relevant.

However, the clinical usage of magnesium-based implants is still in the research and development stage. The metabolism of magnesium ions in bones is not fully understood and therefore requires further research. In particular, there is currently no clear system for the biological assessment of biodegradable fixators. Therefore, the use of magnesium alloy in the clinic remains promising, but not sufficiently studied and requires further researches.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

Infectious exanthemas in clinical practice

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ABSTRACT

Aim: Analyze the causes and mechanisms of development of exanthems, provide a classification of the morphological elements and characterize clinical manifestations of rash in patients with infectious diseases based on a review of available literature data.

Materials and Methods: The authors analyzed the current literature sources, including a description of exanthema syndrome and a discussion of the features of rashes in various infectious diseases.

Conclusions: As a result of the analysis of existing literature data, it was established that exanthema syndrome is associated with various diseases, disorders and pathological conditions, characterized by a variety of clinical manifestations, which requires clinical differential diagnosis and subsequent laboratory confirmation. Comprehensive knowledge of the causes, mechanisms of development, as well as the clinical manifestations of skin rash will contribute to the development of an improved algorithm for diagnosis and treatment of the diseases accompanied by exanthema syndrome, as well as optimization of therapeutic tactics.

KEY WORDS: exanthema syndrome, classification, infectious diseases, diagnostic algorithm

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INTRODUCTION

Exanthema syndrome includes the presence of rashes on the surface and/or in deeper layers of the skin, is very common in the practice of physicians of many specialties and is of key importance in clinical diagnosis, including differential diagnosis, in the context of various diseases and pathologic conditions [1-3]. The war in Ukraine started by russia is an unprecedented stressor that leads to an increased incidence of many diseases that can be accompanied by exanthema syndrome [4, 5].

The relevance of exanthema syndrome also lies in the fact that very often the rash has polymorphic manifestations, and sometimes even atypical signs. This complicates the timely correct diagnosis, since at the prehospital stage the diagnosis is made mainly on the basis of the clinical picture, without available laboratory confirmation [2, 6, 7].

Therefore, systematization and analysis of existing literature data is appropriate for further development of an improved algorithm for diagnosis and treatment of diseases accompanied by exanthema syndrome.

AIM

Analyze the causes and mechanisms of development of exanthems, provide a classification of morphological elements and characterize the clinical manifestations of rash in infectious diseases based on available literature data.

MATERIALS AND METHODS

The authors analyzed the current literature sources, including a description of exentematous syndrome and a discussion of the features of rashes in various infectious diseases.

REVIEW AND DISCUSSION

Usually, exanthema of infectious nature occurs due to growth of the infectious agent in the skin, its transfer by plasma or infected cells (leukocytes, lymphocytes) into the skin vessels, as well as hypersensitivity reactions. In most infectious exanthems, the appearance of the rash indicates the development of immunologic reactions (antigen-antibody interaction). As a consequence, the following processes occur: swelling of collagen fibers, vascular dilation, cellular infiltration and the appearance of various morphological elements of rashes [1, 6, 8]. Depending on the cause of occurrence, exanthemas of an infectious nature are classified as follows [2, 3, 9]:

- viral (measles, rubella, scarlet fever, chicken pox, COVID-19, hemorrhagic fevers, herpes infections

(including infectious mononucleosis), HIV infection, hepatitis B, enteroviral, parvoviral diseases, etc.);
- bacterial (meningococcal infection, typhoid-paratyphoid diseases, rickettsiosis, leptospirosis, ersiniosis, erysipelas, Lyme disease, syphilis, felinosis, staphylococcal infection, molluscum contagiosum, etc.);
- fungal (histoplasmosis, coccidiomycosis, actinomycosis, etc.);
- parasitic (leishmaniasis, ascariasis, enterobiasis, echinococcosis, opisthorchiasis, trichinosis, toxocariasis, scabies, etc.).

In addition, in the context of differential diagnosis, the clinician should remember the following types of exanthems:
- exanthemas in systemic connective tissue diseases (rheumatic fever, systemic lupus erythematosus, systemic scleroderma, dermatomyositis, systemic vasculitis, juvenile rheumatoid arthritis, etc.);
- exanthema in malignant diseases (including during radiation or chemotherapy);
- exanthems associated with insect bites;
- exanthemas of an allergic nature (associated with taking medications, foods, etc.): urticaria, allergic dermatitis, DRESS syndrome, Lyell's syndrome, Steven-Johnson syndrome, etc.;
- exanthemas caused by the administration of vaccines and serums.

There are *primary morphological elements* that arise on intact skin and are classified as non-hollow and hollow. Primary non-hollow elements include include spots, papules, blisters, tubercles, and nodules, and primary hollow elements include vesicles, pustules, and bullae. *Secondary morphological elements* are formed as a result of the evolution of primary elements, their damage or infection. Secondary elements include scales, crusts, scars, erosions, ulcers, abrasions, cracks, vegetations and striae [2, 3].

EXANTHEMA IN INFECTIOUS DISEASES

MEASLES

The main cutaneous syndrome of measles is a maculopapularexanthema with uneven festoon-like edges. This exanthema is prone to fusion, abundant, bright, sometimes has a hemorrhagic component. The rash appears on the unchanged skin on the fourth or fifth day from the onset of the disease against the background of the second wave of fever, increased intoxication and respiratory and catarrhal syndrome. A pathognomonic sign of a rash in measles is staging: on the first day, the rash appears behind the ears, on the nose; then the rash thickly covers the face, neck, upper chest and shoulders; on the second day, the rash spreads to the trunk and proximal areas of the upper extremities; on the third day, the distal areas of the upper and lower extremities are affected by the rash. The peculiar evolution of the elements of the rash is typical: initially, small papules and spots appear (diameter 3-5 mm), which increase very quickly in size up to 10-15 mm; some spots (especially on the face and upper trunk) merge into a continuous erythematous surface. The exanthema begins to regress from the third day in the order of its appearance on the skin with the formation of secondary elements: pigmentation and bran-like peeling. For the clinical diagnosis of measles, the following symptoms should also be taken into account: acute onset with high body temperature; conjunctivitis, scleritis, blepharitis, lacrimation (worldview, up to blepharospasm); cough, runny nose; on the second day of the disease, Koplik's spots occur on the mucous membrane of the cheeks opposite the small molar teeth (white formations with a diameter of 1 mm, surrounded by a zone of hyperemia); enanthema in the form of pink spots on the mucosa of the hard and soft palate may also develop. Spotted exanthema may appear as a variant of the normal post-vaccination period in children vaccinated with live measles vaccine. On days 6-10 after vaccination, in combination with non-confluent exanthema, the following symptoms sometimes occur: low-grade fever, runny nose, cough, conjunctivitis (within 2-3 days). The staging of rashes, like the Koplik's spots, is not typical for post-vaccination exanthema. For specific diagnostics: cytologic examination (cytoscopy) of swabs from oropharynx (detection of multinucleated giant cells typical for measles); serologic methods (increase in the titer of specific IgG antibodies in dynamics by 4 or more times, detection of specific IgM antibodies to measles virus) [7, 9-11].

RUBELLA

The leading skin syndrome of acquired rubella is an exanthema that appears on the first or second day from the onset of the disease, simultaneously on different parts of the body with predominant localization on the face, extensor surfaces of the extremities and buttocks. The rash in rubella is small-spotted, pale pink, abundant, not prone to coalescence, located on a normal skin background. The rash persists for 2-4 days, occasionally for 5-7 days and disappears without trace. For the differential diagnosis of rubella, the following facts should also be taken into account: enlargement of the occipital and posterior cervical lymph nodes (soft, somewhat painful on palpation, size 1-2 cm); no changes in the mucous membranes of the oral cavity and oropharynx. In 20-30% of cases, rubella can develop without a rash. Such forms are not clinically recognized,

but the possibility of such a course should be remembered when monitoring a pregnant woman in a rubella outbreak. The diagnosis of rubella is confirmed using serological methods (an increase in the titer of specific IgG antibodies over time by four or more times, the detection of specific IgM to the rubella virus); molecular methods (detection of rubella virus RNA in biological materials (blood, urine, saliva, cerebrospinal fluid) by PCR [2, 7, 12].

SCARLET FEVER

The leading skin syndrome of scarlet fever is a small-point rash accompanied by some features: detection of rashes on the background of hyperemic skin; mainly on the bending surfaces of the extremities, lateral parts of the chest, abdomen, internal and posterior surfaces of the thighs and in places of natural folds of the skin (axillary, axillary, elbow, knee); pale nasolabial triangle; dryness and roughness of the skin during the rash; persistent white dermographism due to damage to the autonomic nervous system; acute tonsillitis syndrome (catarrhal, follicular, lacunar) in the first hours of the disease; regional lymphadenitis with damage to the anterior cervical lymph nodes; in severe course of the disease - Pastia's symptom (hemorrhagic strips in the natural folds of the skin – an additional symptom for the diagnosis of the scarlet after the disappearance of the small-point rash; after regression of exanthema - fine bran-like peeling of the skin on the trunk and earlobes, as well as leaf-like lamellar peeling on the fingers and toes, palms and soles are observed; ; changes in the tongue (on the first day of illness, the tongue is covered with a thick white coating; starting from the second day, the tongue begins to clear of plaque and becomes scarlet with enlarged papillae, the so-called "raspberry" or "strawberry" tongue). The reduction in the main clinical symptoms of scarlet fever usually occurs in the following sequence: fever and symptoms of intoxication (on day 2-3), lymphadenitis (on day 3-4), rash (on day 1-5), tonsillitis (on day 6-7), changes in the tongue (from day 10). The degree of intoxication syndrome with scarlet fever may vary, but usually the modern course of scarlet fever is not accompanied by intoxication and a significant increase in body temperature. The diagnosis of scarlet fever is confirmed by bacteriological examination of mucus from the oropharynx (isolation of group A β-hemolytic streptococcus), as well as by serological methods [2, 7, 9, 13, 14].

CHICKENPOX

The leading skin syndrome of chickenpox is vesicular exanthema. Vesicles are single-chambered, round or oval, 0.2-0.5 cm in diameter, surrounded by a rim of hyperemia, located superficially on a non-infiltrated base with transparent contents (on the face, hairy part of the head, trunk and limbs), appear on the first or second day of the disease. On the skin, elements of rashes at different stages of development can be observed: macules, papules, vesicles, crusts. For differential diagnosis, the following facts are important: absence of rashes on the palms and soles, localization of vesicles on the hairy part of the head, undulating fever (the appearance of new rashes is accompanied by an increase in body temperature), moderate symptoms of intoxication. The diagnosis is confirmed by detection of the virus antigen in smear from the contents of vesicles); ELISA (detection of specific IgM in blood); PCR method (detection of varicella virus RNA in biological materials: blood, cerebrospinal fluid, vesicle contents) [2, 7, 11, 15, 16].

HSV ½ INFECTION

Herpetic skin lesions are the most common form of the disease caused by the herpes simplex virus ½ types. The rash appears in the form of grouped, small (up to 0.1 cm in diameter), tense vesicles on an edematous, hyperemic base; The rashes are localized mainly on the skin around the mouth, wings of the nose, ears, and red border of the lips. In some cases, rashes may appear on the mucous membrane of the oral cavity, larynx, tonsils, conjunctiva, sometimes in the form of herpetic stomatitis or gingivostomatitis. Skin lesions may be localized or widespread with recurrent rashes. Hyperesthesia, itching, burning and other subjective sensations precede the appearance of rashes. After the vesicles have ruptured or dried, secondary elements in the form of crusts are formed. The foci disappear on days 7-9.

The generalized form of herpes, herpetic eczema, occurs mainly in individuals with atopic dermatitis, dermatoses, and also with HIV infection. Abundant vesicular eruptions appear in areas of eczematous skin and quickly spread to unaffected skin. Often the elements of the rash merge and burst to form a solid crust. Secondary elements of the exanthema after rejection of the crust look like pink spots or scars. High fever with severe general intoxication syndrome is typical for this disease. The diagnosis is confirmed by the isolation of HSV ½ in cell culture (virological method; material - vesicle fluid, cervical smear, vaginal discharge), detection of HSV½ DNA (PCR method; material - vesicle fluid, cervical smear, vaginal discharge, cerebrospinal fluid); serological tests (specific antibodies against HSV½ appear in the blood within a few weeks after infection) [1, 2, 7].

COVID-19

COVID-19 infection primarily causes interstitial pneumonia and respiratory failure [17], but it is often associated with skin manifestations as well. Lesions of the mucous membranes and skin in COVID-19 can occur at the onset of the disease or during its progression. The pathogenesis of skin rashes may include vasculitic and inflammatory skin rashes. They can be associated with both strong immune response and directly high SARS-CoV2 viral load. These skin manifestations can be divided into several groups: skin rashes similar to frostbite (e.g., toes in COVID); skin rashes similar to urticaria; maculopapular lesions; vesicular rashes; purpura, reticular livedo, and necrotic lesions; urticarial vasculitis, and others such as alopecia and herpes zoster. Detection of SARS-CoV-2 RNA by PCR is of leading importance for confirming the diagnosis of COVID-19 [18].

INFECTIOUS MONONUCLEOSIS

Exanthema in EBV-infectious mononucleosis occurs in 16-25% of patients. The rash appears on the 3rd-14th day of the disease and can be polymorphic: spotty, spotty-papular, roseollous. The rash persists on the skin for 4-10 days, with the possible appearance of secondary elements of the rash in the form of pigmentation. For diagnosis, other clinical signs of infectious mononucleosis are also important: prolonged fever and other symptoms of intoxication; puffiness of the face with difficulty breathing through the nose without severe catarrhal phenomena; generalized lymphadenopathy with predominant enlargement of the posterior cervical lymph nodes, which can reach 10-15 mm in diameter and cause neck deformation; hepatosplenomegaly; tonsillitis syndrome; jaundice, pain in the abdominal area along the mesentery can occur with severe and atypical course of the disease; leukocytosis or normocytosis with lymphomonocytosis and atypical mononuclear cells, relative and absolute neutropenia, moderately increased ESR. The diagnosis is confirmed by the detection of specific antibodies to Epstein-Barr virus antigens in the blood by ELISA and EBV DNA in PCR [11, 12, 20].

ENTEROVIRUS EXANTHEMA (HAND, FOOT, AND MOUTH DISEASE)

One of the leading syndromes of these diseases is polymorphic rash (spotty or spotty-papular, pink, may be fine-pointed), which appears simultaneously on an unchanged skin background on the 1-2 day, together with fever or after its reduction (on the 3-5 day). The predominant localization of rashes – the skin of the face and trunk, less often – the extremities. The exanthema persists for 1-2 days, disappears without a trace. This disease is also characterized by high fever, which can be two-wave, and moderate symptoms of intoxication. Specific diagnostics is based on serological (an increase in the titer of specific IgG antibodies over time by four or more times) and molecular methods (detection of enterovirus RNA in blood, urine, feces, cerebrospinal fluid by PCR) [9, 21, 22].

EXANTHEMA SUBITUM («PSEUDO-RUBELLA», SIXTH DISEASE) CAUSED BY HHV6

The rash in this pathological condition is characterized by the appearance of spotty, pale pink elements with a diameter of 2-5 mm, which do not merge with each other and are located on an unchanged skin background, on days 3-5 of the disease against the background of a critical decrease in body temperature. Exanthema occurs simultaneously, mainly on the torso, neck, to a lesser extent on the face and limbs, persists for 2-3 days and disappears without a trace. To diagnose Exanthema subitum, it is necessary to take into account other characteristic signs of infection caused by HHV_6: acute onset, rapid increase in body temperature to 38-40 °C, moderate symptoms of general intoxication, mild respiratory catarrhal syndrome, possible generalized lymphadenopathy (mainly of the cervical, axillary and axillary groups of lymph nodes). ELISA (determination of specific IgM titer for diagnosis of acute or reactivation of chronic infection) and PCR (detection of DNA in peripheral blood lymphocytes or other tissues) are most often used to confirm the diagnosis. [11, 23].

PARVOVIRAL B19 INFECTION (INFECTIOUS ERYTHEMA)

Infectious erythema (fifth disease) is characterized by the appearance of spotty or spotty-papular bright red rashes on the face, which merge on the cheeks, forming a bright erythema, similar to traces of slaps (on the nose in the form of a butterfly). Exanthema appears on the 2-5th day of the disease. After 1-4 days, a secondary erythematous maculopapular rash on the trunk and extremities, including the palms and soles, with itching begins to appear. The exanthema is mainly localized on the extensor (dorsal) surfaces of the limbs, and the red color of the rash quickly turns bluish-red. With the reverse development of secondary erythema, a mesh "lace" pattern appears. Secondary rash elements disappear gradually with possible recurrence of rash elements due to a number of factors: insolation, hypothermia, bathing, etc. For the diagnosis of parvovirus

infection, it is necessary to take into account other clinical signs that appear before the onset of rashes: fever, respiratory catarrhal syndrome, nausea, diarrhea, arthralgia and arthritis. Parvovirus infection is usually confirmed by the determination of titers of specific IgM and IgG using ready-made ELISA kits. Sometimes a virus is isolated from serum or tissues, as well as viral antigens and DNA are detected by PCR [12, 24, 25].

HEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS)

HFRS is characterized by non-extensive, petechial exanthema, which may appear as streaks, reminiscent of whip marks. The rash is localized in the area of the clavicles, axillary folds, sometimes on the neck and face, appears from the 4th day of diseases and disappears without a trace. At the same time, a hemorrhagic enanthema occurs on the mucous membrane of the soft palate. HFRS is accompanied by hyperemia of the face and neck, increased blood flow to the vessels of the sclera and conjunctiva. For clinical diagnosis, the combination of hemorrhagic rash with other clinical symptoms of HFRS is important: sudden rapid onset of the disease, high fever with chills for 5-6 days, severe symptoms of intoxication (headache, pain in the eyeballs, myalgia, arthralgia), visual disturbances, pain in the abdomen and lower back, other signs of hemorrhagic syndrome (nosebleeds, hemorrhages in the sclera, hemorrhages in injection sites, hematuria), decreased diuresis from 4- 5 days, progression of acute renal failure. The absence of respiratory-catarrhal syndrome is typical for HFRS. For the purpose of specific diagnosis, ELISA (detection of specific IgM antibodies already in the early stages of the disease), immunohistochemical testing and PCR are used (detection of the virus or its particles) and, in some cases, virological testing [26, 27].

HEPATITIS B

The most common skin manifestations in hepatitis B include urticaria and Gianotti-Crosti syndrome (papular acrodermatitis, usually in childhood). Urticaria can be the main characteristic feature of the prodromal period of hepatitis B. Rashes (macular-papular or petechial) usually last for several days and precede the development of arthralgia and jaundice. Gianotti-Crosti syndrome is characterized by: erythematous-papular rash; absence of itching; symmetrical location of exanthema elements on the skin of the face, buttocks and extremities. Fresh elements continue to appear for several days and never merge. The exanthema is maintained for about 3 weeks. Within a few months, signs of lymphadenopathymay be observed. Often, the appearance of rashes is associated with a non-jaundice form of hepatitis, and the symptoms of hepatitis may occur simultaneously with the appearance of the rash or several weeks later. This variant is typical for European countries, where the Adw serotype of the hepatitis B virus is common. The specific diagnosis of hepatitis B is based on the detection of HBV DNA in serum (PCR), the determination of the HBs antigen and the spectrum of antibodies to HBV antigens [28, 29].

CONTAGIOUS MOLLUSCUM

Contagious molluscum is a viral disease characterized by the appearance of nodules ranging in size from hairpin to pea, hemispherical, having skin color or slightly pink, translucent, relatively dense. Nodules are located more often on the skin of the face, neck, as well as the trunk and extremities separately; when pressing on them a grayish-white mushy mass is secreted. In doubtful cases, the diagnosis is confirmed by microscopic examination of secretion from an element of the rash or a skin biopsy stained by the Wright or Giemsa method - the presence of inclusions (mollusc bodies) in the cytoplasm [30].

MENINGOCOCCAL INFECTION (MENINGOCOCCEMIA)

Meningococcemia is characterized by a hemorrhagic, stellate, often with necrosis in the center, dense rash on an infiltrated basis, which can be of different sizes (petechiae, purpura, ecchymoses) and does not disappear with pressure. In the fulminant form of meningococcemia, purple-black spots may be observed in the lower abdomen and on the inner thighs. The rash appears 1-2 days from the onset of the disease, in the fulminant form - after 2-12 hours, with typical localization on the buttocks, thighs, legs, and sometimes on the torso, upper limbs and face. Secondary elements of rashes have the form of pigmentation. In places of large skin lesions, necrosis is exfoliated to form ulcers, followed by scarring. The following signs should be taken into account in the diagnostic process: very acute onset of the disease, high fever with chills, severe symptoms of intoxication (pale skin, lethargy, adynamia, vomiting, headache, dyspepsia, tachypnea, tachycardia), appearance of meningeal symptoms, signs of endotoxic shock. Similar hemorrhagic rashes may be typical for hemophilic and pneumococcal infection, which causes certain difficulties in differential diagnosis. To confirm the diagnosis, a culture of meningococcus is isolated from the nasopharynx, blood and cerebrospinal fluid;

visual microscopy of blood smear and cerebrospinal fluid («thick drop» of blood and cerebrospinal fluid) and latex agglutination of blood and cerebrospinal fluid (detecting meningococcus antigens) are performed [9, 12, 31].

TYPHOID FEVER AND PARATYPHOID A, B, C

These diseases are characterized by roseolosis rashes, which appear in the 2nd week of the disease in a small amount on the skin of the abdomen and on the lateral surfaces of the chest. Differential diagnostic signs are high body temperature, enlarged spleen, liver, apathy. The disease most often begins with a gradual increase in body temperature to 39 C, an increase in general weakness, malaise, and lethargy. The diagnosis is confirmed by specific methods: bacteriological examination of blood, feces, urine; positive Widal reaction (agglutination reaction) – diagnostic titer 1:200, detection of specific IgM, increase in the titer of specific IgG antibodies over time by ELISA [32].

PSEUDOTUBERCULOSIS AND YERSINIOSIS

These diseases are accompanied by a polymorphic rash and are characterized by the following manifestations: exanthema appears simultaneously on days 3-5 (spotty, papular) with thickening in the area of the palms, soles and back of the head, around large joints; combination of skin rash with arthralgia, enlarged liver, abdominal pain and enteric stools (2-3 times a day); acute onset with high body temperature (38-39 °C); polymorphism of clinical complaints (pain in the abdomen, joints, muscle pain, loose stools); the presence of moderate hyperemia in the oropharynx with symptoms of pharyngitis without plaque on the tonsils and regional lymphadenitis; in the initial period of the disease, the tongue is coated with a gray-white coating, then the inside is cleared and after 3-5 days it takes on the appearance of a «raspberry» tongue; palpation of the abdomen reveals tenderness in the area of the liver and along the mesentery, but mainly in the right iliac region; 10-15% of patients may have symptoms of parenchymal hepatitis (subicteric or icteric skin and sclera, discoloration of urine and feces, hyperbilirubinemia), a short-term increase in the activity of ALT and AST; from 5-6 days, fine bran-like peeling of the skin appears in the torso area and large bran-like peeling on the palms and soles ; lack of effect from treatment with beta-lactam antibiotics within a week; damage to the heart and kidneys. Bacteriological and serological methods are used for diagnosis. The pathogen can be isolated throughout the disease from the feces, urine, bile, sputum, nasopharyngeal mucosa, as well as from the material obtained during surgery. Starting from the 6-7th day of disease, specific antibodies in an agglutination reaction or indirect agglutination reaction with a typical strain or autoculture over time [1, 2, 7].

STAPHYLOCOCCAL INFECTION WITH SCARLET FEVER-LIKE SYNDROME

The clinical picture is characterized by the appearance of small-point («scarlet fever-like») rashes on the hyperemic background of the skin. Exanthema is located on the inner surface of the arms and legs, in the lower abdomen with thickening of the rashes in the natural folds of the skin. The rash appears on the 3-4th day of the onset of a staphylococcal focus of infection, persists for several days. Secondary rash elements in the form of lamellar peeling of the skin appear at 2 or 3 weeks. In the first 2-3 days, the tongue is covered with a white coating, then it is cleaned and becomes «papillary». The following manifestations are typical: acute onset, fever, pronounced symptoms of intoxication, diffuse hyperemia of the oropharynx, sometimes signs of tonsillitis. The presence of a primary septic focus is crucial for diagnosis: an infected wound, panaritium, cellulitis, osteomyelitis, etc. and bacteriological examination [1, 2, 7].

ERYSIPELAS

The following clinical signs are typical for Erysipelas: limited bright hyperemia with clear edges that rise above the skin surface; the boundaries of the lesion are irregular in shape, reminiscent of flame tongues or a geographical map; the tissues around the affected skin are swollen, especially in the area of the face, ears, and legs; sometimes blisters with serous and hemorrhagic contents form at the site of inflammation, which usually open to expose the eroded surface; from the affected area of the skin to the regional lymph nodes (lymphadenitis) there is lymphangitis, which is especially clearly visible during thermographic examination ;the disease begins acutely with chills, an increase in body temperature up to 39-40 C, headache and muscle pain, with the rapid development of local symptoms: pain, burning and a feeling of tension in the affected area. Diagnosis is mainly based on clinical data, electrical thermometry is used (there is an increase in the temperature of erythema compared to areas of healthy skin). Hematological changes in patients with erysipelas are characterized by neutrophilic leukocytosis, toxic granularity of neutrophils, and accelerated ESR. Sometimes a biopsy of the affected area is performed [33].

SECONDARY SYPHILIS

In secondary syphilis, treponema spreads in the body through the lymphatic and blood vessels. As a result, there are various clinical manifestations in the form of localized or diffuse lesions of the skin and mucous membranes (roseola, papules, pustules), generalized lymphadenopathy and damage to internal organs. The main symptom of secondary syphilis is the occurrence of a rash that spreads throughout the body, including the palms and soles. Common signs of rashes in secondary syphilis are the following: lack of subjective sensations (itching, pain); density of elements; dark red color; clear, regular, rounded outlines of elements without a tendency to merge; almost complete absence of peeling of the surface; tendency to spontaneous disappearance without atrophy and scarring. Rashes on the skin and mucous membranes may be accompanied by influenza-like symptoms: headache, body aches, fever. The diagnosis is confirmed by bacterioscopic examination of material from various areas of the affected skin and mucous membranes for treponema, serological examination of blood (Microprecipitation reaction with cardiolipin antigen, Wasserman reaction, sedimentary reactions, group reactions for treponemas, species-specific protein reactions for treponemas, ELISA, indirect hemagglutination reactions, Western blotting (to detect IgG and IgM) and PCR [34].

LYME DISEASE

The leading symptom of Lyme disease is the development of a migrating annular erythema with a primary affect at the site of the bite of an infected tick in the first stage of the disease (in 60-80% of patients). First, a red spot is formed, which spreads over the skin after an average of 10 days and can reach a diameter of 10-15 cm or more. The erythema may be diffuse or appear as a ring with clearing or induration in the center, not rising above the skin level, with a bright red periphery. The erythema is most often localized on the thighs, buttocks, and groin area.. After a few days or weeks, ring-shaped erythema may appear in other places where there were no bites. In some cases, the rash looks like urticaria or diffuse erythema. In addition to annular erythema, the disease is characterized by migrating arthralgias, myalgias, arthritis, damage to the cranial nerves, the nervous system and changes in the heart in the form of ventricular conduction disorders with the development of blockades, myocarditis and pericarditis. In later stages, large joints may be affected. For the purpose of specific diagnosis, IgG and IgM titers are determined by ELISA. At the second stage (in case of a positive (IgG+, IgM+) or doubtful ELISA result) , a western blot is performed to confirm the diagnosis [35].

FELINOSIS (DISEASE FROM CAT SCRATCHES, BENIGN LYMPHORETICULOSIS)

The primary skin changes in felinosis take the form of red, painless papules that often fester and heal without scarring. This disease is also characterized by: slow healing of a wound caused by cat's claws or teeth; regional lymphadenitis (lymph nodes (more often - axillary, less often - cervical and inguinal) increase to 5-10 cm in diameter, are painless; in about 30% of patients, these lymph nodes may melt); fever. The diagnosis in case of suspected disease from cat scratches is confirmed in the presence of the following criteria: anamnestic data on contact with animals (cat scratches, bites, primary skin lesions), sterile pus in enlarged lymph nodes; positive serological reactions, typical histological changes in lymph nodes [36].

PROTOZOAL INFESTATIONS AND HELMINTH INFECTIONS

Cutaneous *leishmaniasis* is characterized by the formation of itchy papules that increase in size, after 3-6 months transform into a painful, crusty ulcer with a granulomatous base (up to 10 mm or more in diameter) and do not heal for a long time. These changes form on exposed parts of the body, at the site of a mosquito bite. After a few months, recovery spontaneously occurs, but a thin depigmented scar remains at the site of the ulcer. Parasites can spread through the lymphatic vessels and affect new areas of the skin, accompanied by tissue swelling and enlarged lymph nodes.

In patients with *ascariasis, enterobiasis, strongyloidiasis, echinococcosis, opisthorchiasis, toxocariasis,* etc., maculopapular (usually allergic) rashes of varying sizes and shapes may appear. Such exanthemas are often accompanied by severe itching. *Trichinosis* is characterized by: swelling of the eyelids, face, diffuse swelling and skin tension, reminiscent of polydermatomyositis; on the skin of the extremities, a maculopapular and hemorrhagic rash occurs; as well as hemorrhages under the nails may be observed. Microscopic (including parasitoscopic) methods and the detection of specific antibodies to pathogens are usually used to diagnose Protozoal infestations and helminth infections.

In typical cases of *scabies*, rashes appear in the form of spots or vesicles, accompanied by significant itching, on the hands, primarily in the interdigital folds. However, modern scabies is accompanied by damage to many other parts of the body, usually with thin skin: the upper extremities (mainly on the curved surfaces), chest, abdomen, inner thighs, legs, etc. Diagnosis is based on the clinical picture, epidemiologic history, identification of paired rashes and scabies passages in

the lesions, microscopic laboratory examination and positive effect of rubbing 5% sulfuric ointment [37-40].

CONCLUSIONS

Thus, as a result of the analysis of existing literature data, it was established that exanthema syndrome is associated with various diseases and pathological conditions, characterized by a variety of clinical manifestations, which requires clinical differential diagnosis and subsequent laboratory confirmation. Comprehensive knowledge of the causes, mechanisms of development, as well as clinical manifestations of skin rash will contribute to the development of an improved algorithm for diagnosis and treatment of diseases accompanied by exanthema syndrome, as well as optimization of therapeutic tactics.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

Diagnosis and surgical treatment of tracheal cicatricial stenoses: literature review

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ABSTRACT

Aim: To study of the data about the results of tracheal cicatricial stenoses treatment with the goal of further improvement of its results on the basis of diagnosis enhancing, identification of factors of prognosis of the disease and by conducting a differential analysis of tactics of surgical treatment with the use of minimally invasive interventions.

Materials and Methods: We have identified contemporary literature sources on the topic of modern directions of treatment of tracheal cicatricial stenoses and their complications. The studied material has been summarized and represented in the form of literature review in this article.

Conclusions: To increase the quality of treatment of patients with tracheal cicatricial stenoses and their surgical complications an important role is played by objective formation of groups at risk of complications development and prognosis of the disease dynamics. Successful solution of the problem is related mostly to the highly informative diagnostics, reliable determination of severity of disease, as well creation of multidirectional classification of tracheal cicatricial stenoses. These issues may not be considered completely solved, they require further study. All of the above dictates search of new effective methods of treatment of the indicated pathology and proves relevance of the topic. The stated information emphasizes necessity of improvement of surgical tactics of patients with tracheal cicatricial stenoses.

KEY WORDS: surgical treatment, tracheal cicatricial stenoses, endoscopical interventions

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INTRODUCTION

In spite of the progress of surgical techniques the number of patients with tracheal cicatricial stenoses remains high. Tracheal cicatricial stenosis is one of the most serious complications which arise as a result of prolonged orotracheal intubations, tracheostomies, neck injuries with damage to the trachea [1].

Many issues of pathogenesis, diagnostics, surgical treatment and prevention have been largely studied, annual increase of patients with this pathology equals to approximately 5% [2].

It is well known that up to 25-30% of surgical interventions on the trachea are accompanied with various complications, as a result of which 10% of patients die [3].

Patients with tracheal cicatricial stenosis are observed during a long period of time, are repeatedly hospitalized into medical institutions, they undergo complex reconstructive operations which do not always lead to the patient's recovery.

According to the data of a number of authors up to 25% patients remain disabled after the surgical interventions they underwent as a result of chondromalacia, large scars and tissue fibrosis [4].

According to the data of the World Health Organisation (WHO) 93 people per 1000 of population suffer injuries, poisoning and other effects of external causes which make from 7,7 to 8,1% in the general structure of hospital morbidity [5].

The main cause for tracheal cicatricial stenosis is long-term artificial ventilation of lungs (ventilator) as a result of severe combined trauma, major surgical interventions, and severe somatic pathology. Up to 25% of patients in the intensive care unit are on ventilators for more than a week. According to a number of authors frequency of complications of long-term mechanical ventilation through a tracheostomy or intubation tube is up to 80%. Out of all complications tracheal cicatricial stenosis makes up to 25% of cases. The share of post intubation tracheal stenoses in the structure of morbid-

ity makes from 14 to 45%, share of post tracheostomy stenoses makes from 51 to 73% [6].

Neck injuries with damage to the trachea occupy the third place among the causes of tracheal cicatricial stenoses, making from 4,8 to 12% of the number of diseases [6]. Idiopathic tracheal stenosis occurs in 1-2,9% cases [6].

Among the known factors leading to tracheal cicatricial stenosis the main one is the influence of the intubation tube cuff on the tracheal wall. In spite of use of low-pressure cuffs in the modern resuscitation practice, this problem remains acute. The main traumatic factor is considered to be the excess pressure in the cuff of intubation tube in relation to the capillary pressure in the mucous trachea.

A number of factors can be identified related to direct mechanical trauma of trachea leading to formation of cicatricial stenosis. They comprise damage to the cartilage tissue during tracheostomy, trauma to the tracheal wall by the free end of the tracheostomy or intubation tube [7].

Other factors which aggravate damage to the mucous membrane of the trachea and lead to chronic inflammation is reflux of gastric and duodenal contents followed by aspiration, autoimmune process by the type of reaction of the delayed type to cartilage collagen [7].

Under compression syndrome in patients with nodular goiter such complications may arise as difficult intubation caused by the presence of difficult airways. «Difficult airways» (DA) are all the clinical situations under which as a result of various combinations of anatomic or/ and functional changes in patients and/ or irrational actions of a professional predicted and unpredicted difficulties with provision of effective ventilation through a face mask, supraglottic airway device, difficulties with trachea intubation, performing of cricothyroidotomy arise or there are various combinations of the indicated situations, which create potential or direct threat to the development of critical violations of gas exchange. To identify difficult airways in patients including those with compression syndrome, scale El-Ganzouri is applied [8].

AIM

The purpose of the research is study of the data about the results of tracheal cicatricial stenoses treatment with the goal of further improvement of its results on the basis of diagnosis enhancing, identification of factors of prognosis of the disease and by conducting a differential analysis of tactics of surgical treatment with the use of minimally invasive interventions.

MATERIALS AND METHODS

We have studied modern, accessible to us, literary sources for the last ten years on the topic of modern directions of treatment of cicatricial stenosis of the trachea and its complications in adults. The studied material is summarized and presented in the form of a literature review in this article.

In the presented review of literary sources, the opinions of various world authors are cumulative. Own research results are the subject of scientific development and will be further presented in specialized scientific journals.

In this article, the analysis of the course and possibility of treatment of this pathology in children was not the purpose of the work.

REVIEW AND DISCUSSION

ETIOLOGY AND PATHOGENESIS OF TRACHEAL STENOSIS. CLASSIFICATION OF TRACHEAL STENOSIS

The basis of tracheal cicatricial stenosis lies on pathological process leading to the replacement of the normal wall of scarred trachea with the tissue which narrows the lumen of the respiratory tract. This is often accompanied by destruction of the cartilaginous trachea leading to the loss of the framework of the tracheal wall, occurrence of tracheomalacia [8].

The trigger of occurrence of cicatricial stenosis is the development of a purulent-inflammatory process, necrosis of the mucous trachea resulting in rough scar after critical ischemia of the tracheal wall [9].

Frequency of occurrence of narrowing of the trachea against the long-term artificial ventilation of the lungs, according to the data of a number of authors makes from 0,2% to 25%. It is impossible to estimate reliably the frequency of this complication occurrence due to great variability of the terms of its occurrence. Tracheal stenosis is often diagnosed only several months after artificial ventilation or performance of tracheostomy [10].

In the first half of the previous century the main cause for cicatricial stenosis of the respiratory tract occurrence were specific infectious diseases, such as diphtheria croup, tuberculous lesion, scleroma, syphilis, actinomycosis. In the middle of XX century the main etiologic factor in the formation of cicatricial stenosis became mechanical damage to the trachea [11].

Since the mid-1970s due to the development and wide use of ventilator and tracheostomy the leading etiological factor of the disease became iatrogenic damage to the trachea. With the aim of ventilator they used orotracheal and tracheostomy tubes with cuffs that exert pressure on the tracheal wall, which further leads to ischemia, necrosis and finally formation of scar tissue [12].

In some patients cicatricial stenosis of the respiratory tract develops in the absence of an obvious cause: in such cases it is classified as idiopathic. As a rule pathological process in such patients is localized in the subclavian part of the larynx and the cervical part of the trachea [13].

Today the main etiological factor of cicatricial stenosis development is mechanical damage of its wall as a result of trauma under various resuscitation measures and manipulations. Most often nowadays trauma to the trachea has iatrogenic nature and occurs during artificial ventilation through an intubation or tracheostomy tube, or as a result of complications during a tracheostomy or tracheal intubation [12].

According to the data of authors the highest percentage of complications (up to 18,5%) was observed in patients who underwent circular resection due to post-intubation tracheal stenosis [14].

This is related to the presence of chronical inflammatory process in tracheobronchial tree, presence of areas of tracheomalacia, impaired blood supply to the tracheal wall after its injury, including those outside the area of scar process, severe somatic status of patients after a long-term treatment in the intensive care unit. During interventions for idiopathic stenosis the percentage of complications is much lower (6,6%) in spite of the fact that during idiopathic stenosis laryngotracheal resections were also performed [15].

All the processes described above are more pronounced with post-tracheostomy stenosis. Having a history of tracheostomy is an unfavorable factor and it increases frequency of postoperative complications.

At present there is no unified classification of tracheal cicatricial stenosis. The variety of classification is related to the specialization of the author and provided data of surgical interventions. Thus for example in the earliest classifications proposed by otolaryngologists the main attention was paid to localization of scar process in relation to the vocal folds, their involvement to the scarring process. The extent of cicatricial changes has not received close attention due to low prevalence of radical resection operations [16].

A number of foreign authors offered classifications based on localization of cicatricial stenosis in relation to the tracheostomy: narrowing of the trachea, cranial tracheostomies, caudal tracheostomies, narrowing of the tracheal segment at the site of tracheostomy [17].

Otolaryngologists offered a classification which differentiates stenosis by the degree of narrowing of the airway lumen: 1 degree – up to 50% of trachea lumen, 2 degree – 51-70% of trachea lumen, 3 degree - 71-99% of trachea lumen, 4 degree – tracheal atresia. The classification described above does not reflect localization, extent of cicatricial changes; presence of a tracheostomy, areas of tracheomalacia. Nevertheless the provided classification is still most frequently used in the foreign practice [18].

Starting from the mid 60-s treatment of cicatricial stenosis of the trachea became the prerogative of thoracic surgeons, radical intervention came into practice, it allows to reach satisfactory trachea lumen within one operation– circular resection of the trachea. Correspondingly more attention was paid to the extent of cicatricial changes as a main factor which limits performance of resection.

The offered classification based on dividing of stenoses into primary ones caused by a pathological process in the wall of the trachea, and secondary ones caused by compression of trachea from outside. Stenoses were classified into long (more than 2 cm) and limited (up to 2 cm). The author also classified all stenoses by narrowing of trachea lumen into 3 degrees (I degree – narrowing by 1/3; II degree - from 1/3 to 2/3; III degree more than 2/3) [11].

This classification was improved and completed later, with cicatricial stenoses of the trachea classified by: etiology – post-resuscitation, post-intubation, post-tracheostomy, post-traumatic, postoperative, idiopathic; by localization - subfold department (with damage to folds, without damage to folds), cervical trachea, thoracic trachea, combined lesions; by the degree of narrowing - 1 degree (0,9- 0,7 cm), 2 degree (0,7-0,5 cm), 3 degree (less than 0,5 cm); by prevalence– limited (up to 2 cm), long (more than 2 cm); by anatomical form of the lesion - front-side walls, circular narrowing, atresia; by the condition of the walls of trachea: with tracheomalacia, without tracheomalacia; by the presence of a tracheostomy - with a tracheostomy, without a tracheostomy [6].

At present the most complete and convenient in practice are classifications of tracheal cicatricial stenoses with a transition from absolute to relative values, as the length of trachea, its lumen are individual for each patient and depend on anthropometric data, anatomical and physiological features of the body [1].

Stenoses are classified:

1) by etiology: post-intubation, post-tracheostomy, post-traumatic, idiopathic;
2) by localization: larynx (with damage to the subfold, vocal folds), cervical part of the trachea, upper thoracic, middle thoracic, suprabifurcation parts of the trachea, combined lesions;
3) by the narrowing degree: 1 degree (lumen narrowed by 1/3 of diameter of respiratory tract), 2 degree (from 1/3 to 2/3 of diameter), 3 degree (more than 2/3 of diameter);

4) by prevalence: 1 degree – less than 15% of all the trachea length of a particular patient; 2 degree – from 15 to 30%; 3 degree – from 30% to 60%; 4 degree – more than 60%.
5) by the anatomical form of the lesion: anterolateral wall, circular narrowing, atresia;
6) by condition of the walls of trachea: with tracheomalacia, without tracheomalacia;
7) by the presence of a tracheostomy - with a tracheostomy, without a tracheostomy [19].

Lack of a united generally accepted classification of tracheal cicatricial stenosis does not permit to assess reliably the frequency of complications and mortality after surgical interventions for tracheal cicatricial stenosis depending on etiology, localization, length of process, presence of a tracheostomy and areas of tracheomalacia in the patient.

DIAGNOSIS OF STENOTIC LESIONS OF THE TRACHEA

Tracheal stenosis is diagnosed on the basis of anamnesis, complaints of a patient, characteristic clinical picture, radiological and endoscopic examination data. A pathognomonic symptom which allows to judge on the narrowing of respiratory tract is noisy difficult breathing. However, stridor in cicatricial stenosis of the trachea must be differentiated from an attack of bronchial asthma, inspiratory stridor in paralytic and cicatricial stenosis of the larynx. The severity of the clinical picture depends on the degree of the trachea narrowing. Thus, at the I-II degree of narrowing – difficult breathing may arise only during physical exertion, and at III-IV degree – patients more often have stridorous breathing even at rest [19].

Patients complain on shortness of breath of various degrees of expressiveness, hoarseness, sputum which is difficult to evacuate. Stridor at tracheal cicatricial stenoses is mixed as a rule. In case of localization of pathological process in the cervical part of trachea inspiratory component prevails, in case of stenosis of thoracic trachea or in the presence of tracheomalacia – expiratory one [1].

During objective examination condition of a patient even with compensation of tracheal stenosis is unstable as far as asphyxia can occur when the preserved lumen is blocked by bronchial secretions. Breathing is conducted with the help of auxiliary muscles, position of a patient is forced. Retraction of the flexible muscles of the neck and chest is observed. Cough with a metallic hue and difficulty in expectorating sputum attract attention. Inspiratory shortness of breath, stridorous shallow breathing, cyanosis, and heart failure may occur [20].

The most frequent symptom of tracheomalacia is expiratory stridor, which is accompanied by respiratory attacks and cyanosis, which are aggravated during exertion. The given symptoms most frequently arise during 1-6 weeks after extubation, as far as within this time formation of scar tissue in the wall of the trachea with subsequent narrowing of the tracheal lumen takes place [20].

Today the most informative methods of instrumental diagnostics are X-ray, endoscopic methods of research and research of the function of external breathing. To assess the condition of external breathing function standard methods of research are used: spirography, pneumotachometry, and body plethysmography. Decrease of vital capacity of the lungs and speed indicators of forced breathing allow to diagnose tracheal narrowing of I degree, and also roughly determine the degree of tracheal stenosis. However, because of considerable change of bronchial resistance reliable relations between these indicators and the degree of tracheal narrowing cannot be identified [21].

Extremely inconvenient seems to be the examination of patients with a functioning tracheostomy. That is why indicators of the function of external breathing are better studied in patients which undergo rehabilitation after restoration of tracheal lumen [22].

Taking into account all the stated above these methods can be considered to be auxiliary in diagnostics of tracheal stenosis. In the algorithm of examination of patients with tracheal cicatricial stenosis an important place belongs to radiological diagnostics. At present leading role among radiological methods of diagnostics belongs to multispiral computed tomography with three-dimensional image reconstruction. The advantage of computed tomography is fact that it provides information not only on the degree and prevalence of airway lumen narrowing, but also on the condition of walls of trachea and peritracheal space [23].

Computer processing of the image with the help of special programs allows receiving a visual three-dimensional reconstruction of the image of trachea and pathological changes located in it [24].

Number of identified stenosis during multispiral computed tomography corresponds to the data of endoscopic examination. According to the data of authors axial and coronal images obtained during multispiral computed tomography tend to overestimation of stenosis degree, and virtual bronchoscopy and sagittal images tend to underestimation. The indisputable advantage of multispiral computed tomography is safety of the examination, therefore, multispiral computed tomography is the method of choice for patients with

subcompensated breathing with critical narrowing of the trachea [24].

Nowadays, magnetic resonance imaging is actively used in the examination of patients with tracheal stenoses. The technique allows conducting research of patients with subcompensated breathing, but unlike X-ray research methods during magnetic resonance imaging patient does not receive radiation exposure. Relative disadvantages of the research are related to the physical principles of the research: performing of MRI is absolutely contraindicated if patients have implanted pacemakers and other foreign bodies in the body that are made of magnetic materials. Moreover the possibilities of digital processing of the obtained image do not allow building three-dimensional reconstructions. Besides performing of MRI is rather long procedure possible only for patients with compensated and subcompensated stenosis [5].

The standard of diagnostics of stenotic lesions of respiratory tract nowadays is laryngotracheoscopy. Examination of mucosa of the trachea and larynx through an endoscope makes it possible not only to identify the pathology, to determine its degree of narrowing and extent, localization, but also permits to assess the severity of tracheobronchitis, carry out rehabilitation of tracheobronchial tree, to obtain material for bacteriological study. Results of cultures of bronchial secretions and determination of sensitivity of microorganisms to antibacterial drugs allow achieving high efficiency of the conducted antibacterial therapy and decreasing the risk of infectious complications [20].

In presence of critical narrowing of trachea diagnostic procedure can be transformed into a therapeutic manipulation – endoscopic expansion of the airway lumen [25].

SURGICAL TREATMENT OF TRACHEAL CICATRICIAL STENOSES WITH THE USE OF MINIMALLY INVASIVE INTERVENTIONS

Tracheal cicatricial stenosis is life-threatening disease characterized by replacement of normal structures of the tracheal wall with coarse scar tissue which narrows the respiratory tract lumen. For total recovery from this pathological state complex, traumatic surgical interventions are necessary. Many patients are forced to remain disabled, live with a tracheostomy, periodically visiting specialized medical institutions [26].

Nowadays due to achievements of modern medicine, anesthesiology and possibility of long resuscitation, lives of many most seriously ill patients with difficult independent breathing as well as patients in a state of asphyxiation can be saved. To achieve this goal various methods of artificial ventilation of lungs with the use of intubation or tracheostomy tubes are applied [26].

Tracheostomy is perceived as the method of choice for long-term artificial lung ventilation (ventilator) in critically ill patients. However if the rules of medical care for patients during connection to a ventilator are violated, defects of tracheostomy may cause no less life-threatening conditions later. One of such severe complications is tracheal cicatricial stenosis. Due to wide implementation of artificial ventilation of lungs into clinical practice and increase of general injuries number of patients with this pathology increases steadily [27].

Treatment of tracheal cicatricial stenoses at present remains one of the most complicated and not completely solved problems in surgery. Frequency of development of tracheal cicatricial stenoses after long-term artificial ventilation according to different authors varies from 0,1 to 25% on average equals to 2-3% [27].

Etiological factors and pathogenesis of tracheal stenoses are well studied. Nowadays the majority of all cicatricial stenoses are iatrogenic in nature. The main reason of increase of number of acquired tracheal stenoses is ever wider application in clinical practice of artificial ventilations of lungs through intubation or tracheostomy tubes. Ventilator performed through a naso- or orotracheal tube for more than 3 days, with a higher probability leads to persistent cicatricial stenoses and deformations of the trachea and larynx lumen [23].

The basis of pathogenesis of post-intubation and post-tracheostomy stenosis is trauma with inflatable cuff or the end of the tube of tracheal walls with further accession of infection. The trigger of the disease is tracheal wall damage and development of purulent-necrotic process in it [28].

The periods of time between extubation and the onset of symptoms of cicatricial stenoses vary from several hours to several months, but usually do not exceed 6 weeks. Later manifestation of stenosis symptoms is also possible. In clinical practice shortness of breath and coughing are often associated only with lung diseases and special methods of trachea study are not carried out. This is the reason for stenosis to be often diagnosed late [29].

The fact that first endoscopic intervention which is carried out in specialized institutions in many patients (more than 40%) is performed as an emergency, quite often in the first hours, proves the importance of timely diagnosis of the disease at the pre-hospital stage [29].

Instrumental methods of diagnostics comprise radiological, endoscopic and studies of the function of external breathing. The main tasks of these methods are determination of stenosis localization, its extent,

condition of the larynx above and below stenosis, general condition of a patient [3].

Role and place of each method is identified individually depending on the severity of a patient's condition, the level of equipment of a medical institution and training of medical personnel.

In emergency situations when a patient has stridor at rest or with a slight physical exertion the method of choice is diagnostic laryngotracheoscopy. If necessary, diagnostic examination can be transformed into endoscopic operation. In the shortest period of time and with a minimum trauma with the help of special techniques it is possible to restore the lumen of respiratory tract in majority of patients. Such tactics allows refusing from emergency surgical operations accompanied with high mortality or unjustified tracheostomies, which later complicate treatment and worsen the prognosis. The decisive method of diagnostics of tracheal cicatricial stenoses is endoscopic one [30].

Laryngotracheoscopy allows not only to detect stenosis but also evaluate the mucous membrane of larynx and tracheobronchial tree, degree of expressiveness of its inflammatory changes, with the help of endoscopic ruler measure in millimetres the diameter of the narrowed area and determine the degree of stenosis, determine its length with accuracy up to 1 cm, distance to the main anatomical landmarks (bifurcation of the trachea, vocal folds, tracheostomy) [16].

Besides in the postoperative period it is possible to evaluate the healing process of the tracheal anastomosis with the help of tracheobronchoscope and determine the effectiveness of operation treatment [14].

The main method of tracheal cicatricial stenoses treatment is surgical one. The only radical operation which allows restoring airway patency and removes scar tissue is circular resection of trachea with intertracheal anastomosis. Other operations including endoscopic ones are considered to be palliative, and indications for them must be reasonable and restricted. Nevertheless resection of trachea is rather complicated and traumatic intervention, for this reason it requires a great experience from a surgeon. Number of possible complications according to the authors, Murgu S. et al., may reach 15%, and postoperative mortality may vary between 1,5 and 12,4% [24].

For part of patients such operations are impossible or not indicated due to prevalence of scar changes, severity of the condition, high level of operation risk or anatomic peculiarities. One of conditions for their performing is absence of tracheostomy at the time of operation [16].

Wide application of endoscopic method of treatment has given a chance to refuse from emergency operations, and this in turn decreases the frequency of post operation complications and total mortality. Providing quick and adequate restoration of the airway patency, the method allows liquidating chronic hypoxia, which creates favorable conditions for the scheduled examination and choice of optimum tactics of treatment.

One of the first interventions performed on the patient with decompensated tracheal cicatricial stenosis is emergency restoration of airway patency. To carry out this task various endoscopic methods can be applied. They can be classified into two big groups:

- Methods aimed at removing part of scar tissue from the trachea lumen (mechanical, physical).
- Methods aimed at expansion of the narrowed part of trachea.

Physical methods of influence are based on the use of properties of different types of radiation or high-frequency currents to destroy biological tissues. These methods can be applied in two modifications – to destroy scar-granulation tissues in a circle or their dissection along [3].

The main advantage of electrosurgical method lies in the fact that thermal tissue damage is minimal and general availability and low cost of equipment makes it the most attractive method. Serious disadvantages comprise the presence of contact of the electrode with tissues, as a result of which carbon deposits and welding of the electrode occur, which can lead to bleeding. At the application of all the physical methods of destruction of scar-granulation tissues there is high probability of damage to adjacent unchanged areas of trachea. Later it will inevitably lead to development of inflammatory process, which in turn leads to involvement of ever more tissues to the scarring process and can have negative impact on the results of treatment [4].

The safest ways in this regard are methods aimed at expansion of the stenosis - bougie and balloon dilatation. These interventions in patients with critical tracheal stenosis are usually performed first. The most available method of one-time expansion of lumen in patients with cicatricial stenosis is bulging with tubes of a rigid bronchoscope. As a result of such influence local rupture of scar occurs. Consistently using tubes of increasing diameter, one can most quickly (within 0.5-1 minute) and quite effectively restore the lumen of the respiratory tract with the evacuation of the secretion accumulated in the substenotic department. This operation can be performed with plastic bougie and a set of intubation tubes put on the fiberscope. In the presence of tracheostomy bougie or tubes are sometimes passed through the tracheostomy hole under local anesthesia [2].

Currently, expansion of the narrowed area before the introduction of endoprostheses is successfully used [30].

According to some authors balloon dilatation allows restoring the trachea lumen with minimum trauma to the mucous membrane. Apart from temporary expansion of the narrowed part of trachea this method can be applied as an independent method of treating scar stenosis after lung transplantation, circular resection of trachea, radiation therapy [31].

An important advantage of operations of one-time endoscopic expansion of tracheal cicatricial stenoses is the fact that they allow quickly and effectively expand the lumen, eliminate ventilation disorders, and stabilize the patient's condition, at the same time trauma to the intact parts of the trachea is minimal [25].

A significant drawback of the method is unstable effect of the treatment with development of restenosis in a considerable part of patients. So as an independent method of treatment it is applied only in a restricted number of patients according to strict indications. «Bright» period lasts from several hours to several months, most often 7-14 days. Then regardless of the method of endoscopic influence on the scar tissues in a considerable part of patients the lumen of the trachea narrows again. The best results were obtained only when conducting repeated expansion of membrane-like stenosis [3].

At present universal method of restoring airway patency, it forces specialists to resort to combined methods. For example, photodestruction, electrocoagulation or mechanical removal of tissues supplemented with bougie or balloon dilatation. Opinions of specialists regarding application of the endoscopic methods mentioned above in emergency situations differ. Thus some authors believe that the only way to help with decompensated stenoses in seriously ill patients is emergency tracheoscopy with recanalization of the trachea using a laser, they emphasize that endoscopic laser interventions are simple, effective and safe way of restoring tracheal patency [30].

Other researches note that in emergency situations, in the absence of a tracheostomy, when hypoxemia increases after anesthesia, only the tube of a rigid bronchoscope can be used to quickly expand the lumen and restore adequate breathing [15, 22].

Thus different authors apply different methods of endotracheal impact on scar tissue. Each one chooses one or several most convenient ways of restoring airway patency. The choice of the method is affected by the personal experience of a doctor, as well technical capabilities of a clinic as for a specific clinical situation.

The most important and not fully solved problem for today in the surgery of cicatricial tracheal stenoses is maintaining the lumen of the respiratory tract at a level sufficient for adequate breathing. For provision of this task cylindrical structures (stents) are installed in the lumen of the trachea for a long time, preventing its narrowing [20].

The choice of method and way of installation depend on localization of stenosis, presence or absence of tracheostomy, general condition of a patient and personal experience of a doctor. If the situation regarding the severity of a patient's condition allows determining further tactics during the day, then in order to provide reliable patency of the respiratory tract an orotracheal intubation tube is used [2].

In the presence of tracheostomy and low localization of stenosis a split section of thermoplastic intubation tube, extended tracheostomy cannula or T-tubes are used. With "immature" cicatricial stenoses, some authors leave the tube in the trachea for 3-4 months, during which the scar tissue matures in the form of cicatricial compaction of the walls of the trachea itself, which keeps its lumen from collapsing [17].

In the absence of tracheostomy it is advisable to use endoprosthesis. In case of emergency when the stenosis is localized in the cervical and upper thoracic parts of the trachea, the most accessible method was previously considered to be the placement of a tracheostomy below the narrowing and the introduction of a tracheostomy tube. If the narrowing was localized in the middle or lower third of the thoracic part of the trachea, when the length of conventional tracheostomy tubes was insufficient, extended tracheostomy tubes were used. In the absence of tracheostomy tube of the required length sometimes individually split section of thermoplastic tube was sometimes used. Advantage of such constructions lied in the fact that being individually fitted they as a rule did not damage the trachea walls, they were easy to pull out and insert again. However, despite the obvious advantages, all the methods had a significant drawback - the need for the imposition and long-term support of a tracheostomy, which significantly worsens the patient's quality of life and the conditions for performing a subsequent surgical operation [9].

The introduction into clinical practice of prostheses completely located in the trachea, as well as the development and improvement of methods of their installation using endoscopic techniques allowed in many cases to completely refuse from emergency open operations. Nowadays majority of researches believe that in the absence of tracheostomy it is more appropriate to use endoprosthesis [11].

Prosthetic trachea as an organ has been developed for many years all over the world. Those applied at the first stages with the goal of replacing the cartilage framework, grafts, as well as monolithic, fine-porous, mesh

and combined alloprostheses caused granulation and cicatricial stenosis. All the prostheses were inserted into trachea during open surgical operations [19].

Requirements for all the endoprostheses are as follows: non-toxicity, biological inertness, stability in body tissues, sufficient rigidity, strength, elasticity and flexibility, sufficient drainage function, which allows to remove mucus from the respiratory tract, impermeability to liquid, air and bacteria, easy availability and possibility of sterilization [15].

After inserting endoprostheses in all the patients at the beginning (from several days to weeks) problems may arise related to the presence of foreign body in the trachea lumen – reflex cough and increased tracheobronchial secretion. Inner surface of the prostheses contributes to the deposition of sputum, due to this obturation of the lumen may occur. This process can be prevented when using aerosols with regular mechanical removal of overlays during tracheoscopy. Tracheoscopies with sanitation must be performed regularly, and during the first once every three months. Due to the contact of foreign body with the trachea wall granulomas can be formed. In case of their detection granulomas must be removed mechanically or with the help of laser and electrocoagulation. With recurrent granulomas brachytherapy can be used [30].

It is known that dilatation of tracheal cicatricial stenoses with the help of stents allows not only temporarily restore adequate patency of the airways and prepare a patient for the planned operation but also contributes to the formation of reliable fibrous framework around the protector [4].

Nowadays in literature (Bourinet V et al.) there is no consensus as for how long a stent should be kept in the area of stenosis for the lumen to be formed. Authors believe it to be appropriate for endoprosthesis to stay in the trachea for approximately 4 months [15].

There is a report where the duration of endoprosthesis in the tracheal lumen varied from 5 to 24 months. Remote results are traced back to 1 to 5 years. After removal of the stent stable positive (good and satisfactory) results were obtained in 13 patients, unsatisfactory ones in 9 patients. In all the patients with localization of stenosis in the cervical region, as a result of the treatment, a lumen sufficient for breathing was formed. When the stenosis was localized in the chest such a result was obtained in only 1 patient out of 10. Out of this the author makes a conclusion that temporary insertion of a stent of different construction for cicatricial stenosis in the cervical part of trachea allows to achieve stable effect in all the operated patients but for stenosis localized in the thoracic area this variant of treatment is of low efficiency. Thus results of long-term dilatation of cicatricial stenosis on the stents are controversial. The range of positive results is very wide and includes from 20 to 94,6% [30].

This condition must be related to the lack of uniform criteria in the selection of patients and insufficiently large number of patients in whom the technique of treatment by dilatation on the endoprosthesis was used. Besides it should be noted that in the literature researches completely divide the results of treatment with tubular structures which are fully inserted into the tracheal lumen, and therapeutic tracheostomy tubes that require a functioning tracheostomy, which is fundamental.

CONCLUSIONS

Summing the information obtained by the data of the literature review, we can state the lack of generally accepted concept of application of mini-invasive interventions for tracheal stenosis. In the available literature there are no clearly formulated indications and contraindications for their application.

In the publications in this filed there are no researches devoted to the comparative analysis of endoscopic and traditional surgical interventions for of tracheal stenosis. All of it dictates the necessity of conducting further researches in this important section of clinical surgery.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

From efficacy to adversity: understanding the side effects of antidepressants – systematic review

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ABSTRACT

Aim: To investigate how and why antidepressant side effects occur, in order to use them effectively in clinical practice

Materials and Methods: We have studied modern literary sources on the topic of side effects of antidepressant and presented in the form of a literature review in this article. Electronic Scopus and Pubmed databases were searched for articles on the studied topic. The review included original articles, research, and official recommendations from medical associations.

Conclusions: Side effects of antidepressants can reduce treatment adherence and delay recovery. Therefore, it is extremely important to consider possible side effects when choosing a therapy. While there is no perfect antidepressant that works quickly and is completely free of side effects, newer antidepressants are safer, better tolerated, and associated with lower rates of treatment failure. Most side effects occur as a consequence of the basic mechanism of action of the drugs. Therefore, it is important to understand the neurobiology of side effects, their frequency and risks, ways to prevent them or use them to your advantage resources.

KEY WORDS: depression, anxiety disorder, tolerance, side effects, antidepressant

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INTRODUCTION

Antidepressants are among the most commonly prescribed medications. Studies show that the use of antidepressants has been increasing rapidly in recent decades. In some countries, the use of these medications has more than doubled over the past few years. One in ten US residents over the age of 12 takes antidepressants, and two-thirds of them do not have symptoms of depression [1-3]. Anxiety and stress-related disorders, phobias and eating disorders, functional disorders of the gastrointestinal tract and urination, numerous pain syndromes (including migraine, neuropathic and chronic pain), and sleep disorders are not a complete list of indications for the use of antidepressants. Despite the fact that these medications are the third most frequently prescribed and the fourth best-selling, there are some discussions and doubts about the effectiveness and clinical relevance of antidepressants in treating depression symptoms [2,4].

The well-known psychopharmacologist Stephen Stahl noted in this regard: "Whatever the reason for the controversy over efficacy in research, even a short time spent in clinical practice convinces us that antidepressants are powerful therapeutic agents for many patients" [5]. Large-scale meta-analyses in recent years agree with this view and show that antidepressants are more effective than placebo, and that the lack of clinical effect is more likely to be due to imperfect patient selection and poor adherence to treatment [6].

Although modern antidepressants are quite effective and well tolerated by patients, the lack of compliance and the frequency of premature discontinuation of treatment are worrying. Up to 70% of patients have problems with treatment adherence, one third will stop taking antidepressants within the first month and almost half within the first three months [7]. In general, treatment discontinuation is more likely with tricyclic antidepressants (as high as 44%) and serotonin-norepinephrine reuptake inhibitors, although in studies of safer selective serotonin reuptake inhibitors, almost one in four patients discontinue treatment [6].

Despite the emergence of newer, more selective and pharmacologically engineered drugs, antidepressants are still far from perfect. In particular, it takes at least two weeks of use to achieve the desired effects, and side effects occur almost immediately. This feature contributes to a loss of compliance, leading to premature discontinuation of treatment, which in turn makes it

impossible to achieve a clinical effect. It is in the physician's power to anticipate adverse events, convince the patient that they are benign or temporary, and thereby improve adherence to treatment.

The purpose of this article is to familiarize the reader with the current understanding of how and why antidepressant side effects occur, in order to use them effectively in clinical practice. Most studies consider side effects of drugs from a clinical or statistical point of view, but we will try to consider the neurobiological aspects of these effects to better understand the reasons for their occurrence and thus the possibility of preventing, reducing or even using them to the benefit of the patient.

AIM

To investigate how and why antidepressant side effects occur, in order to use them effectively in clinical practice

MATERIALS AND METHODS

We have studied modern literary sources on the topic of side effects of antidepressant. The studied material is summarized and presented in the form of a literature review in this article. The search for literary sources was carried out in two main scientific databases: Scopus and PubMed. The review included original articles, research, and official recommendations from medical associations.

REVIEW AND DISCUSSION

1. ANTIDEPRESSANTS: THE EFFECT AND ITS SHADOWS

The general pathway of antidepressant action can be represented as blocking transporters or reuptake of serotonin, norepinephrine and/or dopamine, which leads to an increase in their levels in the synaptic cleft and prolongation of their effect on postsynaptic terminals. In the 50s of the last century, the antidepressant effect of iproniazide and the "father of tricyclic antidepressants", imipramine, was discovered [8]. In the 1960s, Glowinski and Axelrod showed that these drugs could inhibit presynaptic norepinephrine reuptake [9]. Since then, the "monoamine hypothesis of depression" and the synthesis of molecules that affect the level of certain monoamines have been developing. The synthesis and approval of fluoxetine in 1987 marked the beginning of the explosion of a new generation of antidepressants with selective serotonin reuptake inhibition. The absence of significant effects on other receptors and electrolyte channels, unlike the first generation, significantly improved the safety profile and tolerability of the new drugs.

The monoamine hypothesis in its original form is significantly simplified, as an important factor is not just an increase in the level of certain transmitters, but also the adaptation of the number and sensitivity of receptors due to gene expression, followed by deeper neuroplastic changes. These mechanisms explain the aforementioned feature of antidepressants: mediator levels increase relatively quickly and immediately cause side effects, as it takes time for receptor regulation, gene expression, and neural circuitry to achieve the desired effects [5].

In one of the first large-scale meta-analyses of antidepressant tolerability, which included a review of 84 clinical trials, the authors identified more than fifteen adverse effects and grouped them by frequency of occurrence [10] (Table 1).

Headache, tremor, and hypotension were the most nonspecific and occurred equally frequently regardless of the drug being taken. The second group of side effects was more common among patients taking tricyclic antidepressants: dry mouth, constipation, diplopia, dizziness and palpitations. Symptoms such as nausea, loss of appetite, anxiety, anxiety and insomnia were more common with selective serotonin reuptake inhibitors.

Most side effects can be logically explained by the chemical structure of the drugs and their effect on certain receptors (Fig. 1, Table 2).

Tricyclic antidepressants have the widest range of side effects due to their multimodal effects on cellular structures (Fig. 1). The blockade of M_{1-} acetylcholine receptors causes dry mouth, constipation and urinary retention, diplopia, and, in part, sedation. A powerful, rapid sedative effect, weight gain, and prolongation of the QT interval are the consequences of blocking H_1 -histamine receptors. Effects on voltage-gated sodium channels cause cardiotoxicity, cardiac dysrhythmias, or even death in case of overdose. Orthostatic hypotension is caused by blockade of α -adrenoceptors.

Interestingly, paroxetine, the only SSRI that blocks muscarinic receptors (M_3), has a more pronounced sedative effect in its class and a greater tendency to increase weight. On the other hand, the R-enantiomer of citalopram affects H_1 -histamine receptors, so in high doses it is more dangerous in terms of QT prolongation compared to other SSRIs. The isolation of the S-enantiomer into a separate drug (escitalopram) weakens the antihistamine effect, making this molecule a "perfectly selective" serotonin reuptake inhibitor [5].

No matter how selective modern antidepressants are, it is stated that 80% of patients experience at

Table 1. The most frequent side effects of using "monoamine" antidepressants ("atypical" antidepressants are discussed in the text)

Class of antidepressant	Mechanism of action	Side effects
Tricycle (TCA)	Blockade of serotonin, norepinephrine, H_1 -histamine receptors, α_1 -adrenergic receptors, M_1 -cholinergic receptors, voltage-gated Na^+ channels	Cardiotoxicity, dry mouth, constipation and urinary retention, diplopia, orthostatic hypotension, drowsiness and sedation
Serotonin reuptake inhibitors and norepinephrine (SNRI)	Blockade of serotonin and norepinephrine reuptake	Nausea, sexual dysfunction, increased sweating, arterial hypertension
Selective serotonin reuptake inhibitors (SSRIs)	Serotonin reuptake blockade	Nausea, loss of appetite, sexual dysfunction

Table 2. Side effects of antidepressants depending on the mechanism of action

Mechanism of action	Clinical effect
Serotonin reuptake blockade	Nausea, diarrhea Loss of appetite at the beginning of treatment and weight gain later Decreased libido, anorgasmia Sedation, insomnia, increased anxiety Serotonin syndrome
Blockade of the norepinephrine transporter	Increased blood pressure Anxiety and tremors Tachycardia Increased sweating
Dopamine reuptake blockade	Psychomotor agitation, akathisia Provoking psychosis Parkinsonism
H1 histamine receptor blockade	Drowsiness and easier falling asleep Sedation and anxiolytic effect Increased risk of falls in old age Weight gain
Blockade of muscarinic acetylcholine receptors	The duality of the gaze Sedation, memory and cognitive impairment Dry mouth, constipation Sinus tachycardia Urinary retention Exacerbation of angle-closure glaucoma
Blockade of α1 adrenergic receptors	Orthostatic (postural) hypotension and dizziness Reflex tachycardia Strengthening the effect of antihypertensive drugs

Table 3. Monoamine receptors regulating sexual function (adapted from [26])

System	Receptor	Effect on sexual function
Dopamine	D/D_{12}	Activation
Serotonin	5-NT 1A	Activation (indirect effect through activation of D2 receptors)
	5-NT 1B/1D	Suppresses
	5-NT 2A	Suppresses
	5-NT 2C	Suppresses

least one side effect, and almost every second patient experiences several side effects at the same time. The most frequent side effects of serotonin reuptake inhibitors are, so to speak, a shadow of the main effect. An increase in serotonin levels leads to activation of serotonergic neuronal receptors, a gradual increase in neurotransmission of mediators by these neurons leads to an antidepressant or anxiolytic effect, but activation of "undesirable" receptor subtypes by serotonin causes side effects [11-12] (Fig. 2).

The mechanism of action of antidepressants of other groups is mostly associated not only with the blockade of serotonin and/or norepinephrine transporters, but also with the direct effect on the receptors by the molecule itself. For example, trazodone, in addition to blocking serotonin reuptake, directly blocks 1A/2C sero-

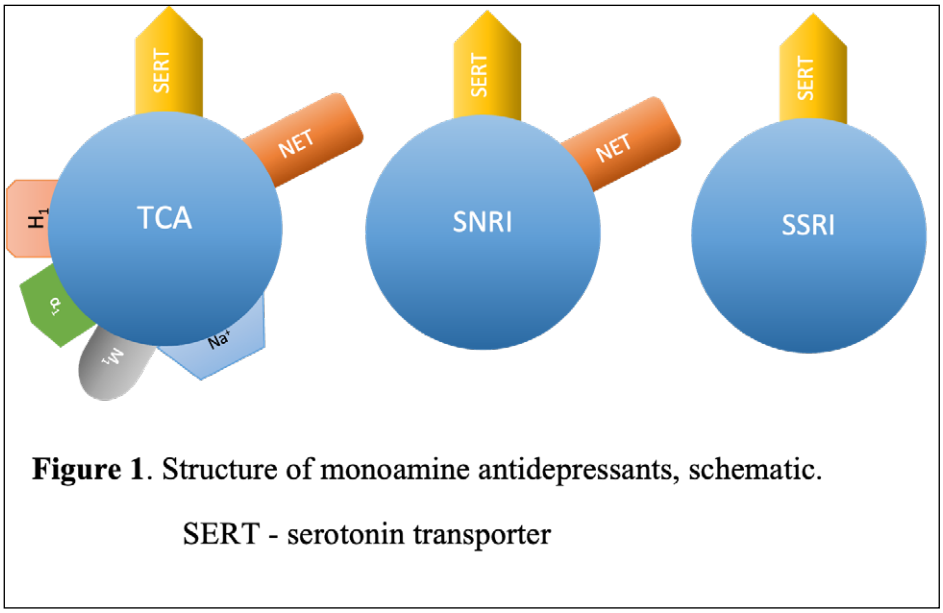

Figure 1. Structure of monoamine antidepressants, schematic.

SERT - serotonin transporter

Fig. 1. Structure of monoamine antidepressants, schematic. SERT - serotonin transporter. NET - norepinephrine transporter.

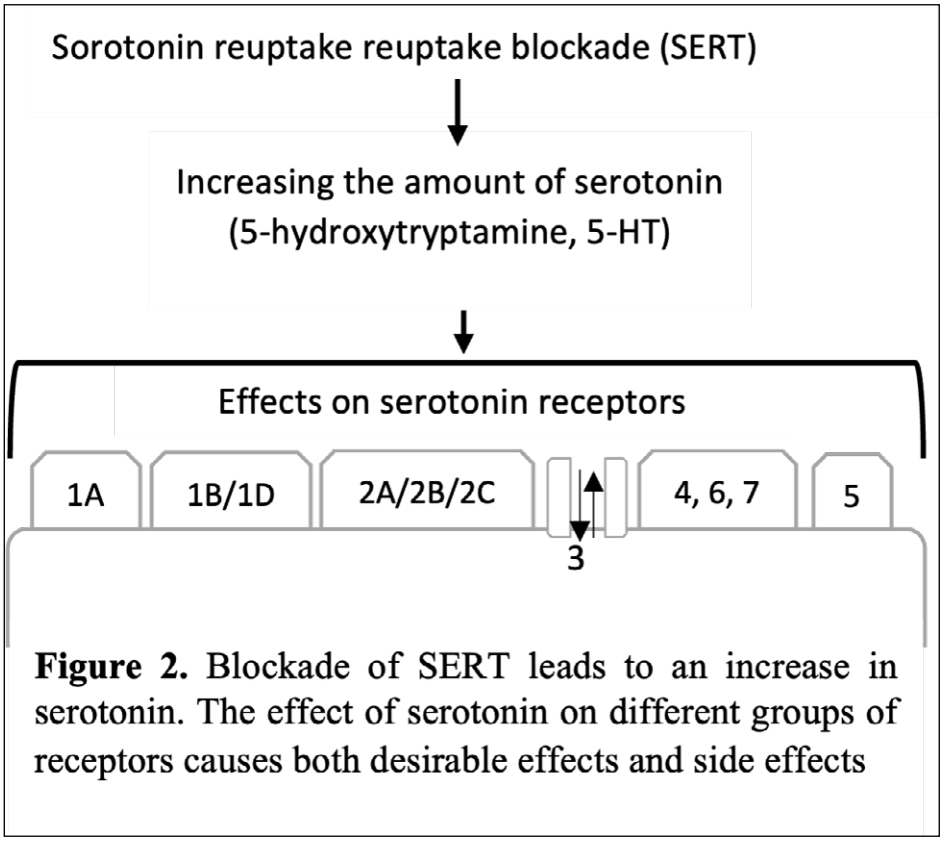

Figure 2. Blockade of SERT leads to an increase in serotonin. The effect of serotonin on different groups of receptors causes both desirable effects and side effects

Fig. 2. Blockade of SERT leads to an increase in serotonin. The effect of serotonin on different groups of receptors causes both desirable effects and side effects.

tonin receptors, as well as α_1 -adreno- and H_1 -histamine receptors, which is the cause of, among other things, hypnotic and anxiolytic effects. Agomelatine is a melatonin receptor agonist and a serotonin receptor antagonist of type 2A/2C, which determines a good hypnotic effect and a mild antidepressant effect. Mianserin and mirtazapine do not affect monoamine transporters at all, but block α_2 and H_1 receptors: the first mechanism provokes the release of norepinephrine and stimulation of serotonergic neurons, the second is already known to improve sleep and reduce anxiety [13].

The effects of these "atypical" antidepressants and the possibilities of using their side effects to your advantage will be discussed in more detail below. We will start with the side effects that are the most common causes of treatment failure and loss of compliance

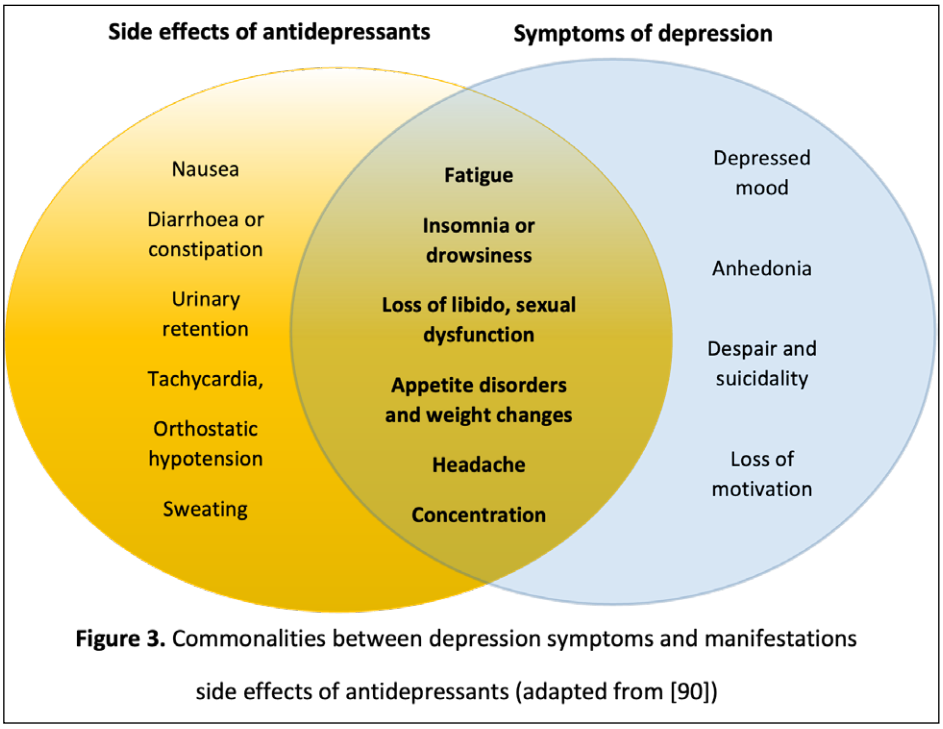

Figure 3. Commonalities between depression symptoms and manifestations side effects of antidepressants (adapted from [90])

Fig. 3. Commonalities between depression symptoms and manifestations side effects of antidepressants (adapted from [40]).

Table 4. Frequency of sexual dysfunction during treatment with different antidepressants (adapted from [29])

	Summary frequency of sexual dysfunction		
	< 30 %	**10-30%**	**> 30 %**
Antidepressant	Agomelatine Mirtazapine Bupropion	Escitalopram Duloxetine Venlafaxine	Paroxetine Sertraline Fluoxetine Fluvoxamine

GASTROINTESTINAL EFFECTS AND NAUSEA

Unpleasant gastrointestinal symptoms are among the most common complaints when taking antidepressants, especially at the beginning of treatment (occurring in more than a third of patients).

Dry mouth and constipation are characteristic of all tricyclic antidepressants and high doses of paroxetine, which is a consequence of a decrease in secretion and slowing of peristalsis (M-cholinergic receptor blockade). The most pronounced anticholinergic effects are inherent in amitriptyline, imipramine, and doxepin, with somewhat lesser effects in nortriptyline and desipramine [14,15].

Nausea is one of the first unwanted symptoms of increased serotonin levels and is common during the initial period of treatment with all selective serotonin reuptake inhibitors and serotonin/norepinephrine reuptake inhibitors. Activation of serotonin receptors of the third type (5-HT3) both in the central nervous system (postrema area) and in the periphery (parasympathetic gut terminals) is a key pathophysiological mechanism of nausea and vomiting. These effects are temporary and dose-dependent: gradual desensitization of 5-HT3 receptors (up to 2 weeks) reduces the negative gastrointestinal effects of serotonin, and their blockade is used, for example, to relieve nausea and vomiting in the postoperative period or during chemotherapy [16].

Sertraline, fluvoxamine, and duloxetine cause nausea and transient diarrhea somewhat more frequently than other antidepressants [17]. Accordingly, a simple recommendation to reduce the dose or take the medication with food is usually sufficient to reduce these side effects.

EFFECTS ON APPETITE/EATING AND WEIGHT

Transient loss of appetite is relatively common at the beginning of treatment with serotonergic medications, although it is not as threatening a side effect as weight gain or metabolic syndrome. Along with nausea, weight gain is one of the most common reasons for discontinuing antidepressant treatment [18]. Increased appetite and weight gain with long-term antidepressant use is

associated with blockade of 5-HT2C and H_1 receptors, while their activation (as well as 5-HT1A, 1B) leads to loss of appetite.

Studies show that weight gain with prolonged use of SSRIs and SSRIs (6-36 months) is observed in half of patients, with the vast majority of patients showing an increase of more than 7% compared to the start of therapy. Escitalopram, paroxetine, sertraline, duloxetine, venlafaxine, and mirtazapine showed the most significant effect, and fluoxetine showed the least [19]. Independent risk factors are low body mass index and a family history of overweight.

There is evidence that antidepressants can provoke the development of metabolic syndrome or diabetes mellitus [20]. While monitoring of metabolic changes has become routine with modern antipsychotics, it is often ignored for antidepressants. Another negative fact from the point of view of clinical management is that weight gain may be delayed. On the other hand, weight gain at the beginning of antidepressant treatment is a reliable predictor of further weight gain [21].

As for other classes of antidepressants, blockade of the H_1 receptor is a powerful additional mechanism for increasing appetite and weight gain, which is typical, for example, for tricyclic antidepressants [21].

The above mechanisms can be used to correct loss of appetite or to increase body weight. Mirtazapine, as a blocker of 5-HT2C and H_1 receptors, is the drug of choice in this case [22]. In children and adolescents with eating disorders, fluoxetine (direct 5-HT2C antagonism, but without effects on H_1) is often recommended due to the availability of a larger evidence base and the absence of antihistamine side effects [23].

SEXUAL DYSFUNCTION

Reduced libido, erectile dysfunction, and delayed ejaculation are common side effects of antidepressants, especially serotonin reuptake inhibitors. Deterioration in quality of life and treatment refusal are much more common among patients with adverse sexual dysfunction [24]. It is worth noting that loss of libido is also one of the characteristic symptoms of depressive disorder, so it is often ignored by many clinicians and is not associated with treatment [25].

In order to understand the cause and ways of correcting sexual disorders when using monoamine antidepressants, it is worthwhile to understand the basics of physiology and neuropharmacology of sexual function (Table 3).

Sexual desire is a complex phenomenon that is controlled by hormones (testosterone and estrogens) and neurotransmitters, mainly dopamine. It is dopamine that increases sexual desire by activating the mesocorticolimbic and tuberoinfundibular pathways. Selective serotonin reuptake inhibitors suppress libido by blocking dopaminergic neurons in the ventral tire area, which give rise to the mesocorticolimbic pathway [27].

Sexual arousal is impossible without the activation of the autonomic nervous system, the release of acetylcholine and nitric oxide (NO) as vasodilatory agents that stimulate blood flow to the genitals. Elevated serotonin levels and anticholinergic effects block NO release, smooth muscle relaxation, and, consequently, arousal [5,27].

Orgasm is caused by activation of descending spinal noradrenergic pathways and sympathetic innervation of the genitals. Dopamine also enhances orgasm. In contrast, activation of descending serotonergic pathways, presumably through 5-HT 1B/2A/2C receptors, suppresses orgasm and slows ejaculation. It is worth noting that serotonin 1A receptor agonists do not have a negative impact on sexual function, as they are often also D2 dopamine receptor agonists.

The overall incidence of sexual dysfunction varies considerably, depending on study design, sponsorship or conflict of interest, control methods (self-report or standardized questionnaires), etc. . Interestingly, the incidence of anorgasmia as a side effect of antidepressants is almost the same in men and women [28].

To understand the general trends, it is convenient to use the adapted data from Kennedy et al. (2007) (Table 4).

Many prospective multicenter studies have confirmed that paroxetine is the most commonly used medication for sexual dysfunction, followed by other SSRIs, duloxetine and venlafaxine, and even less commonly by bupropion and mirtazapine. In the study by Serretti and Chiesa (2009), agomelatine, bupropion, and mirtazapine showed a level of sexual dysfunction comparable to placebo [30].

Trazodone stands out significantly in the issue of sexual dysfunction, as it can be used specifically for its correction. Antagonism of the 5-HT 2A/2C receptor (see Table 3) improves libido and arousal, while adrenolytic properties (α1-antagonism) improve erectile function due to smooth muscle relaxation and improved blood flow [31]. In addition to trazodone, bupropion (inhibits norepinephrine and dopamine transporters), sildenafil (potentiates the peripheral action of NO), amantadine (dopamine receptor agonist), or a change of therapy to an antidepressant with a lower incidence of relevant side effects (bupropion, mirtazapine) are also often used to correct sexual dysfunction provoked by antidepressants. The choice of an "antidote" usually depends on the antidepressant that provoked the side effect, the

immediate type of dysfunction, the patient's preferences, and the clinician's clinical experience.

In some cases, antidepressants can be used as symptomatic therapy for sexual dysfunction in patients without depressive disorder or other indications for these drugs. Activation of 5-HT2C and stimulation of 5-HT1A receptors is stated to be one of the causes of anorgasmia and prolonged ejaculation time in patients taking serotonergic antidepressants. This side effect is used as a therapeutic one, in particular, for premature ejaculation: SSRIs (paroxetine, sertraline), duloxetine, or even clomipramine (as the most serotonergic TCA) are frequent off-label prescriptions in such cases [32].

COMMON CNS SIDE EFFECTS INCLUDE DROWSINESS OR INSOMNIA, ANXIETY, AGITATION, OR, CONVERSELY, SEDATION

As already mentioned, blockade of H_1 histamine and muscarinic acetylcholine receptors (Table 2) causes sedation, drowsiness and facilitates sleep when using many antidepressants, in particular tricyclics. Doxepin has the most pronounced antihistamine effect among all members of the class. Among SSRIs, paroxetine has a slightly greater sedative effect (anticholinergic effect). Other antidepressants that are often used in insomnia and have a powerful "hypnotic" effect are characterized by their effect on H_1 receptors: mianserin, mirtazapine and trazodone. [5]

Agomelatine is somewhat different in terms of its mechanism of hypnotic effect. Agonism of MT_1 and MT_2 melatonin receptors, as well as blockade of 5-HT2C receptors in the hypothalamic suprachiasmal nucleus (which is a brain pacemaker) leads to normalization of the sleep-wake cycle without severe daytime sleepiness and other side effects inherent in antihistamine antidepressants [33].

Insomnia and increased anxiety are fairly common complaints at the beginning of the use of selective serotonin reuptake inhibitors. These effects are mostly temporary and dose-dependent. [34]

CARDIOVASCULAR COMPLICATIONS, BLOOD PRESSURE AND BLEEDING RISK

The risks of antidepressant use in relation to the cardiovascular system have been well known since the widespread use of tricyclics. Of course, the effect on electrolyte channels, adrenergic and cholinergic systems cannot be absolutely safe, and such threatening complications as Q-T interval prolongation and arrhythmias have become a significant limitation to the use of "old generation" antidepressants. Blockade of α-adrenergic receptors with tricyclics often provokes orthostatic hypotension and reflex tachycardia (also typical of trazodone).

Modern selective serotonin reuptake inhibitors are considered safer, although cases of the same side effects have been reported, mostly with high doses. There is a recommendation to perform an ECG in all patients before using antidepressants. Particular attention is paid to elderly patients, as well as during titration and dose increases. [35]

Hypertension, potentiation of pressor effects, and provocation of hypertensive crises have been described as side effects of serotonin and norepinephrine reuptake inhibitors. Venlafaxine has potentially the most unfavorable profile in this group of effects, requires constant blood pressure monitoring and should be used with caution in patients with hypertension [36].

Over the past few decades, there have been reports of an increased risk of bleeding with selective serotonin reuptake inhibitors [37]. A study of the United Kingdom general practice database showed that antidepressants with serotonergic effects significantly increase the risk of gastrointestinal bleeding. The highest risk was observed with sertraline, escitalopram and venlafaxine, which was at least 2 times higher than in the control group. Concomitant use of NSAIDs or steroids doubles the risk, while the use of antacids or proton pump inhibitors has a protective effect. According to the results of this study, the risk of upper gastrointestinal bleeding among patients taking SSRIs is: 1 case per 2000 patients treated, while concomitant use of NSAIDs increases this rate to 1 case per 250 patients, and low-dose aspirin - 1 case per 500 [37].

INSTEAD OF CONCLUSIONS: BEYOND NEUROBIOLOGY

The effect on the serotonergic system, norepinephrine and dopamine, on numerous receptors in the nervous system and beyond determines both the positive therapeutic effect of antidepressants and their side effects. Even the most selective serotonin reuptake inhibitors have numerous side effects that are directly related to their underlying mechanism of action. Perhaps the "antidepressant of the future" should only affect certain receptors without a general increase in mediator levels or impact on "side targets," but even in this seemingly ideal case, in such a holistic system as the brain, imbalances in neural circuits and pathways appear, which inevitably lead to undesirable consequences [38].

Unfortunately, the level of evidence and the ability to translate "direct" research findings into practice is limited by several factors. First of all, many symptoms of the underlying

disorder (e.g. depression) and clinical manifestations of side effects are very similar [39]. Sometimes it is difficult to distinguish between patient complaints and classify them as side effects or symptoms that existed before treatment (Fig. 3).

One of the most unpleasant phenomena in pharmacotherapy is the nocebo effect [40]. Negative expectations of the patient regarding treatment, often based on previous experience, as well as somatic symptoms mimicking side effects, general level of anxiety, somatisation and neuroticism - all these factors significantly affect the success or, in this case, the failure of therapy.

It is important to remember that prescribing a drug, especially one from the psychotropic group, is not treatment, but only a part of the therapeutic relationship between a doctor and a patient. Each of these parties has their own vision of the disease, expectations of success or failure, fear of side effects or confidence in their absence... In these circumstances, not only psychopharmacology or neurobiology, but also the individual psychology of the patient is a crucial factor, because, first of all, the quality of the relationship and adherence to treatment determines its ultimate success.

CONCLUSIONS

Side effects of antidepressants can reduce treatment adherence and delay recovery. Therefore, it is extremely important to consider possible side effects when choosing a therapy. While there is no perfect antidepressant that works quickly and is completely free of side effects, newer antidepressants are safer, better tolerated, and associated with lower rates of treatment failure. Most side effects occur as a consequence of the basic mechanism of action of the drugs. Therefore, it is important to understand the neurobiology of side effects, their frequency and risks, ways to prevent them or use them to your advantage.

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CONFLICT OF INTEREST

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REVIEW ARTICLE

CONTENTS

The right to life

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ABSTRACT

Aim: The article is devoted to the study of the right to life as a key aspect of legal theory. The purpose of the study is to examine the theoretical aspects of the right to life, to analyze its essence, place and significance in the system of general human rights; to outline the main principles and concepts related to the right to life, to consider their evolution in different legal systems and to discuss important ethical aspects related to this topic.

Materials and Methods: The article examines the right to life, defines its place in the modern world, highlights the issues of correlation between this right and the right to abortion and euthanasia, and assesses the prospects for their realization. The empirical basis of the study is grounded on articles by scholars and assessments by leading experts in the field. The authors used general scientific methods, including system analysis, system modeling, and the dialectical method. To demonstrate the relationship between the right to life of an unborn child and a woman's right to abortion, a social survey was conducted among different population groups.

Conclusions: Analyzing the right to life in the context of its interrelationship with other human rights, such as the right to liberty, the right to health and the right to a fair trial, helps to affirm the principles of the rule of law and ensure equality before the law for all. In the modern world, the concept of the right to life has become an important element of international law, which covers not only physical existence, but also the provision of a decent and quality life, health, education and social protection. Cultural and religious aspects play a significant role in shaping the understanding of the right to life. Different religious and cultural traditions have their own interpretations of the beginning and end of life, influencing attitudes to aspects related to medical interventions, abortion, euthanasia and other issues.

KEY WORDS: human rights, right to life, protection of rights, right to health, right to an abortion, right to euthanasia.

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INTRODUCTION

The right to life is a fundamental and inalienable human right that guarantees everyone the non-interference in their life and protection from any form of violence, including crimes committed by the state or private individuals. This right is recognized as a universal principle in all international documents and constitutions of most countriesof the world.

The importance of the right to life lies in the fact that it is the basis for the realization of other human rights and defines the core value on which any society is based. This right ensures the safety and dignity of every person, regardless of their origin, race, gender, social status or other characteristics. The recognition of the right to life is a key point in any legal system, as it puts humanitarian values and human rights at the center of attention.

This right defines the limits of actions of the state and its bodies, providing protection against arbitrary arrests, threats and violence. It is the basis for creating laws and policies aimed at ensuring the safety, health and well-being of everyone.

Thus, the right to life is a fundamental right that provides the basis for recognizing the dignity of every human being and is a key element of any humanitarian and legal system.

For centuries, the right to life has been recognized as a fundamental principle, but its interpretation and application have evolved [1]. From classical interpretations limited to protection against direct violation of life to the modern context, where it extends to aspects of medical interventions, bioethics, technological innovations and social conditions. The notion of the right to life has centuries-old roots that have changed and adapted over time under the influence of cultural, religious and legal perspectives.

The historical aspect of the development of the right to life runs through the stages of the evolution of human rights and the formation of humanitarian principles that are reflected in different cultures and civilizations.

Many ancient civilizations, such as Ancient Egypt, Mesopotamia, Greece, and the Roman Empire, already had formulas or laws that set some limits to the use of violence and guaranteed a certain level of protection for life.

The period of the Middle Ages was difficult for the development of human rights. On the one hand, some concepts about human dignity and inviolability were formulated, on the other hand, political and religious conflicts often led to gross violations of human rights, including the right to life.

The concept of the inviolability of the person and his rights was elaborated and shaped by Enlightenment philosophers such as John Locke and Jean-Jacques Rousseau. This led to the establishment of human rights as inalienable and indisputable.

With the emergence of various international conventions and treaties, such as the Hague Convention of 1899 and the creation of the League of Nations in 1919, the first attempts were made to systematize and protect the rights of nations and individuals.

After the Second World War, the adoption of the Universal Declaration of Human Rights by the UN in 1948 defined the international standard of the right to life [2]. This was the beginning of a broad international movement for the protection of human rights, which continues to this day.

The mentioned stages demonstrate the long and complex history of the formation and development of the right to life, which inspires modern legislation and international norms for the protection of human rights.

The modern world faces significant contradictions in the interpretation and application of the right to life. The growing complexity of medical and technological capabilities raises questions about the limits of its protection and the definition of its scope [3]. Does the right to life cover only the physical aspect, or does it also include the right to a quality and dignified life? How should society balance the right to life with other rights and interests?

AIM

The right to life is one of the fundamental concepts underlying the modern legal order. Recognized as an inalienable right of every person, this concept raises a number of complex ethical, legal and socio-cultural issues that require deeper consideration and analysis.

The aim of the study is to examine the theoretical aspects of the right to life, analyze its essence, place and significance in the system of general human rights; to outline the basic principles and concepts related to the right to life, to consider their evolution in different legal systems and to discuss important ethical aspects related to this topic. The work will examineboth the general principles and norms of international law, as well as the peculiarities of the legislation of individual countries, with a focus on the protection of the right to life. Considering the importance of this topic for all spheres of society, the analysis of the right to life aims to contribute to the improvement of human rights protection and the strengthening of the rule of law as the basis of a just and humanitarian society.

MATERIALS AND METHODS

The article examines the right to life, its medical and legal nature, legal regulation, its place and significance in the system of general human rights, the historical development of the right to life, determines its place in the modern world, and assesses the problems and prospects for its realization. The methodological basis of the study is general scientific methods, which include system analysis, system modeling, and the dialectical method. The historical and legal method was used to study the formation and development of the right to life. The technical and legal method was used to interpret the provisions of the law governing the right to life. The method of legal forecasting was used to determine a complex of possible options for the development of the right to life, taking into account the current development of medicine and its institutions. In order to determine the characteristic features of the right to life, the authors used the following materials: normative legal acts and normative legal agreements that regulate the right to life, articles by scientists, assessments of leading experts in the field on the specified issue.

The article highlights the issues of correlation between this right and the right to abortion and euthanasia, and assesses the prospects for their realization. The empirical basis of the study is grounded on articles by scholars and assessments by leading experts in the field. The right to euthanasia has been the subject of study by many researchers, including Parra Jounou, Triviño Caballero, and Cruz Piqueras. The authors of the scientific article "For, against, and beyond: healthcare professionals' positions on Medical Assistance in Dying in Spain" [4] emphasize that since 2021, Spain has been providing the right to euthanasia and medically assisted suicide. According to the law, the state is obliged to ensure their access through medical services, which means that the participation of medical professionals is crucial. Through a survey of doctors, the authors concluded that there is no agreement among medical workers on the acceptance or rejection of such medical services, and that there is a multiplicity of moral views on this issue.

Carpenter Travis, Vivas Lucasy in the article "Ethical arguments againstcoercingprovider participationin MAiD (medical assistanceindying) in Ontario, Canada"stress that it hashistoricallybeen a crimeinCan adatoprovideassistancetosomeoneinendingtheirownli fe, however, thisparadigmwasinvertedin 2015 when the Supreme Court of Canada ruled that restrictions on this practice, within certain defined parameters, violated the righ to life, liberty, and security of the person [5].

Emanuel Ezekiel, Onwuteaka-Philipsen Bregje, Urwin John, Cohen Joachim in the article "Attitude sand practice sofeu than asia and physician-assisted suicide in the United States, Canada, and Europe" [6] conduct a comparative analysis of the practice of euthanasia in modern countries. The authors conclude that public support for euthanasia and physician assisted suicide in the United States has plate auedsince the 1990s (range, 47%-69%). In Western Europe, an increasing and strong public support for euthanasia and physician assisted suicide has been reported; in Central and Eastern Europe, support is decreasing.

Various aspects of the right to abortion are also considered by contemporary scholars. Among them: Mónica Frederico, Carlos Arnaldo, Peter Decat, Adelino Juga, Elizabeth Kemigisha, Olivier Degomme, Kristien Michielsen, Mulumebet Zenebe, Haldis Haukanes.

In the article "Induced abortion: a cross-sectional study on knowledge of and attitudes toward the new abortion law in Maputo and Quelimane cities, Mozambique" [7] the authors try to establish the relationship between artificial abortions by women of reproductive age in suburban areas of the cities of Maputo and Kelimane and their awareness and attitude towards the laws that allow abortions.The authors conclude that the number of abortions is low compared to other countries in sub-Saharan Africa. This statistic is based on limited knowledge about the possibility of legal abortion, social factors (level of education, religion, living in suburban areas of cities).

Mulumebet Zenebe and Haldis Haukanes in their article "When abortion is not within reach: Ethiopian university students struggling with unintended pregnancies" [8] conclude that the law allowing abortions increased access to safe abortions for young unmarried women studying at universities. In general, many female university students in Ethiopia face pregnancy-relatedproblems.And the reasons for refusing to have an abortion are not always based on a woman's will. Rather, they are caused by the inability to determine the time of pregnancy and missed deadlines for abortions, strong religious beliefs, paralyzing shame and condemnation from society, and, as a result, psychological denial of the situation and the problem. This state of affairs has serious negative consequences for women (actual and psychological) and is caused by the tension between different norms and expectations of students' sexual behavior, gender and the injustice embedded in such norms. Students increasingly believe that it is "modern" and "cool" to be involved in a relationship, and accordingly there is considerable pressure on female students to have sexual relations. At the same time, societal norms based on religious beliefs and family customs are still very strict when it comes to premarital sex for women. Young unmarried women may be stigmatized when they violate these norms and such violation becomes evident to the public in the form of a growing belly. Therefore, universities are recommended to work to increase female students' self-awareness and self-esteem, as well as to transform the culture of masculinity and encourage sexual responsibility among men.

To demonstrate the relationship between the right to life of an unborn child and a woman's right to abortion, a social survey was conducted among different population groups.

In the article, the authors used the results of a survey of various population groups on the issue of the relationship between the right to life of an unborn child and a woman's right to an abortion.

REVIEW AND DISCUSSION

The right to life is one of the fundamental human rights that underlies the modern legal order, international humanitarian law and national legislation of many countries around the world. This issue becomes especially relevant in the context of modern challenges and threats facing humanity, such as conflicts, terrorism, health crises, environmental disasters and many others.

The right to life is one of the most important guarantees that ensure the dignity and security of every person. The study of the theoretical aspects of this right is crucial for understanding its essence and application in practice.

Ongoing threats, such as conflicts, terrorism, violence, environmental disasters and global pandemics, emphasize the importance of effective protection of the right to life. The study of this topic reveals ways to improve international cooperation, legislation and law enforcement in order to ensure the highest level of human rights protection.

The concept of the right to life is an important element in modern legal systems, recognized and protected at the level of both national and international legislation. In many countries of the world, legislation guarantees the right to life as one of the fundamentalhuman rights.

However, the specificity and scope of this right may vary depending on cultural, religious and historical factors. Some legal frameworks emphasize the protection of life from direct harm, while others include a wider range of guarantees, such as social protection, access to healthcare and other aspects that affect the quality and conditions of human life. International legal acts and conventions reflect generally accepted principles and standards on the right to life. For example, the Universal Declaration of Human Rights, the European Convention on Human Rights, as well as many other international documents define and protect this right as one of the fundamental ones. They call on the participating states to ensure the protection of the life of every person and to avoid any actions that may violate this right.

Analyzing the right to life in the context of its relationship with other human rights, such as the right to liberty, the right to health and the right to a fair trial, helps to affirm the principles of the rule of law and ensure equality before the law for all.

This topic is also important in terms of shaping public consciousness and public discourse regarding the importance of respect for human life and dignity [9]. Understanding the essence and significanceof the right to life contributes to raising citizens' awareness of their rights and responsibilities, which is an important step towards building a just and humanitarian society.

Thus, consideration of the theoretical aspects of the right to life becomes an important step in ensuring the security and dignity of every person, contributing to the development of legal culture and strengthening the rule of law as the basis of a sustainable and developed society.

The right to life has some basic legal principles that underlie its conception and realization [10]. These principles are recognized in international law and national legislation of many countries. The inviolability of the person enshrines the inviolability of the life of every person, which means that no one has the right to violate or threaten the life of another person. Prohibition of the use of violence - this principle states that any use of violence or threat of violence is unacceptable and violates the right to life. The state must ensure that the right to life is adequately protected by creating appropriate laws, policies and mechanisms of protection. The priority of humanitarian values - this principle recognizes the primacy of humanitarian values and human rights over other interests and goals. Universality and non-discrimination – these principles emphasize that the right to life is universal and should apply to every person regardless of race, color, sex, language, religion, political or other beliefs, national or social origin, property or other status.Every person has the right to effective protection of his or her life against any form of violence or threats. These principles affirm the importance and inviolability of the right to life in the legal system and define the basic principles and values on which the protection of this right is based.

The general principles of international law relating to the right to life are critical components in a society that strives to ensure the dignity and safety of every person [11]. These principles serve as the basis for international standards and norms aimed at protecting the life and dignity of people regardless of their nationality, race, or other personal characteristics.

One of the most important principles is the inviolability of the person. This principle recognizes that every person has the right to life and cannot be subjected to cruel, inhumanor degrading treatment. It affirms the inadmissibility of any actions or policies that violate this right, and calls on states to comply with this norm in all spheres of life.

Another key principle is the prohibition of the use of violence [12]. International law makes it clear that any use of violence, even in conflicts or wars, is unacceptable [13]. This principle emphasizes the importance of peaceful resolution of conflicts and the use of alternative dispute resolution mechanisms to ensure human life and safety.

State guarantees are another principle that plays an important role in international law. This principle is designed to ensure that states not only adopt the necessary laws and policies to protect the right to life, but also effectively implement them in practice. This includes the establishment of effective legal mechanisms to prevent human rights violations and to properly investigate and punish those responsible for violations of the right to life.

Thus, the general principles of international law relating to the right to life define moral and legal norms designed to ensure the safety, dignity and inviolability of every person. They form the basis for creating just and humanitarian societies that respect and protect the rights of all their members.

International law recognizes the right to life as one of the most important and inalienable human rights. In order to ensure its effective protection and implementation, a number of international agreements and documents were concluded, which determine the norms and standards that states must adhere to in this area.

One of the most important international agreements is the Universal Declaration of Human Rights, which was adopted by the UN General Assembly in 1948. This document defines the right to life as an inalienable right of every person and calls on all states to respect and protect it. It became the basis for further international agreements and documents regulating the right to life.

Another important agreement is the International Covenant on Civil and Political Rights, which was adopted in 1966. This document guarantees the right to life and establishes the obligations of states to ensure it. It also provides mechanisms for monitoring and protecting this right, in particular through a system of appeals to the UN Human Rights Committee.

In addition, the Geneva Conventions and Additional Protocols to them establish norms of humanitarian law that regulate the conduct of parties to conflicts and ensure the protection of the civilian population in wartime. These documents are of great importance for the protection of the right to life in armed conflicts and hostilities.

In general, international agreements and documents regulating the right to life play a key role in the formation of international standards and norms that ensure the protection of this right at the level of the world community. They define the obligations of states and establish mechanisms for monitoring and settling violations of this right, which contributes to the creation of a more just and humanitarian world for all.

International organizations play an important role in ensuring and protecting the right to life both in peace timeand in conditions of conflict and danger. Their actions are aimed at establishing international standards and norms, monitoring compliance with these norms by participating states, as well as providing assistance and protection to victims of human rights violations.

One of the most influential international organizations in the field of protection of the right to life is the United Nations (UN). Through its bodies, such as the UN High Commissioner for Human Rights and the UN Human Rights Council, it contributes to monitoring the human rights situation in various countries around the world and takes measures to prevent violations and protect the rights of people in danger.

Another important organization is the International Committee of the Red Cross (ICRC), which actively operates in the conditions of wars and armed conflicts, providing assistance and protection to the wounded, prisoners of war and the civilian population. They also work to prevent violations of international humanitarian law and provide humanitarian aid in the event of humanitarian crises.

In addition, the International Criminal Court (ICC) plays an important role in prosecuting those who have committed crimes against humanity, including murder, violence and crimes in armed conflict. This helps prevent impunity and ensure justice for victims of such crimes.

Thus, international organizations play a key role in ensuring the protection of the right to life by promoting the establishment of international standards, monitoring compliance with these standards and providing assistance and protection to those most in need of protection in the world. Their actions set modern humanitarian standards and set the foundations for a just and humanitarian world for all people.

The right to life is closely related to the right to health, as ensuring health is a necessary prerequisite for the exercise of the right to life. Guaranteeing access to quality medical care and services is an important element of ensuring the right to life.This means that everyone has the right to access medical care, necessary medicines and other medical procedures that can preserve or improve their health and prolong their lives. The right to health also provides for preventive measures that help prevent diseases and improve the overall health of society.

The right to life has a deep connection with the right to liberty and security of person. Ensuring the right to life implies the inviolability of the person from any violence or discrimination, and guarantees freedom from arbitrary arrest or restriction of liberty without legal basis. The right to liberty and security of person also includes protection from any form of physical and psychological violence that may threaten a person's life and security.

The right to life is also interconnected with other socio-economic and cultural rights [14]. Guaranteeing the right to life involves ensuring access to basic material needs such as housing, food, water and education. Cultural rights are also an integral part of the right to life, as they contribute to the maintenance of the identity and dignity of each person, which in turn positively affects their overall health and well-being.

In general, the right to life has broad implications for other human rights, such as the right to health, liberty and security of the person, as well as socio-economic and cultural rights.

Violation of the right to life is a serious problem in many parts of the world [15]. Examples of such violations include extradition killings, death sentences without fair trials, extremist violence and genocide, which are the source of suffering for thousands of people.These violations often occur in the context of wars, internal conflicts, or as a result of authoritarian regimes that do not adhere to international human rights standards. The analysis of such violations demonstrates the need to raise international standards and monitoring mechanisms to protect the right to life in all parts of the world.

Social and economic factors, such as poverty, inequality, wars and conflicts, affect the realization of the right to life [16]. In poor and vulnerable communities,

access to adequate health care and education is limited, which worsens health and threatens life. Conflicts and hostilities lead to massive violations of the right to life, forcing millions of people to become refugees and displaced persons. These factors require a comprehensive approach to the provision of socio-economic rights that aims to reduce inequality and fight poverty.

Civil society and human rights organizations play an important role in protecting the right to life. Their activities include monitoring human rights violations, publicly condemning unlawful acts, and assisting victims of violations in accessing justice and compensation. They also work to raise public awareness of issues related to the right to life and mobilize public support for action to combat human rights violations.

All these aspects point to the importance of joint work of states, civil society and the international community in ensuring effective protection of the right to life and overcoming the problems that impede its full realization.

The right to life has always been the subject of heated ethical and moral debate, especially in the context of abortion, euthanasia and other essential aspects of human existence.

One of the most controversial issues is abortion, where public opinion is divided due to moral, religious and legal aspects. Some consider abortion to be a violation of the right to life of the unborn, while others support the right of women to make a free choice in this matter.

Euthanasia, carried out at the request of the individual or for medical reasons, also causes heated debate in society. Some support the right to a dignified death, adhering to the principle of personal autonomy, while others consider it a violation of the inalienable right to life.

Social values and beliefs have a great influence on the formation of legislation regarding the right to life. Laws and norms related to this right reflect the opinion and moral values of society. However, in controversial issues such as abortion and euthanasia, it is important to find a balance between the protection of human rights and individual freedom of choice.

This discussion emphasizes the importance of taking into account ethical, moral and societal aspects in the formulation of legislation on the right to life, as well as the need to understand and respect diverse views on these controversial issues.

The right to life emerges as a key component of the field of medicine, bioethics, and other fields full of ethical, moral, and legal dilemmas. Its role is crucial in regulating complex controversies arising in medical practice and bioethical research.

The field of medicine and bioethics faces numerous challenges, such as resolving conflicts between patients and medical professionals, regulating experimental treatments, determining the moment of beginning and end of life, and other ethical issues related to medical practice. The right to life is a fundamental principle in resolving such disputes. It forms the basis for the development of medical standards, codes of ethics and legislation aimed at protecting life and treating it with dignity.

Important decisions related to the right to life are made in court practice. The courts resolve controversial issues related to medical interventions, restrictions based on moral beliefs, decisions to stop treatment, and other areas where human life becomes the object of judicial intervention.

This judicial practice not only responds to specific situations, but also sets precedents that become important guidelines for the medical and legal communities in the future.

The understanding of the right to life largely reflects a variety of views that stem from ethical, moral, religious and legal beliefs. Let us consider different approaches to this concept and analyze their advantages and disadvantages.

1. Absolutist approach: This approach asserts that the right to life is absolute and inviolable, even in cases of conflict with other rights or situations of extreme circumstances.
2. Context-Based approach: This position is that the right to life should be considered in the context of the situation, taking into account the specifics and circumstances of the particular case.
3. Broad interpretation of the right to life: Some opinions recognize that the right to life encompasses not only physical existence, but also a quality life with dignity, including access to basic needs, health care and social protection.

An absolutist approach provides stability and clarity, but there may be conflict with other rights and contradictions in complex situations. A context-based approach allows for consideration of circumstances, but may lead to subjectivity in assessing situations. A broad interpretation of the right to life opens up opportunities for greater protection of the individual, but can be subjectively perceived and ambiguously used.

A critical review of different approaches shows their uniqueness and at the same time their weaknesses. Comparison and analysis of these approaches help to better understand the complexity and contextuality of the right to life in modern society.

The analysis of various aspects of the right to life has revealed its importance as a key legal concept

that determines not only the physical existence of a person, but also the relationship between society and the individual. To summarize, it is important to identify the main arguments and express our own position on the right to life.

The analysis of the concept of the right to life in different eras and cultures has shown its evolution and changing interpretations. The diversity of approaches in different countries and international conventions demonstrates the universality of this concept, but also the complexity of its practical application.

The discussion of ethical and moral aspects related to abortion, euthanasia [17] and other complex issues demonstrates the profound influence of social values on the formation of legislation and regulation of these issues.

Let us imagine that a person has just been diagnosed with an inoperable brain tumor. The doctor says that he or she has less than six months to live and that the remaining time will be extremely painful and the person will likely face intense suffering until her body finally meets death. Would he or she like to have the right to choose how to end this life?

Hundreds of thousands of people around the world suffer from debilitating, fatal diseases and similar diagnoses. However, these people cannot get rid of their pain and suffering with dignity and respect because of the laws of some countries that do not allow euthanasia. Given that some diseases deprive a person of living a decent life, this way becomes inhumane, both for the person suffering from the disease and for his or her family, who have to experience the horror of watching their loved ones suffer for an unknown time.

Euthanasia, the practice of intentionally ending life to alleviate suffering, has been a controversial topic for many years. The idea that a person has the right to end his or her own life in exceptional circumstances, also known as the "right to die," is a matter of both philosophical and legal debate.

Proponents of euthanasia argue that people have the right to make decisions about their own lives, and that everyone should be able to die with dignity, rather than suffer pain and agony. They also argue that euthanasia can save money and resources by reducing the costs associated with prolonged end-of-life care. Opponents of euthanasia argue that it is unethical and contrary to the sanctity of life. They believe that it is wrong to take the life of another person, even if that person is suffering

From a legal perspective, the laws governing euthanasia vary from country to country. In some countries, such as the Netherlands, euthanasia is legal under certain circumstances, while in others, such as Ukraine, it is not recognized. Although the issue of the right to euthanasia is complex and multifaceted, one thing is clear: it is a deeply personal decision that should be made with respect for the individual's autonomy.

The issue of the right to life and euthanasia is closely related to the right to health care, as medical resources in different countries of the modern world are not equal. A disease that is incurable in one country can be treated in another, where the level of medical development is at a higher level (see the movie "King Arthur").

Currently, the issue of legalizing euthanasia has become one of the most pressing in the legal field. Euthanasia is a topic of scientific debate and research that lies on the borderline between law and medicine. Euthanasia is defined as a type of behavior of a medical worker (action, inaction or decision) that is committed consciously and intentionally, and is aimed at ending unbearable suffering (physical, mental, moral, etc.), is carried out at the repeated and unambiguous request of the patient (and in cases provided for by law, his or her legal representative), provided that he or she is fully, timely and objectively informed of the consequences of such intervention, which results in death.

If we analyze the historical development of the concept of "euthanasia," this term was first characterized and explained in the 16th century by the English philosopher Francis Bacon. He noted that the doctor's duty is not only to restore health, but also to alleviate the suffering and torment caused by the disease. Such pain relief should occur not only when it leads to recovery, but also when there is no hope for salvation. In this case, it is necessary to make death itself easy and peaceful, and in this regard, euthanasia is already happiness.

From a historical point of view, the development of the concept of euthanasia was influenced by the period of Antiquity, when people could be deprived of their lives due to birth defects or particularly serious illnesses. During the Second World War, a significant number of people were killed through "medical procedures," but unlike euthanasia, these "procedures" were inhumane. And only with the creation in 1935 of the first societies that advocated the legalization of euthanasia, the humanization of this process and reflections on the further legitimate nature of euthanasia began.Perhaps, the observance of the principle of humanism is the beginning of finding an answer to the question of depriving a person of his life with the help of another person's intervention. We must find an answer to the question whether any existence is life or life is only a worthy human existence?

If we classify the types of euthanasia and analyze their differences, we can note that there are different types of euthanasia according to the will of the person:

• Voluntary, which is carried out with the consent of the patient and at his request. Active voluntary euthanasia is legal in Belgium, Luxembourg and the Netherlands. Passive voluntary euthanasia is legal in the United States. When a patient accepts death independently, but with the assistance of a doctor, the term "assisted suicide" is often used. Assisted suicide is legal in Switzerland and the US states of California, Oregon, Washington, Montana and Vermont.

• Non-voluntary, that is performed when the patient's consent is not possible. For children it is illegal worldwide, but decriminalized under certain circumstances in the Netherlands under the Groningen Protocol.

• Forced, that is carried out against a person's will. It can be considered a form of punishment for a criminal offense. However, the inclusion of this type of euthanasia in this classification is controversial, since in this case we are talking about the death penalty, which is currently prohibited in Europe, except for the Republic of Belarus. The relationship between the right to life and the death penalty is the subject of a separate consideration.

The second classification is determined by the criterion of the method of achieving death and accordingly divides euthanasia into:

• Active, that involves administering to a dying person drugs or using other actions that cause rapid death. Active euthanasia is often considered suicide with medical assistance (providing the patient with drugs that shorten life at his or her request).

• Passive euthanasia, which involves the intentional termination of the patient's maintenance therapy. Here, the question remains open regarding the existence of the institution of euthanasia in the absence of an adequate level of medical services. In countries with underdeveloped medicine, failure to provide medical service in itself causes the death of a person.

Today, euthanasia is still not a legitimate process in most countries of the world, but there are a number of countries in which euthanasia has become legal. In particular, euthanasia has been legalized in Switzerland, Australia, the Netherlands, Belgium, France and some US states. It should be noted that Dutch courts have de facto allowed assisted suicide to terminally ill patients since 1984.

The European Court of Human Rights has repeatedly heard cases regarding the prohibition of euthanasia in European countries. The first euthanasia case was Sanles Sanles v. Spain [18]. After an accident in 1968, a relative of the applicant suffered from tetraplegia. In January 1998, he committed suicide with the support of a third party, while his request for recognition of the right to die with dignity was pending. The applicant was his legal successor, whom he officially appointed to manage the case. In particular, she sought recognition of the right to a dignified death for the applicant. The court rejected her request, explaining that the Spanish authorities cannot be held responsible for not adopting a law on the decriminalization of euthanasia. In this case, the European Court of Human Rights attributed the institution of euthanasia to the internal national legal system of the state. Although the case was lost, the very process of consideration of the case by an international authority created prerequisites for the further formation of the institution of euthanasia.

The next case, Pritty v. United Kingdom, received a lot of publicity [19]. The applicant was dying of motor neuron disease, an incurable progressive disease affecting the muscles. Given that the final stage of this disease is painful and humiliating, she wanted to decide for herself how and when to die. Due to her illness, the applicant was not able to commit suicide on her own and wanted her husband to help her in this. However, based on English law, suicide is not a crime, assisting suicide is a crime. As the authorities refused her request, the applicant complained to the European Court of Human Rights that her husband was not guaranteed immunity from liability if he helped her die. The court refused.

An interesting case is Haas v. Switzerland [20]. The applicant, who had been suffering from severe affective bipolar disorder for about 20 years, decided that he could no longer live a decent life. After two suicide attempts, the applicant tried to obtain a substance which, if taken in a certain amount, could help him to commit suicide in a safe and dignified manner. Since the substance was only available by prescription, the applicant tried to obtain it from several psychiatrists, but his efforts were unsuccessful. In the European Court of Human Rights, the applicant argued that Article 8 of the Convention imposed a positive obligation on the state to create conditions for safe and painless suicide. The authorities rejected his complaint, so he believed that his right to commit suicide in a safe and dignified manner had been violated by Switzerland. Haas lost the case.

The case of the boy Alfie Evens (Great Britain) or the other name of the case "euthanasia at the initiative of the state" also referred to the institution of euthanasia as its subject of consideration. Never before has the United Kingdom given consent to applicants for euthanasia (passive or active). Alfie's case was unique in that after the doctors examined Alfie, they ruled that he could no longer be cured and the court ordered that Alfie be taken off life support. Alfie Evans was in a semi-comatose state for over a year, but the boy's

parents refused to accept that his illness was terminal. The conflict between the parents and the doctors drew public attention when Alfie's parents filed a lawsuit challenging the decision of the medical board to remove the child from life support.

The boy's parents fought to have him transferred to a hospital in Rome, where hospital officials pledged to provide the necessary medical care and improve his condition. The UK Supreme Court rejected the application for consent to transfer the boy to Italy, leaning towards the opinion of doctors that irreversible changes had occurred in his brain, depriving him of the senses of sight, hearing, taste and touch, and that further therapy was not in his best interests and could be inhumane. Alfie died in hospital as a result of the life support being switched off (passive euthanasia). An interesting fact in this case is that 4 months after Alfie's death, his parents gave birth to a second boy.

Therefore, we can observe a certain pattern: the European Court of Human Rights has not satisfied any of the above cases on the recognition of the institution of euthanasia, noting that the recognition or prohibition of the institution of euthanasia is within the internal interests of the state. However, the fact that applicants from different countries have applied to the European Court of Human Rights since 1993 states that social and legal relations regarding euthanasia exist in modern society, and that failure to regulate them and to recognize their existence will lead to criminalization of this institution. The issue of legalizing euthanasia is a subject of debate among representatives of medicine, law, churches, and religious denominations at the international and national levels. Some states legalize passive euthanasia within their national systems, providing a vivid example of the comprehensive nature of human rights.

The debate about the ethics and necessity of legalizing the right to abortion also continues. In order to answer the question "Does a woman have the right to decide on her own to give birth to a child or not?"we need to consider possible views on the specified problem.

With regard to the regulation of the right to abortion in modern law, it should be noted that abortion is an involuntary or artificial termination of pregnancy before the fetus can be considered viable. Views on the moment when fetal viability is determined can be divided into two groups. The first is that an embryo is viable from the moment of conception, at any stage of pregnancy it is already a human being. These thoughts give birth to completely different views on abortion. Different views on the moment of determining the viability of the fetus lead to today's controversies about the possibility of termination of pregnancy at different times.The second view is reflected in the idea that an embryo acquires human significance when all the organs necessary for life are formed (12 weeks after conception). These opinions give rise to very different views on abortion. Different views on the moment of determining the viability of the fetus lead to today's controversy over the possibility of abortion at different stages of pregnancy.

In 2020, more than 42.6 million abortions were performed worldwide, slightly more than in 2019 (42.3 million abortions). 1st place is occupied by China (more than 9 million annually); 2nd place - USA (1 million 213 thousand visits to the doctor); 3rd place - the Russian Federation (more than 1 million 208 thousand); 4th place – India (about 642 thousand); 5th place – Vietnam (332 thousand); 6th place – Japan (more than 242 thousand); 7th place – France (198 thousand); 8th place - England (190 thousand); 9th place – Turkey (more than 177 thousand); 10th place - Azerbaijan (160 thousand). It is worth noting that these statistics are not divided into abortions for medical reasons and those at the request of the woman herself. Among the leaders are countries with a large population (e.g., China - 1.4 billion people, India - 1.380 billion people, the United States - 350,585,880 people), which justifies the high number of abortions in these countries. In Ukraine, according to the Ministry of Health, 46552 pregnancy terminations were performed in 2018. Of these, 18115 were legal for medical reasons after 12 weeks of pregnancy and 28437 were legal by the woman's decision and with medication. Statistics on abortions in the world show that their number does not depend on the level of economic and technical development, the type of legal regime of the country, territorial affiliation, or religious preferences.

A woman's right to an abortion and free disposal of her body often comes into conflict with social condemnation, so the problem may not only be to have an abortion, but that the woman will then suffer negative consequences from members of society. A woman who has had an abortion can become a victim of persecution and suffer psychological and even physical violence from society or relatives.

A survey conducted in Ukraine among people of different ages and statuses to understand the public's position on abortion allowed us to reflect public opinion on this issue. Here are some of the answers of the respondents:

Girl, 18 years old, student: "I believe that this is a woman's choice. It is not right to force a woman to give birth to a child under social pressure if she does not have the desire and financial means to support the child. In my opinion, it is better to terminate the

pregnancy than to have the child's life crippled by an unloving mother."

Girl, 17 years old, student: "If a woman has difficulties with the material or psychological support of a child, I am in favor of her having an abortion, but if the reason is her personal opinion or some other reason, I am against abortion, because after all it is life, it is a future human being."

Boy, 18 years old, student: "A woman should make the decision to have an abortion, first of all from the point of view of health, because not every woman can bear a child and not every woman wants to have an impact on her health after pregnancy. It is up to the woman to decide whether to give birth or not."

Boy, 17 years old, student: "I have a negative attitude towards abortion, I believe that every child has the right to life, even the one in the mother's womb. And abortion violates these rights. It is possible to terminate a pregnancy only if the pregnancy threatens the life of the mother or the child. In the case of rape, a woman has the right to choose, but she will be responsible for this act in front of her conscience."

Woman, 45 years old, housewife: "I have an ambiguous attitude towards abortion, but to the question of who should decide the fate of the embryo, I will answer that the father and mother of the child should make this decision together. If a woman does not want to give birth because she is "not ready" or 'doesn't want to", then after the birth of the child her opinion will change, it is the baby who will change her opinion when she holds her native little miracle in her arms."

Man, 56 years old, private entrepreneur: "Only a woman has the full right to decide the fate of her pregnancy: to keep the child or abandon it. She is an independent person and is able to choose what to do. Of course, children are happiness and a miracle, but not everyone is ready for it."

A total of 100 people were interviewed. 49% of the respondents said that a woman has the right to an abortion, 27% have the opposite opinion - a woman does not have such a right, and 24% have an ambiguous position, in their opinion, it all depends on the circumstances.

As can be seen, most people believe that the right to an abortion belongs exclusively to a woman. The opinions of the interviewed persons are based on upbringing, faith and moral views. Each of us has an attitude towards anything that stems from the environment in which we live.

The practice of the European Court of Human Rights does not stay away from the issue of the relationship between the right to life and the right to have an abortion by a woman. In particular, in the case of "R.R. v. Poland" (application no. 27617/04) [21] the applicant was informed about the possibility of fetal malformation of the fetus after an ultrasound examination conducted on the 18th week of pregnancy.She immediately expressed her desire to have an abortion. She was recommended to undergo genetic testing by amniocentesis at 23 weeks of pregnancy. After several refusals to perform an amniocentesis by her doctor and a number of other doctors, the study did take place and confirmed that the fetus suffered from Turner syndrome. The test results were obtained after the 24th week of pregnancy, and according to Polish law, abortion on the grounds of abnormal fetal development is possible only during the first 24 weeks of pregnancy. The applicant was awarded compensation in connection with the doctors' failure to conduct timely genetic tests.

But the main thing is that the court emphasized the relevance of the information that the applicant tried to obtain through genetic testing in order to make a decision regarding the continuation of her pregnancy. Polish law allows abortion until the fetus can survive on its own outside the mother's body and only when prenatal tests or other medical reports indicate a high risk that the fetus is seriously and irreversibly damaged or will suffer from an incurable disease after birth. Therefore, access to complete and reliable information about fetal health is not only important for the comfort of a pregnant woman, but also a prerequisite for the possibility of a legally permitted abortion.

Another case is "Tysiąc v. Poland" (Application no. 5410/03) [22], which was heard by the European Court of Human Rights, is that the applicant had suffered from severe myopia for many years. After she became pregnant for the third time, she sought medical advice because she was worried about the impact the pregnancy might have on her health. The three ophthalmologists she consulted concluded that the pathological changes in her retina posed a serious risk to her vision if she carried the pregnancy to term. Despite the applicant's requests, they refused to issue her a certificate for termination of pregnancy. The applicant also consulted a general practitioner and received a certificate from him listing the risks posed by the pregnancy in terms of retinal problems, as well as the consequences of two previous cesarean sections. Thus, the applicant could not terminate the pregnancy and gave birth by caesarean section.In the second month of pregnancy, the applicant's myopia progressed significantly in both eyes. She was examined by the head of the obstetrics and gynecology department of a public hospital, who did not see any medical grounds for an abortion on medical grounds. Thus, the applicant was unable to terminate her pregnancy and gave birth by caesarean section. After giving birth, her vision

deteriorated further due to a retinal hemorrhage. She was informed that because the retinal changes were advanced, they could not be corrected with surgery. The medical board concluded that her condition required treatment and daily care and found her largely disabled.

In this case, the Court noted that the legislation governing termination of pregnancy touches the sphere of personal life, because when a woman is pregnant, her private life becomes closely related to the developing fetus. The term "private life" is broad and covers, among other things, the physical and social aspects of a person's identity, including the right to personal autonomy, personal development and to establish and develop relationships with other people and the outside world. Although the Convention on the Rights and Freedoms of Man and Citizen does not guarantee a specific level of medical care, the state has a positive obligation to ensure the protection of its citizens, their right to private life.The case "Tysiąc v. Poland" (Application no. 5410/03) concerned a special combination of various aspects of private life. If the law on abortion concerns the traditional balancing of privacy and public interest, in the case of therapeutic abortion, the positive obligations of the state to ensure the physical integrity of expectant mothers must be taken into account.

Case of A, B AND C v. Ireland (Application no. 25579/05) [23] concerned applicants resident in Ireland who are women aged 18 and over. All three applicants traveled to England to have an abortion because they believed they did not have the right to have an abortion in Ireland. The first applicant was nine and a half weeks pregnant and accidentally became pregnant, believing her partner to be infertile. At that time, she was unmarried, unemployed and living in poverty.She had four young children. The youngest was disabled, and all four children were in foster care because of the problems she faced as an alcoholic. She was depressed during her first four pregnancies and struggled with depression during her fifth pregnancy.In the year leading up to her fifth pregnancy, she remained sober and was in regular contact with social workers to regain custody of her children. She believed that having another child at this point in her life (with the attendant risk of postpartum depression and her sobriety) would jeopardize her health and the successful reunification of her family. She went to England and had an abortion there.After submitting this application, the applicant became pregnant again and gave birth to a fifth child. She struggled with depression and cared for three of her children, while two (including a disabled child) remained in foster care. She claimed that abortion was the right decision for her in 2005. The second applicant was seven weeks pregnant and became pregnant unintentionally. She took "morning after pills" but they didn't work.The third applicant was in her first trimester of pregnancy. Before that, she had been undergoing chemotherapy for three years for a rare form of cancer. The cancer went into remission and the applicant unintentionally became pregnant. She was unaware of this when she underwent a series of cancer tests. Given the uncertainty of the risks involved, the third applicant traveled to England to have an abortion. She claimed that she wanted to have a medical abortion (miscarriage induced by medication) because her pregnancy was in the early stages, but she could not find a clinic that would provide this treatment. Therefore, she had to wait another eight weeks before a surgical abortion became possible. Due to the difference in factual circumstances, the court considered the complaints of the first two applicants and the third applicant separately.

The court noted that the concept of "private life" is a concept that covers, among other things, gender identification, sexual orientation and sex life, the physical and psychological integrity of a person, as well as the decision whether or not to have a child or to become a genetic parent. At the same time, a woman's right to respect for her private life should be compared with other competing rights and freedoms, in particular with the right of the unborn child.

CONCLUSIONS

Summarizing the main theses allows us to draw certain conclusions about this key legal concept. The right to life is one of the fundamental human rights that guarantees every person the right to inviolability and security. This right recognizes the inherent value of human life and is intended to ensure its protection from all forms of violence, discrimination and violations.

In ancient civilizations, the right to life and its protection reflected the general moral standards of society and the belief in the natural rights of man. Over time, this concept has received formal recognition in legal systems, becoming a key aspect of legislation.

In the Middle Ages and early modern times, the right to life was often seen in the context of the power of church and state, where religious doctrines and sociocultural norms played a decisive role.

In today's world, the concept of the right to life has become an important element of international law, covering not only physical existence, but also ensuring a decent and high-quality life, health, education and social protection.

Cultural and religious aspects play a significant role in shaping the understanding of the right to life.

Different religious and cultural traditions have their own interpretations of the beginning and end of life, influencing attitudes to aspects related to medical interventions, abortion, euthanasia and other issues. For example, some religions see the right to life as inviolable and sacred, while other religious traditions may allow certain exceptions depending on the context or circumstances.

Guaranteeing the right to life is key to any just and humanitarian society. This right is the basis for ensuring the dignity, peace and security of every human being. Its protection contributes to the strengthening of social order, the maintenance of justice and the development of democratic values.

First of all, it is necessary to strengthen the legislation that guarantees the protection of the right to life at the level of the state and the international community. Next, it is important to develop mechanisms for monitoring and supervising the observance of this right, to promote education and increase public awareness of its importance. It is also important to support the development of human rights organizations and promote their active participation in the protection of this right.

To summarize, the right to life is an integral part of human rights, which is important for ensuring a dignified, peaceful and secure way of life for humanity. Guaranteeing this right is crucial for any society, as it creates the preconditions for ensuring harmonious development and support for all other human rights. Its full protection and implementation requires not only effective laws, but also the active participation of the public and human rights organizations in the process of ensuring it.

The right to life is a fundamental right that is reflected in the laws of numerous countries and international acts. The right to life is an inalienable right, but its understanding and application should be flexible and take into account the specifics of each situation. Our own position is that the right to life should be guaranteed, but with due regard to the specific circumstances and interests of the individual.

Its understanding and application is a complex problem that requires consideration of ethical, moral, religious and legal aspects.

The call for further research and development of the topic means the need to further study the impact of the right to life on modern medical and bioethical practices, as well as to develop more flexible and balanced legal solutions to controversial issues such as abortion, euthanasia, and others.

When it comes to the relationship between the right to life of an unborn child and a woman's right to an abortion, the decision must be made individually in each case, based on the actual circumstances, such as the health of the woman, the condition of the fetus, the circumstances of the pregnancy, etc. It is impossible to derive general formulas in this matter, because the actual circumstances of a case are significantly different from each other.

When comparing the right to life and the right to euthanasia, it should be assumed that these rights do not contradict each other. If we consider the right to life (the right of the first generation of human rights) from the standpoint of a dignified human existence without unbearable torment, then the right to euthanasia (the right of the 4th generation of human rights) is its logical continuation, and its legalization in the developed countries of the world is fully justified.

Through further research and development of the topic, society will be able to better understand how to ensure the protection of human rights, avoid conflicts and find compromise solutions in difficult situations related to the right to life.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

Extrinsic black staining of teeth: a review

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ABSTRACT

Aim: Discovering of the prevalence, causes and consequences of the Extrinsic black staining(EBS) of teeth in pediatric population

Materials and Methods: Upon completion of the scientific search, the review included 47 scientific articles from the electronic databases, reference lists of articles, and selected textbooks in the time interval from 1976 to 2023.

Conclusions: The results show that EBS is a possible protective factor against early childhood caries. Dental plaque and gut microbiome may be related to EBS in the temporary dentition. The literature suggests a decreased caries prevalence in the presence of EBS which associated with low incidence of caries in children. The nature of the black pigmentation is suggested to be a form of bacterial plaque with an insoluble ferric salt. Tabaco smoke, food and antibiotics that can be also a risk factors for EBS. There are some clinical and localization features that dentist have to pay attention for. Many diseases of various systems and organs are directly related to black plaque.

With this review, we wanted to encourage dentists to identify the problem in childhood and collaborate in a multidisciplinary team to improve treatment efficiency and speed up the selection of the right tactics for the most person-centered approach to avoid worsening the problem in adulthood.

KEY WORDS: dental plaque, black stain, systematization of colored dental plaques, the class of diseases

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INTRODUCTION

The topic of teeth staining is often given insufficient attention by scientists and dentists, especially in the pediatric field. According to the International Classification of Diseases (ICD-11), deposits on the teeth (code DA08.4), in particular, the extrinsic black staining (EBS) of teeth, belong to the Diseases of the hard tissues of the teeth (DA08) of the class: Diseases or disorders of the orofacial complex of Chapter XIII. Diseases of the digestive organs [1]. Plaque can be different in colors, including black, green, orange, brown, etc. EBS is another problem in pediatric dentistry, along with dental caries [2, 3]. The accumulation of EBS leads to a visual discoloration of the teeth, which usually causes aesthetic problems and is a common problem for patients. EBS is a common problem affecting patients, often causing aesthetic concerns. Its unattractive appearance, which is common in children, can have a negative impact on a child's self-esteem [4]. EBS of teeth prevalence varies from 2.4 to 18% [2,3,5-7], at the same time, the research amoung adults is few [8].

AIM

Discovering of the prevalence, causes and consequences of the extrinsic black staining of teeth in pediatric population.

MATERIALS AND METHODS

The search strategies included electronic databases, such as Pubmed, Cochrane Library and Science Direct, reference lists of articles, and selected textbooks. Articles and textbooks used in this study were mainly reached by using the following keywords: «pediatric», «dental plaque», «black stain», «colored dental plaque». Upon completion of the scientific search, the review included 47 scientific articles in the time interval from 1976 to 2023.

REVIEW AND DISCUSSION

PREVALENCE

On the continent of Eurasia, in its European part, the prevalence of black plaque was 7.54% in children

aged 4-11 years (n=1100) in the city of Valencia (Spain) [3], 3.1% at 6-year-old children (n=3272) in Oviedo (Spain) [7], 6.3% at 6-12 years old (n=1086) in Potentsa (Italy) [3], 2.4% at 3-5.5 years old ochildren (n=950) in Thessaloniki (Greece) [9], high prevalence of up to 19.9% among school-age children from Switzerland (aged 7-15 years) [10].

At the Asian part of Eurasia the prevalence of a EBS was 16% at children of 10–12 years old (middle age 11,7±1,1 years) (n=1748) [3], 18% at children of 6-12 years old (middle age 9.4±1.9 years) (n=1472) in Udaipur (India) [11], 9.9% at children (middle age 4.55 years) (n=1937) in Shanghai (China) [5].

On the continent South America prevalence of a EBS is 3.5% at children of 5 years old (n=1120) of (n=1120) [6] and 14.8% at children of 6-12 years old (n=263) in Pelotas (Brazil) [12], and 4.16% at the children of 3-10 years old (n=433) in Rosario (Argentina) [3].

In our studies, EBS was diagnosed in 2.94 to 4.55% of 6 to 17 year olds (n=562) m. Kyiv, Ukraine [5].

We found that there is no apparent sex predilectio n [5, 13].

ETIOLOGY

The cause of EBS is not entirely understood. Ultrasound examination suggests that this stain is caused by a certain type of plaque with a tendency to calcification [13, 14]. Its microbial composition is believed to consist of chromogenic bacteria such as *actinomyces* and *prevotella melaninogenic* [12, 15]. The microbiome was characterized by various microbial biomarkers, such as *Pseudomonas fluorescens, Leptotrichia sp.*_HMT_212, *Actinomyces sp.*_HMT_169 and *Aggregatibacter sp.*_HMT_898 in the plaques from the BTS group. Functional analysis of microbial species suggested the existence of a hyperactive metabolic state on the surfaces of teeth with black plaques and showed that trivalent iron, ferrous iron complex transport system, and iron (III) transport system were more abundant in the plaque samples [16]. Higher numbers of *Lautropia* and *Pesudopropionibacterium* species were observed in the EBS group compared to the non-EBS group ($p<0.05$). *Lactobacillus* species were found in the feces of the EBS group. The results show that EBS is a possible protective factor against early childhood caries. Dental plaque and gut microbiome may be related to EBS in the temporary dentition [17].

Given the changes in the oral microbiome in EBS, when comparing the intestinal and oral microbiomes using 16S rRNA sequencing, it was shown that bacteria inhabiting the oral cavity are capable of penetrating the intestine and modulating intestinal dysbiosis [18, 19]. This may suggest a relationship between oral dysbiosis and intestinal dysbiosis and is closely related to the manifestation of inflammatory bowel disease and colorectal cancer [20, 21], thus, by a feedback mechanism, intestinal dysbiosis contributes to the demineralization of hard tooth tissues, thereby ensuring the formation of caries [22]. In addition, it was shown that a greater similarity of taxonomic units (OTUs) (n=378) among fecal samples was found between the EBS group and the healthy group, but 124 OTUs were unique to the EBS group. In addition, children with both caries and EBS showed an alteration in the gut microbiome, as the same microbial pattern was present in both plaque and fecal samples, suggesting a link between the oral and gut microbiome. In conclusion, the EBS group had a high OTU richness but lower uniformity compared to a group of healthy volunteers [23, 24]. There were only a few significant differences in α- and β-diversity, but potential organisms in the microbiota associated with EBS and caries in children were identified. In particular, the most common genera in plaque in patients with EBS were *Streptococcus*, *Actinomyces*, and *Leptotrichia* [25, 26]. The most common genera in the plaque of patients with caries were *Streptococcus, Leptotrichia, Actinomyces* and *Porphyromonas*.

Patients with EBS had a characteristic composition of the oral microbiome dominated by *Actinomyces* (10.82%), thus associating their presence with the appearance of colored biofilms on children's teeth [27, 28], while it was determined that the microbiome in the EBS group was characterized by various microbiome biomarkers, such as *Pseudomonas* species.

Agathobacter gram-positive anaerobes synthesizing butyrate to modulate anti-inflammatory functions of the gut were more common in the caries group than in EBS. *Escherichia* and *Shigella* species were associated with activation of proinflammatory status through the IL-6 signaling pathway and the pyrin domain of the NLR family, which has a positive correlation with chronic and persistent infections with adhesive and invasive characteristics [29, 30]. It was found that the enrichment of the oral microbiome with *Escherichia* and *Shigella* species correlated with patients who had EBS without caries than in the control group among children who had both caries and EBS [23, 24].

These results show that children with both caries and EBS may have gut dysbiosis and peripheral inflammation. Thus, the need for further research is obvious. The literature suggests a decreased caries prevalence in the presence of EBS [31] which associated with low incidence of caries in children [6, 32, 33].

It has been suggested that black stain on teeth is associated with low levels of dental caries in children

with temporary teeth. However, it remains questionable whether black stain has a protective effect against dental caries or whether children at low risk of caries are more likely to develop black stain because their oral microbiome favors the microorganisms that form black stain [34].

RISK FACTORS AND ENVIRONMENTAL IMPACT

The nature of the black pigmentation is suggested to be a form of bacterial plaque with an insoluble ferric salt, most likely ferric sulphide, which is formed by the reaction between the hydrogen sulphide produced by the bacteria and iron in the saliva or gingival exudate [13]. It is perhaps for this reason that some studies suggest an increased prevalence in EBS with the consumption of particular vegetables, dairy products and fruits high in iron [3].

In a study conducted among schoolchildren, 5 students were scraped for black spots and analyzed for trace elements. The trace elements were analyzed using inductively coupled photospectrometry (ICP). Out of the 5 scrapings, 3 showed the presence of iron ions of approximately 2.56%, calcium ions of 17.15% and magnesium ions of 0.72%, while the remaining 2 samples showed calcium ions of 14.86%, magnesium ions of 0.82% and no iron ions [35].

There are a profeccional impact that can cause EBS. The characteristic EBS of teeth in iron factory workers is well documented [36]. The effect of manganese and silver on the formation of EBS. The combustion products of coal tar in smokers penetrate into the pits and fissures, soaking enamel and dentin with tobacco juice. A number of other metals, such as potassium permanganate, can produce other dark colors of EBS ranging from purple to black when used in mouthwashes [32]. Black and red plaque. The use of betel leaves and nuts is commonly seen in adults and children in the Eastern Hemisphere, where betel leaves and nuts are used as stimulants. Sometimes a black and red coating may occur. The use of betel leaves and nuts is commonly seen in adults and children in the Eastern Hemisphere, where betel leaves and nuts are used as drug stimulants [37].

Poor dental care and consumption of certain beverages or food can be associated with discoloration or staining of teeth. In the literature there was a case of a linezolid (powerful antimycrobal and antimycobacterial drug that used in the short- and long-term treatment regimens of multidrug resistant tuberculosis and mycobacteriosis) black dental hyperpigmentation. Tooth discolorations of linezolid that have been mostly reported in children. There are two types of tooth discoloration. The stains that are present on the outer surface of the teeth and can be removed manually are known as extrinsic stains, whereas those which are deposited within the enamel of the tooth during its development are known as intrinsic stains. The teeth discoloration found with linezolid are of extrinsic type and reversible (i.e., they can be removed with extensive cleaning) [38, 39]. The characteristic EBS of teeth in people using iron supplements is well documented [36]. The dark brown to black discoloration was observed in people taking iron supplements, and oral iron salts in liquid form could cause the teeth to appear greenish black [40]. Artificially induced caries increased the structural porosities of the teeth and led to greater iron uptake and, consequently, higher discoloration. The maximum structural changes and subsequent staining occurred in the ferrous sulfate group followed by ferrous ammonium citrate, ferrous fumarate, and ferrous gluconate. Therefore, it is mandatory to consider implementing a uniform global policy for improving the quality of the iron supplements and the benefit of their considerable effects, although there is no scientific evidence that they play a key role in dental prevention [41].

Also, drugs can cause the teeth discoloration. In the operational study Jun Wang and all analyzed 1188 AE were reported from 2004 to 2021 The top reported drug was tetracycline (n=106), followed by salmeterol and fluticasone (n=68), amoxicillin (n=60), chlorhexidine (n=54), and nicotine (n=52). Cetylpyridinium (PRR=472.2, ROR=502.5), tetracycline (PRR=220.4, ROR=277), stannous fluoride (PRR=254.3, ROR=262.8), hydrogen peroxide (PRR=240.0, ROR=247.6), and chlorhexidine (PRR=107.0, ROR=108.4) showed stronger associations with tooth discoloration than the remaining drugs. Of 625 AE reports involving 25 drugs with positive risk signals, tooth discoloration was mostly reported in patients aged 45–64 (n=110) and ≤18 (n=95), and 29.4% (192/652) of the reports recorded serious outcomes [38].

CLINICAL AND LOCALIZATION FEATURES

EBS affects both temporary and permanent dentition [4]. The EBS is tightly adhered to the enamel surface and cannot be removed with a probe [2].

Studies show that the places of the highest prevalence are the lingual surfaces of the mandibular teeth [4]. This is probably due to its close proximity to the mandibular salivary glands and the role of saliva in the etiology of black staining [5]. Also it affects cervical third of the tooth crown [4] and buccal and lingual surfaces [4, 5].

Usually EBS follow the gingival margin [3, 4] does not extend to the proximal areas [13]. Sometimes black plaque covers the entire surface of the tooth. It can be deposited in the form of streaks on the tooth along the gum margin or spots on the vestibular and lingual surfaces [2].

CLASSIFICATION

EBS can be classified based on the surface area of the tooth affected [6]: score 1 corresponds to the presence of pigmented dots or thin lines with incomplete coalescence parallel to gingival margin; score 2 corresponds to continuous pigmented lines and limited to half of the cervical third of the tooth surface; score 3 corresponds to the presence of pigmented stains extending beyond half of the cervical third.

The classification of stained plaque was published in 2020 [2], a systematization of stained plaque was proposed, which takes into account the color of plaque, its localization, the degree of cariogenicity and the presence of classes of diseases (according to the international classification of diseases 10 revision - ICD 10).

MANIFESTATIONS OF PLAQUE IN OTHER DISEASES

The occurance of EBS is described in pediatric population with some infectious and parasitic diseases (ascariasis) [2], blood disorders (iron deficiency anemia) [7], with endocrine, nutritional and metabolic diseases (malnutrition) [42], mental and behavioral disorders (autism spectrum disorders) [2], disorders of expressive speech, mild mental retardation, general underdevelopment of speech, mental retardation [2], onychophagia [43], hearing and mastoid bone dieseases (infectious processes in the internal or middle ear) [2], with respiratory diseases (influenza and pneumonia [44] acute tonsillitis, bronchial asthma, chronic rhinitis [2, 7] breathing through mouth disorders [43]), with diseases of the digestive system (enterocolitis, functional disorders of the intestine, unspecified ones) [2, 7], maxillofacial abnormalities (malocclusion due to sucking on the tongue, lips or finger and maxillofacial anomalies) [43], with skin diseases (allergic contact dermatitis) [2].

In the study [2], black plaque was observed in 8 out of 19 (42.11%) children with respiratory diseases, in 6 out of 19 (31.58%) children with parasitic diseases, in 5 out of 19 (26, 32%) of children with diseases of the digestive system, and in 3 of 19 (15.79%) children with diseases of the blood and hematopoietic organs.

CARIESOGENITY IN PATIENTS WITH DENTAL PLAQUE

The assessment of dental plaque cariogenicity was carried out according to the procedure proposed by J.L. Hardwick, E.B. Manly [45]; the acid activity was determined with the use of the colorimetric method of indicator's color changing from yellow to pale orange (0-30%); from pale orange to red (40-60%); from red to dark red (70-100%); methylene red was used as a color indicator for dental plaque. One percent of glucose solution was applied for several minutes on the tooth enamel; 0.1% aqueous solution of methylene red was applied on the surface of lower incisors. The results of staining were interpreted in the following way: the indicator was positive if the color of the stained plaque changed to red; the indicator was negative if the color did not change.

When black plaque was stained with methylene red, the color of the indicator did not change, so these types of plaque can be considered as having a low degree of caries susceptibility. After removing black plaque, the surface of the tooth enamel underneath was shiny, without damage or traces of demineralization. After professional cleaning of teeth, patients with black plaque were observed to re-form after two months [44].

Also, black dental plaque should be considered dense in consistency.

CONCLUSIONS

External black plaque is a common problem among patients that begins in childhood and continues into adulthood, causing aesthetic discomfort and certain difficulties in social interaction. Correct diagnosis of EBS is essential to provide appropriate advice on oral hygiene and subsequent treatment of this external staining. It can often indicate an aesthetic concern of the patient or parents of a pediatric patient, and simple dental procedures, including professional oral hygiene, can significantly improve the patient's self-esteem.

The dentist should be careful to avoid iatrogenic damage to immature tooth enamel in pediatric patients with an overbite when attempting to remove the stain.

There is a clear correlation between black plaque and concomitant conditions and caries susceptibility, which requires careful attention of the dentist to patient management and detailed history taking. With this review, we wanted to encourage dentists to identify the problem in childhood and collaborate in a multidisciplinary team to improve treatment efficiency and speed up the selection of the right tactics for the most person-centered approach to avoid worsening the problem in adulthood.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CASE STUDY

CONTENTS

Rhinocerebral form of mucormycosis in a patient with post-COVID-19 syndrome and type 1 diabetes mellitus: clinical and morphological analysis of the case from practice

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ABSTRACT

Mucormycosis is a serious life-threatening opportunistic infection which is characterized by various clinical and morphological manifestations, rapid progression, unpredictable course and high mortality. The development of mycormycosis depends on the metabolic and immune status of the human body. The authors conducted a clinical and morphological analysis of the case of rhinocerebral mucormycosis in a patient who was diagnosed lifetime, but despite a set of therapeutic and surgical measures led to death, which was due to the severity of the pathology, late hospitalization of the patient and the presence of comorbidities (type 1 diabetes mellitus and post-COVID-19 syndrome). The clinical case presented by the authors raises the issue of the need to increase physicians' awareness about the rhinocerebral form of mucormycosis, improve early diagnostic methods and find effective approaches to therapeutic and surgical treatment of this pathology.

KEY WORDS: mucormycosis, rhinocerebral form, comorbidity, clinical and morphological analysis, case from practice

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INTRODUCTION

Mucormycosis is a serious life-threatening opportunistic infection caused by ubiquitous molds belonging to the order Mucorales [1]. Today mucormycosis is the third most frequent invasive mycosis, following candidiasis and aspergillosis [2]. The most frequently reported pathogens in mucormycosis are Rhizopus spp, Mucor spp, and Lichtheimia spp, followed by Rhizomucor spp, Cunninghamella spp, Apophysomyces spp, and Saksenaea spp [3]. Rhizopus oryzae being the most common species. It is responsible for approximately 60% of mucormycosis cases and 90% of rhinocerebral form in humans.

The incidence of mucormycosis in developed countries is lower than that in developing countries [4]. Mucormycosis pathogens are widespread in the environment in different climatic zones. They enter the human body through inhalation of infected dust, ingestion of food, and traumatic damage to the eyes and skin. There are cases of mucormycosis infection in case of skin damage as a result of tattoos, drug addicts, and the use of medical instruments contaminated with fungal spores [5]. Nosocomial infections caused by Mucorales commonly develop as complications in healthcare settings, particularly intubation and mechanical ventilation, in surgical wounds or at intravenous catheter insertion sites. [6].

Mucormycosis is characterized by various clinical and morphological manifestations, rapid progression, unpredictable course and high mortality [7]. There are five main clinical and morphologic forms of mucormycosis: rhinocerebral, cutaneous, pulmonary, gastrointestinal and disseminated. Some scientists also identify rare forms characterized by damage to the kidneys, heart, joints, etc. The most common clinical presentation of mucormycosis is the rhinocerebral and pulmonary forms [8]. The mortality rate of mucormycosis has been reported as 96% for disseminated form, 85% for gastrointestinal form, 76% for pulmonary form, 69% for rhinocerebral form [9].

The development of mycormycosis depends on the metabolic and immune status of the human body. Nu-

merous studies have proven that a predisposing factor for the development of mucormycosis in a patient is the presence of an immunodeficiency state. The reasons for the latter may be the use of glucocorticosteroid drugs, immunosuppressive therapy after organ transplantation, irrational use of antibiotics, oncohematological diseases, HIV/AIDS, severe diabetes mellitus [10]. Diabetic ketoacidosis is a major predisposing comorbid condition that can help Mucorale [11]. During ketoacidosis, iron disengages from transferrin, and the released free iron impairs the function of neutrophils and undermines their ability to damage and kill Mucorales hyphae [12]. In recent times, tuberculosis and chronic renal failure have also become more prominent as risk factors [13]. Malnourishment and neonatal prematurity are also referred to as risk factors for the development of mucormycosis [4].

COVID-19 infection increases the incidence of COVID-19-associated mucormycosis and post-COVID-19 mucormycosis. It's remarkable that before the pandemic, mucormycosis was reported at a rate of 0.5 to 1.5 cases per million, but with the advent of COVID-19, this rate has surged to 26.7% [14]. Research indicates that mucormycosis typically develops around 10 to 15 days after a COVID-19 diagnosis [15].

The ability of SARS-CoV-2 to damage not only lung tissue but also vascular endothelium, activate nonspecific cellular response and production of proinflammatory cytokines, causing, on the one hand, a cytokine storm, and on the other hand, enhancing immunosuppression by reducing the levels of $CD4^+$-, $CD8^+$-lymphocytes, creates favorable conditions for the development of mucormycosis [11, 16, 17]. Our previous study identified the bacterial and fungal genesis of rhinosinusitis in patients in post-COVID-19 period. Among the bacteria, it was noted Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumonia, Streptococcus pneumonia and Enterococcus faecalis. Among fungi, there were Aspergillus, Candida, Mucor and Coccidioides [18].

The diagnosis of mucormycosis, especially in the setting of unfavorable comorbidity, is extremely difficult, requires an interdisciplinary approach and occurs during morphological examination of the material. The purpose of this work was to conduct a detailed clinical and morphological analysis of a fatal case of rhinocerebral mucormycosis in a patient with post-COVID-19 syndrome and type 1 diabetes mellitus.

CASE STUDY

Patient M., female, 42 years old, was hospitalized on September 3, 2023. The anamnesis revealed that the patient had a long history of type 1 diabetes mellitus, and in August 2023 she was hospitalized for severe coronavirus infection (COVID-19). From August 28 to September 2, 2023, the patient was treated for a cavernous sinus thrombosis, but the treatment did not give the desired result, the patient's condition deteriorated, and she was transferred to another hospital.

At the time of the last hospitalization, the patient was in an extremely serious condition. During the examination, the skin of the face on the left side in the infraorbital region, in the lower eyelid, and nose was necrotic, black in color. The nasal mucosa was black, with fragments of necrotic tissue and bloody clots in the nasal passages. Cytological examination of nasal discharge revealed abundant mixed flora and inflammatory cells. On September 6, 2023, the patient underwent enucleation of the left eye and necrectomy of the soft tissues of the left face. On September 8, 2023, a computed tomography scan diagnosed multiple foci of infarction in the left temporal, parietal and frontal lobes, left cerebellar hemisphere, and brainstem. Over time, the patient's condition progressively deteriorated. On September 12, 2023, the patient died.

During the autopsy, the woman's left eye was absent. The skin of the face on the left side in the infraorbital region, in the lower eyelid, nose, as well as the mucous membrane of the nose and the soft and hard palate on the left were necrotic and black in color. The dura mater was tense and full of blood. The soft meninges looked swollen, dull and sharply hemorrhagic. The brain was edematous and moist, the furrows were smoothed, and the gyri were flattened. Multiple small abscesses and ischemic infarcts were recorded in the left temporal, parietal and frontal lobes, left cerebellar hemisphere, and brainstem. In areas of infarcts the brain acquired a mushy consistency and was whitish-gray in color. Macroscopic examination of other organs and systems did not reveal any pathology.

Histologic examination of the surgical material in the eyeball revealed purulent panophthalmitis, acute thrombosis of dilated choroid and iris vessels. In the removed skin fragments with underlying soft tissues, severe necrotic and hemodynamic changes, moderate inflammatory changes, and a proliferation of plexiform fungal hyphae growing through the walls of blood vessels and filling their lumens, and in some places growing into nerve trunks were detected (Fig. 1).

Histologic investigation of the autopsy material revealed hemodynamic disorders in the soft meninges, severe diffuse infiltration of neutrophilic leukocytes and lymphocytes, which in some visual fields extended to the brain tissue (Fig. 2). Alternative and hemodynamic disorders were noted in the brain tissue, diffuse leuko-

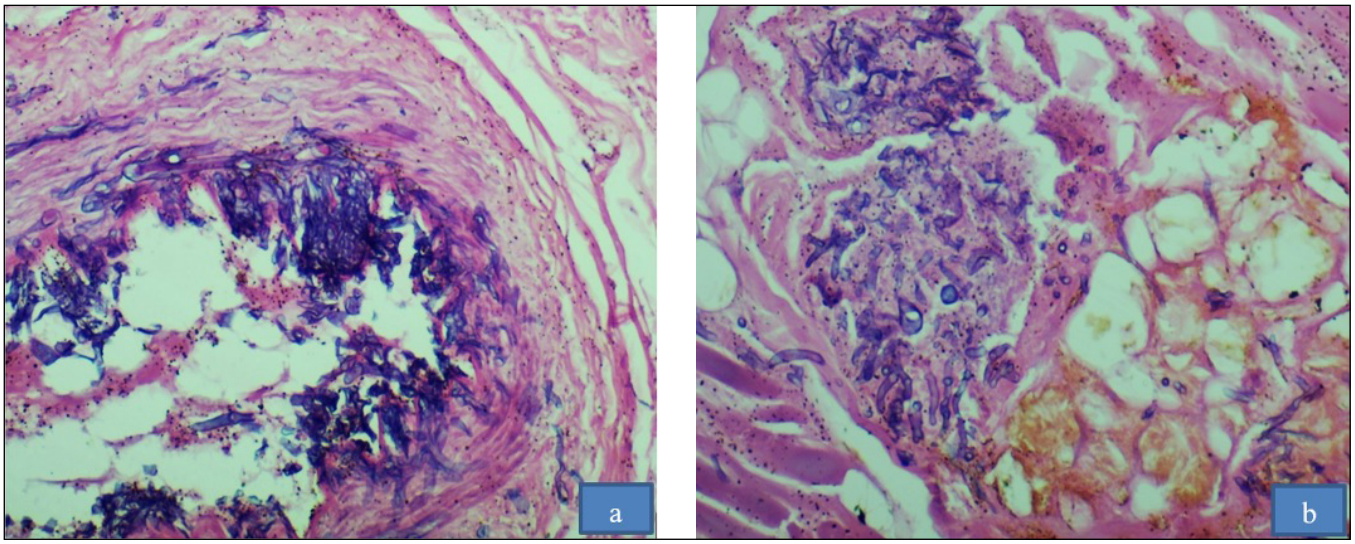

Fig. 1. Disseminated fungal hyphal plexuses in the skin with underlying soft tissue. Hematoxylin and eosin staining, a) ×200, b) ×200.

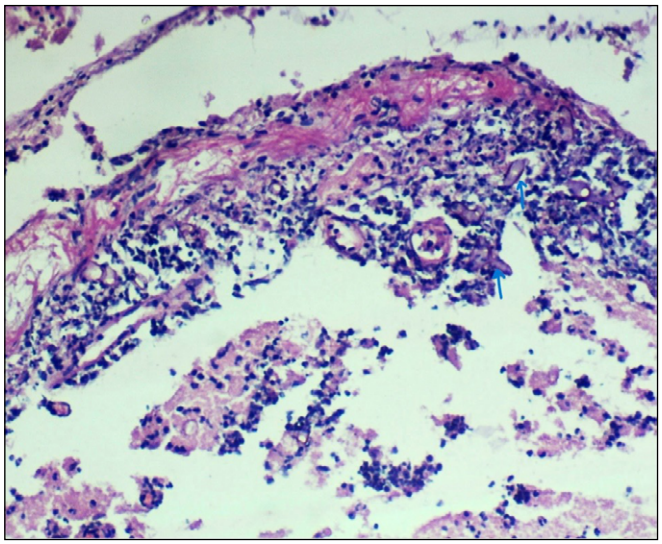

Fig. 2. Fungal hyphae (marked with a blue arrow), diffuse infiltration with neutrophilic leukocytes, lymphocytes of the dura mater and brain tissue. Hematoxylin and eosin staining, ×200.

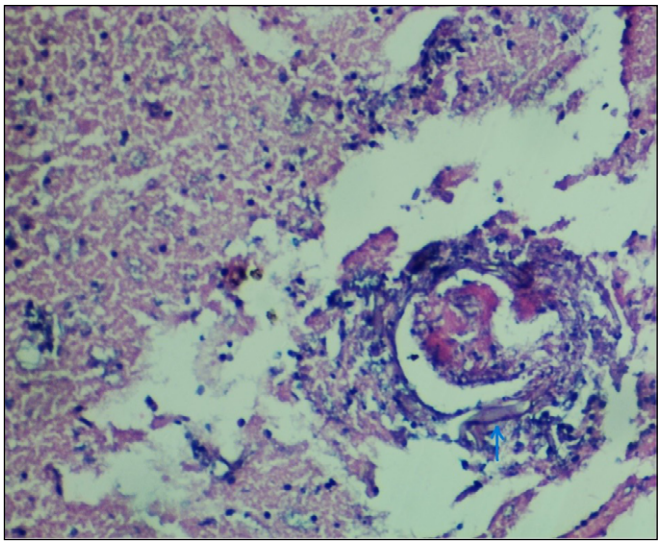

Fig. 3. Alternative, hemodynamic and inflammatory changes in the brain tissue. Accumulation of fungal hyphae in the brain tissue, in thrombotic masses that obstruct the lumen of the vessel. Fungal hyphae (marked with a blue arrow) sprouting through the vessel wall. Hematoxylin and eosin staining, ×400.

cyte infiltration in some visual fields, and multiple acute abscesses with the presence of coenocytic (aseptate) hyphae in some visual fields (Fig. 3). The cerebral vessels were characterized by plethoricity and the presence of acute thrombi with coenocytic hyphae in the lumen (Fig. 3). In some of the fields of view, fungal hyphae sprouted through the vessel wall (Fig. 3). The mucous membrane of the paranasal sinuses on the left showed severe necrotic changes.

Thus, the results of the autopsy, histological examination of the surgical and autopsy material indicated that the patient died as a result of intoxication and brainstem dislocation caused by the rhinocerebral form of mucormycosis.

DISCUSSION

Rhinocerebral mucormycosis used to be a rare fungal infection, which, taking into account the literature and the results of our previous studies, is now quite common in patients of different ages, especially against the background of comorbid pathology, manifested by weakened immunity [18, 19]. In rhinocerebral form of mucormycosis the fungus penetrates the nasal cavity through the skin-mucosa junction, spreads to the palate, sinuses, and orbit, and finally causes intracranial infection [20, 21].

Major insights in mucormycosis pathogenesis have brought into focus the host receptors (glucose-regulated protein 78 (GRP78)) and signaling pathways (epidermal growth factor receptor activation cascade)

as well as the adhesins used by Mucorales for invasion [4, 22]. Notably, GRP78 also serves as a shared entry route for both the SARS-CoV-2 virus and Mucor fungi into the nasal and paranasal sinus mucosa [23].

In the article, the authors conducted an analysis of the clinical and morphological features of the rhinocerebral form of mucormycosis in a patient with type 1 diabetes mellitus and post-COVID-19 syndrome. The observed case of rhinocerebral mucormycosis was characterized by typical clinical manifestations and diagnosed during life, but despite the complex of therapeutic and surgical measures, this pathology led to death, which, in our opinion, was due to the severity of the pathology, late hospitalization of the patient and the presence of unfavorable comorbidity (type 1 diabetes mellitus and post-COVID-19 syndrome).

Early diagnosis of mucormycosis is extremely important, which is possible during morphological examination of biological material from the lesion. During the examination of the material, fungal hyphae are characterized by uneven thickness. The mycelial filaments branch at different angles, are unseptate, with a double-circular membrane and coarse-grained cytoplasm. Clusters of mycelium form disorderly plexuses [22].

The pathognomonic morphological feature of mucormycosis is the lesion of blood vessels, which is important in the manifestation and generalization of the pathological process. Some scientists characterize mucormycosis as an angioinvasive disease [24]. The lesions of blood vessels, as shown by our morphological examination of the surgical and autopsy material, were characterized by the fact that fungal hyphae germinated the vessel wall, formed clusters of threads and thrombi in their lumen. Changes in the vessel walls caused trophic disorders and, accordingly, the development of severe necrotic changes in the patient's skin of the face on the left side in the infraorbital region, in the lower eyelid, nose, as well as the mucous membrane of the nose and the soft and hard palate on the left. The literature describes cases of rhinocerebral mucormycosis manifested by necrosis of the bone tissue of the facial skull [25].

Multiple ischemic infarctions in the left temporal, parietal, and frontal lobes, left cerebellar hemisphere, and brainstem, which were found during the autopsy, are most likely caused by cerebral vascular thrombosis. According to the literature, as a result of angioinvasion in mucormycosis, not only ischemic infarctions occur in the brain, but also intracerebral hemorrhages [24].

CONCLUSIONS

Mucormycosis is a life-threatening pathology characterized by various manifestations, severe course and high mortality. This disease, which was previously considered rare, shows a significant increase in the incidence, especially among patients with comorbid conditions, manifested by weakened immunity. The authors conducted a clinical and morphological analysis of a case of rhinocerebral mucormycosis in a patient who was diagnosed lifetime, but despite a set of therapeutic and surgical measures led to death, which was due to the severity of the pathology, late hospitalization of the patient and the presence of comorbidities (type 1 diabetes mellitus and post-COVID-19 syndrome). The clinical case presented by the authors raises the issue of the need to increase physicians' awareness about the rhinocerebral form of mucormycosis, improve early diagnostic methods and find effective approaches to therapeutic and surgical treatment of this pathology.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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