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Memory of
dr Władysław
Biegański

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Abdominal wall endometriosis in Ukraine: A multicenter study

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ABSTRACT

Aim: To estimate the incidence of abdominal walls endometriosis in Ukraine and review the clinical findings, imaging results, and histopathology of patients who have had cesarean scar endometriosis.

Materials and Methods: We performed multicentre retrospective cohort study from January 2020 to December 2024. The study involved 27 hospitals from 10 regions of Ukraine and included 9,157 reproductive women who had a painful mass in their previous abdominal surgery scar area. Definitions of endometriosis were adapted from the WHO.

Results: Among 9,157 patients, 387 (4.2%) abdominal walls endometriosis (AWE) were observed.

Of all cases 82.2% women had cesarean scar endometriosis and 17.8% had scar endometriosis related to gynecologic surgical procedure. The incidence of AWE associated with history of cesarean section, gynecological abdominal hysterectomy and laparoscopic procedures was 6.3%, 2.3%, and 1.2%, respectively. The main symptoms of scar endometriosis were palpable abdominal mass (100%) and cyclic pain (86.8%). The latency period between cesarean section procedure and of cesarean scar endometriosis (CSE) symptom onset was 31.8 ± 23.6 months. The duration between the onset of symptoms of CSE and this surgery was 28.7 ± 25.4 months. The diagnosis of CSE was made through a histopathological examination.

Conclusions: In Ukraine abdominal wall endometriosis (AWE), is a relatively uncommon entity that usually develops at the site of a surgical scar that occurs after obstetric or gynecologic surgeries. In our study the most frequently of all AWE cases was cesarean scar endometriosis.

KEY WORDS: incidence, scar endometriosis, abdominal walls endometriosis, cesarean section, cesarean scar endometriosis, Ukraine

INTRODUCTION

Endometriosis is a common disease among women worldwide. This disease negatively affects women's reproductive health, leads to a loss of productivity at work and causes significant socio-economic damage. Due to its nonspecific symptoms of endometriosis, misdiagno-

sis or delayed diagnosis are usually happened which can lead to adverse outcomes including infertility, ectopic pregnancy, and chronic pelvic pain. Despite heightened attention from the scientific community in world, the causes of endometriosis and the pathogenesis of endometriosis has not been fully elucidated to date.

Previous studies have shown that the most common of endometriosis include ovarian endometriomas, superficial peritoneal disease, and deep infiltrating endometriosis [1, 2]. Less frequently, the disease may extend beyond the pelvis (extra-pelvic endometriosis). The extra-pelvic sites of endometriosis include the gastrointestinal tract, urinary tract, upper and lower respiratory tract, umbilicus, inguinal region, and surgical scars of the abdominal wall, and brain [3-6].

According to the literature, in women who had obstetric, gynecological (abdominal hysterectomy or laparoscopy) and other abdominal surgical procedures may cause endometriosis [7-10]. Robert Meyer reported that postoperative scar endometriosis, can be caused by the dissemination of endometrial tissue to the wound at time of surgery [11]. According to the literature, abdominal wall endometriosis in women can occur after cesarean section, hysterectomy, salpingostomy, episiotomy, amniocentesis, laparoscopy and other surgeries. The endometrial tissue can be found at the abdominal wall musculature, or most commonly in the subcutaneous tissue [5].

The reason why some women develop postoperative scar endometriosis after surgical procedure do not is not fully understood. According to the literature, the risk factors for scar endometriosis (abdominal wall endometriosis) are not well known, it has been shown that previous cesarean section, is the most common risk factor for this condition [7, 12, 13]. The reported that, the occurrence of scar endometriosis has been increasing together with the increase of cesarean section incidence [14].

Cesarean section is one of the most common obstetric surgical procedures performed on women in Ukraine. However, studies focused on cesarean scar endometriosis have not been conducted in Ukraine. A previous study has focused on the prevalence of surgical site infection associated with Obstetric and gynecological surgical procedures, and surgical site infections as risk for the development of endometriosis [10, 15, 16].

AIM

The aim of this study was to estimate the incidence of abdominal walls endometriosis in Ukraine and review the clinical findings, imaging results, and histopathology of patients who have had cesarean scar endometriosis.

MATERIALS AND METHODS

STUDY DESIGN, SETTING AND PATIENTS

We performed multicentre retrospective cohort study based of surveillance data on endometriosis in 2020-

2024. This study was included women with abdominal wall endometriosis. The study involved 27 hospitals from 10 regions (Kharkiv, Kherson, Zaporizhzhia, Odessa, Kyiv, Poltava, Vinnytsya, Lviv, Ternopil, Lutsk) of Ukraine. Inclusion criteria: women who had obstetric, gynecological (abdominal hysterectomy or laparoscopy) and other abdominal surgical procedures, and had suggestive symptoms during anamnesis and the presence of abdominal wall close to the surgical scar should raise suspicions of endometriosis. Exclusion criteria: hernia (inguinal or incisional), abdominal wall tumours of other causes, lipomas, haematomas, granulomas, metastases from distant tumours, and desmoid tumours, and women who refused to sign the informed consent form.

DEFINITION

Scar endometriosis, also referred to as abdominal wall endometriosis (AWE) is a disease in which tissue similar to the lining of the uterus (endometrium) grows on the abdominal wall close to the surgical scar. In this study the scar endometriosis was defined as visible evidence of postsurgical residual of unresectable endometriotic lesions. Asymptomatic or latent period was defined as the time interval between the previous surgery and the onset of the symptoms. Cesarean section scar endometriosis is a one form of extra-pelvic endometriosis where endometrial tissue implants in the surgical incision. Endometriosis was identified using Abdominal ultrasonography (USG), computed tomography (CT), magnetic resonance imaging (MRI), and fine-needle aspiration (FNA) biopsy.

DATA COLLECTION

We analyzed the inpatient data medical records patients with scar endometriosis, also referred to as abdominal wall endometriosis (AWE) to identify and describe demographic characteristics, clinical features, types of previous obstetric (cesarean section), gynecological (abdominal hysterectomy or laparoscopy) and other abdominal surgical procedures, symptoms at the time of presentation, imaging methods used for diagnosis, including both USG, CT and MRI, and pathology reports, and type of surgery for endometriosis.

ETHICS

The protocol for the study project has been approved by a suitably constituted Ethics Committee of the Shupyk national healthcare university of Ukraine (Kyiv, Ukraine) and that it conforms to the provisions of the Declaration of Helsinki.

Table 1. Characteristics, symptoms, diagnostic tools, and cesarean scar endometriosis sites of the patients (n=387) in Ukraine (2020-2024)

Variable	CSE	
	n	%
Age of the patients (years), mean $\pm$ SD (range)	32.2 $\pm$ 3.4 (21.2-43.1)	
Age at cesarean section (years), mean $\pm$ SD (range)	27.3 $\pm$ 3.1 (19.4-37.1)	
Latency period (months), mean $\pm$ SD (range)	31.8 $\pm$ 23.6 (3-118)	
Duration between symptoms and surgery (months), mean $\pm$ SD (range)	28.7 $\pm$ 25.4 (1.5-176)	
Body mass index		
≥ 25	238	61.5
< 25	149	38.5
Parity		
1	346	89.4
2	41	10.6
Number of cesarean section		
1	358	92.5
2	29	7.5
Main complaint (symptoms)		
Painful abdominal mass	387	100.0
Cyclic pain	336	86.8
Noncyclic pain	51	13.2
Swelling	101	26.1
Dysmenorrhea	112	28.9
Onset of complaints		
1 year after the cesarean section	82	21.2
2 years after the cesarean section	204	52.7
3 years after the cesarean section	81	20.9
4 years after the cesarean section	20	5.2
Incision type		
Pfannenstiel	320	82.7
Vertical midline	67	17.3
Scar endometriosis site		
Right side of the scar	205	53.0
Left side of the scar	139	35.9
Middle line of the scar	43	11.1
Prediagnosis		
AWE	332	85.8
Incisional hernia	55	14.2
Radiology diagnostic tools		
USG	387	100.0
CT	92	23.8
MRI	48	12.4
USG-guided needle biopsies	45	11.6
Admission		
Gynecology	177	45.7
General surgery	210	54.3
Treatment		
Surgical resection	387	100.0

CSE, Cesarean scar endometriosis USG, Ultrasonography, CT, Computed tomography, MRI, Magnetic resonance imaging

Source: compiled by the authors of this study

STATISTICAL ANALYSIS

In present study, statistical analyses were performed using SPSS Statistics for Windows, Version 20.0. (IBM Corp., Armonk, NY, USA). The demographic and clinical characteristics of women were analyzed using descriptive methods (means, $\pm$). All data are presented as numbers and percentages. χ^2 test was performed to compare the differences between groups for categorical variables. The Cox model calculated 95% confidence interval (CI) of scar endometriosis (abdominal wall endometriosis) in women undergoing obstetric and gynecological, and other abdominal surgical procedures compared to the comparison group. In this study p value <0.05 was considered statistically significant.

RESULTS

The study included 9,157 reproductive women who had a painful mass in their previous abdominal surgery scar area. These patients had 5,047 obstetric (cesarean section), 1,857 hysterectomy and 2,253 laparoscopic procedures. Among 9,157 patients, 387 (4.2%) abdominal walls endometriosis (AWE) were observed. Of all AWE cases 318 (82.2%) women had cesarean scar endometriosis and 69 (17.8%) women had AWE related to gynecologic surgical procedure. The incidence of AWE associated with history of cesarean section, gynecological abdominal hysterectomy and laparoscopic procedures was 6.3% [95% confidence interval (CI), 6.1–6.6], 2.3% (95% CI, 2.1–2.5), and 1.2% (95% CI, 1.1–1.3), respectively. In this study all patients had a history of at least one endometriotic nodule of the abdominal wall, and a histological diagnosis of endometriosis.

The most AWE cases (82.2%, 318/387) was diagnosed in cesarean section scar area. The mean age of the patients with AWE was 32.2 ± 3.4 years (range 21.2–43.1). All patients with AWE had a history of at least one cesarean section procedure. The mean age at cesarean section was 27.3 ± 3.1 years (range 19.4–37.1). The body mass indexes (BMI, kg/m^2) of 61.5% patients with cesarean scar endometriosis were ≥ 25 , and those of 38.5% < 25 . The mean BMI was 26.95 ± 3.59 kg/m^2 (range from 22.3 to 33.5 kg/m^2). The common complaint of the patients with cesarean scar endometriosis was a palpable mass (100%) under the incision scar and cyclical pain (86.8%). Noncyclic pain was 13.2% patients. Swelling in the incision scar area and dysmenorrhea had 26.1% and 28.9% patients, respectively. In present study the latency period of cesarean scar endometriosis in women ranged from 3 to 118 months, with a mean of 31.8 ± 23.6 months. The duration between the onset of main symptoms of cesarean scar endometriosis and surgery was 1.5–176 (mean 28.7 ± 25.4) months (Table 1).

In this study, a majority (82.7%) of the patients had undergone a Pfannenstiel incision. The vertical midline incision was 17.3% patients. In total, 387 abdominal wall endometriomas associates with cesarean section were excised. A majority of the endometriomas were located in corner sites, including right side of the scar (53%) and left side of the scar (35.9%). Endometrioma in middle line of the scar was 11.1% patients. Table 1 presents the main characteristics, symptoms, diagnostic tolls, and cesarean scar endometriosis sites of the patients.

The latency period between cesarean section procedure and symptom onset was 31.8 ± 23.6 (range 3–118). Latency period based on patients' characteristics, symptoms, and cesarean scar endometriosis sites showed in Table 2.

In present study, of the 387 endometriomas, 89.9% were located between the adipose layer and the fascia layer, and 8.3% were located between the adipose layer and the muscular layer. Only 1.8% of all endometriomas were located between the muscular layer in cesarean section area. All patients who had a painful mass in their previous abdominal surgery scar area underwent preoperative abdominal ultrasound (USG), 23.8% underwent computed tomography (CT), 12.4% magnetic resonance imaging (MRI), and 11.6% received ultrasound-guided needle biopsies (Table 1). In this study, the diagnosis of cesarean scar endometriosis was made through a histopathological examination. All of the patients with cesarean scar endometriosis were treated surgically and endometriomas were excised easily.

DISCUSSION

The aim of this study was to estimate the incidence of abdominal walls endometriosis (AWE) in Ukraine and review the clinical findings, imaging results, and histopathology of patients who have had cesarean scar endometriosis. This study expands upon the previous reports on endometriosis [2, 10] and is the first study to publish incidence of cesarean scar endometriosis (CSE) in Ukraine. In present study, among 9,157 patients, 387 (4.2%) AWE were observed. Of all cases 82.2% women had cesarean scar endometriosis and 17.8% had AWE related to gynecologic surgical procedure. The incidence of AWE associated with history of cesarean section, gynecological abdominal hysterectomy and laparoscopic procedures was 6.3%, 2.3%, and 1.2%, respectively. The main symptoms of AWE were palpable abdominal mass (100%) and cyclic pain (86.8%). The latency period between cesarean section procedure and of cesarean scar endometriosis (CSE) symptom onset was 31.8 ± 23.6 months. The duration between the onset of symptoms of CSE and this surgery was

Table 2. Latency period based on patients' characteristics, symptoms, and cesarean scar endometriosis sites in Ukraine (2020-2024)

Variable	CSE		Latency period	
	n	%	months	median (quartiles)
Age at cesarean section (years)				
≤ 24	99	25.6	24	12–48
25–34	271	70.0	24	12–40
≥ 35	17	4.4	30	10–48
Parity				
Nulliparous	326	84.2	24	12–36
Multiparous	61	15.8	21	6–48
One previous cesarean section				
Yes	53	13.7	24	6–48
No	334	86.3	24	12–36
Dysmenorrhea				
Yes	112	28.9	19	12–36
No	275	71.1	24	12–36
Incision type				
Pfannenstiel	320	82.7	24	12–36
Vertical midline	67	17.3	33	24–60
Location of the scar endometriosis				
Right side of the scar	205	53.0	24	12–36
Left side of the scar	139	35.9	24	12–48
Middle line of the scar	43	11.1	30	24–38
Bound of the endometriomas				
Adipose layer	348	89.9	24	12–39
Fascia layer	32	8.3	24	18–49.5
Muscular layer	7	1.8	48	48–56

CSE, Cesarean scar endometriosis

Source: compiled by the authors of this study

28.7 ± 25.4 months. The diagnosis of CSE was made through a histopathological examination.

According to the literature, pelvic endometriosis in women involves organs or tissues in the located on the outer walls of the uterus, the ovaries, the pelvic peritoneum, and the uterosacral ligaments cavity [1, 2, 10] and extra-pelvic endometriosis involves organs or tissues located outside the pelvic cavity [4-9, 11,12]. Case reports in the literature include findings of extra-pelvic endometriosis in virtually every organ system and tissue in the body, including the CNS, lungs, pleura, heart, diaphragm, gallbladder, liver, small bowel, appendix, colon, rectum, kidney, bladder, ureter, the umbilicus, episiotomy scars, abdominal wall incisions, biceps muscle, bone, and peripheral nerve are other reported sites in the literature. The true prevalence or incidence of extra-pelvic endometriosis is unknown. The literature reports that although endometriosis predominantly involving pelvic structures, extra-pelvic

manifestations of endometriosis account for 1–12% of all form (pelvic and extra-pelvic) endometriosis cases, with the abdominal wall representing the most frequent extra-pelvic site [18].

Scar endometriosis, also referred to as abdominal wall endometriosis (AWE) is a disease in which tissue similar to the lining of the uterus (endometrium) grows on the abdominal wall close to the surgical scar. Scar endometriosis is estimated to represent 0.03%–2% of extra-pelvic forms of the disease [19, 20]. In present study, among 9,157 patients, 387 (4.2%) scar endometriosis were observed.

According to the literature, scar endometriosis most commonly arises in or adjacent to surgical scars following obstetric or gynecologic surgery involving laparotomy or laparoscopy [10, 21]. Incidence of scar endometriosis has a very low and varied from 0.03–0.45% [6] to 3.5% [22]. This can be explained due to inconsistent epidemiological data reports and due to

diagnostic difficulties. Kaplanoglu M., et al reported that the incidence of scar endometriosis of 0.03–0.4% following cesarean sections, 1.08–2% after hysterectomies, and 0.06–0.7% post-episiotomy [23]. However, Mishin I, et al [24] and Thanasa A, et al [25] reported that in approximately 20% of cases without a history of abdominal surgery. In our study of all cases 82.2% women had cesarean scar endometriosis and 17.8% had AWE related to gynecologic surgical procedure. The incidence of scar endometriosis associated with history of cesarean section, gynecological abdominal hysterectomy and laparoscopic procedures was 6.3%, 2.3%, and 1.2%, respectively.

According to the literature, scar endometriosis associated with cesarean section in women manifests as a firm, painful lump near the scar that may cause cyclic pain with the menstrual cycle and this symptoms onset usually occurs within three months to 9–10 years after surgery [7, 8, 11, 12].

In our study the main symptoms of cesarean scar endometriosis (CSE) were palpable abdominal mass (100%) and cyclic pain (86.8%). The latency period between cesarean section procedure and of CSE symptom onset was 31.8 ± 23.6 months. The duration between the onset of symptoms of CSE and this surgery was 28.7 ± 25.4 months. The diagnosis of CSE was made through a histopathological examination.

STRENGTHS AND LIMITATION

Our report is the first on the diagnosis of extrauterine endometriotic lesions and the prevalence of abdominal wall endometriosis associated with cesarean section in Ukraine. The strengths of our study lay in having included a highly patients who had obstetric and gynecological surgical procedures association with abdominal scar endometriosis. A limitation our study is that the mostly ultrasound (USG) was used of diagnosis. However, using USG alone without a subsequent computed tomography (CT) or magnetic resonance imaging (MRI) would not produce a definitive diagnosis of abdominal scar endometriosis and would involve the risk of missing

out other pathologies. Despite the abovementioned limitations, our report contributes to the search for the best approach for pathology of AWE. Further studies be valuable in contributing to findings this study.

CONCLUSIONS

Our study showed that in Ukraine scar endometriosis, also referred to as abdominal wall endometriosis (AWE), is a relatively uncommon entity that usually develops in the skin, subcutaneous tissues, and abdominal wall musculature at the site of a surgical scar that occurs after various obstetric or gynecologic surgeries. Despite its association with prior cesarean section, scar endometriosis in women remains under-recognized. In our study the most frequently of all abdominal endometriosis cases was cesarean scar endometriosis. Abdominal wall endometriosis should be suspected in all women with a history of cesarean section who had palpable, painful abdominal mass associated with the menstrual cycle. Ultrasound (transabdominal) imaging in the clinical setting is a valuable tool to identify endometriotic foci inside the superficial tissues of the abdominal wall. The subcutaneous and intramuscular endometrioses of the abdominal wall are not rare gynecological conditions. The clinically diagnose of cesarean section scar are quite difficult to clinically diagnose if the mass is not easily palpable. The use of computed tomography, magnetic resonance imaging, and fine-needle aspiration biopsy (FNA) may improve the timely diagnosis of ectopic endometriotic lesions associated with cesarean section. Multimodal imaging (USG, MRI, and CT) aids differentiation of scar endometriosis, but histopathology remains definitive. Early diagnosis of scar endometriosis and intervention are paramount to prevent complications, including malignancy. Future studies are needed on the features of endometriotic masses, using with all methods (USG, CT, MRI, and FNA) for screening of the abdominal wall, in the neighboring sites, underlying the cesarean section scar to highlight small non-palpable endometriotic foci.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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Neurobiological correlates of PTSD chronicity: Integrating stress biomarkers and brain morphometric changes

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ABSTRACT

Aim: This study aimed to investigate how the chronicity of post-traumatic stress disorder (PTSD) shapes neurobiological functioning by integrating biochemical stress-related biomarkers (serotonin, cortisol, noradrenaline, IL-12, IGF-1, CASP-9, nNOS, IL-10) with morphometric and microstructural brain parameters assessed using magnetic resonance imaging (MRI). A further objective was to determine whether early PTSD (≤ 5 years) and long-term PTSD (> 5 years) exhibit distinct profiles of neuroinflammatory, neurodegenerative, and neuroplastic alterations, with particular focus on brain regions involved in emotion regulation, memory, and cognitive control (hippocampus, amygdala, insular cortex, and prefrontal cortex).

Materials and Methods: The study included 92 adult male miners and mine rescue workers exposed to life-threatening events. Participants were assigned to three groups: PTSD ≤ 5 years ($n = 33$), PTSD > 5 years ($n = 31$), and controls without PTSD symptoms ($n = 28$). PTSD diagnosis was confirmed using the Clinician-Administered PTSD Scale for DSM-5 (CAPS-5). MRI was performed on a 1.5 T General Electric (GE) Optima 360 scanner and included volumetric T1-weighted imaging, T2-weighted FLAIR, SWAN, and diffusion tensor imaging. Assessed brain parameters included ventricular width, amygdala dimension, corpus callosum thickness, insular cortex thickness, and lateral fissure width. Biochemical markers were quantified using standard laboratory assays.

Results: Significant differences across all groups were observed for nearly all biomarkers and structural brain measures ($p < 0.001$). Early PTSD was characterized by elevated neuroinflammatory markers, reduced anti-inflammatory and neurotrophic activity (lower IL-10 and IGF-1), early ventricular enlargement, thinning of the corpus callosum and insular cortex, and reduced amygdala dimensions. Long-term PTSD demonstrated pronounced cortical atrophy, including marked widening of the lateral fissures, significant third-ventricle enlargement, and persistent thinning of the insular cortex and callosal body. Cortisol showed the strongest positive correlation with structural degeneration. Noradrenaline exhibited a potential compensatory effect, demonstrating negative correlations with ventricular width. The control group displayed physiological, homeostatic correlation patterns.

Conclusions: The chronicity of PTSD substantially modulates both biochemical and structural brain profiles. Early PTSD reflects acute neuroinflammation and impaired neuroprotection, whereas long-term PTSD is dominated by progressive neurodegeneration associated with chronic dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis. Integrating biomarker profiles with MRI-derived measures provides a robust framework for identifying markers of disease progression and for developing phase-specific therapeutic strategies aimed at mitigating atrophy, modulating stress-response systems, and supporting neuroplasticity.

KEY WORDS: post-traumatic stress disorder, stress biomarkers, brain morphometry, Neuroinflammation, neuroplasticity

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INTRODUCTION

Post-traumatic stress disorder (PTSD) is a complex condition characterized by persistent impairments in stress regulation, heightened activation of inflammatory pathways, increased oxidative stress, and diminished adaptive capacity. These changes affect both the central nervous system and psychological functioning, influencing emotional processing, behavior, and overall quality of life [1, 2]. PTSD is among the most frequently diagnosed trauma-related disorders, developing as a consequence of exposure to events that exceed the organism's adaptive threshold. Its pathophysiology involves multiple interacting biological processes that, over time, lead to lasting functional and structural alterations within the central nervous system.

A central mechanism underlying PTSD is the sustained activation of the hypothalamic–pituitary–adrenal (HPA) axis, which regulates the stress response. Individuals with PTSD exhibit disturbances in cortisol secretion, resulting in heightened limbic system sensitivity, impaired emotional regulation, and increased vulnerability to anxiety-related reactions [3, 4]. Chronic inflammation also plays a critical role, maintained by persistent microglial activation, elevated levels of proinflammatory cytokines, and dysregulation of anti-inflammatory pathways. These processes accelerate the loss of volume in regions essential for memory, learning, and emotional integration, including the hippocampus and prefrontal cortex. Concurrently, oxidative stress – arising from excessive production of reactive oxygen species

combined with impaired antioxidant defense - leads to neuronal damage, mitochondrial dysfunction, and reduced synaptic plasticity [5–7].

PTSD is therefore a systemic disorder involving the interaction of biological, psychological, and environmental factors. Neurobiological alterations manifest directly in emotional and behavioral functioning. Individuals with PTSD frequently experience affective dysregulation, chronic hyperarousal, irritability, sleep disturbances, and difficulties with memory retrieval and organization. Many of these symptoms stem from dysfunction and overload of limbic structures, particularly the amygdala, which plays a key role in fear responses and threat detection [8, 9].

Equally important are alterations in brain networks involved in the integration of internal and external stimuli. Dysfunction of the prefrontal cortex - especially its medial and dorsolateral subdivisions - compromises cognitive control and emotional inhibition, leading to difficulties in decision-making, anticipation of consequences, and planning. Disturbances within the insular cortex impair interoceptive processing, promoting somatization and increasing the severity of dissociative symptoms [10, 11].

The impact of chronic stress on brain function is reinforced by extensive neurobiological evidence showing that prolonged exposure to trauma and its psychological aftermath leads to microstructural changes affecting both gray and white matter. Brain regions responsible for memory consolidation, emotional integration, executive control, and interoceptive perception demonstrate increased susceptibility to degeneration and reorganization. While some of these processes may be partially reversible due to neuroplasticity, their trajectory is strongly influenced by PTSD duration, age, environmental factors, and individual cognitive reserve [12, 13].

PTSD therefore represents a multidimensional disorder in which neuroinflammatory, hormonal, metabolic, and psychological processes converge. Understanding these interrelations in the context of morphometric and biochemical changes within the brain allows for deeper insight into the underlying pathophysiological mechanisms and facilitates the identification of therapeutic targets - particularly within HPA axis modulation, attenuation of neuroinflammation, and enhancement of neuroplasticity [14, 15].

The duration of PTSD has a significant influence on the structure and function of key brain regions - including the hippocampus, amygdala, and prefrontal cortex. In the present study, brain structure was assessed using magnetic resonance imaging (MRI); including diffusion tensor imaging (DTI) [16, 17]. The length of PTSD duration is one of the primary factors differentiating

the extent and nature of neurobiological alterations observed in limbic and cortical structures.

Neuroimaging studies consistently show that chronic exposure to trauma-related stress leads to progressive neuronal changes involving both volumetric parameters and the microstructure of white matter and communication pathways within the brain [18]. The hippocampus—highly sensitive to cortisol and oxidative stress - undergoes gradual volumetric reduction as PTSD persists, reflecting neurodegenerative processes and impaired neuroplasticity. Reduced hippocampal volume contributes to memory impairments, disrupted consolidation of memory traces, and diminished capacity to inhibit fear responses, thereby perpetuating PTSD symptoms [19, 20].

The amygdala, a central hub for emotional processing, fear responses, and aversive learning, exhibits more complex patterns of alteration [21, 22]. Early stages of PTSD may be associated with transient volume increases due to heightened neuronal activation and metabolic demand. However, chronic PTSD is characterized by gradual amygdala shrinkage, likely attributable to prolonged cytotoxic stress and overload of limbic pathways. These changes contribute to persistent hyperreactivity to emotional stimuli, impaired emotion regulation, and exacerbated intrusive symptoms [23].

The prefrontal cortex (PFC) - critical for executive function, impulse control, stress modulation, and integration of emotional information - also demonstrates stage-dependent alterations. Early in the disorder, functional impairments of the PFC may occur in the absence of pronounced structural changes, whereas later stages are characterized by cortical thinning, disruptions in white-matter integrity, and dysregulated connectivity with limbic regions [24, 25].

MRI-based techniques, including diffusion tensor imaging, enable measurement of fractional anisotropy (FA) and mean diffusivity (MD), which provide sensitive indices of microstructural neural integrity. These measures allow detection of subtle early-stage neuronal abnormalities that may not be apparent in standard volumetric imaging. The incorporation of advanced MRI modalities into PTSD research has been essential for elucidating how disorder duration shapes the trajectory of neurobiological alterations and which adaptive or degenerative mechanisms predominate at different stages of illness progression [26].

AIM

The aim of the study was to determine the impact of PTSD duration on selected morphometric, brain parameters assessed using magnetic resonance imaging

(MRI). A multiparametric MRI protocol was employed for this purpose.

A further objective of the study was to compare the MRI-derived parameters with biochemical markers, including serotonin, cortisol, noradrenaline, interleukin-12 (IL-12), insulin-like growth factor-1 (IGF-1), caspase-9 (CASP-9), neuronal nitric oxide synthase (nNOS), and interleukin-10 (IL-10). These biochemical markers had been previously quantified and analyzed by the author in two independent studies: *Neurobiological Correlates of Coping Strategies in PTSD: The Role of IGF-1, CASP-9, nNOS, and IL-10 Based on Brief-COPE Assessment* (doi: 10.3390/cimb47100868) and *Neurobiological and Existential Profiles in Posttraumatic Stress Disorder: The Role of Serotonin, Cortisol, Noradrenaline, and IL-12 Across Chronicity and Age* (doi: 10.3390/ijms26199636) [27, 28].

MATERIALS AND METHODS

SUBJECTS

The study sample consisted of 92 adult males aged 18–50 years who had been exposed to life-threatening events, including occupational hazards associated with mine rescue operations and mining-related work. Participants were stratified into three groups: (1) PTSD duration ≤ 5 years (early phase; $n = 33$), (2) PTSD duration > 5 years (late/chronic phase; $n = 31$), and (3) a trauma-exposed control group without PTSD symptoms ($n = 28$).

Diagnostic evaluation was conducted by a board-certified psychiatrist through a structured clinical interview, review of medical records, and administration of the Clinician-Administered PTSD Scale for DSM-5 (CAPS-5). Individuals were eligible for inclusion if they met DSM-5 diagnostic criteria for PTSD and had a documented Criterion A traumatic exposure. Exclusion criteria included the presence of any other psychiatric disorders (e.g., major depressive disorder, bipolar disorder, substance abuse), significant somatic illnesses, and current use of psychotropic or hormonal medications. Additional exclusion criteria encompassed employment in uniformed services and a history of substance dependence.

The control group was matched to the PTSD groups with respect to age, educational attainment, and occupational characteristics. All participants provided written informed consent prior to participation.

ETHICAL APPROVAL

The study procedures were conducted in accordance with the ethical standards outlined in the Declaration

of Helsinki. The study protocol was approved by the Bioethics Committee of the Silesian Medical Chamber in Katowice, Poland (Decision No. 39/2018 of 10 October 2018, Annex No. 1 of 28 October 2019, Annex No. 2 of 19 June 2023, Annex No. 3 of 31 March 2025).

MRI

Brain structural assessments were acquired on a 1.5 T General Electric (GE) Optima 360 MRI system using a standardized neuroimaging protocol. The protocol included high-resolution T1-weighted volumetric imaging (3D T1 BRAVO; fast spoiled gradient-recalled echo [SPGR], isotropic slice thickness 2 mm); volumetric FLAIR imaging (3D CUBE FLAIR; 3D fast spin echo [FSE], 1.6-mm slices); susceptibility-weighted angiography (SWAN; 3D gradient-echo T2*-weighted sequence, 3-mm slices); and diffusion tensor imaging (DTI; 32 noncollinear directions, $b = 1000 \text{ s/mm}^2$, 4-mm slice thickness). Additionally, axial T2-weighted turbo spin echo (TSE) images (5-mm slices) were obtained. The total acquisition time was approximately 40 minutes.

All datasets were reviewed and post-processed on a GE AW workstation using the Brain View platform. Quantitative morphometric analyses included: the width of the third ventricle (WTV), defined as the maximal transverse diameter in the axial plane at the level of the thalami; the thickness of the left amygdala (TLA) and the right amygdala (TRA), quantified as the maximal mediolateral extent in the coronal plane at the level of the infundibular recess of the third ventricle; the thickness of the corpus callosum body (TBCC), measured in the craniocaudal axis in the mid-sagittal plane halfway between the genu and the splenium; the thickness of the left insular cortex (TLIC) and the right insular cortex (TRIC), assessed as the mean cortical thickness in the axial plane at the level of the basal ganglia, the width of the left lateral cerebral fissure (WLLCF) and the width of the right lateral cerebral fissure (WRLCF), defined as the maximal separation between the insular cortex and the temporal lobe in the axial plane; and the width of the interhemispheric fissure in the frontal region (WIFFR), calculated as the maximal distance between the frontal lobes in the coronal plane anterior to the genu of the corpus callosum. measurements of third-ventricle width, corpus callosum thickness (body), and the width of the lateral fissures. All measurements represent the mean of two independent raters [29].

STATISTICAL METHODS

Analyses were conducted using the R Statistical language (version 4.3.3; [30]) on Windows 11 Pro 64 bit

(build 26100), using the packages *report* (version 0.5.8; [31]), *GGally* (version 2.2.1; [32, 33]), *gtsummary* (version 1.7.2; [34]), *corrplot* (version 0.94; [35]), *reshape2* (version 1.4.4; [36]), *ggplot2* (version 3.5.0; [37]), *dplyr* (version 1.1.4; [38]) and *tidyr* (version 1.3.1; [39]). [30-39].

RESULTS

To provide a comprehensive comparison of the neurobiological profile across individuals with different durations of PTSD, we analyzed a range of stress-related, neurodegenerative, and immunological biomarkers, along with morphometric measurements of key brain structures. Table 1 presents a summary of these biochemical and structural indicators in three study groups: early PTSD (≤ 5 years), chronic PTSD (> 5 years), and controls without PTSD symptoms. The data reveal distinct and statistically significant differences among groups, reflecting both altered biomarker patterns and structural brain changes associated with the chronicity of PTSD. This comparison highlights characteristic biological signatures that may differentiate the stages and progression of post-traumatic stress disorder (Table 1).

NEUROSTRUCTURAL ALTERATIONS ACROSS PTSD GROUPS

Statistical analyses of brain morphometry revealed significant group differences across the majority of structural parameters ($p < 0.001$), providing robust evidence for the impact of PTSD on neural architecture. The neurostructural consequences of PTSD manifested as measurable alterations in brain morphology, with distinct patterns corresponding to the chronicity of the disorder. PTSD exerted widespread effects on structures implicated in emotional regulation, cognitive processing, and stress responsivity (Fig. 1) depicts the distribution of brain measurements across groups).

The third ventricle was significantly wider in individuals with recent PTSD than in controls, with a median difference of 2.3 mm ($p < 0.001$; CLD: $A > B > C$). This enlargement is consistent with atrophy of surrounding periventricular tissue, and may reflect prolonged activation of the hypothalamic–pituitary–adrenal (HPA) axis and glucocorticoid-induced neurotoxicity in stress-sensitive regions. The amygdala, essential for threat detection and fear processing, displayed reduced left and right transverse diameters in the recent PTSD group relative to controls (median differences: 3.8 mm and 4.0 mm; $p < 0.001$; CLD: $C > B > A$). Such reductions may reflect excitotoxic damage associated with sustained hyperarousal, contributing to heightened emotional reactivity and impaired fear extinction - core PTSD features.

Similarly, the corpus callosum, critical for interhemispheric information integration, was significantly thinner in the recent PTSD group compared with controls (median difference 1.7 mm; $p < 0.001$; CLD: $C > B > A$), suggesting reduced white-matter integrity and potentially underlying impairments in attention, memory, and cognitive flexibility. The insular cortex, a region central to interoception, emotional salience, and autonomic integration, was also thinner bilaterally by approximately 0.5 mm in recent PTSD relative to controls ($p < 0.001$; CLD: $B > A$), which may exacerbate dissociative experiences and emotional dysregulation. Marked widening of the lateral cerebral fissures (median difference 3.5 mm between recent PTSD and controls; $p < 0.001$; CLD: $A > B > C$) further supports the presence of cortical atrophy consistent with stress-induced neurotoxicity. Collectively, these statistically significant deviations demonstrate PTSD's broad and clinically meaningful impact on limbic, cortical, and white-matter structures (Table 1).

IMPACT OF PTSD DURATION ON STRUCTURAL BRAIN CHANGES

The chronicity of PTSD significantly modulated these structural abnormalities. Across several measurements, the long-term PTSD group (> 5 years) displayed intermediate values between the recent PTSD group (≤ 5 years) and controls, suggesting partial yet incomplete recovery over time.

For the third ventricle, the long-term PTSD group displayed a median width 1.0 mm smaller than the recent group ($p < 0.001$), suggesting attenuation of periventricular atrophy, potentially reflecting reduced HPA axis instability or adaptive neuroplastic modifications. Amygdala measurements in the long-term group approached control values, with median differences of 1.3 mm (left) and 1.0 mm (right), compared with 3.8 mm and 4.0 mm in recent PTSD ($p < 0.001$), implying partial restoration of limbic integrity. The corpus callosum was 0.7 mm thicker in long-term PTSD compared with recent PTSD ($p < 0.001$), indicating improvement in white-matter coherence. Likewise, insular cortex thickness in the long-term group closely approximated that of controls ($p < 0.001$), in contrast to pronounced thinning in early PTSD. Although the lateral fissures remained wider in long-term PTSD relative to controls, they were 1.8–2.0 mm narrower than in recent PTSD ($p < 0.001$), indicating partial mitigation of cortical atrophy. No significant differences were observed in interhemispheric fissure width ($p = 0.955$), suggesting relative resilience of this region to PTSD-related changes.

Taken together, these patterns support a model in which early PTSD is associated with acute stress-driven structural disruption, whereas long-term PTSD reflects a mixed profile of persistent neurodegeneration and partial compensatory adaptation.

Table 1. Comparison of selected biochemical biomarkers and morphometric measurements of key brain structures in the study cohort (N = 92), stratified by PTSD duration (≤ 5 years – early PTSD; > 5 years – late/chronic PTSD) and a trauma-exposed control group without PTSD symptoms

Characteristic	N	Overall (N = 92)	Past PTSD (≤ 5 y) (N = 33)	Past PTSD (> 5 y) (N = 31)	No PTSD (Control) (N = 28)	p-value	CLD Relations
Age (years)	92	34.0 (28.8, 41.0)	34.0 (31.0, 41.0)	36.0 (29.5, 41.0)	33.5 (24.2, 41.5)	0.524	-
Biomarkers							
IGF-1 (nmol/ml)	92	10.8 (5.2, 32.3)	4.9 ^A (4.0, 5.8)	13.6 ^B (7.6, 19.7)	37.1 ^C (32.5, 44.6)	< 0.001	C > B > A
CASP9 (ng/ml)	92	10.2 (3.0, 22.9)	23.8 ^A (20.8, 26.5)	6.8 ^B (3.7, 16.6)	2.7 ^C (1.8, 3.3)	< 0.001	A > B > C
nNOS (ng/ml)	92	25.7 (7.0, 57.9)	62.3 ^A (46.6, 82.1)	19.1 ^B (9.5, 35.7)	5.7 ^C (4.8, 7.1)	< 0.001	A > B > C
IL10 (ng/l)	92	806.9 (319.2, 2411.8)	279.0 ^A (231.0, 345.3)	833.7 ^B (523.0, 1448.5)	3159.0 ^C (2814.0, 3516.3)	< 0.001	C > B > A
Serotonin (ng/ml)	92	144.8 (120.2, 203.4)	109.9 ^A (96.6, 122.2)	147.4 ^B (139.6, 153.2)	225.2 ^C (209.6, 296.7)	< 0.001	C > B > A
Cortisol (ug/dl)	92	14.0 (10.9, 36.2)	9.8 ^A (6.8, 11.6)	47.5 ^B (36.4, 57.6)	13.5 ^C (12.0, 15.4)	< 0.001	B > C > A
Noradrenaline (pg/ml)	92	371.3 (220.3, 544.8)	271.7 ^A (210.7, 406.3)	271.7 ^A (189.0, 399.7)	580.2 ^B (449.1, 830.2)	< 0.001	B > A
IL-12 (pg/ml)	92	21.6 (8.5, 54.7)	62.4 ^A (51.8, 69.1)	17.0 ^B (11.2, 33.9)	7.7 ^C (6.3, 8.6)	< 0.001	A > B > C
Brain Measurements							
Width of the Third Ventricle (WTV)	92	6.6 (5.5, 7.7)	7.7 ^A (7.1, 8.4)	6.7 ^B (5.8, 7.6)	5.4 ^C (4.4, 6.0)	< 0.001	A > B > C
Width of the Left Amygdala (TLA)	92	17.5 (16.0, 18.1)	15.0 ^A (14.0, 18.0)	17.5 ^B (16.5, 18.0)	18.8 ^C (18.0, 20.0)	< 0.001	C > B > A
Width of the Right Amygdala (TRA)	91	18.0 (15.0, 19.0)	15.0 ^A (13.8, 15.0)	18.0 ^B (17.0, 19.0)	19.0 ^C (19.0, 20.0)	< 0.001	C > B > A
Thickness of the Body of the Corpus Callosum (TBCC)	91	5.4 (4.5, 6.2)	4.6 ^A (4.2, 5.4)	5.3 ^B (4.7, 5.8)	6.3 ^C (5.9, 7.0)	< 0.001	C > B > A
Thickness of the Left Insular Cortex (TLIC)	91	2.5 (2.2, 2.9)	2.3 ^A (2.0, 2.5)	2.6 ^B (2.4, 3.0)	2.8 ^B (2.5, 3.0)	< 0.001	B > A
Thickness of the Right Insular Cortex (TRIC)	91	2.5 (2.1, 2.9)	2.2 ^A (1.9, 2.5)	2.7 ^B (2.2, 3.0)	2.7 ^B (2.4, 3.0)	< 0.001	B > A
Width of the Left Lateral Cerebral Fissure (WLLCF)	91	4.7 (3.1, 5.8)	6.0 ^A (5.4, 6.3)	4.2 ^B (3.3, 5.2)	2.5 ^C (2.1, 3.2)	< 0.001	A > B > C
Width of the Right Lateral Cerebral Fissure (WRLCF)	91	4.9 (3.0, 5.8)	6.0 ^A (5.5, 6.4)	4.0 ^B (3.4, 5.0)	2.5 ^C (2.0, 3.1)	< 0.001	A > B > C
Width of the Interhemispheric Fissure in the Frontal Region (WIFFR)	91	2.4 (2.0, 3.0)	2.4 (2.0, 3.0)	2.5 (2.0, 3.0)	2.6 (2.0, 2.9)	0.955	-

Notes: Continuous variables are presented as median (interquartile range, IQR); categorical variables as n (%). Values were rounded to one decimal place. p-values were calculated using the Kruskal–Wallis test for continuous variables and the chi-square test or Fisher's exact test for categorical variables. A p-value < 0.05 indicates statistical significance. Groups labeled with different letters (A, B, C) differ significantly ($p < 0.05$) according to compact letter display (CLD) notation following significant Kruskal–Wallis or chi-square tests. Groups sharing the same letter do not differ significantly. The letter order corresponds to PTSD ≤ 5 years, PTSD > 5 years, and No PTSD (Control).

Source: Own material

BIOMARKER – MORPHOMETRY CORRELATION PROFILES

Correlation analyses (Fig. 2) integrating eight biochemical markers (IGF-1, CASP-9, nNOS, IL-10, serotonin,

cortisol, noradrenaline, IL-12) with nine brain morphometric variables (WTV, TLA, TRA, TBCC, TLIC, TRIC, WLLCF, WRLCF, WIFFR) revealed distinct neurobiological profiles across recent PTSD, long-term PTSD, and controls.

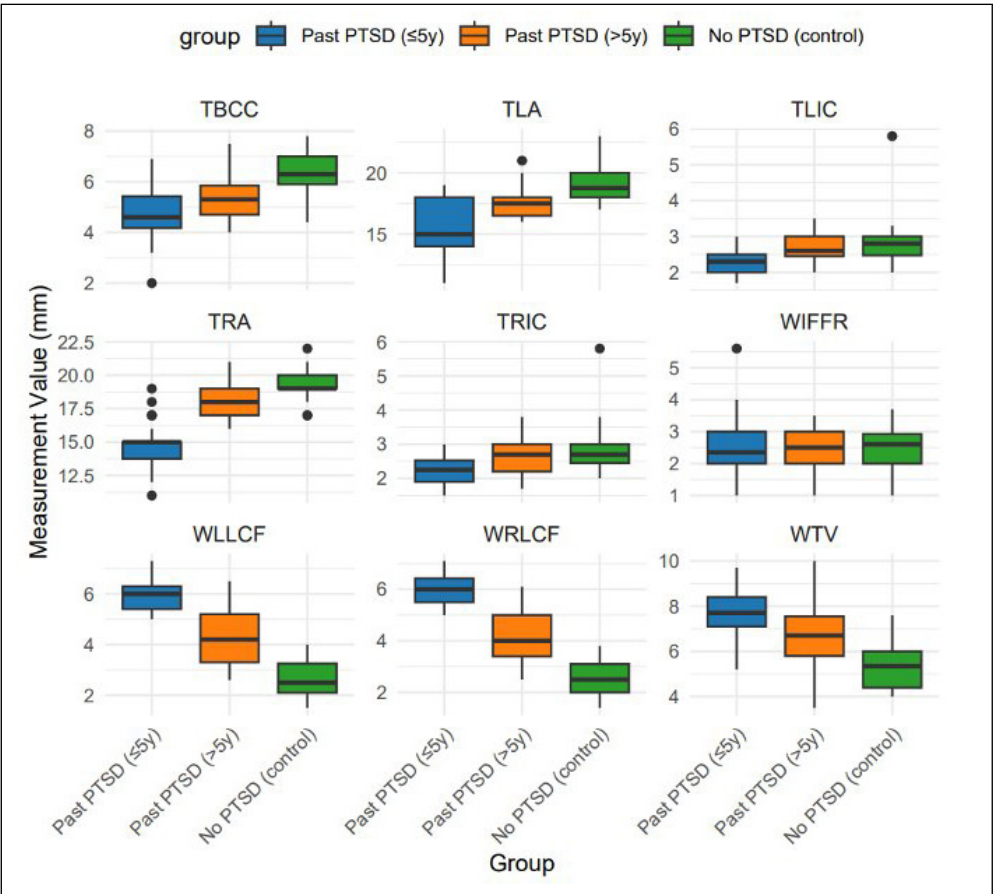


Fig. 1. Distribution of the brain measurements across PTSD groups
Notes: Distribution of morphometric brain parameters across the three study groups: PTSD ≤5 years, PTSD >5 years, and the trauma-exposed control group without PTSD symptoms. The presented measurements include: WTV (width of third ventricle), TLA and TRA (width of left/right amygdala), TBCC (thickness of body of the corpus callosum), TLIC and TRIC (thickness of the left/right insular cortex), WLLCF and WRLCF (width of the left/right lateral cerebral fissure), and WIFFR (width of the interhemispheric fissure in the frontal region). All values are expressed in millimeters.
Source: Own material

RECENT PTSD (≤5 YEARS)

Recent PTSD was characterized by correlations indicative of acute neuroinflammatory and neurodegenerative processes:

IGF-1 negatively correlated with WRLCF ($\rho = -0.42$, $p = 0.017$), linking lower neurotrophic activity with frontotemporal cortical atrophy.

IL-10 negatively correlated with WTV ($\rho = -0.44$, $p = 0.011$), suggesting that diminished anti-inflammatory signaling contributes to ventricular enlargement.

CASP-9 negatively correlated with WTV ($\rho = -0.36$, $p = 0.037$), consistent with regulated apoptotic activity exerting a potentially protective effect during early pathology.

Cortisol positively correlated with TLA ($\rho = 0.35$, $p = 0.045$), indicating that elevated glucocorticoids may transiently preserve amygdala structure, thereby maintaining heightened emotional reactivity and hyperarousal.

LONG-TERM PTSD (>5 YEARS)

Correlation patterns shifted toward markers of chronic neurodegeneration and compensatory neurobiological responses:

Cortisol exhibited strong positive correlations with WRLCF ($\rho = 0.78$, $p < 0.001$) and WLLCF ($\rho = 0.74$, $p < 0.001$), underscoring its role in sustaining frontotemporal atrophy through chronic HPA axis dysregulation.

Noradrenaline correlated with WTV ($\rho = 0.51$, $p = 0.006$), suggesting activation of compensatory mechanisms that may preserve neural perfusion and suppress proinflammatory microglial activity.

CONTROL GROUP

Patterns in controls reflected physiological homeostasis: CASP-9 negatively correlated with WTV ($\rho = -0.39$, $p = 0.042$), consistent with regulated apoptosis supporting healthy neuronal turnover.

Serotonin positively correlated with TRIC ($\rho = 0.51$, $p = 0.006$) and TLIC ($\rho = 0.38$, $p = 0.044$), indicating preserved insular integrity essential for emotional and interoceptive stability.

Cortisol correlated with WIFFR ($\rho = 0.39$, $p = 0.042$), reflecting normative stress responsivity.

Noradrenaline correlated with WRLCF ($\rho = 0.41$, $p = 0.039$), indicating physiological variability rather than pathological change.

CLINICAL AND NEUROBIOLOGICAL INTERPRETATION

PTSD significantly disrupts normal biomarker–brain coupling, with distinct clinical implications. In recent

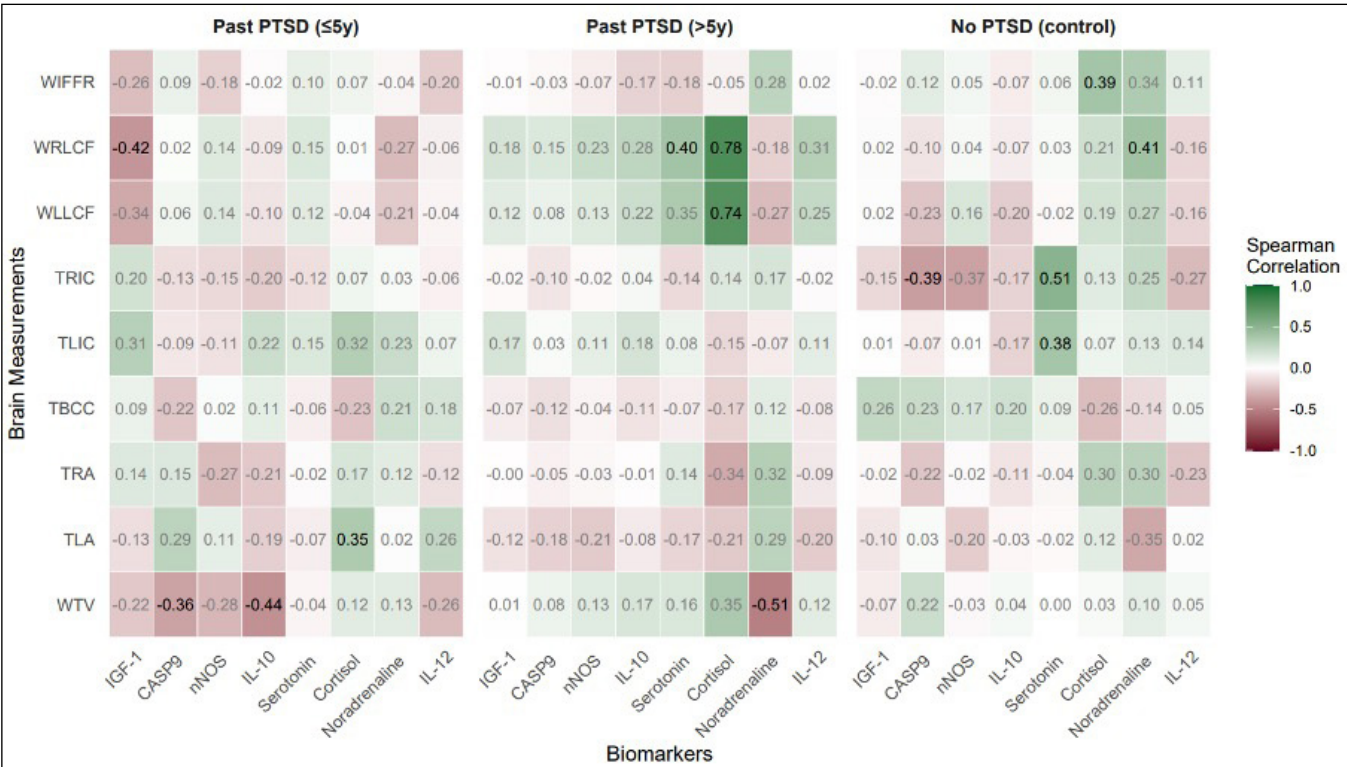


Fig. 2. Spearman Correlations: Biomarkers vs. Brain Measurements by PTSD Group

Notes: Correlation coefficients represent Spearman's rank correlations between biomarkers (x-axis) and brain measurements (y-axis) across three groups: Past PTSD (≤5 years), Past PTSD (>5 years), and No PTSD (Control). Values range from -1 (red, strong negative correlation) to 1 (green, strong positive correlation), with white indicating no correlation. Significant correlations ($p < 0.05$) are shown in black, while non-significant correlations are in grey. Brain measurements (all in mm) include WTV (width of the third ventricle), TLA/TRA (width of left/right amygdala), TBCC (thickness of the body of the corpus callosum), TLIC/TRIC (thickness of left/right insular cortex), WLLCF/WRLCF (width of left/right lateral cerebral fissure), and WIFFR (width of the interhemispheric fissure in the frontal region). Biomarkers include IGF-1 (nmol/ml), CASP9 (ng/ml), nNOS (ng/ml), IL-10 (ng/l), Serotonin (ng/ml), Cortisol (ug/dl), Noradrenaline (pg/ml) and IL-12 (pg/ml)

Source: Own material

PTSD, reduced IGF-1 and IL-10 levels correspond with cortical atrophy and ventricular enlargement, respectively, suggesting heightened vulnerability to cognitive impairment and accelerated neuronal loss. CASP-9 patterns imply partial neuroprotection, while cortisol-related amygdala preservation contributes to hyperarousal and emotional dysregulation.

In long-term PTSD, strong cortisol-morphometry correlations reveal persistent neurodegeneration driven by chronic stress hormone exposure, consistent with progressive cognitive decline, impaired decision-making, visuospatial deficits, and increased susceptibility to mood disorders. Noradrenaline's protective correlations suggest compensatory neurobiological mechanisms that may slow structural deterioration.

In healthy controls, correlations predominantly reflect homeostatic regulation of apoptosis, serotonergic support of insular integrity, and normative stress responsiveness. However, baseline cortisol- and noradrenaline-related patterns indicate subtle predispositions that, under pathological stress, may increase vulnerability.

OVERALL SUMMARY

The duration of PTSD acts as a critical risk factor that amplifies structural and biochemical dysregulation over time. Recent PTSD is marked by acute inflammatory and stress-driven neurobiological disruption, whereas long-term PTSD exhibits persistent cortical atrophy and maladaptive stabilization of chronic stress pathways. Compared with the predominantly protective patterns seen in controls, prolonged PTSD is associated with accelerating structural deterioration, emphasizing the need for early, targeted interventions to prevent long-term clinical consequences such as cognitive decline, emotional instability, and diminished treatment responsiveness.

DISCUSSION

Previous publications by the author [27, 28] have described neurobiological disturbances and associations among stress-related biomarkers as a function of PTSD duration and patient age, providing an interpretive framework for the present findings. The current study

extends these analyses by demonstrating that biochemical alterations not only reflect underlying pathophysiological processes but are also directly linked to quantifiable morphometric changes in the brain. This integration advances the understanding of PTSD neurobiology by bridging molecular markers with structural imaging indicators [40].

Correlation analyses between biochemical markers and brain morphometric parameters revealed a heterogeneous pattern of neurobiological alterations depending on PTSD chronicity, supporting the hypothesis that the disorder's pathophysiology evolves over time. Early PTSD (≤ 5 years) was characterized predominantly by disruptions in neuroinflammatory balance, whereas chronic PTSD (> 5 years) exhibited structural brain changes consistent with progressive cortical atrophy. In the control group, the observed associations were physiologically normative and reflected intact neurotransmitter modulation and homeostatic regulation of neuronal plasticity.

In individuals with early PTSD, diminished anti-inflammatory activity played a key role, exemplified by the strong negative correlation between IL-10 levels and third-ventricle width. Reduced IL-10 - an essential cytokine inhibiting proinflammatory M1 microglial activation—was associated with enlargement of periventricular structures, suggesting neurodegenerative processes [41]. The negative correlation between IGF-1 and the width of the lateral fissures (WRLCF, WLLCF) indicates that lower IGF-1 levels are linked to greater tissue loss in frontotemporal regions, consistent with reduced neuroprotective capacity.

The negative correlation between caspase-9 and third-ventricle width in early PTSD suggests that early-stage apoptotic activation may serve a protective role by containing excessive degradation of neural tissue. Conversely, the positive correlation between cortisol and amygdala dimensions reflects heightened limbic reactivity, which clinically manifests as hyperarousal, intrusive symptoms, and deficits in emotional regulation [42, 43].

Morphometric findings further corroborated these biochemical associations. In early PTSD, the most pronounced atrophic tendencies were observed in third-ventricle width (WTV), lateral fissure width (WLLCF, WRLCF), and reductions in corpus callosum thickness (TBCC) and insular cortex thickness (TLIC, TRIC). These patterns suggest that neuroinflammatory processes during the initial years of PTSD contribute to early atrophic changes. Alterations in the amygdala - relatively mild in this phase - may reflect a state of emotional overload rather than advanced structural deterioration [44].

In chronic PTSD (> 5 years), a distinct profile emerged, indicating progressive structural damage, particularly

in the frontotemporal cortex. The strongest correlations involved cortisol, which exhibited a robust positive association with lateral fissure width. This finding is consistent with extensive evidence demonstrating the neurotoxic effects of prolonged HPA axis activation, leading to loss of gray matter volume and reduced neuronal connectivity [45, 56]. The magnitude of this correlation - the largest among all observed associations - underscores cortisol's central role in the pathomechanisms of late-stage PTSD and supports its utility as a biomarker of neurodegenerative progression [45, 46].

Morphometric data reinforce this interpretation: individuals with chronic PTSD exhibited widening of the lateral fissures and third ventricle, significant thinning of the corpus callosum and insular cortex, and moderate reductions in amygdala volume. The negative correlation between noradrenaline and third-ventricle width may indicate activation of compensatory mechanisms. Noradrenaline, by suppressing proinflammatory microglia and supporting cerebral perfusion, may mitigate the progression of atrophy, suggesting its potential as a neuroprotective biomarker in chronic PTSD. The positive association between IGF-1 and morphometric variables in this group may reflect attempts to re-engage regenerative processes [47].

The control group exhibited associations characteristic of physiological neurotransmitter regulation and homeostatic neuronal plasticity. The positive correlation between serotonin and insular cortex thickness supports its role in modulating interoception, affective processing, and autonomic regulation. Noradrenaline showed moderate correlations with lateral fissure width, reflecting normative anatomical variability in healthy individuals. The negative correlation between CASP-9 and insular thickness suggests regulated, homeostatic apoptosis typical of healthy neuronal remodeling.

Taken together, these findings indicate that PTSD duration is a crucial factor shaping both the direction and magnitude of associations between biochemical and morphometric brain parameters. The early phase of the disorder is dominated by neuroinflammation and dysregulated neuroendocrine function, whereas the chronic phase is characterized by progressive neurodegeneration, particularly within frontotemporal networks. The integration of biochemical and structural data underscores the complexity of PTSD pathophysiology and highlights the need to tailor therapeutic strategies to specific disease stages. Neuroinflammatory and neuroprotective mechanisms appear to be promising targets for early interventions, while in chronic PTSD, therapeutic priorities shift toward slowing atrophy and supporting residual compensatory mechanisms. Understanding these associations may inform the development of prognostic biomarkers and personalized

therapeutic approaches aimed at modulating HPA axis activity, noradrenergic signaling, and neuroinflammatory pathways [48, 49].

LIMITATIONS

This study has several limitations that should be considered when interpreting the findings. First, the study population consisted exclusively of male participants employed in the mining industry, which restricts the generalizability of the results to women and to individuals who have experienced trauma from other sources. Second, both the MRI assessments and the biochemical measurements were conducted at a single time point, which precludes evaluation of the temporal dynamics or longitudinal progression of the observed changes.

FUTURE DIRECTIONS

Future research should aim to address several important gaps identified in the present study. Longitudinal designs, incorporating repeated MRI measurements and serial biomarker assessments, are essential to clarify the temporal dynamics of neuroinflammatory, neuroendocrine, and structural changes across the trajectory of PTSD. Such approaches would enable the identification of early markers predictive of transition to chronicity as well as mechanisms underlying potential recovery.

Expanding the study population to include women and individuals exposed to diverse forms of trauma would increase generalizability and allow examination of potential sex-specific or trauma-type-specific neurobiological pathways. Given the known influence of sex hormones on stress reactivity, neuroinflammation, and neuroplasticity, exploring sex differences may yield critical insights into personalized treatment strategies.

Future studies would also benefit from integrating multimodal neuroimaging approaches - including functional MRI, resting-state connectivity, MR spectroscopy, and advanced diffusion models - to obtain a more comprehensive characterization of neuronal integrity, microglial activation, and network dysfunction. Combining imaging data with detailed neuropsychological assessments could further elucidate the clinical significance of structural and biochemical abnormalities, particularly in domains such as memory, interoception, emotional regulation, and executive functioning.

Another promising direction involves investigating therapeutic modulation of the biomarkers identified in

this study, such as cortisol, IGF-1, noradrenaline, IL-10, and CASP-9. Interventional studies - pharmacological, psychotherapeutic, behavioral, or neuromodulatory (e.g., tDCS, TMS, neurofeedback) - could determine whether targeted modulation of neuroinflammatory and neuroendocrine pathways translates into measurable neuroprotective or restorative effects on brain structure.

CONCLUSIONS

The findings of this study demonstrate that the duration of PTSD is a key determinant of both biochemical and structural alterations within the brain. Early PTSD is characterized primarily by neuroinflammatory imbalance and disturbances in neuroendocrine homeostasis, which manifest as early morphometric deviations - particularly reduced hippocampal and amygdalar volumes, thinning of the insular cortex and corpus callosum, and enlargement of periventricular and frontotemporal spaces. These changes are consistent with emerging neurodegenerative processes during the initial phase of the disorder.

In contrast, chronic PTSD (>5 years) is associated with more pronounced and progressive structural abnormalities, including widening of the lateral fissures and third ventricle, consistent with advancing cortical atrophy. These structural changes strongly correlated with elevated cortisol levels, supporting the role of sustained HPA axis activation as a major contributor to long-term neurotoxicity. At the same time, partial microstructural reorganization was observed, suggesting engagement of adaptive neuroplastic responses. Compensatory mechanisms - reflected in increased IGF-1 levels and the neuroprotective influence of noradrenaline - may represent endogenous attempts to counteract ongoing neuronal injury.

The control group exhibited patterns characteristic of physiological neuronal homeostasis, further emphasizing the pathological nature of the biochemical and morphometric alterations identified in PTSD.

Altogether, these results indicate that PTSD is not a static disorder but rather a dynamic condition with evolving neurobiological mechanisms. Early phases are dominated by neuroinflammation and dysregulated neuroendocrine signaling, whereas chronic stages are marked by progressive neurodegeneration alongside limited compensatory plasticity. Integrating biochemical profiling with MRI-derived parameters provides strong evidence that PTSD involves complex interactions between neuroinflammatory, neuroendocrine, and neuroplastic pathways.

These findings highlight the importance of stage-specific therapeutic strategies: interventions targeting neuroinflammation and promoting neuroprotection may be most

effective in early PTSD, while approaches aimed at slowing cortical atrophy and supporting residual compensatory mechanisms may be essential in chronic stages. Understanding these dynamic relationships may contribute to

the development of prognostic biomarkers and guide the implementation of personalized, mechanism-based treatments focused on modulating the HPA axis, noradrenergic signaling, and neuroinflammatory processes.

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CONFLICT OF INTEREST

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Dynamics of stress symptoms manifestation in law enforcement officers in the process of performing their service and combat missions during the war

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ABSTRACT

Aim: To study the dynamics of stress symptoms manifestation in police officers of different ages before and after performing their service and combat missions of varying duration.

Materials and Methods: The research, conducted in 2024-2025, involved 71 Master's-level student officers (officers) (male, up to 25 years old) from Odesa State University of Internal Affairs (OSUIA) and 103 serving officers of various ages (male, up to 30 and older) who are on staff at OSUIA in multiple positions. Research methods: theoretical analysis of literature sources, psycho-diagnostic methods, statistical methods.

Results: The most pronounced manifestation of all stress symptoms was found in serving officers, regardless of age, after returning from their rotations lasting 3-4 months. After performing service and combat missions, the stress level (14.8-15.4 points) among serving officers of all age groups was significantly ($p \leq 0.001$) higher than among student officers (10.1 points). No significant differences were found between the stress levels of serving officers across different age groups ($p > 0.05$), indicating that the level of stress does not depend on the age of law enforcement officers, but rather on the duration of their exposure to stressful conditions.

Conclusions: The research indicates the need to develop stress resilience in law enforcement officers to ensure the effectiveness of their service and combat activities in conditions of martial law, as well as to develop skills in the use of available means for stress prevention in the process of prolonged service and combat activities to restore mental health indicators.

KEY WORDS: stress, stress symptoms, mental health, police officers, law enforcement officers, war

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INTRODUCTION

The service activities of law enforcement officers often occur in extreme conditions, particularly during the current legal regime of martial law in the country [1, 2]. The professional environment of operational and service activities requires exceptional mental resilience from police officers. Police officers are constantly under psychological stress, undertake risky actions while performing their professional duties, and are subjected to high levels of emotional and physiological tension. Law enforcement officers frequently encounter individuals who engage in negative actions, either directly or indirectly [3, 4]. Police officers interact not with the best representatives of society, who are often characterized by heightened emotionality, rudeness, aggressiveness, and inadequacy. Negative emotions distort the per-

sonality traits of police officers and, in the most severe cases, cause psychosomatic illnesses [5, 6].

Scientists [7-10] argue that constant professional stress can lead to deformation of professional personality, increase the proneness to conflict within the team, reduce work efficiency, and negatively impact the health of staff and their loved ones. The lack of adequate psychological training and adaptive stress management skills among police officers can often lead to negative consequences for their mental and physical health.

AIM

The aim is to study the dynamics of stress symptoms manifestation in police officers of different ages before

and after performing their service and combat missions of varying duration.

MATERIALS AND METHODS

PARTICIPANTS

The research involved 71 Master's-level student officers (officers) (male, up to 25) from Odesa State University of Internal Affairs (Ukraine, Odesa, OSUIA) majoring in "Law" specialty and 103 serving officers under the age of 30 ($n = 35$), under the age of 40 ($n = 41$), and over the age of 40 ($n = 27$), who are on staff at OSUIA in various positions. The student officers were assessed for stress symptoms before and after their internship in practical (combat) police units (duration: 4-6 weeks), while the serving officers were assessed before and after their utilization tours (rotation) to the combat zone (duration: 3-4 months). The research was conducted in 2024-2025, and all respondents were male. The criteria for inclusion in the experiment were as follows: for student officers, age up to 25 years, male gender, and participation in an internship in practical police units lasting at least 4 weeks; for serving officers, male gender, and participation in a rotation to a combat zone lasting at least 3 months. Medical condition, marital status, and positions held (for serving officers) were not inclusion criteria and were not taken into account. The exclusion criterion was the participant's voluntary desire to withdraw from the research at any time.

RESEARCH METHODS

theoretical analysis and generalization of literature sources, psycho-diagnostic methods (test of Yu. V. Shcherbatykh), 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 (21 sources (2010-2025) from MedLine, Scopus, Web of Science databases were analyzed).

To study the peculiarities of stress symptoms manifestation in law enforcement officers during the performance of their service and combat tasks under martial law, we used a test of Yu. V. Shcherbatykh, which allows the study of various signs of stress – intellectual, behavioral, emotional and physiological according to the answers to the questionnaire consisting of four groups of questions of 12 symptoms each [11]. For the presence of one of the intellectual and behavioral symptoms, the respondents rated it at 1 point; emotional – 1.5 points, and physiological – 2 points. The total maximum score for the entire list can theoretically reach 66. A score of

0 to 5 points is considered good, meaning there is no significant stress at this point in life. A score of 6 to 12 points means that a person is experiencing moderate stress, which can be compensated for by rational use of time, periodic rest, and finding the best way out of the situation. A score of 13 to 24 points indicates a fairly pronounced tension of the emotional and physiological systems of the body, which arose in response to severe stress that could not be compensated for. In this case, unique methods of coping with stress are required. A score of 25 to 40 points indicates a state of severe stress, which requires the help of a psychologist or psychotherapist to overcome it successfully. This stress level suggests that the body is already close to the limit of its ability to resist stress. A score of more than 40 points indicates that the body is moving to the third, most dangerous stage of stress – the depletion of adaptive energy reserves.

STATISTICAL METHODS

The methods of mathematical statistics were used to process the data obtained. The compliance of the sample data distribution with the Gauss' law was assessed using the Shapiro-Wilk W test. The reliability of the difference between the indicators was determined using the Student's t-test. The reliability of the difference was set at $p < 0.05$. All statistical analyses were performed using SPSS software, version 10.0, adapted for medical and biological research.

ETHICS

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 OSUIA. 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.

FRAMEWORK

This scientific article was carried out according to the plan of the research work of the National Academy of Internal Affairs for 2020-2026 "Psychological, pedagogical and sociological support of law enforcement officers» (state registration number 0113U008196).

RESULTS

Analysis of the dynamics of intellectual stress symptoms in student officers during their 4-6 week internship in

Table 1. Dynamics of intellectual stress symptoms in student officers (n = 71) and serving officers (n = 103) during their service and combat activities in a state of martial law (in student officers – before and after their internship, in serving officers – before and after their rotation), %

Intellectual symptoms	Student officers (n = 71)		Serving officers under the age of 30 (n = 35)		Serving officers under the age of 40 (n = 41)		Serving officers over the age of 40 (n = 27)	
	Before	After	Before	After	Before	After	Before	After
Prevalence of negative thoughts	23.9	45.1	17.1	45.7	14.6	46.3	14.8	44.4
Difficulty concentrating	11.3	21.1	8.6	25.7	9.8	24.4	11.1	25.9
Deterioration of memory performance	12.6	19.7	5.7	17.1	7.3	19.5	7.4	18.5
Constant, fruitless revolving of thoughts around the problem	25.3	45.1	8.6	54.3	9.8	53.6	7.4	51.8
Increased distraction	14.1	35.2	11.4	48.6	12.2	48.8	11.1	48.1
Difficulties in decision-making	19.7	33.8	5.7	51.4	4.9	46.3	3.7	44.4
Bad dreams, nightmares	18.3	32.4	14.3	57.1	12.2	56.1	18.5	59.3
Frequent errors in calculations	7.1	18.3	2.9	25.7	4.9	24.3	3.7	25.9
Passivity, desire to shift responsibility	11.3	39.4	5.7	28.6	7.3	29.3	7.4	33.3
Violation of logic, confused thinking	5.6	8.5	2.9	14.3	4.9	9.8	3.7	11.1
Impulsiveness, unreasonable decisions	19.7	32.4	11.4	42.9	12.2	46.3	11.1	44.4
Narrowing the "field of view", possible options for action	7.1	12.7	5.7	31.4	4.9	29.3	3.7	29.6

Source: compiled by the authors of this study

practical police units and in serving officers during their 3-4 month utilization tours to perform special and combat missions in a combat zone (rotations lasting 3-4 months) indicates a significant deterioration in the level of intellectual stress symptoms manifestation, especially in serving officers (Table 1). It was found that the most pronounced intellectual symptoms of stress in student officers and serving officers are: bad dreams – in student officers, the level of manifestation of this symptom worsened from 18.3 % to 32.4 %, in serving officers – from 14.3-18.5 % to 57.1-59.3 % depending on age; constant, fruitless rumination on a problem – among student officers from 25.3 % to 45.1 %, among serving officers – from 7.4-9.8 % to 51.8-54.3 %; increased distraction – among student officers from 14.1 % to 35.2 %, among serving officers – from 11.1-12.2 % to 48.1-48.8 %; difficulties in decision-making – among student officers from 19.7 % to 33.8 %, among serving officers – from 3.7-5.7 % to 44.4-51.4 %, and others.

Symptoms that characterize cognitive functions (memory, concentration, logical thinking) also tend to worsen during service and combat activities under martial law. Still, the magnitude of changes in their levels of manifestation is somewhat smaller compared to the intellectual symptoms listed above. It should be emphasized that no significant differences in the levels of intellectual stress symptoms were found among serving officers of different age groups, which indicates an equally negative impact of stress on law enforce-

ment officers of various ages; the leading indicator of the adverse effect of stress factors is the duration of exposure to stressful conditions. In addition, based on the conclusions of many scientists, it should be added that the degree of influence of stress factors on the body of law enforcement officers is also determined by the strength of the factors, the level of stress resilience of the individual, their individual psychological characteristics, as well as their abilities and skills to apply available means and methods of coping with stress in the conditions of a specific activity.

The assessment of behavioral symptoms of stress in student officers and serving officers shows that, like intellectual symptoms, they tend to worsen in the course of their service and combat activities (Table 2). At the same time, all symptoms are more pronounced in serving officers than in student officers, which is due to the duration of their exposure to constant stress factors and the complexity of the missions they perform. Among the behavioral symptoms of stress in student officers and serving officers, the most pronounced are: sleep disturbances, insomnia – in student officers, the level of manifestation of this symptom worsened from 4.2 % to 9.9 %, in serving officers – from 11.4-14.8 % to 48.6-51.9 %, depending on age; loss of appetite or over-eating – among student officers from 5.6 % to 12.7 %, among serving officers – from 3.7-7.3 % to 45.7-48.1 %; low productivity – among student officers from 7.0 % to 35.2 %, among serving officers – from 8.6-11.1 % to 34.3-

Table 2. Dynamics of behavioral stress symptoms in student officers (n = 71) and serving officers (n = 103) during their service and combat activities in a state of martial law (in student officers – before and after their internship, in serving officers – before and after their rotation), %

Behavioral symptoms	Student officers (n = 71)		Serving officers under the age of 30 (n = 35)		Serving officers under the age of 40 (n = 41)		Serving officers over the age of 40 (n = 27)	
	Before	After	Before	After	Before	After	Before	After
Loss of appetite or overeating	5.6	12.7	5.7	45.7	7.3	46.3	3.7	48.1
Increase in errors when performing common actions	7.0	18.3	2.9	20.0	4.9	19.5	7.4	18.5
Faster or slower speech	0	2.8	0	5.7	0	7.3	0	7.4
Voice trembling	0	0	0	8.6	0	4.9	0	3.7
Increase in conflict situations	5.6	19.7	5.7	28.5	4.9	29.3	7.4	29.6
Chronic lack of time	12.7	21.1	17.1	31.4	17.1	31.7	14.8	37.1
Less time to spend with friends	2.8	9.9	14.3	22.9	19.5	26.8	22.2	29.6
Loss of attention to one's appearance, untidiness	0	2.8	0	5.7	4.9	9.8	7.4	11.1
Antisocial, conflict behavior	2.8	12.7	5.7	37.1	7.3	39.0	7.4	37.0
Low productivity of activities	7.0	35.2	8.6	34.3	9.8	40.0	11.1	40.7
Sleep disturbances, insomnia	4.2	9.9	11.4	48.6	12.2	51.2	14.8	51.9
More intensive smoking, alcohol consumption	8.5	11.3	8.6	25.7	9.8	26.8	11.1	29.6

Source: compiled by the authors of this study

40.7 %; antisocial, conflict behavior – among student officers from 2.8 % to 12.7 %, among serving officers – from 5.7-7.3 % to 37.0-39.0 %; chronic lack of time – among student officers from 12.7 % to 21.1 %, among serving officers – from 14.8-17.1 % to 31.4-37.1 %; more intense smoking and alcohol consumption – among student officers from 8.5 % to 11.3 %, among serving officers – from 8.6-11.1 % to 25.7-29.6 %; an increase in errors when performing routine actions – among student officers from 7.0 % to 18.3 %, among serving officers – from 2.9-7.4 % to 18.5-20.0 %.

In addition, the severity of particular behavioral symptoms' manifestation, primarily among serving officers, before their utilization tours, indicates that they experience a certain level of stress while performing their daily duties under martial law. During long rotations, with the constant influence of stress factors from service and combat activities, the severity of symptoms enhanced significantly, indicating an increase in stress levels during special and combat missions. As with intellectual symptoms, the level of behavioral stress symptoms manifestation in serving officers of different age groups is practically the same, which confirms our previous conclusions.

The analysis of emotional stress symptoms in student officers and serving officers during service and combat activities under martial law confirmed the negative dynamics of most emotional symptoms' manifestation during their internship and utilization tours (Table 3).

The most pronounced emotional symptoms of stress were: gloomy mood – among student officers, the level of manifestation of this symptom worsened from 14.1 % to 30.9 %, among serving officers – from 21.9-22.9 % to 54.3-59.3 %, depending on age; irritability, anger attacks – among student officers from 12.7 % to 28.2 %, among serving officers – from 17.1-22.2 % to 51.4-55.5 %; feelings of constant longing, depression – among student officers from 8.5 % to 11.3 %, among serving officers – from 12.2-14.8 % to 37.1-40.7 %; anxiety, restlessness – among student officers from 19.7 % to 29.6 %, among serving officers – from 20.0-25.9 % to 41.5-44.4 %; decreased life satisfaction – among student officers from 4.2 % to 8.5 %, among serving officers – from 9.8-14.8 % to 37.0-39.0 %; decrease in self-esteem, dissatisfaction with oneself – among student officers from 2.4 % to 8.5 %, among serving officers – from 8.6-11.1 % to 20.0-25.9 %. At the same time, it was found that the level of emotional symptoms in serving officers after returning from their rotations is significantly more pronounced than in student officers.

The study of the dynamics of physiological stress symptoms manifestation in student officers during their military internship and in serving officers during their rotations shows that, after returning from the combat zone, the number of serving officers with physiological stress symptoms increased significantly (Table 4). The number of student officers exhibiting signs of stress after their internship also increased,

Table 3. Dynamics of emotional stress symptoms in student officers (n = 71) and serving officers (n = 103) during their service and combat activities in a state of martial law (in student officers – before and after their internship, in serving officers – before and after their rotation), %

Emotional symptoms	Student officers (n = 71)		Serving officers under the age of 30 (n = 35)		Serving officers under the age of 40 (n = 41)		Serving officers over the age of 40 (n = 27)	
	Before	After	Before	After	Before	After	Before	After
Worry, anxiety	19.7	29.6	20.0	42.9	21.9	41.5	25.9	44.4
Suspicion	2.8	4.2	8.6	17.1	7.3	19.5	7.4	18.5
Gloomy mood	14.1	30.9	22.9	54.3	21.9	56.1	22.2	59.3
Feeling of constant longing, depression	8.5	11.3	14.3	37.1	12.2	39.0	14.8	40.7
Irritability, bouts of anger	12.7	28.2	17.1	51.4	19.5	53.7	22.2	55.5
Emotional "stupidity", indifference	4.2	5.6	5.7	14.3	4.9	12.2	7.4	14.8
Cynical, inappropriate humor	5.6	9.9	8.6	17.1	9.8	19.5	7.4	22.2
Decreased sense of self-confidence	2.8	7.1	2.9	11.4	7.3	12.2	11.1	14.8
Decreased life satisfaction	4.2	8.5	11.4	37.1	9.8	39.0	14.8	37.0
Feelings of alienation, loneliness	11.3	14.1	14.2	28.6	12.2	29.3	14.8	33.3
Loss of interest in life	0	0	0	5.7	4.9	9.8	7.4	11.1
Decreased self-esteem, dissatisfaction with oneself	2.4	8.5	8.6	20.0	9.8	24.3	11.1	25.9

Source: compiled by the authors of this study

but the increase is considerably smaller compared to serving officers. The most pronounced physiological symptoms of stress in student officers and serving officers, the manifestation of which has a negative dynamic in the process of service and combat activities, are: weight gain or loss – in student officers, the level of manifestation of this symptom worsened from 5.6 % to 12.7 %, among serving officers – from 11.4-14.8 % to 45.7-59.3 % depending on age; decreased immunity, frequent malaise – among student officers from 9.9 % to 12.7 %, among serving officers – from 8.6-14.8 % to 54.3-59.3 %; increased fatigue – among student officers from 12.7 % to 28.2 %, among serving officers – from 14.3-22.2 % to 45.7-48.1 %; digestive disorders – among student officers from 4.2 % to 14.1 %, among serving officers – from 14.3-18.5 % to 42.9-44.4 %; pain of undetermined nature, headaches – among student officers from 5.6 % to 9.9 %, among serving officers – from 8.6-18.5 % to 40.0-51.9 %; feeling of muscle tension – among student officers from 9.9 % to 16.9 %, among serving officers – from 8.6-14.8 % to 34.1-37.0 %.

Based on the study of stress symptoms in student officers and serving officers during their service and combat activities under martial law, it was found that serving officers have a significantly higher level of manifestation of all stress symptoms compared to student officers. It has also been found that there were no significant differences in the levels of stress symptoms manifestation among serving officers of different age

groups. This allows us to conclude that the leading indicator of the negative impact of stress factors is the duration of the subjects' stay in stressful conditions. It has also been confirmed that the degree of influence of stress factors on the bodies of law enforcement officers is determined by their level of stress resilience, their individual psychological characteristics, as well as their abilities and skills to apply available means and methods of coping with stress in the conditions of service and combat activities.

The study of stress levels among student officers and serving officers using Yu. V. Shcherbatykh's test shows that all groups studied exhibited moderate stress levels (ranging from 6 to 12 points) before performing their service and combat missions under martial law (Table 5).

After performing service and combat missions, a statistically significant decrease in stress levels ($p \leq 0.05$ - 0.001) was observed in all groups of subjects. However, while the difference between stress levels before and after their internship was 3.4 points for student officers, it was 6.8 to 7.1 points for serving officers.

It is essential to note that, before the commencement of the missions under martial law conditions, no statistically significant differences were found among all subject groups ($p > 0.05$). After that, the stress level of serving officers of all age groups was higher than that of student officers, with a statistically significant probability ($p \leq 0.001$). A dependence of the stress level on the duration of law enforcement

Table 4. Dynamics of physiological stress symptoms in student officers (n = 71) and serving officers (n = 103) during their service and combat activities in a state of martial law (in student officers – before and after their internship, in serving officers – before and after their rotation), %

Physiological symptoms	Student officers (n = 71)		Serving officers under the age of 30 (n = 35)		Serving officers under the age of 40 (n = 41)		Serving officers over the age of 40 (n = 27)	
	Before	After	Before	After	Before	After	Before	After
Pain of an uncertain nature, headache	5.6	9.9	8.6	40.0	17.1	41.5	18.5	51.9
Increase or decrease in blood pressure	2.8	7.0	8.6	28.6	12.2	34.3	14.8	33.3
Rapid or irregular pulse	1.4	5.6	11.4	37.1	12.2	39.0	18.5	40.7
Disorders of digestive processes	4.2	14.1	14.3	42.9	14.6	43.9	18.5	44.4
Impaired breathing	0	0	0	5.7	0	7.3	3.7	11.1
Feeling of tension in the muscles	9.9	16.9	8.6	34.3	14.6	34.1	14.8	37.0
Increased fatigue	12.7	28.2	14.3	45.7	17.1	46.3	22.2	48.1
Hand tremors, cramps	0	2.8	0	8.6	4.9	9.8	11.1	18.5
Appearance of allergies, other skin rashes	0	0	0	5.7	4.9	7.3	7.4	11.1
Increased sweating	2.8	4.2	0	8.6	4.9	9.8	7.4	11.1
Increase or loss of body weight	5.6	12.7	11.4	45.7	14.6	48.8	14.8	59.3
Decreased immunity, frequent ailments	9.9	12.7	8.6	54.3	12.2	58.5	14.8	59.3

Source: compiled by the authors of this study

officers’ stay in stressful conditions was revealed. After an internship in practical (combat) units, the stress level of student officers was lower than that of serving officers of different ages. The highest stress level was found among serving officers after they returned from rotations lasting 3-4 months. At the same time, while the stress level of student officers after their internship was within 12 points and corresponded to a moderate level, serving officers who had performed their service and combat missions showed a pronounced stress level (13-24 points).

DISCUSSION

As experts point out [2, 3, 9, 12], the accumulation of negative stress factors in law enforcement officers can lead to mental illnesses, such as depressive disorder, which can vary in severity. It is wrong to say that it is easy to get out of depression, that it is enough to go through a difficult period in life simply, and then everything will be fine. It is also wrong to think that you can get out of depression simply by talking to your family or friends over a glass of wine, or by taking antidepressants, the effect of which is incorrectly imagined to be similar to the impact of energy drinks, stimulants, alcohol, or drugs on our body, which supposedly make it possible to quickly get rid of insomnia, feelings of fear, anxiety, panic attacks, negative mood, apathy, unfounded pessimism, and pain that is inadequate to a person's physical condition [8, 13].

The development of stress in law enforcement officers is influenced by: 1) personal anxiety, personal adaptive potential, nervous and mental stability, communication skills, moral normativity; 2) intellectual, behavioral, emotional, physiological signs of stress and a comprehensive assessment of the manifestation of stress; 3) general erudition, breadth of interests, stability and consistency of behavior, attention to others, and productivity of behavior; 4) self-analysis of personality [14]. This is also supported by the data we have obtained, as the symptoms of stress in law enforcement officers during their service and combat activities in conditions of martial law can manifest in various ways, including intellectual, behavioral, emotional, and physiological signs, and can be observed at different levels.

According to scientists [15], the action of stressors (stress factors) on the body causes an increase in the production of adrenal hormones – catecholamines, which largely determine the manifestations and consequences of stress. The manifestations of stress are highly polymorphic, and the very occurrence of a stressful situation causes negative consequences: stomach pain, frequent urination, severe headaches, and inability to breathe deeply because something is interfering. Signs of stress include: increased anxiety, a feeling of crisis or major obstacle; inability to concentrate; too many mistakes at work; memory impairment; frequent feelings of fatigue; high-speed speech; feeling of loss of control; frequent changes of mind; frequent pain (head, back, stomach); increased excitability, irritability; work no lon-

Table 5. Dynamics of stress level in student officers (n = 71) and serving officers (n = 103) before and after performing tasks of their academic training and service activities under martial law, points

Cadets' training years	Stress level		Δ	t / p
	Before	After		
Student officers (n=71)	6.7±0.64	10.1±0.82	3.4	3.27/≤0.01
Serving officers under the age of 30 (n = 35)	7.9±0.91	14.8±1.06	6.9	4.94/≤0.001
Serving officers under the age of 40 (n = 41)	8.5±0.85	15.4±1.01	6.8	5.15/≤0.001
Serving officers over the age of 40 (n = 27)	8.3±0.98	15.1±1.19	7.1	4.61/≤0.001
t / p 1-2	1.08/>0.05	3.51//≤0.001		
t / p 1-3	1.69/>0.05	4.07//≤0.001		
t / p 1-4	1.37/>0.05	3.46//≤0.001		

Notes: Δ – difference between the studied indicators; t – value of Student's t-test; p – level of statistical significance of differences; t / p 1-2, 1-3, 1-4 – value of Student's t-test and level of significance between the indicators of student officers and serving officers of different age groups

Source: compiled by the authors of this study

ger brings the same joy; loss of sense of humor; sharp increase in the number of cigarettes smoked; alcohol addiction; constant feeling of hunger; loss of appetite; inability to finish work on time. Our research confirms the conclusions of many scientists [4, 6, 9, 11, 16] regarding the manifestation of various stress symptoms in law enforcement officers while performing service and combat missions during wartime. The most significant indicators of these symptoms are objective conditions, including the duration of law enforcement officers' exposure to stressful situations and the intensity of the stress factor. Therefore, subjective indicators (such as age, gender, temperament, and sphere of activity) are of secondary importance.

Scientists [17, 18] indicate that stress is currently the cause of almost 90 % of human diseases. Chronic psycho-emotional tension and post-traumatic stress disorder (PTSD) are the causes of neurocirculatory dystonia, hyperventilation syndrome, functional stomach disorders, biliary tract dyskinesia, preclinical forms of ischemic heart disease, arterial hypertension, hypertensive disease, chronic gastroduodenitis, non-ulcer dyspepsia, etc. According to the WHO, the number of diseases caused by the adverse effects of stress has increased 25-fold over the past 60 years [19]. Stress can undermine the immune system, making us more vulnerable to infections. The main pathologies caused by stress include depression, anxiety, heart attack, stroke, weakening of the immune system, and, as a result, vulnerability to various infections, ranging from common colds and herpes to such dangerous diseases as AIDS, certain forms of cancer, and autoimmune diseases such as rheumatoid arthritis or multiple sclerosis. Stress often causes skin reactions (rashes, itching, various dermatitis,

etc.), gastrointestinal disorders, insomnia, neurological disorders, and disturbances in human sexual and reproductive function. Stress is increasingly recognized as a factor in the etiopathogenesis of various modern diseases such as allergies [20, 21]. The results of our research complement the above information on the negative impact of stress factors in service activities on the manifestation of various stress symptoms and, accordingly, on the mental health of law enforcement officers. Moreover, the longer law enforcement officers remain in stressful conditions, the more pronounced the symptoms of stress become. This necessitates the search for effective means and methods of stress prevention during service and combat activities in wartime conditions, as well as guidance on the early development of stress resilience in law enforcement officers throughout their daily service activities.

CONCLUSIONS

The dynamics of the manifestation of intellectual, behavioral, emotional, and physiological symptoms of stress in law enforcement officers (including student officers and serving officers across different age groups) during their service and combat activities under martial law conditions were studied. The most pronounced manifestation of all stress symptoms was found in serving officers, regardless of age. Among the most pronounced symptoms of stress in law enforcement officers after performing their service and combat missions, the following were identified: intellectual: bad dreams – up to 59.3 %, fruitless rumination on a problem – up to 54.3 %, difficulty making decisions – up to 51.4 %; behavioral: sleep disturbances, insomnia

– up to 51.9 %, loss of appetite or overeating – up to 48.1 %, antisocial, conflictual behavior – up to 39.0 %, more intense smoking, alcohol consumption – up to 29.6 %; emotional: gloomy mood – up to 59.3 %, irritability, anger attacks – up to 55.5 %, anxiety – up to 44.4 %; physiological: weight gain or loss – up to 59.3 %, decreased immunity, frequent malaise – up to 59.3 %, increased fatigue – up to 48.1 %, pain of an unspecified nature, headaches – up to 51.9 %.

It was found that after performing service and combat missions, all groups of subjects showed a statistically significant increase in stress levels ($p \leq 0.05-0.001$). At the same time, while the difference in stress levels between student officers before and after their internship was 3.4 points, for serving officers it was 6.8 to 7.1 points. In addition, after performing service and combat missions, the stress level (14.8-15.4 points) among serving officers of all age groups was significantly ($p \leq 0.001$) higher than among student officers (10.1 points). The stress level of student officers after their internship corresponds to a moderate

level, whereas serving officers who have performed service and combat missions exhibit a pronounced level of stress. No significant differences were found between the stress levels of serving officers from different age groups ($p > 0.05$), indicating that the stress level does not depend on the age of law enforcement officers, but on the duration of their exposure to stressful conditions.

The research highlights the need to develop stress resilience in law enforcement officers to ensure the effectiveness of their service and combat activities in times of war, as well as to cultivate skills in utilizing available means to prevent stress during service and combat activities.

PROSPECTS FOR FURTHER RESEARCH

It is planned to investigate the dynamics of stress symptoms manifestation in female law enforcement officers before and after performing their service and combat missions during the war.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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Circulating Cytotoxic T lymphocytes associated antigen-4 (CTLA-4) levels and CTLA-4 gene polymorphism role in head and neck squamous cell carcinoma

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ABSTRACT

Aim: To determine the function of soluble CTLA-4 and the CTLA-4+49A/G gene polymorphism in patients with head and neck squamous cell carcinoma.

Materials and Methods: The current study recruited 180 subjects (90 patients with HNSCCs and comparable number apparently healthful persons as reference) from February 2024 to February 2025. These subjects selected from Medical City, Baghdad, Iraq. All individuals' genomic DNA was extracted from blood samples. To evaluate soluble (sCTLA-4), an enzyme-linked immunoassay kit was used. The Tetra-Primer Amplification Refractory System-Polymerase Chain Reaction (T-ARMS-PCR) was used to genotype and amplification of the CTLA-4 gene using specific primers.

Results: As opposed to controls (median = 389.5 ng/ml, range = 160-1966 ng/ml), the median serum level of sCTLA-4 in patients was 1593.5 ng/ml (range = 1118-2000 ng/ml), which was noticeably higher. There was a significant difference ($p=0.024$) in the frequency of patients with the AA genotype compared to those did not (67.78% vs. 50%). Conversely, controls had a significantly higher frequency of the heterozygous genotype (AG) than patients (37.78% vs. 28.89%) (OR = 0.20, 95%CI = 0.05-0.76, $p=0.018$). At allelic level, G allele was far more frequent in controls than patients (31.11% versus 17.78%).

Conclusions: The G allele and the heterozygous genotype (AG) may be regarded as protective factors from HNSCC, and AA genotype is risk factor for HNSCC. Soluble CTLA-4 rise remarkably in patients with HNSCC suggesting a function for this protein in pathogenesis of this disease.

KEY WORDS: Head and neck squamous cell carcinoma, CTLA-4+49A/G, sCTLA-4, polymorphism

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INTRODUCTION

Squamous cell carcinoma is the most common histological type of head and neck malignancies, which are a diverse group of tumors that arise in the head and neck area. The majority of these malignancies originate from the squamous cells lining the mucosal surfaces of the upper aerodigestive tract, collectively referred to as head and neck squamous cell carcinoma [1]. It accounted for about 890,000 new cases and 450,000 fatalities, making it the seventh most prevalent cancer globally. Tobacco and alcohol use, and HPV infections are the primary risk factors for HNSCC [2]. This cancer type's oncogenesis and evolution are significantly influenced by the immune system. Interestingly, the immunosuppressive tumor microenvironment causes immunological escape via a

number of methods [3]. In HNSCC immunosuppression, PD-1/PD-L1 and Cytotoxic T lymphocytes antigen-4 (CTLA-4) are the main immunological checkpoints. It is well known that Cytotoxic T lymphocyte-Associated Antigen-4 (CTLA-4; CD152), a homologue of CD28, contributes to the reduction in T cell activation and clonal proliferation. Antigen-presenting cells' ligands, CD80 and CD86 can normally bind to either CD28 or CTLA-4, causing a costimulatory or co-inhibitory response, respectively [4]. Cytotoxic T lymphocytes antigen-4 (CTLA-4) is only expressed on the surface of activated T cells, down-regulates the stimulatory signals from CD28 and competes for binding to B7 family members i.e. B7.1 and B7.2. This co-inhibitory molecule also interferes with signals delivered by T cell receptor (TCR). There are two types of CTLA-4 inhibitory tech-

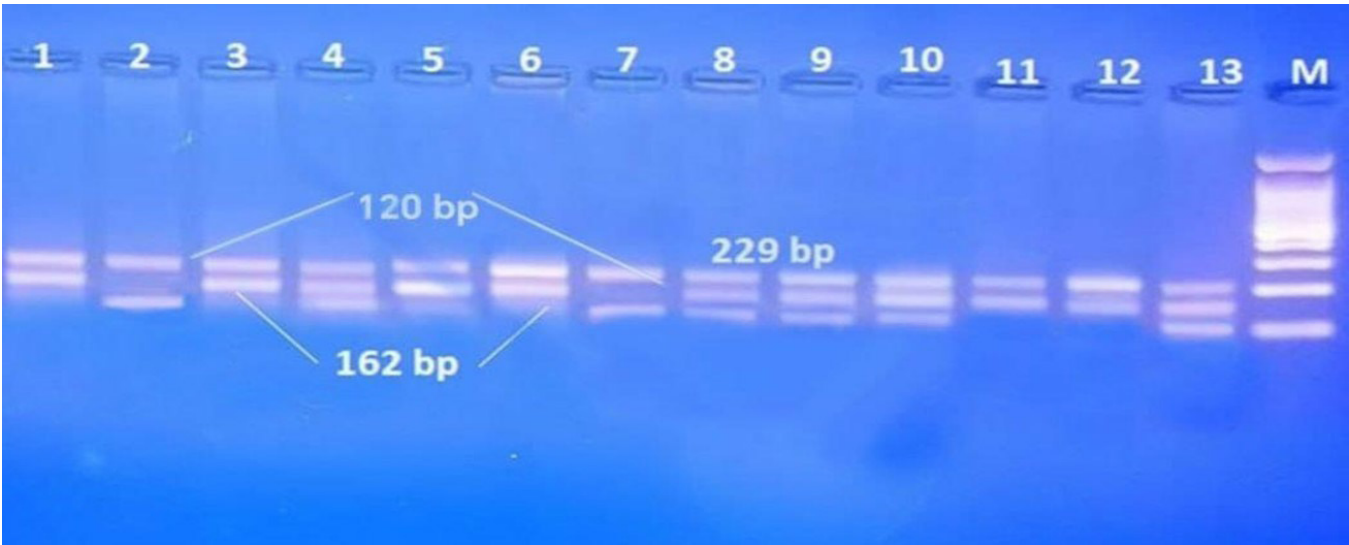


Table 1. The sequences of CTLA-4 +49A/G primers and the length of the resultant fragments employed for T- ARMS-PCR amplification of genes [15]

Primers	Sequence (5'-3')	Fragment length
Outer primers	Forward:49 Fo	229bp
	5'-GTGGGTTCAAACACATTTCAAAGCTTCAGG-3'	
	Reverse:49 Ro	
Inner primers	5'-TCCATCTTCATGCTCCAAAAGTCTCACTC-3'	A162bp
	49Fi(G)	
	5'-GCACAAGGCTCAGCTGAACCTGGATG-3'	
	49Ri(A)	G 120bp
	5'-ACAGGAGAGTGCAGGGCCAGGTCCTAGT-3	

Source: Own material

niques: cell-intrinsic (which target the T-cell that expresses CTLA-4) and cell-extrinsic (which target secondary cells) [5]. Chromosome 2q33 is dedicated to the CTLA-4 gene, which has four exons [6]. The CTLA-4+49A/G genotype has been connected to a number of cancer disorders risk e.g., malignant bone tumors [7], nasopharyngeal carcinoma [8], oropharyngeal carcinoma [9-10]. As an alternative, cytotoxic T-lymphocyte protein 4 (s CTLA-4) is produced in a soluble form by spliced mRNA. Serum levels of sCTLA-4 are modest in normal human serum, but they have been discovered to be raised in a number of malignant illnesses [11-12]. Reduced surface expression of CTLA-4 protein and lower amounts of soluble CTLA-4 mRNA are linked to the G allele, a functional polymorphism of CTLA-4 [13-14].

MATERIALS AND METHODS

STUDY POPULATION

180 subjects registered in this case- control study, encompass 90 patients with HSCCs and 90 healthy participants. All subjects enlisted at National Al-Amal Hospital for Oncology and Baghdad Center for Radiation – Medical City (Baghdad, Iraq) during period from February 2024 to February 2025. Diagnosis was executed by examined clinically and assert by histopathological diagnosis.

ETHICAL APPROVAL

Ethical approval to perform the research acquired from scientific and ethical committees/College of Medicine / University of Diyala (No: 2025HHR901 Date: 19/3/2025).

DATA AND SAMPLE COLLECTION

Every individual who has previously participated in the study gave their written consent. Each subject's age, gender, and smoking status were obtained through direct interview using a pre-made formula. Two halves of five milliliters of venous blood were taken out. Serum was separated in a gel tube comprising the first portion (3 ml) that was employed for detection of s CTLA-4 by Enzyme-Linked Immunosorbent Assay. Two milliliters of the second component were placed in an EDTA tube and kept at -80 °C, till needed for DNA extraction for amplification of CTLA-4+49A/G.

SERUM PREPARATION AND STORAGE

After being stored at room temperature (15–25°C) for 20 minutes to guarantee complete clotting, the tubes were centrifuged for 10 minutes at 1900 x g at 4°C. Lastly, to detect sCTLA-4, the supernatant was cautiously moved to a new tube.

Table 2. Demographic features of the study population

Variables	Patients (n=90)	Controls (n=90)	p-value
Age, years			
Mean ± SD	55.26±15.85	51.12±10.59	0.041
Range	9.0-85	16.0-82	
Sex			
Male	74(82.22%)	53(58.89%)	0.001
Female	16(17.78%)	37(41.11%)	
BMI (kg/m ²)			
Mean ± SD	25.03±3.81	25.13±3.76	0.819
Range	18.78-34.37	20.34-31.46	
Smoking			
Yes	41(45.56%)	82(91.11%)	<0.001
No	49(54.44%)	8(8.89%)	

BMI: Body Mass Index; SD: Standard Deviation
Source: Own material

Table 3. Clinical characteristics of patients with HNSCC

Variables	Frequency	Percentage
Type of cancer		
Laryngeal	30	33.33%
Nasopharyngeal	41	45.56%
Tongue	9	10%
Oropharyngeal	6	6.67%
Others	4	4.44%

Source: Own material

DETERMINATION OF CYTOTOXIC T LYMPHOCYTES ASSOCIATED ANTIGEN-4 (CTLA-4) BY ELISA

Enzyme Linked Immunosorbent Assay (ELISA) (Cat. No: ELK1815, ELK biotechnology, USA) kit for quantitative assessment of human serum CTLA-4. A microtiter plate that has already been coated with an antibody specific to human CTLA4 is included in this kit. After standards or samples have been added to the proper microtiter plate wells, a biotin-conjugated antibody that is specific to human CTLA4 is added. Then, horseradish peroxidase (HRP) conjugated to Avidin is added to each microplate well, and the plates are incubated. When TMB substrate solution is added, only the wells that contain human CTLA4, Color changes will occur in enzyme-conjugated Avidin and biotin-conjugated antibody. A sulfuric acid solution is added to interrupt the enzyme-substrate combination, and the color shift is measured using spectrophotometry at a wavelength of 450 nm ± 10 nm. To determine the amount of human CTLA4 present in the samples, the optical density (OD) of the samples is compared to the standard curve.

ISOLATION DNA AND TETRA-PRIMER AMPLIFICATION REFRACTORY SYSTEM - POLYMERASE CHAIN REACTION (ARMS)

Gsync™ DNA extraction kit fast organizer (Cat. No. GS100, Geneaid, Taiwan) is a commercial kit was used,

as instructed by the manufacturer, to extract human genomic DNA. A260/A280, the extracted DNA concentration, was determined by the Biospec Nano spectrophotometer. Using certain primers, the CTLA-4 gene was generated and genotyped (Table 1). The Tetra-Primer Amplification Refractory System-Polymerase Chain Reaction (T-ARMS-PCR) is being used. The PCR settings included a 10-minute initial denaturation at 95°C, 35 cycles of denaturation 94° for 30 sec C, 30 seconds of annealing at 62°C, and a 45-second extension at 72°C. An elongation for seven minutes at 72°C was the last phase [15]. PCR products were appreciated by electrophoresis on a 2% agarose gel and Ethidium bromide was used to stain this results. Following gel electrophoresis, a digital camera and a UV transilluminator were used to read the data, sequences of outer and inner primers and length of product illustrated in figure 1.

STATISTICAL ANALYSIS

The statistical software SPSS 25.0 (SPSS, Chicago) was employed to carry out the analyses. Constant statistics were presented as mean and standard deviation unless specified. Student t-test, Mann Whitney U test was used to compare means as required. Categorical variables were expressed as number and percentage and analyzed with Chi-square test. Binary logistic regression was applied to gauge the correlation between HNSCC and the CTLA-4+49A/G gene polymorphism. Based on this test, the odds ratio (OR) and related 95% CI were estimated. Any variation with a p-value below 0.05 was regarded as statistically significant.

AIM

This study's goal was to assess the role of the CTLA4 +49A/G gene variants and soluble CTLA-4 in HNSCC Iraqi population.

Table 4. Median level of sCTLA-4 in patients with HNSCC and controls

Variables	Patients (n=90)	Controls (n=90)	p-value
sCTLA-4, ng/ml			
Mean $\pm$ SD	1593.4 $\pm$ 228.76	470.5 $\pm$ 310.0	
Median	1593.5	389.5	<0.001
Range	1118-2000	160-1966	

Source: Own material

Table 5. The incidence of various genotypes and alleles in patients and controls with CTLA-4 polymorphism

CTLA-4 Polymorphism	Patients (90)	Controls (90)	p-value	OR(95%CI)
Genotypes				
AA	61(67.78%)	45(50%)	0.024	1.0
AG	26(28.89%)	34(37.78%)	0.018	0.20(0.05-0.76)
GG	3(3.33%)	11(12.22%)	0.142	0.36(0.09-1.41)
HWE	0.909	0.260		
Dominant model				
AA+AG	87(96.67%)	79(87.78%)	0.037	1.0
GG	3(3.33%)	11(12.22%)		0.25(0.07-0.920)
Recessive model				
AA	61(67.78%)	45(50%)		1.0
AG+GG	29(32.22%)	45(50%)	0.016	0.48(0.26-0.87)
Alleles				
A	148(82.22%)	124(68.89%)	0.004	1.0
G	32(17.78%)	56(31.11%)		0.47(0.29-0.77)

Source: Own material

RESULTS

THE STUDY POPULATION'S CLINICAL AND DEMOGRAPHIC FEATURES

Table 2 displays the association of demographic factors with HNSCC. Patients were 55.26 $\pm$ 15.85 years old on average, which was significantly older than the controls (51.12 $\pm$ 10.59 years old). The male-to-female ratio is more equal in controls (58.89% male, 41.11% female), while the majority of HNSCC patients are male 82.22%. The significance of this difference is strong p=0.001. The mean BMI of HNSCC patients 25.03 $\pm$ 3.81 kg/m² and controls 25.13 $\pm$ 3.76 kg/m² does not differ significantly p=0.819. There was a highly significant difference p<0.001 between the percentage of smokers among HNSCC patients 45.56% and controls 8.89%.

CLINICAL CHARACTERISTICS OF THE PATIENTS WITH HNSCC

Nasopharyngeal cancer 45.56% is the most common sort among the patients, which is followed by laryngeal cancer 33.33%. The less frequently occurring cancers include tongue 10%, oropharyngeal 6.67%, and other types 4.44% (Table 3).

SERUM LEVEL OF SCTLA-4

It was discovered that the data pertaining to the blood levels of sCTLA-4 was not regularly distributed. As a

result, the median and range of these data were used, and the non-parametric Mann Whitney U test was used for analysis. In patients with HNSCC, the median serum level of sCTLA-4 was 1593.5 ng/ml (range = 1118-2000 ng/ml), considerably greater than in controls (median = 389.5 ng/ml, range = 160-1966 ng/ml) (Table 4).

MOLECULAR ASSAYS

This research looked at The SNP CTLA-4+49A/G and HNSCCs: A relationship was examined using the T- ARMS technique. AA, AG, and GG are the three SNP genotypes that were found in both patients and controls (Fig. 1.

CTLA-4 +49A/G GENE POLYMORPHISM WITH HEAD AND NECK SQUAMOUS CELL CARCINOMA

The AA genotype of this polymorphism was more frequent patients than controls 67.78% vs. 50% with a significant difference p=0.024. In contrast, the heterozygous genotype (AG) was more frequent in controls than patients (37.78% vs. 28.89%) with a significant difference (OR= 0.20, 95%CI=0.05-0.76, p=0.018). Similarly, the mutant homozygous genotype (GG) was more frequent in controls than patients (12.22 vs. 3.33%) although the difference was not significant. The G allele was considerably greater in prevalence

in controls than in patients at the allelic level 31.11% versus 17.78%, with an extremely significant variance (OR=0.47, 95%CI=0.29-0.77, $p=0.004$) (Table 5).

DISCUSSION

This case-control study was showed the HNSCC patients were significantly older than the controls with mean of age 55 years. In Iraq previous study was demonstrated that head and neck cancer patients increased in the ages from 41 to 60 years old [16]. Globally past report was observed the HNSCCs mean age was in sixth decade of life. Research on carcinogenesis in elderly persons led to the hypothesis that tumor cell transformation is caused by a sequence of spontaneous mutations rather than by increasing exposure to carcinogenetic substances [17]. Males make up a significant part of HNSCC sufferers than females, which agree with another studies substances [18-19]. This may be related to tobacco and alcohol usage. The disparity in working patterns observed in our state may also be a contributing cause to the higher incidence of HNSCC in men than in women. The vast majority of men are engaged in outdoor activities because they work as laborers or farmers, which entails long hours in blazing sun [20]. In comparison to controls, a highly significant difference $p<0.001$ was found in the percentage of smokers among HNSCC patients. This report agrees with other previous studies [19-21]. Numerous carcinogens, including nitrosamines, aromatic amines, aldehydes, and polycyclic aromatic hydrocarbons, are found in tobacco. These compounds, which are created by burning at high temperatures, are known to damage the DNA of oropharyngeal cells, potentially leading to cancer [22]. In current study, the most prevalent cancer type among patients is nasopharyngeal cancer, which is followed by laryngeal cancer, cancers of the tongue, oropharynx, and other forms are less common, this finding comparable with other study in Iraq It revealed that the floor of the mouth had the lowest prevalence and the tongue had the highest [18]. Another study announced the oral cavity accounted for 58.4% of all cancer cases observed in HNSCC patients, with the oropharynx, larynx, hypopharynx, and nasopharynx following closely behind [19]. One of the main elements that stops the autoimmune damage connected to T-cell hyperactivation is the CTLA-4 protein, which acts as an immunological checkpoint. The current study investigated the connection between HNSCC manifestation and one of the circulating T-cell brake and polymorphism rs231775 (c.49A>G) linked to HNSCC susceptibility. This finding demonstrated that the median serum levels of sCTLA-4 in patients were significantly higher than those in controls. Thus, it may be said that sCTLA-4 levels are essential for controlling the immune system. Although sCTLA-4 levels in normal human serum

are modest, increased or high levels of the protein are linked to number of cancers [23]. On the other several studies worldwide have shown elevated levels of sCTLA-4 in the patients with certain cancers, like breast cancer acute lymphoblastic leukaemia [24-25] because it accumulates in a wide range of other malignancies, the examination may not be an accurate diagnostic marker even with its relatively high sensitivity and specificity. This result is in contrast to an Iraqi study that involved 45 women with breast cancer diagnoses and 45 healthy women who were paired with patients as a control group. The study indicated a non-significant increase in the serum level of CTLA-4 under $p>0.05$ in patients compared to control in mean $\pm$ SD (3.17 ± 0.82 ng/ml) [26]. In the present study, the frequency of various genotypes and allele of CTLA-4+A/G polymorphism in patients and controls revealed significantly more frequent heterozygous AG genotypes in control than patients. Patients were more likely to have the AA genotype than controls, while controls were more likely to have the mutant homozygous genotype (GG), however not statistically considerable distinction was seen. The G allele was much more prevalent in controls than in patients at the allelic level. This study partially agree with previous case-control study discovered single nucleotide polymorphisms (SNPs) in the CTLA-4 gene were genotyped on 301 patients with prostate cancer (PCa) and 301 controls, Carrier of the CTLA4c.49A>G[A] allele and AA genotype were overrepresented in PCa compared to controls ($p=0.082$ and $p=0.13$, respectively) [27]. Globally, another study conducted in Turkey compatible with this finding that observed the homozygous AA genotype for CTLA-4+49A>G were more frequent in patient than controls [23]. Nonetheless, these investigations presented contentious findings regarding the correlation between different cancer types and CTLA-4 polymorphisms [28]. For example, Qi et al. found that a lower risk of colorectal cancer was linked to the CTLA4 +49 AA genotype [29]. Conversely, Cozar et al., [30] found a link between the CTLA4 gene and renal cancer susceptibility and the CTLA4 AA genotype, but no correlation between the CTLA4 gene and colon cancer. According to Sun et al., [31] found Chinese women who carry the A allele are more likely to develop breast cancer and other cancers. Another study by Ghaderi et al. on Iranian women with breast cancer showed that, in comparison to controls, the frequency of the GG genotype was much lower [32]. The CTLA-4+49A/G polymorphism occurs when guanine is substituted for adenine at position 49 in the CTLA-4 gene. Consequently, GCC which encodes alanine, replaces codon 17 (ACC), which encodes threonine. By regulating the total intensity of T-cell activation, the CTLA-4 receptor fulfills a crucial regulatory role during the im-

munological response [33]. Actually, this regulating effect has been explained by two different processes. The first mechanism involves CTLA-4 engaging with its ligands B7.1 and B7.2 to deprive the homologous receptor CD28 of its ligands. The subsequent system involves the signal transduction pathway inhibiting T-cell activation by down regulating T-cell receptor dependent signaling [34]. Numerous phenotypic alterations that impact one or both of these two processes occur when alanine is substituted for threonine. The CTLA-4 protein, which contains alanine, was thought to have a changed spatial structure that results in poor handling of the protein in the endoplasmic reticulum and less effective N-glycosylation [35]. The dimerization and subsequent activation of CTLA4's inhibitory activity depend heavily on this glycosylation [36]. Additionally, some data indicated that patients with autoimmune illnesses had a markedly lower mRNA for the CTLA-4 protein linked to the GG genotype [37]. In addition to downregulation CTLA-4 synthesis, Sun et al., [31] proposed that the G allele produces a protein with a reduced affinity for B7. There is nearly universal consensus that G allele-bearing CTLA-4 has a lower capacity to regulate T-cell activation than A allele-bearing CTLA-4, regardless of the exact mechanism by which this polymorphism influences the immune response. However, this has two sides. The carriers of the +49G variant have the advantage of a strong immunological response and sustained T-cell activation, which may shield them from various infectious agents and even some types of cancer. On the other hand, this variation puts its carriers at risk for a variety of autoimmune conditions. Because it alters the CTLA-4's inhibitory function and results in a Thr/Ala substitution in the leader peptide,

the A>G transition at position 49 in exon 1 (CTLA-4c.49A>G) may contribute to antitumor immunity. The presence of [AA] genotype was shown to be associated with significantly lower activation and proliferation of T lymphocytes than [GG] genotype. The protein product coded by CTLA-4c.49A>G[AA] genotype CTLA-4 Thr has more capacity to bind B7.1 and a stronger inhibitory effect on T-cell activation compared with CTLA-4 Ala. The CTLA-4c.49A>G polymorphism in the leader sequence has also been hypothesized to affect intracellular/surface partitioning, CTLA-4 glycosylation, and endocytosis or surface trafficking rates, all of which could change the molecule's inhibitory activity [14, 31, 38, 39].

CONCLUSIONS

CTLA-4 gene polymorphism was significantly associated with HNSCC: CTLA-4 AA genotype consider risk factor for HNSCCs susceptibility. Elevated of serum levels CTLA-4 participate in pathogenesis of HNSCCs. Use sCTLA-4 as Biomarkers: This immune marker shows strong diagnostic value and could assist in early detection or monitoring of HNSCC. Expanding screening methods should be the main focus in future work since it is crucial to better therapy.

RECOMMENDATIONS

Recommended for using another primer in another region of gene for obtain the additional risk factors for HNSCCs and Recommended for diagnose another gene. Our findings should be addressed by requiring additional research on a bigger patient population.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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Lectin histochemistry of bone regeneration dynamics in the rabbit mandible following transplantation of natural mineral and organic osteoplastic materials

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ABSTRACT

Aim: To reveal the applicability of lectin histochemistry methods for the evaluation of bone tissue injury recovery.

Materials and Methods: The research was accomplished on 35 mature rabbits aged 6–7 months weighting 2.5–3 kg subdivided into three groups. Control group included 10 rabbits with post traumatic mandibular bone injury site healed under the blood cloth. First experimental group consisted of animals with damaged bone recovery supplemented with an osteotropic material based on native octacalcium phosphate (OCP-N). Second experimental group consisted of rabbits with bone defect filled with natural collagen cone (Col-C). Monitoring of the post-traumatic processing. was carried out for 84 days using panel of three lectins (WGA, LABA and CNFA) with different carbohydrate specificities.

Results: The lectins demonstrated high variability of binding with constituents of posttraumatic bone tissue. In particular, it was revealed rather selective WGA reactivity with microvascular endothelium, LABA reactivity with macrophages and CNFA with fibroblaststs and osteogenic cells. Injured bone in control and both experimental groups demonstrated distinct rearrangement of lectin receptor sites with drift towards posttraumatic recovery being more prominent after application of OCP-N osteotropic material. Traumatic bone injury induced accumulation of macrophages in the site of inflammation, as well as redistribution of lectin receptors in microvascular endothelium.

Conclusions: Lectins of different carbohydrate specificities can be recommended for monitoring bone tissue regeneration after posttraumatic injuries.

KEY WORDS: bone tissue, lectin histochemistry, osteoregeneration, octacalcium phosphate, natural collagen

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INTRODUCTION

The relevance of present research is due to the growing number of patients and victims of war who need adequate augmentation of bone defects, a significant number of relevant surgical interventions, and is confirmed by a huge number of specialized studies and publications in this area. However, the vast majority of studies are aimed on finding the so-called «perfect» material or justifying the optimal way to use it, in other words, at determining a certain rating of osteoplasty methods. Therefore, it is justified to study the dynamics of histoarchitectural remodeling during osteoregeneration depending on the osteoconductive and osteoinductive properties of osteotropic materials that differ in their physicochemical composition. The urgency of finding effective methods for stimulating bone tissue regeneration after injuries is due to the fact that extensive and infected post-traumatic defects,

despite the natural ability of the bone to self-heal, significantly complicate the healing process and cause a number of complications [1, 2].

It should be noted that this is not the first study devoted to solving numerous issues related to ways to increase the effectiveness of osteoplasty using various osteotropic materials. However, when it comes to morphological transformations of bone and soft tissue components of the dento-maxillary apparatus when using certain osteoplastic materials, we are faced with a complete lack of ideas in this direction. Those fragmentary information and individual publications that try to cover it sometimes turn out to be contradictory or even antithetical in their general conclusions. In this aspect, the problem of our research is clearly distinguished precisely by the fact that it is aimed on studying specific morphogenetic mechanisms that ensure the osteoregenerative process

Table 1. Lectins used and their carbohydrate specificities

Lectins full names and abbreviated forms	Carbohydrate specificities	Complementary oligosaccharide
Clitocybe nebularis fungus agglutinin, CNFA	DGalNAc	DGalNAc(β14)GlcNAc
Wheat germ agglutinin, WGA	DGlcNAc > NeuNAc	Man(β1-4)GlcNAc(β1-4)GlcNAc > NeuNAc(α2-6)Gal(β1-4)GlcNAc
Laburnum anagyroides bark agglutinin, LABA	LFuc	Gal(β1-4)Fuc(β1-3)Glc

Source: compiled by the authors of this study

at the tissue, cellular and subcellular levels when it is initiated using osteotropic materials with different properties, thereby providing the opportunity to choose the optimal material depending on the specific clinical situation. Extensive and infected post-traumatic bone defects significantly slow down the rate of remodeling, often requiring surgical intervention using osteoplastic materials [3].

In large bone defects, autologous bone remains the «golden standard», but it also has a number of disadvantages [4-6]. In the treatment of small and medium bone defects, alternative osteoplastic materials are increasingly used, which minimizes the risk of complications associated with autotransplantation. Effective integration of these materials with the bone bed without the formation of fibrous tissue is a determining condition for their successful use. Xenogenic materials, in particular, based on octacalcium phosphate, demonstrate good osteoconductive properties due to their structural similarity to human bone tissue and the optimal ratio of calcium to phosphorus. Biodegradation of octacalcium phosphate with subsequent replacement by new bone makes it a valuable material in dental regenerative surgery [7-10].

Also in our study, a comprehensive analysis of the effectiveness of a modified natural collagen matrix was conducted. This substance is widely used for post-extraction preservation of the alveolar process and promotes the formation of a blood clot with its subsequent reorganization into bone tissue. Collagen-based materials have high biocompatibility and a predictable result of preserving the height of the alveolar process. The main characteristics of natural collagen implants include: high ability to resorption processes, ensuring blood clot stabilization and effective local hemostasis. Collagen cones have found wide application in various fields of dentistry, in particular, in implantology, periodontology and maxillofacial surgery. They are used to control bleeding and hemostasis after tooth extraction, during biopsy and when performing closed sinus lifting [11]. Collagen-based materials in various forms, including membranes, sponges or matrices, hydrogels, and composite scaffolds, are also widely used in vivo to support bone regeneration in various clinical applications [12].

AIM

To reveal the applicability of lectin histochemistry methods for the evaluation of bone tissue injury recovery.

MATERIALS AND METHODS

The study was conducted on 35 mature male rabbits aged 6-7 months, weighing 2.5-3 kg. The animals were divided into three groups: a control and two experimental groups (10 animals each). Another 5 intact animals were used to study the normal structure of the bone tissue of the studied area of the lower jaw (LJ). The control group included animals with a bone tissue defect healed under a blood clot. The first experimental group consisted of rabbits in which the bone defect was filled with the osteotropic material Compact BoneB («Dentegris», Germany), the main crystalline element of which is native octacalcium phosphate (OCP-N). The second experimental group consisted of rabbits in which bone defect was filled with the material Collacone («Botiss dental», Germany), which is a natural collagen cone (Col-C). Animals of the control and experimental groups were given general anesthesia by intraperitoneal injection of Thiopenate (Bropharma, Ukraine) at a rate of 25 mg/kg of animal body weight at the level of the edentulous area of the alveolar part of the lower jaw using a dental bur, causing a bone-destroying injury in the form of a shaft 4 mm deep and 3 mm wide. Post-traumatic condition was monitored 1, 7, 14, 21, 28, 35, 56 and 84 days after the injury.

The dynamics of histoarchitectural rearrangements of bone tissue in the area of an experimental defect of the lower jaw were studied using the lectin histochemical method, which occupies a significant place among modern methods of morphological analysis [13,14]. To identify carbohydrate determinants of bone tissue structures, a panel of lectins conjugated with horseradish peroxidase was used (Table 1). All used lectins were purified and conjugated with peroxidase by Professor V.O. Antonyuk, a Faculty member of Lviv Medical University Pharmacy Department.

Serial sections of 5-7 μm thick were made from the obtained paraffin blocks of decalcified bone fragments on a sled microtome. Processing of sections with lectins was carried out according to the standard scheme de-

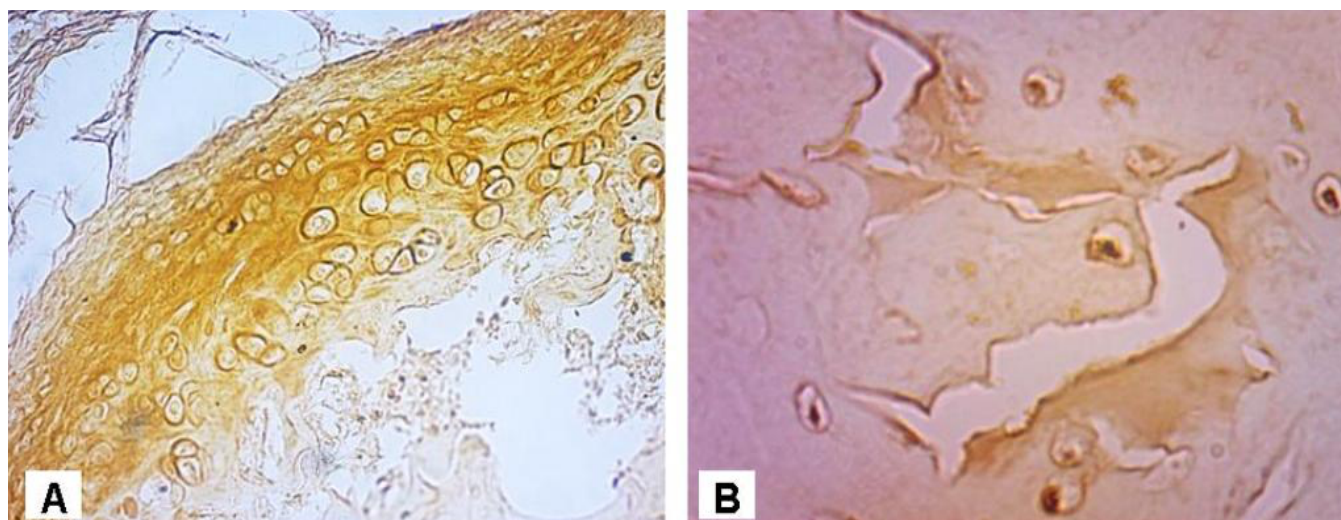


Fig. 1. Regenerate deep zone on the 3rd week post surgical treatment of control group rabbit as visualized by WGA-HRP. A). Destruction of chondroid during cartilage osteogenesis. $\times 400$. B) Intense WGA labeling of osteoclasts and surface lining of the Howship's lacunae. $\times 600$
Picture taken by the authors

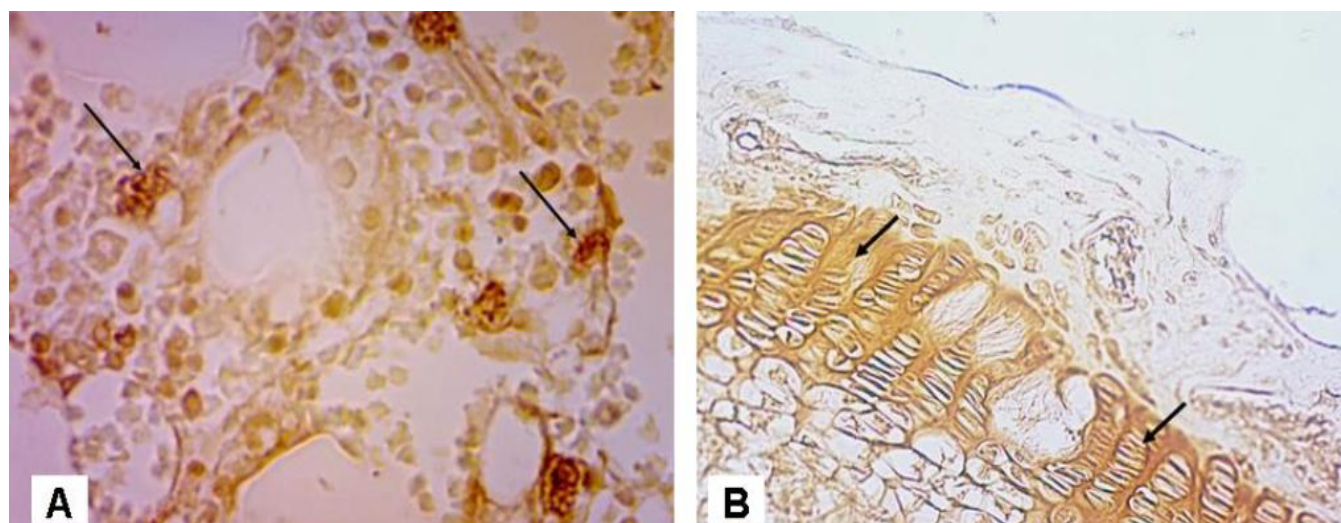


Fig. 2. Deep zone of the regenerative island: 2nd week post surgical treatment; control group rabbits; carbohydrate visualization by LABA-HRP conjugate. A). Accumulation of macrophages in the inflammatory zone (arrows). $\times 600$. B). Islet of chondrohistogenesis: columns of chondrocytes subjected for resorption are highlighted by arrows. $\times 400$
Picture taken by the authors

scribed elsewhere [15]. The lectin panel is presented in Table 1. Stained histological slides were studied using a UlabXSP-137TLED photomicroscope (PRC). Pictures were taken with an XCAM-1080 P camera (PRC).

All animals were kept in standard vivarium conditions and procedures related to housing, care, marking and all other manipulations were carried out in compliance with the provisions of the «European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes» (Strasbourg, 1985);, the «General Ethical Principles of Animal Experiments» adopted by the First National Congress on Bioethics (Kyiv, 2001), the Law of Ukraine No. 3447-IV «On the Protection of Animals from Cruelty to Animals» in accordance with the EU Council

Directive 2010/63/EU on the implementation of the regulations, laws and administrative provisions of the EU Member States concerning the protection of animals used for scientific purposes. The Bioethics Commission of Danylo Halytsky Lviv National Medical University approved the design and methodology of research project with final conclusion that conducted research meets ethical requirements in accordance with the Order of Ministry of Health of Ukraine № 231 of November 1, 2000 (protocol № 1 of January 20, 2025).

RESULTS

A study conducted using peroxidase-labeled lectins in the control group showed significant variability in the intensity

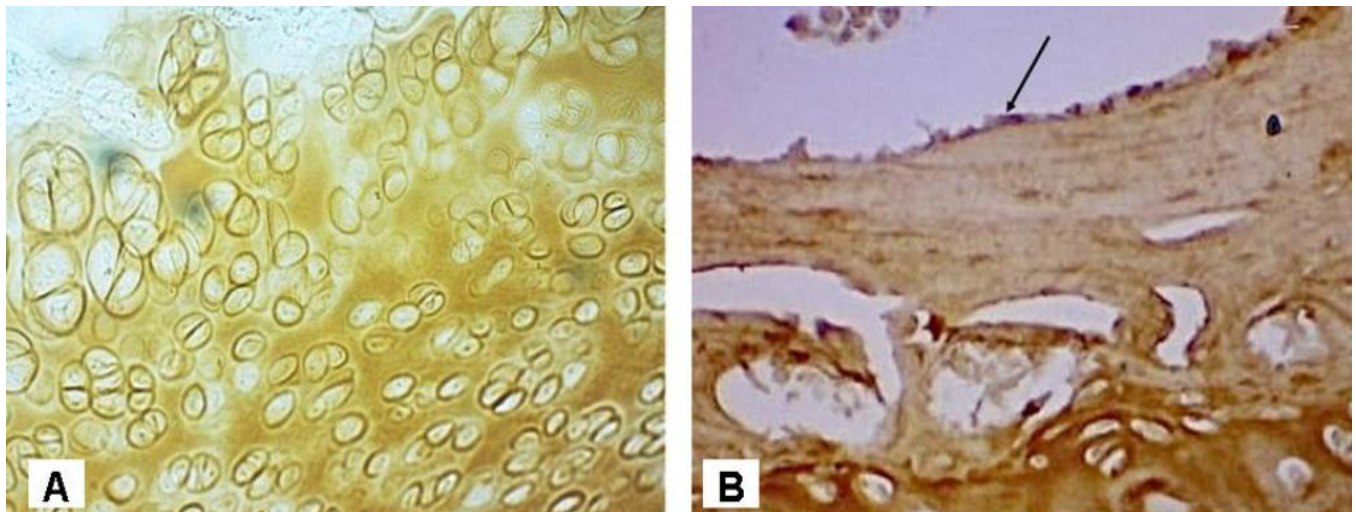


Fig. 3. Regenerate deep zone on the 3rd (A) and 7th (B) weeks post surgical treatment: control group rabbits subjected to CNFA-HRP conjugate. A). Islet of chondrohistogenesis in progress. $\times 400$. B). Intense reactivity of osteoblasts covering bony trabecule (arrow); osteocytes and osteomucoid less reactive. $\times 400$

Picture taken by the authors

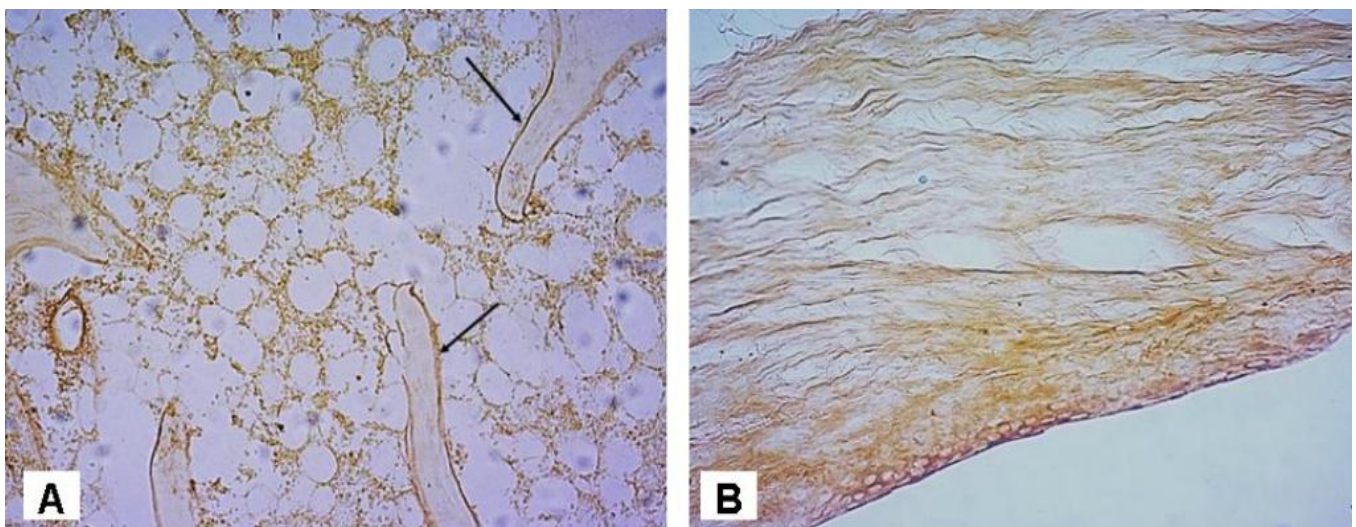


Fig. 4. Deep zone of osteoregenerate after 8 (A) and 5 (B) weeks of traumatic damage with subsequent tissue healing under octacalcium phosphate. A). Bony trabecules covered by WGA reactive glycoconjugates (arrows). $\times 100$. B). LABA reactivity of enosteal cover of bony trabecule, collagen fibers of newly formed bone are faint positive. $\times 200$

Picture taken by the authors

of binding to carbohydrate determinants of structural elements of bone tissue of the alveolar processes of the mandible, periodontium, gums and blood clot structures. In particular, when using WGA (DGlcNAc > NeuNAc), high reactivity was detected at the early stages of the study in the microcirculatory bed endothelium, individual cellular elements of the periodontal ligament and in the fibroblasts of the gingival lamina propria. Strong WGA labeling was also characteristic for the surfaces of blood cells. Within 1-3 weeks after injury, the highest intensity of WGA binding was observed on the periphery of cartilage osteogenic islets, in the deep zone in the regenerate, close

to the areas of chondroid destruction (Fig. 1A). From the 6th to the 12th week of the experiment, a slightly different activity of WGA lectin binding to the structures under investigation was observed. The vascular endothelium was mostly unresponsive, but individual areas of WGA binding were detected in the basement membranes of small blood vessels (Fig. 1B). The reactivity of cellular elements during this period did not show significant changes.

LABA reactivity (LFuc specific) was detected on individual cells in the studied samples. At the end of the day 1st of experiment, on the background of osteolytic injury, strong LABA reactive cells were identified in the

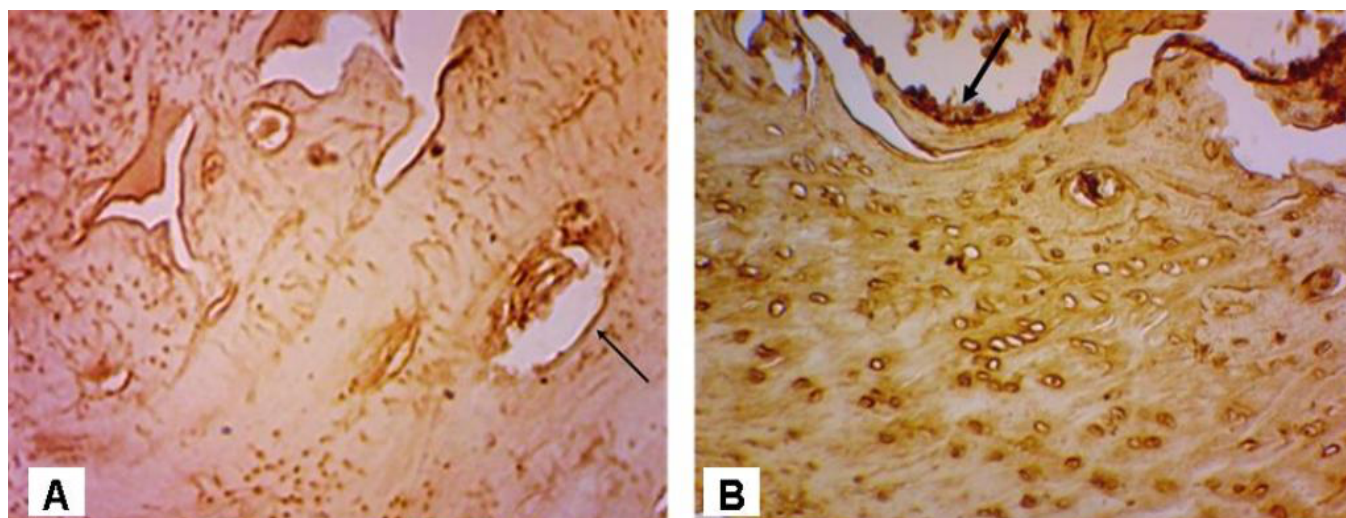


Fig. 5. CNFA lectin labeling of osteoregenerate peripheral trabecule on week 8th post traumatic damage and subsequent healing under octacalcium phosphate (A) and natural collagen treatment (B). In both cases CNFA strongly labeled osteoblastic cover of bony trabecules (arrows), cell bodies of early osteocytes, surface of their housing lacunas, surface of Howship's lacunas with detryt deposits inside. A×400; B. ×200

Picture taken by the authors

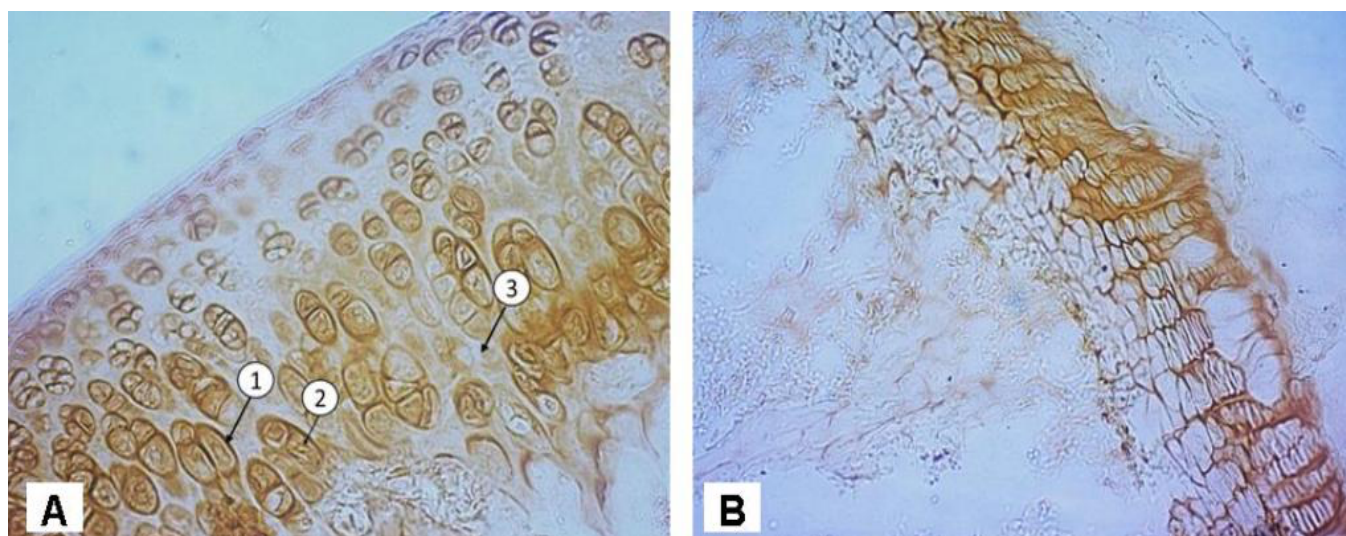


Fig. 6. Lectin reactivity with deep zone of osteoregenerate of the lower jaw of a rabbit. A. Two weeks after injury and subsequent filling of the defect with octacalcium phosphate: intense WGA reactivity with surface of chondrocytes and of their residence lacunae (1); cytoplasm of chondrocytes (2) and adjusting chondromucoid (3) are far too less reactive. ×200. B. Three weeks after injury and filling of the defect with natural collagen: staining with LABA-HRP conjugate. ×200

Picture taken by the authors

damaged tissues, apparently representing macrophages accumulating in inflammatory affected post traumatic tissues (Fig. 2A). Other cell populations, in particular, osteogenic cells, numerous fibroblasts and migratory cells, did not show LABA reactivity throughout the study. In the deep zone of the regenerate, from the 1st to the 4th week after injury, there was an intensive exposure of fucose-specific determinants in the matrix of hyaline cartilage and on the surface of chondrocytes between the islands of cartilagenous osteogenesis (Fig. 2B).

Beginning from the 4th week of experiment and until its completion, LABA lectin did not expose noticeable re-

activity, though endothelium and subendothelial layer of small arteries, as well as the basement membrane of the gingival epithelium were faintly positive.

Treatment of preparations with lectin CNFA (specificity DGalNAc) revealed heterogeneity of binding to carbohydrate determinants of individual structures. In the period from 1st to 5th weeks after injury, considerable CNFA binding was identified between chondroblasts and isogenic groups in the foci of cartilage osteogenesis in the deep zone of the regenerate (Fig. 3A). Also lectin CNFA demonstrated rather selective binding with cellular elements of

periodontal ligament (2nd-3rd weeks), as well as with hemocapillary bed (5th-8th weeks). Additionally CNFA labeled cells of the fibroblastic series located in the periodontal ligament and gums. It is noteworthy that CNFA, unlike other used lectins, selectively labelled carbohydrate determinants of bone trabeculae endosteum, this notion becoming especially evident in the latest stages of experiment (8-12 weeks) (Fig. 3B).

While studying lectin reactivity of lower jaw decalcified sections with injury in the form of bone defect and its subsequent replacement with osteoplastic material OCP-N, a number of changes associated with both the development of injury consequences the appearance of signs of reparative regeneration were found. In particular, while using WGA it was documented variable intensity of binding to carbohydrate determinants of the structural components of bone tissue in the area of surgical intervention. The affected sites included alveolar processes, periodontium and gums. WGA demonstrated strong reactivity with glycoconjugates of the microcirculatory bed endothelium, cellular elements of the dental ligament close to the apex of the tooth root. Such picture was typical for the 14th and 21st days of observation, while later (56th and 84th days), WGA reactivity decreased. At the same time, a slightly positive reaction was detected with the individual fragments of microvascular bed basement membranes of small blood vessels. Lectin reactivity of cellular elements of the studied structures did not expose significant changes. In the composition of the bone regenerate 56th days post OCP-N material implantation, a rather uniform distribution of WGA reactive glycoconjugates between newly formed trabeculae was observed (Fig. 4A).

While using LABA lectin after osteoplasty with OCP-N material, in contrast to the control group and intact animals, noticeable reactivity with cellular elements was documented mainly in the early stages of experiment (from 1st day to the 4th week). Since it is known that LABA lectin is a specific marker of rat macrophages, we relay this observation to the presence of large number of macrophages in the focus of the inflammatory process, which was naturally caused by bone-destructive trauma. When analyzing the newly formed trabeculae of the bone regenerate in the period from 4th till the 8th week of the experiment, uniform distribution of fucosoglycans was detected both on the periphery of the osteoregenerate and in its deep zone (Fig. 4B).

While labeling histological sections of the first experimental group animals with CNFA, this lectin binding unexpectedly was observed with carbohydrate determinants of damaged structures already on the first day of

experiment. Similar pattern was observed in animals of control group. Regarding further terms of observation in animals whose bone defect was filled with OCP-N, on the 5th-8th weeks CNFA lectin selectively labeled surface of single cells in the periodontal ligament and within the walls of hemocapillaries. Similar pattern was inherent at the end of the experiment; however, the reactivity of the endothelium of the microcirculatory bed was comparatively higher in comparison with animals of control group (Fig. 5A). This observation can be related to enhanced vasculogenesis intensity in the area of defect filled with OCP-N material. It is noteworthy that CNFA lectin exposed rather selective binding to carbohydrate residues of the structural components of the endosteum enveloping bone trabeculae, which was most clearly manifested at the end of the observation. At the same time, we did not observe similar CNFA reactivity in the animals of the control group and in intact rabbits.

Results of lectin histochemistry investigation of Col-C treated animal series revealed significant differences in the intensity of lectins binding to carbohydrate determinants of structural components of the alveolar processes of lower jaw, periodontium, and gums. Namely, intensive exposure of WGA receptor sites was observed in the early (2-3 weeks) stages of experiment by the microcirculatory bed endothelium, single cells of the periodontal ligament, and cells of the fibroblastic origin of the gingival plate. Within 1-3 weeks after implantation in the regenerate, the highest intensity of WGA binding was observed on the periphery of the islands of cartilage osteogenesis (Fig. 5B).

In later stages of the experiment (5-12 weeks), WGA lectin showed certain differences of binding. In particular, vascular endothelium was predominantly areactive, though single loci of WGA-specific carbohydrate determinants were still observed in the small blood vessels basement membranes. This lectin reactivity of cellular elements did not tend to change significantly in both the experimental and control groups of animals.

While studying LABA lectin reactivity after osteoplasty using the Col-C material, mostly single cellular elements were detected in the slides under investigation. At the end of the first day of experiment on the background of bone-destroying trauma, clusters of LABA-positive cells were observed in the closely adjacent tissue. To our current thinking these were macrophages, involved by an acute inflammatory process. At the same time, other cell populations, such as osteogenic cells and fibroblast were LABA-areactive throughout the entire investigation. The total count of macrophages in the dynamics of the experiment tended to decrease, which apparently can be due to the slowing down of macrophage migration

into the focus of inflammation, or due to masking or loss of the corresponding carbohydrate determinants. In the composition of bone regenerate in the first three weeks of the experiment, the highest content of LABA receptor sites were observed on the periphery of the islands of cartilage osteogenesis (Fig. 6A). Starting from the 4th week of experiment and until its completion, the LABA lectin did not show noticeable reactivity and the results obtained did not differ from the studied objects of the control group. However, it should be noted that LABA lectin exposed faint reactivity with endothelium and subendothelial layer of small arteries, as well as with the basement membrane of gingival epithelium.

Treatment of tissue sections of rabbits from Col-C series with CNFA lectin demonstrated certain mosaicism of its binding to carbohydrate determinants of individual structures. Namely this lectin unexpectedly bound to the destroyed material (detritus) of tissues one day after the application of the Col-C material. In the dynamics of the experiment, CNFA exposed certain selectivity of binding towards cellular elements of the periodontal ligament (2-3 weeks), hemocapillary wall (5-8 weeks). Other CNFA-positive cellular elements included primarily cells of the fibroblastic series in the periodontal ligament and gums in both the experimental and control groups of the study. It should also be noted that the CNFA lectin, unlike the others studied by us, selectively conjugated with carbohydrate residues of the endosteum of bone trabeculae, which was most clearly manifested in the late stages of the experiment (8-12 weeks) (Fig. 6B), while the endosteum of bone trabeculae in intact animals was completely unreactive.

DISCUSSION

Bone grafting is an integral part of modern dental practice, used in periodontal surgery, implantology, sinus lifting and alveolar augmentation [16, 17]. As a promising alternative to auto- and allotransplantation, in recent years, xenogenic materials have attracted increasing attention, which, due to their properties, promote bone tissue regeneration and are advantageously distinguished by a less invasive procedure for material collection [18, 19]. Wide availability, provided by the possibility of mass production, is a significant advantage of xenogenic materials, which meets the growing demand for bone reconstruction. Due to this, experimental and clinical studies aimed at the development and implementation of various types of xenogenic materials, especially those based on calcium phosphate, have been intensified [20].

According to the obtained data, these materials, promoting the penetration and growth of bone tissue

into the framework, are effective in the treatment of small and medium bone defects, sinus lifting and alveolar appendages augmentation [21-23]. However, their use in large defects is limited by the lack of osteoinductive properties [24]. It should be noted that the use of collagen in bone regeneration has made a remarkable step forward [25]. Improving methods of production and purification of modern collagen preparations ensures the preservation of their natural structure and minimizes the risk of immune reactions. Further modification of collagen, which increases its strength and resistance to cleavage, opens up new prospects for its use for medical purposes [11,12].

Natural collagen preparations are a resorbable material that is a natural matrix for the formation of new osteogenic cells. This substance is widely used for post-extraction preservation of the alveolar process and promotes the formation of a blood clot with its subsequent reorganization into bone tissue [25,26].

To clarify a number of morphogenetic events that accompany the osteoregenerative process under the conditions of osteotropic materials, our work used the lectin histochemical method, which occupies an important place among modern methods of morphological analysis, since the terminal carbohydrate residues of glycopolymers, which are lectin receptors, form the so-called «glycocode» of a living organism, provide mutual recognition and various forms of interaction of cells with their microenvironment [27].

Results of de Sousa GF et al. [28], who were studying the role of plant lectins in cellular and molecular processes of skin wound repair, have demonstrated that lectins are good candidates for the role of healing agents. Cell surfaces are rich in glycoproteins (glycosidic receptors) that can potentially interact with lectins. This lectin-cell interaction is the molecular basis for triggering various changes in biological organisms, including healing mechanisms. A review of relevant studies indicates the presence of promising potential of plant lectins for wound healing and tissue regeneration processes. Also, when studying bone tissue contacts with a titanium implant using the lectin histochemistry method, similar results of lectin binding to structural components of bone tissue were obtained by Violin K et al. [29]. Lectin-histochemical studies of the structural components of bone and periodontal tissues allowed us to establish the features of the distribution of their carbohydrate determinants, both in normal conditions and under the experimental use of osteoplastic materials replacement.

Regarding the WGA lectin, the most indicative was the fact of its binding to carbohydrate determinants of histological structures directly adjacent to the

site of injection of OCP-N plastic material. This concerned, first of all, the endothelium of microvessels. The detected enhanced expression of vascular endothelium glycoconjugates may be evidence of active transendothelial transport, which, as is known, is one of the links in the activation of regenerative processes. In the case of using the Col-C plastic material, the expression of endothelial glycoconjugates was less intensive, which may indicate a lower level of regenerative processes. Regarding the lectin reactivity of individual fragments of microvascular basement membrane, which was detected by WGA, we found a rather noticeable expression of carbohydrate residues in experimental groups of animals. This fact may also indicate intensive transcytosis, and, therefore, an increased level of tissue metabolism [30].

While using lectin LABA, it was documented intense labeling of macrophages in experimental group animals post OCP-N plastic material administration. Such expression indicates an increase in the signaling function of macrophages and activation of their phagocytic activity. In addition, it can be argued that such macrophages create a cleared field for the implementation of future regenerative processes on it. Therefore, it can be assumed that the used plastic material has, at the same time, a stimulating effect on the positive course of restoration of the histoarchitectonics of damaged structures. In addition, confirmation of the acceleration of tissue restoration is also a decrease in the expression of macrophage receptors and a decrease in their content at the final stages of the study. The same lectin in the conditions of natural collagen application was less informative, its reactivity was barely noticeable and sporadic and concerned only the endothelium and subendothelial layer of small arteries, the basement membrane of the gingival epithelium. Regarding the mentioned cells of macrophage nature, the dynamics of the experiment showed that the content of such cells tended to decrease, which is probably due to the slowing down of the migration of macrophages into the focus of inflammation, primarily, or the masking or loss of the corresponding carbohydrate determinants.

In our opinion, the information we obtained when using the lectin CNFA as a carbohydrate-specific

marker is valuable. Although, to date, its affinity to various carbohydrate determinants has not been fully elucidated, we have established a certain informativeness. This, first of all, concerns the active binding of CNFA to destroyed structures in the area of bone-destroying injury at the end of the first day of the experiment. In the group of animals where the material OCP-N was used, the most indicative was the fact of enhanced expression of glycoconjugates of the endosteum of the bone trabeculae of the jaw in the late stages of the experiment, starting from the 8th week until its completion. The obtained result can obviously be interpreted as the restoration and activation of the stromal component of the red bone marrow and, as a consequence, the enhancement of hematopoietic function. In the remaining groups of animals studied, the informativeness of CNFA was insignificant or absent, which does not allow us to state the above.

Another interesting fact that was established by the lectin CNFA is its conjugation with the determinants of fibroblast cells in the periodontal ligament. We registered this phenomenon in all groups of animals. Obviously, the obtained data indicate the activation of the synthetic function of fibroblasts aimed at enhanced synthesis of intercellular substance, and therefore, the restoration of the periodontal structure of the teeth in the area of bone replacement surgery. Thus, the use of these lectins allows us to assert that they are an auxiliary marker in the study of the course of regenerative processes after bone-destroying trauma and the use of osteoplastic materials to eliminate it. The results obtained by us give reason to recommend the used lectins in such studies.

CONCLUSIONS

The peroxidase-labeled lectins we used demonstrated a sufficiently high variability in the intensity of binding to carbohydrate determinants of the structural components of the bone tissue of the alveolar processes of the mandible and are an auxiliary marker in the study of the course of regenerative processes after osteodestructive trauma and the use of osteoplastic materials to eliminate it.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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Effectiveness of an educational program on nurses' knowledge toward massage therapy of infant with colic pain

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ABSTRACT

Aim: This study aims at evaluating the effect of a structured instructional program on improving nurses' knowledge regarding the use of massage therapy as a non-drug approach in managing colic-related discomfort in infants.

Materials and Methods: A quasi-experimental study was performed. Work was done at Al-Hussein Teaching Hospital, which works under the Directorate of Health in Diwaniyah. All nurses who participated in the study $n=72$ were divided into two groups: an experimental group consisting of 36 nurses who received the educational intervention and a control group consisting of the remaining 36 nurses who did not receive any intervention. The researcher created an inclusive training program that emphasized on increasing the knowledge of nurses about the nature, causes, and symptoms of infant colic, normal crying versus colic episodes, diagnosis of colic, pain physiology, mechanism of massage therapy, practical advice for dealing with colicky infants, and both pharmacological and non-pharmacological treatment options.

Results: The findings improved the knowledge of nurses in the intervention group about infant massage therapy in a statistically significant way following the program. This group attained a higher average score than the control group 1.78 vs. 1.34 with standard deviations of 0.09 and 0.14, respectively; $p=0.000$, effect difference was meaningful.

Conclusions: This study identified the real improving effect of educational intervention by demonstrating a significant enhancement in the knowledge and practices of nurses after they underwent the training program. Nurses with high education responded more positively to the program.

KEY WORDS: knowledge, colic pain, massage therapy, educational program

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INTRODUCTION

Infant colic pain is a very popular physiologic digestive disorder that normally disturbs babies in their first months of life. The major symptoms of it include frequent and long periods of crying, fussiness, or irritability of the baby without any obvious cause and not easily comforted or managed by caregivers [1]. Colic episodes refer to otherwise healthy babies less than three months old with uncontrollable crying for at least three hours per day on at least three days per week for three weeks in a month [2]. The word 'excessive crying' is used interchangeably with infant colic and thus represents this most typical behavioral problem of early infancy characterized by persistent crying spells [3]. Management strategies for colicky pain are usually classified into the following: drug therapies (dicyclomine hydrochloride, cafetorium bromide, and simethicone), probiotics, herbal preparations and sucrose as complementary remedies, manual techniques like acupuncture, dietary modifications, and behavioral interventions directed

at parents [2-4]. Non-drug treatments for colic include tactile techniques which initiate reflex pathways in the body and serve to achieve physical as well as emotional relaxation. Such methods have evolved potential in the reduction of discomfort with positive effects on vital signs—heart rate and oxygen saturation of infants [5]. Massage therapy means manipulation of soft tissues by hands in a manner that promotes health and emotional well-being. It is an old practice with a keen interest in its development for thousands of years. Records indicate that massage was integrated into the healing systems of ancient civilizations as early as 2000 BC and this includes the Chinese, Egyptians, Greeks, Hindus, Japanese, and Romans; it was then considered to be a therapeutic practice [6]. Present-day massage therapy falls under complementary and alternative treatment and has surfaced as popular within the trend toward holistic health care. Infant abdominal massage can be seen as one complementary intervention whereby healthy development of both physical and emotional

systems in infants is plausible through the comforting tactile stimulation provided [3]. Massage for infants is the methodical use of touch on the skin of a baby. This practice is started in many cultures soon after the birth of a child and it varies around the globe with different aspects like duration, pressure, oils, and parents' involvement. NICUs are generally places associated with high stress and low physical contact; infant massage has been introduced there as a supportive therapy. It has been better to result in improved weight gain, shorter stays in hospitals, and fewer postnatal health issues [7]. In spite that the evidence on massage therapy for colicky infants is promising, much aspects remain unknown. Specifically, large-scale studies have yet to focus on the long-term effects of infant massage as well as the role of related nursing education programs [8]. Further research is needed to develop standard protocols for clinical use of the therapy and assess the impact of an educational program on a nurse's practice and patient outcomes.

AIM

This study was designed to investigate the impact of a structured educational program on improving nurses' knowledge about the use of massage therapy as a non-pharmacological therapy for infants suffering from colic pain.

MATERIALS AND METHODS

STUDY DESIGN AND PROGRAM IMPLEMENTATION

The study followed a quasi-experimental design, implementing an educational program to assess its effect on improving nurses' knowledge about infant massage therapy for colic pain. It was carried out from 1/3/2024 to 10/5/2025 at the Al-Hussein Teaching Hospital in Diwaniyah, Iraq. A purposive sample of 72 nurses was recruited based on predetermined inclusion criteria, having at least one year of experience in the pediatric ward and a pre-test knowledge score below 60%. Participants were equally divided into two groups; study group $n=36$ and control group $n=36$, data Collection and Instrumentation.

DATA COLLECTION AND INSTRUMENTATION

The data were collected through a questionnaire by self-administration. The questionnaire comprised two parts: Demographic data (education level, years of experience, gender, age, etc.) and Knowledge assessment,

developed to assess the nurses' knowledge of infantile colic and massage interventions. The knowledge section was formatted into four domains: Domain 1: Knowledge about infant colic pain (10 MCQs) Domain 2: Symptoms of colic pain (10 questions) Domain 3: Warning signs and red flags (10 questions) Domain 4: Massage techniques for Colic Relief (10 questions) A study was done with eight pediatric nurses to see if the questions were clear, useful, and took an appropriate amount of time. These participants did not take part in the main study. This pilot helped us confirm face and content validity for the tool, and based on it, we made revisions.

EDUCATIONAL PROGRAM STRUCTURE

The educational program is four structured theoretical lectures given in two weeks. The content covers: Causes and signs of infantile colic, Ways to manage without using medicine Evidence-based massage techniques for the relief of symptoms, Nursing' roles in educating and encouraging caregivers. Pre-intervention (pre-test) assessments were carried out before implementation. After the program, a post-intervention (post-test) assessment was done with the same tool to measure knowledge gains.

SCORING AND KNOWLEDGE LEVELS

Each multiple-choice question was scored as follows: Correct response: 2 points incorrect response: 1 point Mean scores (MS) were used to categorize knowledge levels: Poor: 1.00–1.33 MS Moderate: 1.34–1.67 MS Participants were given 45 to 60 minutes for questionnaire completion. Expert review proved adequate content validity CVI=0.88, and reliability was supported by the pilot study.

LIMITATIONS

Major weaknesses of the study are the relatively small sample size and the fact that most participants were diploma nurses, which limits generalizability. Further studies with more varied and bigger samples should be undertaken to validate these findings and bring new insights.

ETHICAL CONSIDERATION

Before collecting data, the basic ethical requirement was met by making sure the participants' rights, dignity, and personal values were protected. The study got approval from the Scientific Research Ethics Committee at the

Table 1. General Characteristics of study and control groups

Demographic data		Control Group		Study Group		χ ² P value
		Freq. (N=36)	Percent.	Freq. (N=36)	Percent.	
Age / Years	24-32	23	63.9	23	63.9	2.48
	33-41	5	13.9	9	25.0	0.28
	42-50	8	22.2	4	11.1	NS
Gender	Male	11	30.6	8	22.2	0.64
	Female	25	69.4	28	77.8	0.42 NS
Educational Status	School of Nursing	9	25.0	6	16.7	3.87 0.27 NS
	Institute of Nursing	15	41.7	18	50.0	
	College of Nursing	10	27.8	6	16.7	
	Postgraduate	2	5.6	6	16.7	
Years of Experience	< 10	22	61.1	22	61.1	1.47
	10-20	8	22.2	11	30.6	0.47
	> 20	6	16.7	3	8.3	NS
Years of experience in the pediatric ward	1-6	23	63.9	26	72.2	1.33
	7-12	10	27.8	6	16.7	0.51
	≥ 13	3	8.3	4	11.1	NS
No. of Training Sessions	0	7	19.4	9	25.0	3.0
	1-4	24	66.7	26	72.2	0.22
	≥ 5	5	13.9	1	2.8	NS

NS: Non-Significant at P>0.05

Source: Own material

Faculty of Nursing, University of Kufa (135# in 27\2\2024). Also, informed consent was obtained from all nurses who took part in the research before their participation.

STATISTICAL ANALYSIS

SPSS Version 26.0 (IBM Corp., SPSS Inc.) was utilized for statistical analysis of the current data. The normality of scale measurements was assessed by the Kolmogorov-Smirnov test. Variables that exhibited normal distribution were reported in mean ± standard deviation and analyzed by paired t-test to compare pre- and post-assessments. Significance was set at p<0.05.

RESULTS

Table (1) shows descriptive statistics on demographic characteristics for both the study and control groups. Variables include age, sex, level of education, overall work experience, years in pediatric wards, and attended training sessions. Results from a chi-square test indicate no significant differences between the two groups with respect to all measured variables P>0.05. Age distribution is fairly consistent; most respondents fall into the 24-32 age bracket 63.9%. The female percentage

is higher in both groups—69.4% in the control group and 77.8% in the study group. More than half of them have a diploma from a nursing institute (41.7% in the control group and 50.0 %in the study group). Most nurses indicated that they have practiced for less than ten years and that their experience specific to pediatrics most commonly fell within one to six years. Most of the participants attended between two to four training sessions. The non-significant P-values confirm the demographic comparability between both groups that would inherently minimize bias in later comparisons.

Table 2 presents the mean scores of nurses’ baseline knowledge of massage therapy as a non-pharmacological intervention in managing pain due to infant colic, comparing the study and control groups at pre-test. The study group’s score was 1.32(SD=0.12), and the control group’s was 1.33(SD=0.13). An independent samples t-test value of 0.32 with 78 degrees of freedom; P-value = 0.75, indicates no statistically significant difference between the two groups at the levels of knowledge before intervention, emphasizing further educational programs on massage therapy for infant colic.

The differences in the mean scores of nurses’ knowledge regarding massage therapy for infants with colic pain in the control group, pretest and post-test, are illustrated

Table 2. Paired t test for the overall nurses' knowledge about massage therapy of infant with colic pain between study and control groups at pre-test assessment

	Pre-Test Comparison	Mean	SD	Independent T-Test	df	P-value
Overall Knowledge	Study	1.32	0.12	0.32	78	0.75 NS
	Control	1.33	0.13			

SD: standard deviation, df: degree of freedom, NS: Non-Significant at $P > 0.05$

Source: Own material

Table 3. Paired t test for the overall nurses' knowledge about massage therapy of infant with colic pain between (pre-test and post-test) assessments for the control group

	Control Group Tests	Mean	SD	Paired T-Test	df	P-value
Overall Knowledge	Pre-test	1.33	0.13	0.47	39	0.64 NS
	Post-test	1.34	0.14			

SD: standard deviation, df: degree of freedom, NS: Non-Significant at $P > 0.05$

Source: Own material

in table 3. From pre-test to post-test, there was a slight increase in knowledge (Mean for Pre-test=1.33, SD=0.13; Mean for Post-test=1.34, SD=0.14). The paired t-test value of 0.47 with 39 degrees of freedom indicates no statistically significant difference $P=0.64$, which confirms non-significance (NS) at $P > 0.05$. These findings imply that unlike the study group, the control group did not attain significant knowledge gains, thereby underlining the effectiveness of the intervention applied to the study group.

The (pre-test and post-test) comparisons for nurses' knowledge (study group) about massage therapy on infants with colic pain deduced that there was a highly significant increase in knowledge, moving from the pre-test (Mean = 1.32, SD = 0.13) to the post-test (Mean = 1.78, SD = 0.09). The paired t-test value of 25.82 with 39 degrees of freedom denotes a statistically significant improvement ($P = 0.000$), indicating high significance (HS) at $P < 0.01$. This confirms that the training intervention impacted positively on increasing nurses' knowledge about massage therapy for infant colic pain (Table 4).

The differences in mean scores of overall nurses' knowledge about massage therapy for infants with colic pain between the study and control groups at post-test is illustrated in table 5. The study group showed a greater improvement, with a mean score of 1.78(SD=0.09), compared to the control group's mean score of 1.34(SD=0.14). The value of t computed was 16.33 with a degree of freedom (df) of 78 and a P-value of 0.000 which proves that a difference, what-so-ever, is highly significant $P < 0.01$. These outcomes demonstrate that the educational intervention successfully improved nurses' knowledge in the study group, whereas the control group experienced only slight changes.

The regression model was statistically significant $F(6, 65)=3.66$, $p=0.003$; it explained about 27.3% of the variance in nurses' post-test knowledge scores with an

adjusted R^2 of 0.198, which is the explanatory power in a moderate degree after considering the number of predictors. Of the demographic variables that were tested, educational status was a significant predictor; this implies that nurses who had higher academic qualifications performed better in terms of what they learned from the intervention. In this study, other factors such as training, years of experience, gender, age did not make a significant contribution to predicting knowledge levels; thus formal education proves to be most important in evidence-based nursing practice (Table 6).

DISCUSSION

This demographic and social research sample comprises 72 male and female nurses from Al-Hussein Children's Hospital in Diwaniyah, between the ages of 24 and 50 years. Thirty-six nurses constitute the experimental group, while an equal number makes up the nurses' group with no intervention. The chi-square χ^2 test indicates that there are no statistically significant differences $P > 0.05$ between both groups; therefore, statistically, no significant difference existed between the study and nurses' group with no interventions. This concurs with earlier findings [8-9], which indicated non-significant differences in demographic data between groups at a p-value greater than 0.05. The researcher would attribute this finding to the fact that all participants were drawn from the same hospital and worked within the same units, hence applying to variables such as age, gender, educational status, years of experience, pediatric ward experience, and number of training sessions. According to the age groups, therefore, the greater percentage of both groups of participants fell within the 24-32 range; 63.9% in both control and study groups. This finding is consistent with other studies [8, 10] where they reported a similar age distribution. Results may indicate that

Table 4. Paired t test for the overall nurses' knowledge about massage therapy of infant with colic pain between (pre-test and post-test) assessments for the study group

	Study Group Tests	Mean	SD	Paired T-Test	df	P-value
Overall Knowledge	Pre-test	1.32	0.13	25.82	39	0.000 HS
	Post-test	1.78	0.09			

SD: standard deviation, df: degree of freedom, HS: high significance at $P < 0.01$

Source: Own material

Table 5. Paired t test for the overall nurses' knowledge about massage therapy of infant with colic pain between study and control groups at post-test assessment

	Post -Test Comparison	Mean	SD	Independent T-Test	df	P-value
Overall Knowledge	Study	1.78	0.09	16.33	78	0.000 HS
	Control	1.34	0.14			

SD: standard deviation, df: degree of freedom, HS: high significance at $P < 0.01$

Source: Own material

Table 6. Multiple linear regression analysis predicting nurses' post-test knowledge scores from demographic variables

Variable	B	SE B	β	t	p-value
Age (years)	-0.003	0.007	-0.056	-0.421	0.675
Gender (Male = 1, Female = 0)	0.012	0.034	0.045	0.353	0.725
Educational Status (ref = secondary)	0.094	0.031	0.342	3.032	0.004
Years of Experience	0.006	0.009	0.073	0.667	0.507
Pediatric Ward Experience (years)	0.008	0.010	0.083	0.800	0.427
No. of Training Sessions	0.019	0.014	0.123	1.379	0.173

B: The unstandardized coefficient, SE B: The standard error of that coefficient, β : (beta) coefficient

Source: Own material

younger nurses have more desire to participate in educational courses to gain more experience than older nurses who may rely more on their accumulated knowledge. In addition children's units require high degree of activities skill performance speed and concentration which is found more among youngsters or newly appointed nurses. Females formed the majority in both groups. In the nurses' group with no intervention, they were 69.4%, and in the study group, 77.8%. This finding is consistent with previous studies [11-12], where they also found a high female representation among the nursing staff. Concerning education, more than half of the participants graduated from nursing institutes - 41.7% in the nurses' group with no intervention and 50.0% in the study group - which confirms previous findings [13] that a considerable portion of nursing staff had similar educational backgrounds. In those terms, most of the participants were in fact individuals who had less than 10 years of work history, which has been previously reported in other studies. Related to training sessions, it appears that most of the participants attended 2 to 4 sessions-72.2% in the study group and 66.7% in the control group which is also previously reported. Nurses' participation in training sessions was reported at a rate of 84% in the study group and 68% in the nurses' group with no intervention. The results indicate a very significant increase

in the knowledge of nurses regarding massage therapy for infants with colic pain in the study group. The mean pre-test score was 1.32(SD=0.13), and it raised to 1.78(SD=0.09) in the post-test. The paired t-test value of 25.82 at 39 degrees of freedom, $P=0.000$, is an indication of extremely statistically significant improvement; that is, at the level $P < 0.01$. In the nurses' group with no intervention, knowledge increased slightly from a pre-test mean of 1.33(SD=0.13) to a post-test mean of 1.34(SD=0.14). The paired t-test value was 0.47 at $df=39$ with $P=0.64$ which is non-significant. During the pre-test, both groups had almost equal levels of knowledge (mean of study group=1.32, SD=0.12; mean of nurses' group with no intervention=1.33, SD=0.13). The value of the independent t-test was 0.32 ($df=78$, $P=0.75$), indicating no significant difference $P > 0.05$, however the posttest comparisons showed a very marked improvement in the study group as compared to the nurses' group with no intervention (mean=1.78, SD=0.09 vs. mean=1.34, SD=0.14). The t-value was 16.33 with $df=78$ and $P=0.000$ which confirms a highly significant difference $P < 0.01$. This therefore implies that the educational program had a strong positive impact on knowledge mean of scores. This is supported by studies that showed similar results in their experimental groups after educational interventions [10, 13, 16]. For example, the study indicated that the experimental

group's mean post-test score was increased significantly more than that of the nurses' group with no intervention [16]. Another study showed statistically significant differences in nurses' knowledge before and after an educational intervention [13]. Similarly, improved patient knowledge was reported following a lifestyle education program [17]. The researcher is of the opinion that the educational program helped in upgrading the nurses' knowledge by providing them with a theoretical and practical foundation, which in turn enhanced their skills, built confidence, created awareness regarding non-pharmacological interventions, and encouraged continuous learning and engagement. The findings suggest that educational programs for nurses on therapeutic infant massage will positively change their knowledge and practice towards better outcomes for infants. This is also the case in previous studies [6, 8, 18] that proved effective behavioral methods such as massage compared to other methods in relieving colic in infants. The present study indicated that formal education is most paramount to evidence-based nursing practice. Research on pain management in pediatric nursing shows that educational interventions can successfully fill the knowledge gaps. Therefore, the tremendous increase in the use of non-pharmacological strategies of pain relief after the intervention proves that education plays a key role in improving nursing practices in emergency and intensive care

units [19-20]. In other words, this indicates how systemic structures need to support nurses to effectively apply newly acquired knowledge. This study further reinforces the notion that nurses with higher educational qualifications are more likely to implement learned techniques, thereby improving patient care outcomes. That systematic review on interventions aimed at multiple risk behaviors with young people further lends itself to educational status as a predictor of success intervention. It revealed well-structured educational delivered by competent educators interventions that significantly improved students' knowledge and behavior change [21]. This puts forward the fact that it is not only important that the educators have an educational background but also that effective teaching strategies elicit engagement levels. Therefore, for pediatric nurses who engage in educational interventions, these findings imply that healthier educational qualifications will translate into more effective health education for children.

CONCLUSIONS

Based on the current results, it was concluded that following the intervention, the study group demonstrated a very high key and practical improvement in the aspect of knowledge, thus verifying the effect of the training program.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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Qualitative and quantitative analysis of rat liver sinusoids under condition of a long-term experimental exposure to cannabidiol oil

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ABSTRACT

Aim: The study aimed to determine the structural organization and conduct a quantitative analysis of the cellular composition of rat liver sinusoids under condition of a long-term (10 weeks) experimental exposure to cannabidiol oil.

Materials and Methods: An experimental study was conducted on 20 white non-linear male rats weighing 180–230 g, in compliance with ethical principles. The modeling of prolonged cannabidiol oil exposure in 14 white rats was performed through daily per os administration at a dose of 10 mg/kg/ for 10 weeks, the control group was 6 sexually mature white male rats, that oral administration the CBD carrier solvent – hemp seed oil. Histological, immunohistochemical and morphometric studies of the cellular composition of liver sinusoids were performed with statistical processing. In all comparisons, the difference was considered statistically significant at a minimum significance level of $p < 0.05$.

Results: After 10 weeks exposure to CBD oil administration pathohistological changes in the rat liver sinusoids were not observed. There is a significant increase in the average number of Kupffer cells in the sinusoidal wall in all zones of the lobule $p < 0.001$, especially near the portal zone (1.58 times) compared to the control group, which can be explained by their activation in response to long-term exposure to CBD. Perisinusoidal cells in the experimental series in all areas of the liver lobule were the least compared to endothelial cells and Kupffer cells, $p < 0.001$.

Conclusions: Histological, immunohistochemical study with quantitative analysis of the cellular composition of rat liver sinusoids indicates the safety of long-term experimental exposure to CBD oil.

KEY WORDS: liver, rats, sinusoids, endothelial cells, Kupffer cells, perisinusoidal cells, CBD, safety

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INTRODUCTION

Cannabidiol (CBD) is a phytocannabinoid found in the hemp plant (*Cannabis sativa* L.) of the Cannabaceae family [1]. As a drug, CBD is approved at appropriate therapeutic doses by the US Food and Drug Administration, the European Medicines Agency, and the European Food Safety Authority [2–4] and is currently marketed as the oral drug Epidyolex in the EU and Epidiolex outside the EU for the treatment of severe childhood epilepsy (Lennox-Gastaut syndrome, Dravet syndrome) and tuberous sclerosis [5]. In addition, pre-clinical and clinical trials evaluating the efficacy of CBD as a therapeutic agent have been identified for a variety of conditions, including anxiety, pain, inflammation, various substance use disorders, post-traumatic stress disorder, sleep disorders, and others [6].

In Ukraine, CBD is a legal substance and is excluded from the list of narcotic and psychotropic substances

(CMU Resolution of May 6, 2000, No. 770, CMU Resolution of April 7, 2021, No. 324) [7].

Today, most consumers use CBD in the form of non-medicinal over-the-counter preparations, so-called CBD oils, which contain from 5% to 40% CBD extract dissolved in edible oil and are advertised with various health benefit claims [8]. In 2022, the European Medicines Agency and the European Food Safety Authority identified the risks associated with the use of CBD as a dietary supplement or food ingredient and summarized the possible adverse effects of CBD on human health. Possible side effects from the liver, gastrointestinal tract, endocrine and reproductive systems, metabolic disorders, genotoxicity, neurological and psychiatric disorders were noted. Regarding side effects from the liver, increased liver weight, hypertrophy of liver hepatocytes, increased levels of liver enzymes (ALT, AST, alkaline phosphatase), increased bilirubin, potential drug-induced liver damage were indicated [9, 10].

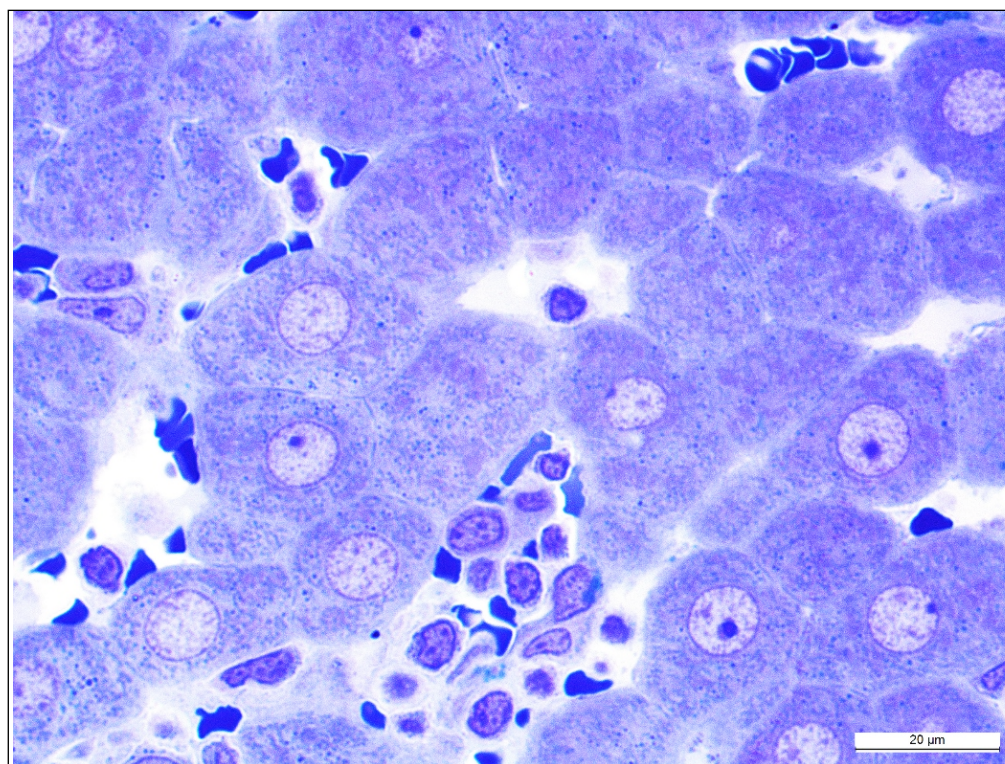


Fig. 1. Rat liver after 10 weeks of experimental use of CBD oil. Preservation of the structure and shape of hepatocytes. Individual sinusoidal capillaries are moderately dilated, full of blood. Semi-thin preparation (1 μ m), staining with methylene blue – basic fuchsin, $\times 1000$ (immersion) *Picture taken by the authors*

Thus, experimental studies of the liver are needed to establish the nature and severity of possible liver damage, including the stromal-vascular compartment, hemodynamic features and to determine the safety of CBD use.

AIM

To determine the structural organization and conduct a quantitative analysis of the cellular composition of rat liver sinusoids under condition of a long-term (10 weeks) experimental exposure to CBD oil.

MATERIALS AND METHODS

A series of experimental studies was conducted in vivarium conditions on 20 white non-linear male rats weighing 180-230 g, 5-7 months old at the beginning of the experiment after ethical approval by the Bioethics Commission at the Danylo Halytskyi Lviv National Medical University (protocol No. 7 dated August 29, 2022) in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), Council of Europe Directive 2010/63/EU, Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty to Animals" [11, 12].

The main group included 14 rats that received 10% CBD oil orally once a day at a dose of 10 mg/kg for 10 weeks. The manufacturer of the product under study is

the licensed company "Aroma Extract Labs s.r.o." (Praha, Czech Republic). To administer the dose, a laboratory single-channel pipette dispenser 20 μ l MicroPette Plus was used. The control group included 6 sexually mature white male rats, that oral administration the CBD carrier solvent – hemp seed oil. During the experimental study, the general condition of the rats was observed. At the end of the experiment, after euthanasia, material was collected for morphological study. The liver was used as the material for the study. Paraffin blocks were made from liver tissue samples according to the standard method [13]. Deparaffinized histological sections with a thickness of 5 ± 1 μ m were stained with hematoxylin-eosin, after which the general histological structure of the liver was examined. In addition, for a targeted study of the sinusoids of the liver lobules, serial semi-thin sections with a thickness of 0.5-1 μ m were made from epoxy blocks according to the generally accepted method, stained with methylene blue–basic fuchsin, and studied under a light microscope at a magnification of $\times 1000$ (immersion) [14].

We also performed immunohistochemical studies of the liver sinusoids. For this purpose, histological sections 5 ± 1 μ m thick were prepared from paraffin blocks, which were applied to highly adhesive "Super Frost" slides, and then dried according to the standard method. Unmasking was performed in citrate buffer, pH-6.0. Visualization of the IHC reaction was performed using the DAKO EnVision+System detection system with DAB chromogen (diaminobenzidine) [15]. We used

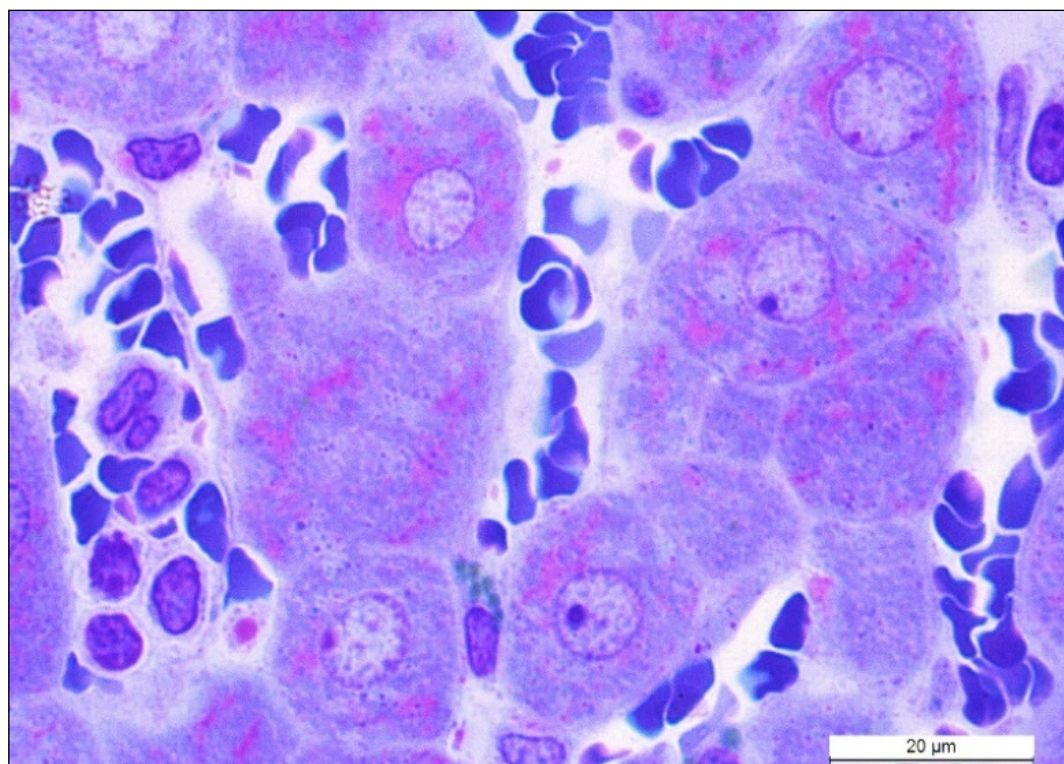


Fig. 2. Marked dilatation of sinusoids and deposition of blood in them. Semi-thin preparation (1 μm), stained with methylene blue – basic fuchsin, ×1000 (immersion)
Picture taken by the authors

monoclonal antibodies for macrophages CD68 (Clone KP1, DAKO), vascular endothelium CD31 (Clone JC70A, Thermo Fisher scientific).

The studies were performed according to the manufacturer's protocol with the necessary controls. To study histological preparations, we used a Leica DM 2500 light-optical microscope (Leica Microsystems GmbH, Germany) with a Leica DFC450 C digital camera (Germany) and licensed Leica Application Suit Version 3.8 software. For quantitative analysis, we counted the main cells of the sinusoid wall in different areas of the liver lobules (around the central vein, near the portal tracts and in the intermediate zone between the triad and the central vein) on an area of 0.01 mm² of the histological preparation (100 μm×100 μm).

Data processing was carried out by applied statistical methods used in medicine using the R Commander program (version 2.7-2, GNU General Public License) based on the Windows operating system. Data are presented as mean values: arithmetic mean with standard deviation ($M \pm SD$), the reliability of the difference between these indicators was determined by the Mann-Whitney (U) test. In all comparisons, the difference was considered statistically significant at a minimum significance level of $p < 0.05$ [16].

RESULTS

Microscopically, the liver tissue after 10 weeks of experimental use of CBD oil is represented by lobules in the

form of hexagonal prisms, the lobular and trabecular structure is not disturbed. Hepatocyte dystrophy was not observed. Sinusoids were located between the hepatocyte beams, some of them were moderately dilated and hyperemic. The structural microscopic organization of hepatic beams and sinusoids is presented in a semi-thin section (Fig. 1).

In some histoslides, sinusoidal capillaries are significantly dilated, although unevenly (Fig. 2). The diameter of single dilated and hyperemic sinusoids reached 20 μm. The median diameter of sinusoids in the variation series was 9.96 μm. The average diameter of sinusoids was 9.78 ± 0.58 μm and significantly exceeded the parameter of the control group (7.66 ± 1.00 μm) ($p < 0.001$). At the same time, the parameter of the average diameter of sinusoids is averaged and relative, since in different parts of the lobule the lumen of sinusoids is uneven.

The sinusoidal wall was lined with elongated endothelial cells with hyperchromic nuclei. Individual endothelial cells protruded into the lumen of the sinusoidal capillaries, which could be well visualized by immuno-histochemical typing using the CD31 marker (Fig. 3).

Kupffer cells, which were significantly larger than endothelial cells, were visualized in the wall of sinusoidal capillaries and partially at their bifurcations. IHC typing using the CD68 marker made it possible not only to visualize Kupffer cells in different areas of the liver lobule (Fig. 4), but also to conduct a quantitative study and compare them with the control group.

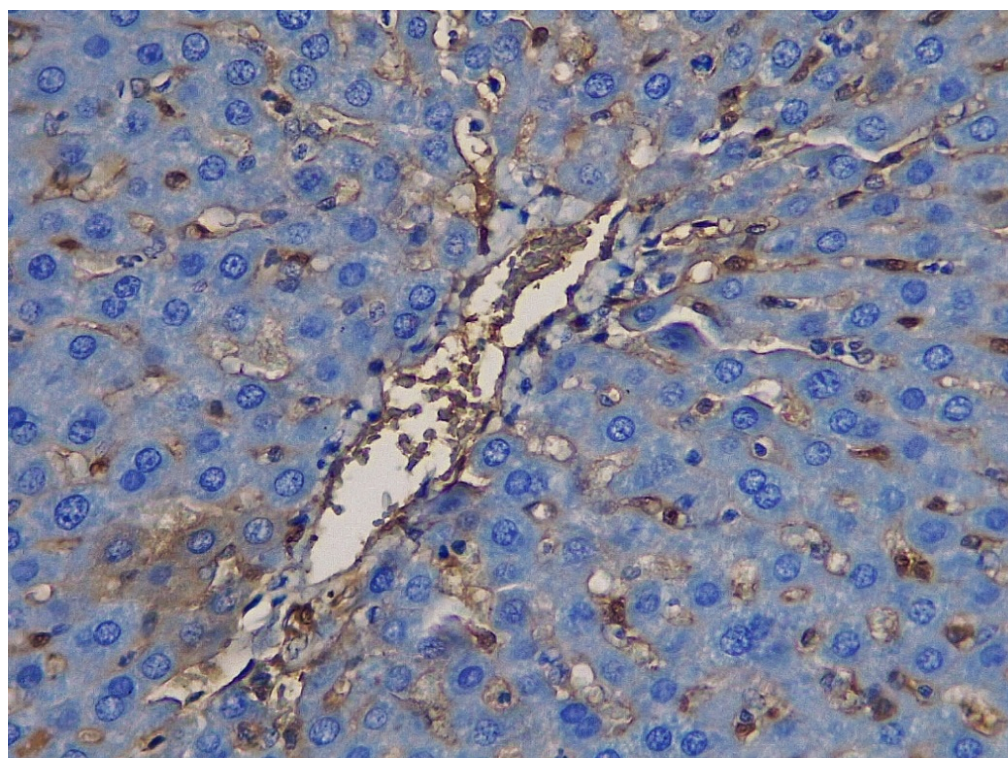


Fig. 3. Rat liver tissue after 10 weeks of experimental CBD oil administration. Representative immunohistochemistry results for CD31. CD31-positive endothelial cells of the sinusoids and central vein are stained brown. IHC typing of endothelial cells using the CD31 marker (Clone JC70A, Thermo Fisher scientific), $\times 400$
Picture taken by the authors

Ito fat-storing cells were in the perisinusoidal space of Disse in the so-called "pockets" between hepatocytes and endothelial cells, had long processes, a rounded, slightly elongated or irregular shape, a large nucleus, and lipid droplet-like inclusions, indicating their inactive state.

For quantitative analysis of the cellular composition of rat liver sinusoids under condition of a long-term (10 weeks) experimental exposure to CBD oil, we counted the main cells of the sinusoidal wall on an area of a histological preparation of 0.01 mm^2 ($100 \mu\text{m} \times 100 \mu\text{m}$) in different zones of the liver lobule, using the traditional classical concept, where the central vein is the central zone, the triads are the peripheral zone, and the intermediate zone is the interlobular. According to the Rappaport model, the liver acinus is divided into three acinar zones (1, 2, and 3) - periportal (1), intermediate (2), and pericentral (3) [17]. The quantitative characteristics of endothelial cells, Kupffer cells, and perisinusoidal cells in the classical liver lobule in the experimental series after 10 weeks of CBD exposure and in the control group are presented in Fig. 5, Fig. 6, Fig. 7.

Comparison of the average CD31 cell counts on the 0.01 mm^2 histological preparation area of the experimental CBD group at the end of week 10 ($M \pm SD$) with the control group is presented in Fig. 5. Analysis of the average endothelial cell counts on the 0.01 mm^2 area demonstrated a significant difference in different zones of the liver lobules. In the experimental group, in zone 1 of the liver lobules near the triads, the average number of endothelial cells was the lowest compared to other

zones, amounting to 4.88 ± 0.12 and significantly different from the control group (5.22 ± 0.17), $p < 0.001$. In the intermediate (midzone) zone 2, the average number of endothelial cells was 5.24 ± 0.07 , which was also lower than the control group (5.79 ± 0.18), $p < 0.001$. Around the central vein (zone 3), the average number of endothelial cells was 11.77 ± 0.56 , significantly exceeding the indicators in other zones and significantly different from the control group (13.12 ± 0.30), $p < 0.001$.

The average CD31 cell counts (in an area of 0.01 mm^2) in different areas of the liver lobule, both in the experimental series and in the control group, significantly differing from each other, which can be explained by the different average diameter of sinusoids. Thus, around the central vein, the average diameter of sinusoids is smaller than in the midzonal zone and near the triads, as a result, the density of endothelial cells in an area of 0.01 mm^2 around the central vein in the lobule was significantly higher (11.77 ± 0.56) than near the triads (4.88 ± 0.12) and in the midzonal zone (5.24 ± 0.07). The average number of CD31-positive cells around the central vein exceeded the average number near the triads by 2.41 times ($p < 0.01$). When comparing the average CD31 cell counts in an area of 0.01 mm^2 in the experimental series with the control group, it was found that in all areas of the liver lobule, the average CD31 cell count was significantly lower than the analogical parameter in the control group, which is explained by the larger diameter of the sinusoids and improved blood flow to the liver lobule under long-term exposure to CBD oil.

In the experimental series of long-term exposure to

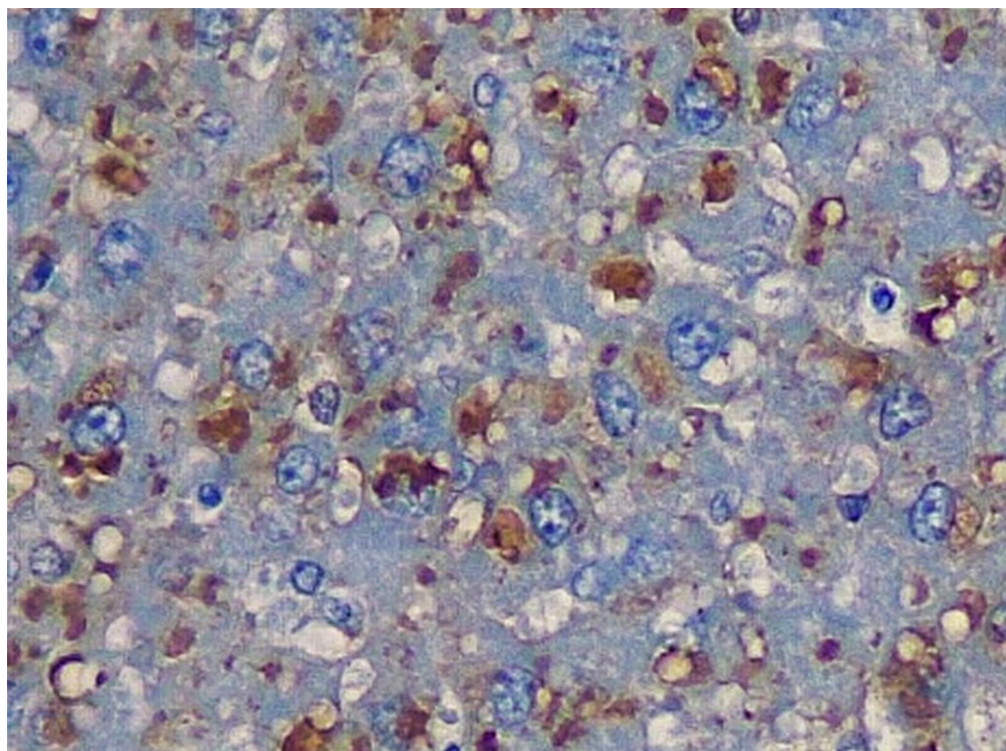


Fig. 4. Rat liver tissue after 10 weeks of experimental CBD oil administration. Representative immunohistochemistry results for CD68. Hyperplasia and hypertrophy of Kupffer cells in the sinusoids of the hepatic lobule. IHC typing of Kupffer cells using the CD68 marker (Clone KP1, DAKO), $\times 600$

Picture taken by the authors

CBD oil, the highest average number of Kupffer cells per 0.01 mm^2 area ($100 \mu\text{m} \times 100 \mu\text{m}$) was determined in the liver triad zone and was 6.12 ± 0.13 cells, and significantly ($p < 0.001$) 1.58 times higher than the analogical parameter in the control group (3.88 ± 0.10 cells). Also, the average number of Kupffer cells in the liver triad zone in a comparative analysis exceeded the parameter in the intermediate zone by 1.17 times (5.22 ± 0.06 cells) and around the central vein by 1.79 times (3.42 ± 0.13 cells) within the experimental group. The highest average levels of CD68-positive cells in the liver triad zone in both the experimental and control groups can be explained by the key role of Kupffer cells in immune regulation, protecting the liver from various harmful effects, toxins, and harmful substances that can enter the bloodstream from the intestine.

In the intermediate zone of the liver lobule of the experimental series, the average CD68 cell count was 5.22 ± 0.06 and significantly exceeded the appropriate value in the control group (2.90 ± 0.16), $p < 0.001$. In the zone of the lobule around the central vein, the average CD68 cell count was the lowest (3.42 ± 0.13) compared to other zones of the liver lobule of the experimental series but exceeded the value of the control group (1.06 ± 0.12) by 3.23 times, $p < 0.001$. As in the triad zone, in the intermediate zone of the lobule and around the central vein, the average CD68 cell counts significantly exceeded the appropriate values in the control, which is explained by hyperplasia of specialized macrophages and their important importance for the immune function of the liver (Fig. 6).

Perisinusoidal cells in the experimental series in all zones of the liver lobule were the least compared to endothelial cells and Kupffer cells. When comparing between zones, the average number of cells per 0.01 mm^2 area ($100 \mu\text{m} \times 100 \mu\text{m}$) was the highest near the triads (3.02 ± 0.08) and significantly exceeded the appropriate value in the control group (2.13 ± 0.08), $p < 0.001$. In the intermediate zone and around the central vein, the average number of perisinusoidal cells was 2.33 ± 0.09 and 1.37 ± 0.06 , respectively, and significantly differed from the control group, $p < 0.001$. In the intermediate zone of the control group, the average number was 1.31 ± 0.11 , and around the central vein – 0.67 ± 0.07 (Fig. 7).

Thus, conducted histopathological, immunohistochemical and morphometric study with quantitative analysis of the cellular composition of the liver sinusoids allowed to establish their structural microscopic organization after long-term experimental exposure to CBD oil for 10 weeks. In some fields of view, the sinusoidal capillaries are unevenly dilated. The average diameter of the sinusoids was $9.78 \pm 0.58 \mu\text{m}$ and differed from the control group ($7.66 \pm 1.00 \mu\text{m}$) ($p < 0.001$).

In rare cases, in the intermediate and pericentral zones of hepatic lobules sinusoids were dilated and hyperemic, which can be explained by increased blood flow to the liver triad and a compensatory-adaptive process under the conditions of experimental exposure to CBD. We did not observe any hemodynamic disturbances, and we did not diagnose any impaired blood outflow through the central vein. In this case, slight hyperemia was part of the normal physiological process.

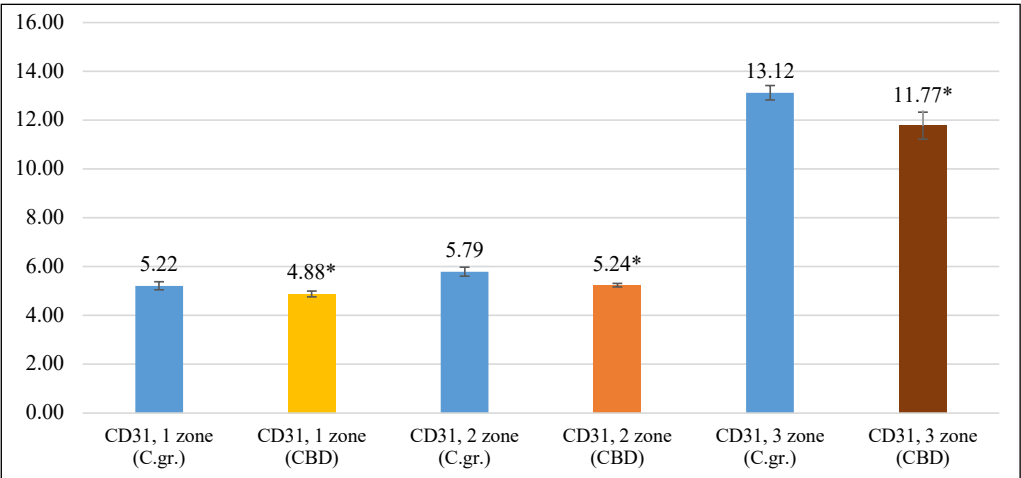


Fig. 5. Comparison of average indicator CD31 cells (in an area of 0.01 mm²) at week 10 of experimental exposure to CBD with the control group (C.gr.) (M±SD)
Note: * - significant difference (p < 0.05) with the control group
Picture taken by the authors

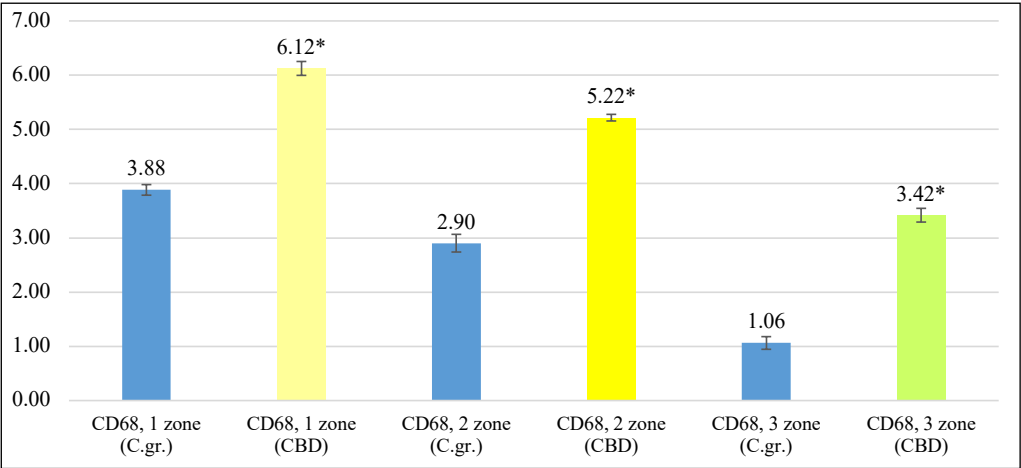


Fig. 6. Comparison of average indicator CD68 cells (in an area of 0.01 mm²) at week 10 of experimental exposure to CBD with the control group (C.gr.) (M±SD)
Note: * - significant difference (p < 0.05) with the control group
Picture taken by the authors

Comparative analysis of the average values of endothelial cells, Kupffer cells and perisinusoidal cells in different zones of the lobule on the area of the histological preparation of 0.01 mm² with long-term use of CBD oil and in the control group demonstrated that the trend of cell distribution in different zones of the lobule was preserved. In the experimental series, in the zone near the triads (zone 1), Kupffer cells prevailed, the average value of which was 6.12±0.13, there were slightly fewer endothelial cells - 4.88±0.12 per 0.01 mm² of the preparation area and 3.02±0.08 - perisinusoidal cells. When compared with the control group, there were fewer endothelial cells, which can be explained by an increase in the diameter of sinusoids. However, there were significantly more Kupffer cells than in the control group, which is associated with their proliferation in response to CBD oil. There were also more perisinusoidal cells than in the control group due to their activation and proliferation. In the area around the central vein of the lobule (zone 3), endothelial cells (11.77±0.56) predominated among all the studied cells, however, the average number of endothelial cells was significantly lower (p < 0.001) than in the control group, which can be explained by the larger average diameter of sinusoids in the experimental group. There were fewer Kupffer

cells around the central vein than in the area near the triads in the experimental series, but more than in the control group. Among the perisinusoidal cells present, there were the fewest (1.37±0.06) in the experimental series, but significantly (p < 0.001) more than in the control group (0.67±0.07). In the intermediate zone of the lobule (zone 2), among all the studied cells, the average number of endothelial cells (5.24±0.07) and Kupffer cells (5.22±0.06) did not differ significantly from each other (p > 0.05) but significantly differed from the control group (p < 0.001). Perisinusoidal cells were the least in zone 2 of the lobule (2.33±0.09) among all cells, but significantly more than in the control group (p < 0.001).

DISCUSSION

CBD is a cannabinoid with significant therapeutic potential among many non-psychoactive compounds. CBD has a broad spectrum of pharmacological actions in conditions such as pain, inflammation, epilepsy, anxiety, and others, in the treatment of various neurodegenerative diseases and neuropsychiatric conditions [18, 19]. In rodent models, CBD has also been shown to have, among others, anxiolytic, antioxidant, and

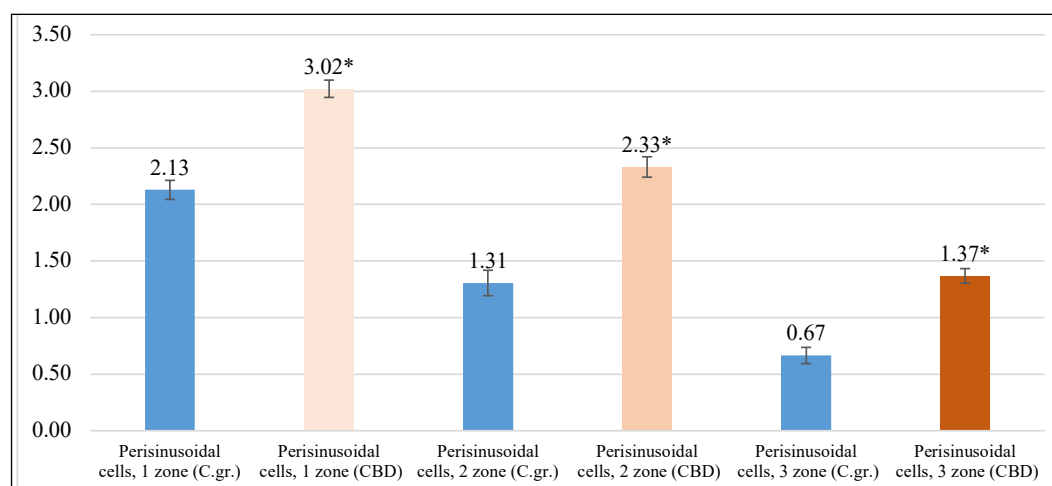


Fig. 7. Comparison of the average indicator of perisinusoidal cells (on an area of 0.01 mm²) at week 10 of experimental exposure to CBD with the control group (C.gr.) (M±SD)

Note: * - significant difference ($p < 0.05$) with the control group

Picture taken by the authors

anti-inflammatory effects [20, 21]. The potential therapeutic effects of CBD in various liver diseases have been reported in vivo and vitro models [22]. However, in addition to the results with the therapeutic effect of CBD, there are studies in both animals and humans that have found negative effects associated with the use of CBD, including changes in liver function. Henderson R.G. et al. (2023) evaluated the toxicity of a CBD extract derived from hemp administered for 14 and 90 days to male and female Sprague Dawley rats. The first series of animals were orally administered doses of 0, 30, 70, and 150 mg/kg CBD for 14 days, and the second series of rats were administered doses of 0, 50, 80, 120, and 140 mg/kg CBD for 90 days. In neither of the first or second series of studies were there any biochemical changes in the blood associated with CBD administration, including changes in AST, ALT, alkaline phosphatase, and total bilirubin. However, histopathological examination of the liver showed dose-dependent hypertrophy of hepatocytes during the 14-day study, consistent with an increase in liver weight. Similarly, liver hepatocyte hypertrophy was also diagnosed after a 90-day experimental study, however, these histological changes disappeared after a 28-day recovery period when CBD was discontinued [23]. In our study, we did not observe hepatocyte hypertrophy, nor did we observe dystrophic-necrotic changes.

Previously, Ewing L.E. et al. (2019) investigated the hepatotoxic potential of CBD in male B6C3F1 mice after varying durations of oral administration of the cannabinoid [24]. The authors noted that a total dose of 246 mg/kg CBD extract is equivalent to 20 mg/kg CBD per day, the maximum recommended human maintenance dose of CBD in Epidiolex. In an acute toxicity study, mice were given single oral doses of 0, 246, 738, or 2460 mg/kg CBD extract, equivalent to 0, 20, 60, and 200 mg CBD/kg/day, respectively, and observed for 24 hours. Clinical and biochemical analysis revealed

a modest but statistically significant ($p < 0.01$ – 0.001) dose-dependent increase in serum levels of both AST and ALT at single doses of 738 mg/kg and 2460 mg/kg. Administration of CBD at a dose of 2460 mg/kg resulted in a significant increase in total bilirubin (> 20 -fold, $p < 0.001$). In a subacute toxicity study, animals of different series were administered single doses of 61.5, 184.5 or 615 mg/kg, which are equivalent to 5, 15 and 500 mg CBD/kg/day of CBD extract, respectively, daily for 10 days. Histopathological evaluation revealed swelling of the cytoplasm of hepatocytes in mice administered 184.5 and 615 mg/kg of CBD. Clinical and biochemical analyses showed that mice treated with 615 mg/kg CBD extract had significantly elevated total bilirubin, moderately elevated ALT and AST levels, but no significant increase in alkaline phosphatase. In contrast, lower doses of CBD extract did not significantly alter these parameters. Thus, according to the authors, oral CBD may cause liver damage, especially at high doses [24].

CONCLUSIONS

Based on the conducted experimental study, it was found that long-term exposure to 10% CBD oil at a dose of 10 mg/kg/day does not lead to the development of pathomorphological changes in the liver sinusoids. Characteristic morphological manifestations were dilation and hyperemia of single sinusoids, as a compensatory-adaptive process. Destructively altered endothelial cells and Kupffer cells were not detected.

Comparative analysis of the average values of endothelial cells, Kupffer cells and perisinusoidal cells in the sinusoidal wall on an area of histological preparation of 0.01 mm² in the experimental and control groups demonstrated that the trend of cell distribution in different zones of the lobule was maintained.

There is a significant increase in the average number of Kupffer cells in the sinusoidal wall in all areas of the

lobule $p < 0.001$, especially near the portal triads (1.58 times) compared to the control group, which can be explained by their activation in response to long-term exposure to CBD. When comparing the average numbers of endothelial cells (CD31 positive cells) in an area of 0.01 mm^2 in the experimental series with the control group, it was found that in all areas of the liver lobule the average number of CD31 cells was significantly lower than the corresponding number in the control group

$p < 0.001$, which is explained by the larger diameter of the sinusoids. Perisinusoidal cells in the experimental series in all areas of the liver lobule were the least compared to endothelial cells and Kupffer cells, $p < 0.001$. Identification of perisinusoidal liver cells and study of their morphological features is an important task of pathomorphology and pathophysiology in predicting the consequences of experimental exposure to CBD oil on the liver.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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C-reactive protein and ferritin levels associated with chronic periodontitis

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ABSTRACT

Aim: This study aims to determine relationship between level of serum C-reactive protein and serum ferritin in chronic periodontitis disease.

Materials and Methods: Blood samples were collected from (125) patients of chronic periodontitis disease and (125) participants as healthy individuals, ranging in age from 25 to 60 year. serum ferritin and C-reactive protein levels were measured using enzyme-linked immunosorbent assay (ELISA) kits.

Results: The results indicate that the expression of CRP and serum ferritin level was remarkably increased in patients as in contrast to healthy control ($P < 0.001$). The results showed that hypertension, diabetic and chest pain had a significant correlation with the CRP patients in contrast with healthy individuals.

Conclusions: This study concluded that serum ferritin and C-reactive protein level can be used as a biomarker in evaluating the effectiveness of periodontitis patients.

KEY WORDS: periodontitis, CRP, serum ferritin

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INTRODUCTION

The illness of inflammation known as periodontitis is complex. An historical idea that is now coming to light claims that various systemic illnesses may be caused by periodontitis or that it may act as a modulator of those diseases. One of the explaining theories centers on the idea that mediators like interleukin-6, C-reactive protein and tumor necrosis factor spread as a result of periodontitis [1]. This type of reaction is stimulated by bacteria that its either systemically diffusion of it or its toxin or locally infection lead to inflammatory harm to periodontal tissue. Several biopsy techniques to liver or bone marrow biopsy can detected the iron stores in addition to serum ferritin level to discovered the iron load and inflammation [2]. The expression of (periodontal diseases) include a broad expansion conditions of chronic inflammatory to gingiva, bone and ligament that supporting the teeth. Periodontal disease starts from gingivitis which known as legalized gingival inflammation that is begins from bacteria in dental plaque, (pockets) occurred when gingivitis progress and without treating led to loss of gingiva, ligament

and bone which is a hallmark of chronic gingivitis that may lead to loss of tooth. periodontal disease could be participated to total body inflammation burden like atherosclerosis and diabetes mellitus [2]. Between all the possible explanations, one is centered on the idea as the consequence of the proliferation of mediators such C-reactive protein, interleukin-6, and tumor necrosis factor-alpha, periodontitis may have an effect [1]. This kind of reaction is induced by bacteria that it's may be diffused systemically itself or by its toxin or may be locally infection lead to inflammatory damage to periodontal tissue [2]. Different types of inflammatory cytokines led to secretion of CRP from liver [1]. The CRP level is rapidly increased as a replay to inflammation, infection and trauma while its decreased rapidly when these conditions are removed. So the CRP measurements is used widely to control of deferent state of inflammation [2, 3]. In spite the deficiency singular diagnostic specificity, the CRP is very useful clinically because the changes in their plasma or serum levels inverted its strength in different inflammatory process. The assessment could be help to distinguished between the inflammatory

and non-inflammatory conditions. So many of immunosuppressive and anti-inflammatory drugs are not affect directly on CRP unless it effect on disease activity [4]. Recent research has demonstrated that periodontal disease causes an increase in their levels. These papers might demonstrate variations in the development of destructive periodontal disease or severity in various study populations [5, 6]. Patients with some types of inflammatory oral illness have elevated CRP levels in their serum. Evidence suggests that as compared to the general population who are not afflicted, people with severe periodontitis have higher serum levels of CRP. However, they are insufficient to prove that the observed serum CRP levels were brought on by periodontitis because CRP levels fluctuate due to multiple complicating factors like increase blood pressure, aging, alcohol use, low level of physical activity, smoking, chronic fatigue, also Triglycerides are increased when you drink coffee, diabetes type II due to insulin resistance, use of estrogen, consuming a diet high in protein, depression and exhibiting sleep disturbances [5]. Due to mediators of inflammation, several investigations have discovered a connection among chronic periodontitis and increased CRP levels in the blood. C-reactive protein, interleukin-6, and tumor necrosis factor- α are biologically plausible to be secreted during periodontitis and have the ability to activate hepatocytes to create CRP [7]. A ferritin is a substitution dietary iron source" and stressed the critical role it will most likely play in the global control and treatment of IDA. Ferritin is an iron protein storage and mineralization carrier. Iron is kept inside the protein cage in the form of reversible hydrated iron oxide. In plants, animals, and prokaryotes iron release and regulation are maintained by ferritins (which are storage proteins). The previously documented hazardous consequence of an imbalance in iron levels in both plants and mammals, submit that cellular iron concentrations must be tightly managed to minimize cellular harm, as optimal labile iron levels [8] are important to preserved iron homeostasis. It also underlines the importance of maintaining a precise iron balance, as well as the critical control and moderating functions of iron storage. Biomarkers, either created by normal people in good health or by people suffering from particular systemic diseases, are molecules that could potentially be utilized for tracking a person's health, start of disease, responses to treatment, and prognosis. Periodontal disease oral fluid indicators can be linked to inflammation of soft tissue, bacterial products, and proteins of antibacterial. Acute phase reactive ferritin is high in inflammatory conditions, persistent infections, autoimmune disease and liver disease. It is widely known that many chronic inflammatory illnesses

including rheumatoid arthritis and multiple sclerosis are accompanied by elevated blood ferritin levels. Ferritin is crucial for the recycling and storage of iron. A non-toxic, soluble form of iron is stored in ferritin, which then discharges it gradually over time. Apoferritin is the name given to ferritin with no corresponding iron [1]. A 450 kDa soluble protein, ferritin. Although it is present in every cell of the body, it is particularly abundant in the liver, spleen, and marrow macrophages. In a secure and convenient form, it offers intracellular storage of bioavailable iron. The body's cells and tissue fluids contain ferritin in various amounts. Iron and cytokines that cause inflammation are two important factors that are capable of regulating the production of ferritin. The discharge which are indicators of systemic inflammatory reactions, is significantly increased by oral infections. CP is an inflammatory disease, and an increase in serum ferritin levels is correlated with the intensity or duration of the inflammation. The main result of a large number of studies indicates that CP patients' ferritin levels in their blood significantly decreased 1 month following non-surgical treatment for periodontal disease [8, 9]. We came to the conclusion that serum ferritin levels might have a significant role in the etiology of the disease [10]. Therefore, this study is among the first to look into variations of the level of serum ferritin between patients with periodontal disease and patients without this disease.

AIM

This study aims to determine relationship between level of serum C-reactive protein and serum ferritin in chronic periodontitis disease.

MATERIALS AND METHODS

SAMPLES COLLOCATION

The 250 patients in this study, aged 25-60 years of both genders reporting to department of periodontics, Faculty of Dentistry College, University of Kufa from March 2021 to October 2022. This study was classified into groups: Group 1 as healthy individuals (No. 125) and Group 2 as chronic periodontal patients (No. 125). Detailed medical and dental case history were performed by dentist. Plaque Index (Tureskey et al. modification of Quigley Hein Index 1970), Gingival Index (Loe and Silness 1963), Modified Sulcular Bleeding Index, Probing Pocket Depth, and CALs were recorded. All information about this study of the population's statistical characteristics, which included age, sex, socioeconomic status, smoking, hemodialysis induration. Hypertension, diabetes mellitus and chest pain disease that recorded

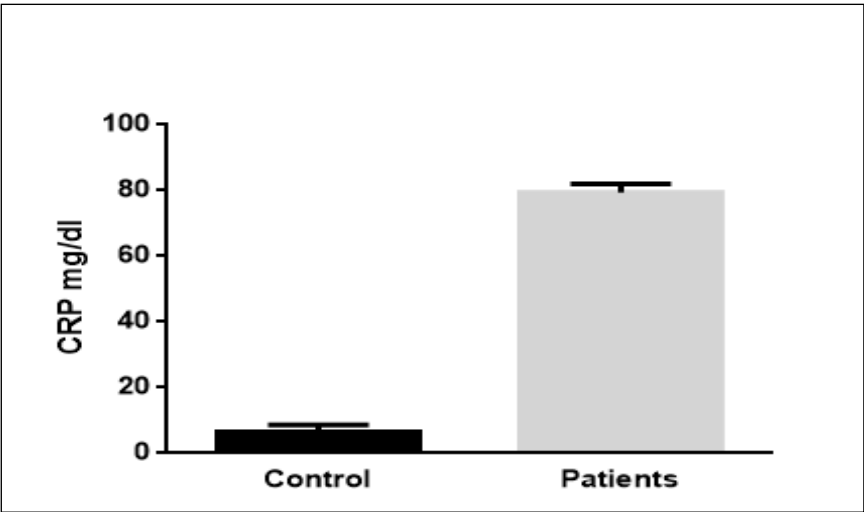


Fig. 1. CRP expression in periodontitis and gingivitis patients
The values plotted one-way ANOVA test among these groups. Non-significant is (ns), (*) is $p < 0.05$, (**) is $p < 0.01$, (***) is $p < 0.001$ and (****) $p < 0.0001$ vs Control.
Source: Own material

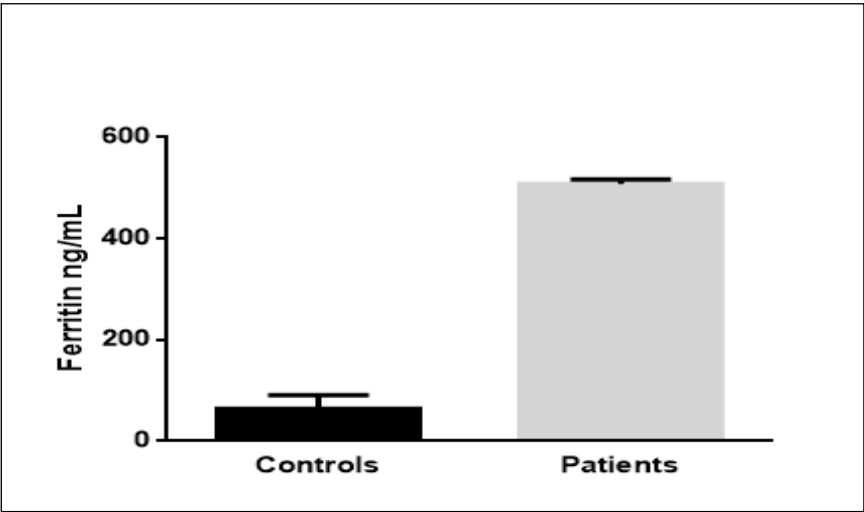


Fig. 2. Ferritin expression in periodontitis and gingivitis patients
The values plotted one-way ANOVA test between these groups. Non-significant which is (ns), (*) is $p < 0.05$, (**) is $p < 0.01$, (***) is $p < 0.001$, (****) is $p < 0.0001$ vs Control.
Source: Own material

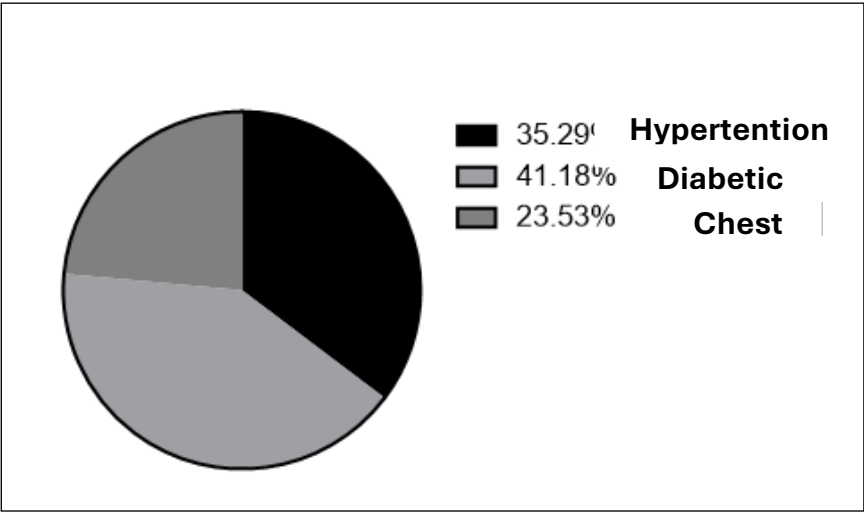


Fig. 3. Associated some disease with periodontitis patients
The pie chart showed the diabetic patients recorded high percentage compare with other disease. The values plotted one-way ANOVA test among two groups. Non-significant which is (ns), (*) is $p < 0.05$, (**) is $p < 0.01$, (***) is $p < 0.001$, (****) is $p < 0.0001$ vs Control.
Source: Own material

from history periodontitis patients , participants signed informed consent forms. Additionally, patients with a history of using steroids or bisphosphonates as well as women who had recently used antibiotics, contraceptives, or anti-inflammatories were all excluded. Blood samples were taken from the patients and the control group. For the purpose of measuring ferritin and CRP,

it was gathered. Patients' 3 ml of blood was drawn into a gel tube, and the serum was then extracted using a centrifuge. The serum will be kept for analysis at -20C.

DETECTION OF CRP

CRP-Turbilatex is a quantitative turbidimetric test for the

detection of C-reactive protein (CRP) in human plasma or serum. The examine was approved out in agreement with the producer's instructions (Mindray BC-2800VET Auto Hematology Analyzer).

DETECTION OF FERRITIN LEVEL

The assay was conducted in agreement with guidelines provided via the manufacturer as outlined in the assay approach. It called for a two-step immunoassay. Antiferritin-coated microparticles bind to ferritin that is present in the sample. Antiferritin acridinium conjugate was added after washing. Relative light units (RLUs) were used to measure the chemiluminescent reaction after Pre-Trigger and Trigger Solutions were added to the reaction mixture.

STATISTICAL ANALYSIS

The statistical application Prism was used for data analysis (Prism version is 9.3.1 (Windows and Mac)). We compared characteristics of participants in each group, by using analysis of variance (ANOVA on way), Non-significant (ns), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ vs Control.

RESULTS

EXPRESSION OF CRP PARAMETER IN PERIODONTITIS

To test the CRP level in patients of chronic periodontitis, the CRP assay was performed. These outcomes show that the appearance of CRP level was remarkably increased in patients as in contrast to healthy control ($P < 0.001$), as illustrated in Figure 1.

EXPRESSION OF FERRITIN PARAMETER IN PERIODONTITIS

There are greater concentrations in serum ferritin in the patients than that of healthy controls ($P < 0.001$). significant correlation and positive was detected between the levels of serum ferritin and number of the sites with periodontal disease (PD) ≥ 6 mm at baseline ($P < 0.001$) compared to healthy control (Fig. 2).

CORRELATION BETWEEN SYSTEMIC DISEASE AND PERIODONTITIS PATIENTS

This data was evaluated the correlation of hypertension, diabetic and chest pain disease with periodontitis patients. The results showed that hypertension, diabetic

and chest pain had a significant correlation with the CRP patients in contrast with healthy individuals. However, the diabetic patients were recorded high percentage compare with hypertension and chest pain, these data may be the increased CRP and ferritin levels in patients related with these diseases. As illustrated in Figure 3.

DISCUSSION

It has been generally accepted that periodontitis could be an important risk factor for some disease depend on the two points: (1) periodontal inflammation have effect on systemic inflammation and (2) periodontal bacteria with their outcome may accede the bloodstream. also, the poor condition of patients led to insufficient oral hygiene that made them to subjected to dialysis therapy [10]. This study will have estimated the attachment in the periodontal disease and the factors serum such as ferritin and CRP. The effects appeared that serum levels of ferritin with CRP had a strongly attachment with the Periodontitis. In addition, hypertension, diabetic and chest pain were significantly correlated with periodontitis and gingivitis patients especially diabetic disease [10, 11]. Multiple studies have a try to confirm a relation between renal disease and periodontal disease, but still understood until now. It has noted that the significant inflammatory marker and most sensitive markers is C reactive protein (CRP), which is used to estimate the individual inflammatory status [9]. Few studies have notified higher levels CRP in the patients that have Chronic Periodontitis than in the healthy persons [11]. However, one study mentioned that there was no considerable difference in serum CRP levels between chronic periodontitis and healthy, our study suggested that the response to inflammatory processes was only local but worsen within seven days because IFN- γ levels were increased. On other hand, in this project, estimated the interconnection of the periodontal disease and the serum factor ferritin in those patients, it's showed that level of serum ferritin was significantly related with gingivitis and periodontitis. In this project, periodontal disease features were obviously greater in those periodontitis patients in contrast toward healthy individual. In these two studies [12, 13] the iron insufficiency is a common finding in patients that subjected to haemodialysis, also in those patients the serum ferritin correlated significantly with iron content in bone marrow. So, the serum ferritin is the common indicator for estimation the patients of iron deficiency that undergo to haemodialysis [9, 14] Furthermore, serum level raise at systemic inflammation. However, the reports of some studies on patients who undergoing haemodialysis showmen the influence of the inflammation on the

ferritin serum level based on iron contented in the body besides that effect of inflammation effect on ferritin serum exclusively when there is sufficient content of iron. This means that the serum ferritin level could be affected by non-surgical treatment of periodontal disease. Hence, it can be concluded that these serum ferritin levels may be an essential factor in the pathogenesis of the illness [14-15].

Though causal association between serum ferritin and enlarged susceptibility to periodontitis is not recognised, it is possible that a failure of adequate host defence to plaque bacteria may be a predisposing factor and may end in periodontal disease, like the acquisition of infection, in general, is dependent on host-related issues of where serum ferritin levels are, but one of the many contributing factors [4]. Hence, evaluating serum ferritin levels is a valuable adjunct to the clinical and radiographically diagnosis of periodontal lesion and predicts the future disease progression and evaluates response to periodontal therapy [6].

CONCLUSIONS

From the current confirmation it be able to be concluded that periodontitis effects in higher levels of CRP and ferritin. The elevated inflammatory factors and some disease may increase periodontitis compared with healthy individual. Although, causal relationship between serum ferritin and CRP increased susceptibility to periodontitis, is not established until now.

RECOMMENDATIONS

Use PCR process for detection genes related with periodontal disease for present work.

Detection of CRP and Ferritin will be evaluated for value as a histological markers of the periodontitis disease.

Should consider larger and more heterogeneous samples of metacentric populations with different severities of periodontal disease, a greater number of assorted risk and confounding factors, and increased control over them.

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Experimental justification of the optimization of modern adhesive protocols ceramic restoration

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ABSTRACT

Aim: The aim of our study is to optimize modern adhesive protocols through experimental investigation.

Materials and Methods: During the study, we used the following methods: laboratory, experimental, scanning electron microscopy, morphological studies and analysis of the elemental composition of structures (samples) were carried out using a scanning electron microscope and statistical.

Results: Scanning electron microscopy of ceramic samples after etching showed changes in the surface structure, which consisted in an increase in micro-spaces, more frequent and deeper relief of irregularities with increasing etching time, acid concentration and activation of the etching gel. On the surface of samples etched for 60 seconds in 9% HF, irregularities of the cellular structure with a size of 5 to 18 μm were detected. The differences between statically and dynamically etched samples are clearly visible.

Conclusions: Thus, our study confirms the safety of using sandblasting to clean ceramics, the importance of dynamic etching at the stage of preparing ceramics for fixation, and the effectiveness of using an alcohol adhesive protocol. The results obtained, based on the principles of evidence-based medicine, can be used by dental professionals to improve the quality of treatment of patients with defects in the hard tissues of the teeth.

KEY WORDS: surface aeroabrasion, ceramic restorations, scanning electron microscopy (SEM), adhesive protocol, acid etching

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INTRODUCTION

A significant development in contemporary aesthetic dentistry is associated with the introduction of adhesive restorative materials into clinical practice. The achievement of reliable fixation through micromechanical retention has enabled minimally invasive tooth preparation and maximum preservation of dental hard tissues [1–4]. The application of adhesive techniques has facilitated the fabrication of ceramic restorations that demonstrate several advantages, which, according to both national and international literature, improve treatment quality and extend clinical longevity [4,5]. At the same time, the increasing use of this technology has highlighted treatment complications related to adhesive techniques, arising from insufficient theoretical knowledge and the unwarranted expansion of their indications [2,3,6]. Errors during adhesive fixation reduce the bond strength between ceramics, zirconia, and dental hard tissues, and may lead to frequent complications such as debonding, fracture of restorations, marginal discrepancies, and secondary caries, ultimately resulting in unfavorable outcomes [2,6].

According to a number of authors, these issues are associated with inadequate understanding of adhesion mechanisms and the influence of enamel, dentin, and ceramic (zirconia) pretreatment procedures on adhesive fixation [1,5–7]. Furthermore, there is no consensus among clinicians regarding the sequence of prosthetic treatment with ceramic restorations and the selection of adhesive systems. This has drawn considerable scientific interest to the subject of adhesive fixation, making it one of the most debated topics in dentistry [7–9].

At present, a unified methodological approach to the protocol and algorithm of adhesive fixation has not been established [3,5,9]. Insufficient knowledge of the factors contributing to the weakening of the adhesive bond and of the underlying mechanisms indicates the need for both theoretical substantiation and experimental research [7,10]. Therefore, this issue represents not only an important scientific direction but also a practical challenge, and thus remains highly relevant.

AIM

The aim of our study is to optimize modern adhesive protocols through experimental investigation.

MATERIALS AND METHODS

To obtain optimal air-abrasion characteristics and to evaluate their effect on the ceramic surface, ceramic specimens were subjected to sandblasting using a Renfert Basic Classic unit (Renfert, Hilzingen, Germany) with a nozzle diameter of 0.8 mm. The sandblasting distance was standardized at 3 cm by means of a custom-made holder fabricated from Triad photopolymer resin (USA). Each specimen was treated for 5 seconds. Surface roughness parameters were determined using a TR 200 profilometer (Time Group Inc., China). Subsequently, the ceramic specimens were etched with either a 4.5% HF solution *Etchant Gel* (Ivoclar Vivadent, Germany) or a 9.5% HF solution *Porcelain Etchant* (BISCO, USA). In part of the specimens, the etching gel was actively distributed with a disposable microbrush, while in others it remained static; following the exposure time, the gel was removed with an air–water spray and the surface dried. Roughness parameters were re-evaluated using the TR 200 profilometer.

Scanning electron microscopy (SEM) was performed with a ZEISS EVO 50 XVP microscope (Carl Zeiss, Germany) to assess ceramic surface morphology and to confirm the digital micro-roughness parameters after air-abrasion and etching. Elemental composition analysis was conducted by electron probe X-ray microanalysis using an INCA analytical attachment with an X-Max detector. The analysis was performed with a focused electron beam at an accelerating voltage of 15 kV and a probe current of 0.5 nA. Morphological and elemental composition studies of the samples were also carried out using a JEOL JSM-IT300LV scanning electron microscope equipped with energy-dispersive and wavelength-dispersive spectrometers. The spatial resolution in the secondary electron detection mode (high vacuum) was no greater than 3.0 nm at an accelerating voltage of 30 kV and no greater than 15.0 nm at 1 kV.

Experimental testing was performed on ceramic restorations fabricated from Vita Mark II material and on zirconia restorations produced from Dental Zirconia Blank Ø98 mm TT-GT-M Functional (1030–1300 MPa) (Multi-Layered). The restorations were luted with a dual-cure resin cement (*Duolink*, BISCO, USA) and a fourth-generation dental adhesive (*Optibond FL*) using two bonding protocols:

1. the wet-bonding protocol;
2. the ethanol-based bonding protocol.

Statistical analysis of surface roughness parameters was carried out using software for the calculation and

statistical processing of experimental data, as well as the built-in statistical and mathematical functions of Microsoft Excel. Prior to statistical evaluation, the roughness values obtained for each specimen group were pooled into datasets. Each dataset was tested for normal distribution using the Mann–Whitney criterion; mean values, standard deviation (SD), and coefficient of variation (CV) were calculated. Homogeneity of variances and mean values of Ra and Rz parameters across different groups was verified using tests appropriate for small sample sizes, including Fisher's test, Student's *t*-test, and the approximate *t*-test at a significance level of $\alpha = 0.05$, with the number of degrees of freedom determined according to the requirements of each test. Statistical processing of the results was performed using methods of descriptive and inferential statistics; mean values, SD, and CV were computed. The significance of differences between means was determined by Student's *t*-test using Microsoft Excel 2022.

RESULTS

The aim of our study was to identify approaches for improving the effectiveness of prosthetic treatment in patients with defects of dental hard tissues using ceramic restorations, including the refinement of the adhesive fixation protocol and its implementation in clinical practice. Based on this aim, the objectives were directed toward investigating the microstructure of ceramic surfaces prepared for fixation and evaluating their microroughness parameters using different techniques, as well as improving the application technique of adhesive systems on the surface of dental hard tissues.

The assessment of specimen microroughness demonstrated that the Ra and Rz parameters, both without treatment and after sandblasting, showed no statistically significant differences across the groups (Fig. 1).

The next stage of the study was devoted to ceramic etching. Many previous investigations have evaluated different exposure times of etching gels on ceramic surfaces and reported findings similar to ours: an increase in surface microroughness with longer exposure times and the presence of morphological changes expressed as widening of intercrystalline spaces [9,11,12].

A distinctive feature of our study was the evaluation of differences between active and passive ceramic etching. The initial hypothesis assumed that continuous distribution of the etching gel with a microbrush would enhance the etching effect and accelerate its onset. However, the obtained results did not confirm a significant effect of active etching with 4.5% HF, but

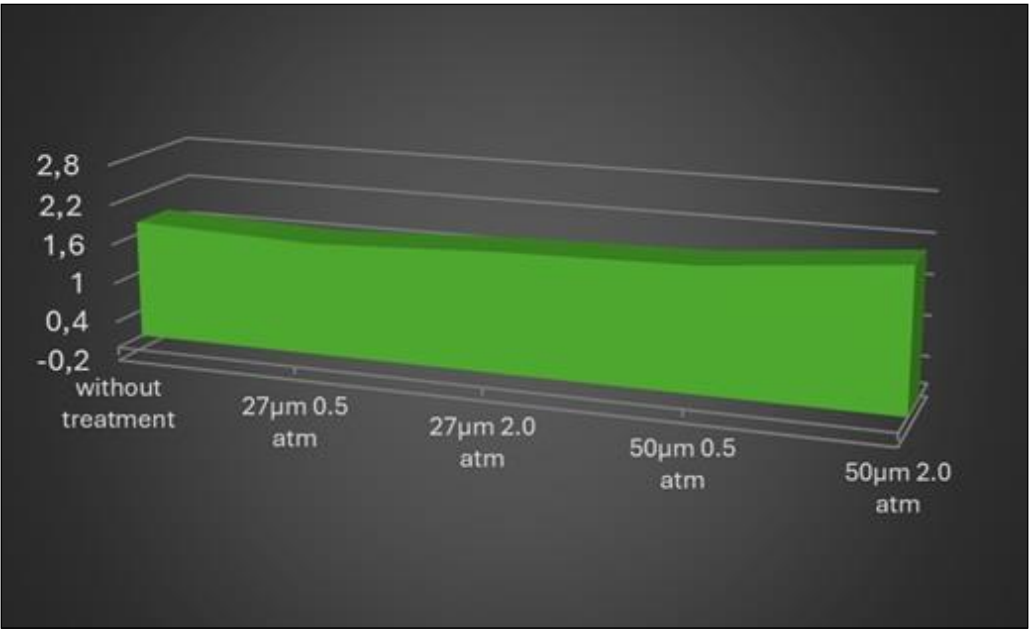


Fig. 1. Microroughness results after air-abrasion treatment
Picture taken by the authors

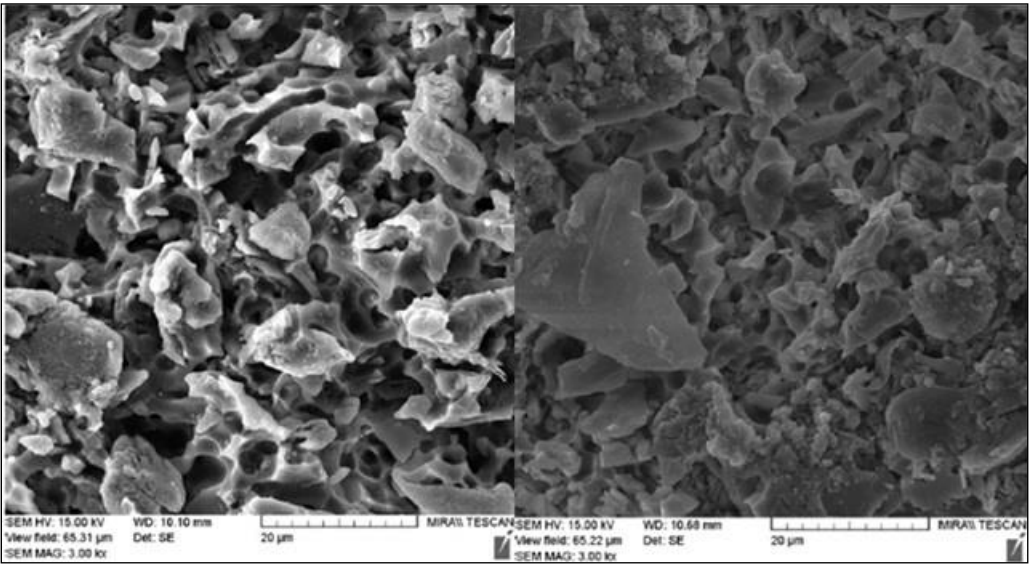


Fig. 2. Left – ceramic specimen etched dynamically for 60 s in 9% HF; right – specimen etched statically for 60 s in 9% HF
Picture taken by the authors

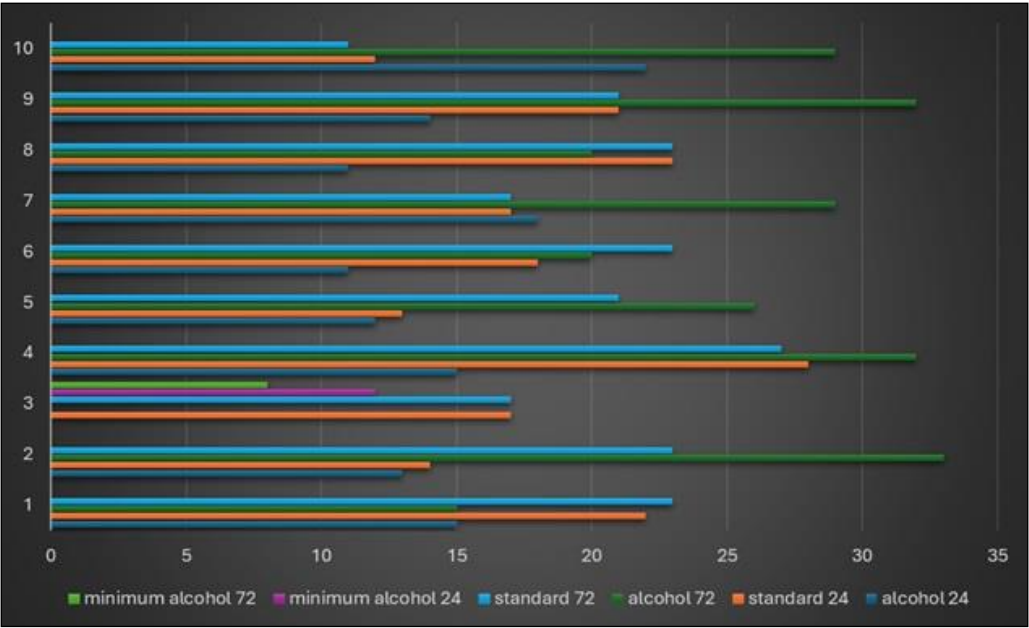


Fig. 3. Comparison of tensile bond strength values among the four groups
Picture taken by the authors

did substantiate the effect of 9% HF on microroughness parameters. The use of 4.5% HF for etching feldspathic ceramic Vita Mark II is therefore not recommended.

Differences in exposure time with 4.5% HF were statistically insignificant with respect to microroughness parameters. In contrast, when using 9% HF gel, a statistically significant difference was observed between the dynamic and static etching methods ($p > 0.05$).

For the analysis of adhesive interface morphology under standard and ethanol-based adhesive protocols, ten second and third molars from both jaws were used, extracted for orthodontic or surgical reasons, without carious lesions, cracks, restorations, or structural defects. The teeth were randomly divided into two groups; each tooth received an adhesively fixed ceramic block made of Vita Mark II with Duolink dual-cure composite cement (Bisco, USA). In the first group ($n = 5$), adhesive protocol No. 1 was applied; in the second group ($n = 5$), adhesive protocol No. 2 was used.

After fixation, the specimens were stored in distilled water for 24 hours. The adhesive interface area was then processed with a turbine handpiece (Dentsply Sirona, Germany) and fine-grit burs (Meisinger, Germany) under water and air cooling. The surface was etched with 37% phosphoric acid for 150 seconds, rinsed with distilled water, and subsequently immersed in sodium hypochlorite solution for 10 minutes, followed by additional rinsing with distilled water. The specimens were stored in distilled water for another 24 hours prior to electron microscopy analysis.

Morphological studies and elemental composition analyses were carried out using a JEOL JSM-IT300LV scanning electron microscope equipped with energy-dispersive and wavelength-dispersive spectrometers. The spatial resolution in secondary electron detection mode (high vacuum) was ≤ 3.0 nm at 30 kV and ≤ 15.0 nm at 1 kV. In low-vacuum mode, the spatial resolution at 30 kV did not exceed 4.0 nm. Under these conditions, the measurement range for linear dimensions extended from 0.03 to 1000 μm with a maximum permissible relative error of 10%. The magnification range was from $5\times$ to $300,000\times$ based on a 10×12 cm image size.

Scanning electron microscopy of ceramic specimens after etching revealed surface structural changes characterized by an increase in microporosities, as well as a more frequent and deeper surface relief with longer etching times, higher acid concentrations, and activation of the etching gel. Consistent with the findings of Ravikumar Ramakrishnaiah et al. (2016), specimens etched for 60 seconds with 9% HF exhibited honeycomb-like surface irregularities ranging from 5 to 18 μm in size. Clear differences were observed between spec-

imens subjected to static and dynamic etching (Fig. 2).

Elemental composition analysis of the etched ceramic specimens, conducted in addition to SEM evaluation, confirmed that the qualitative elemental composition corresponded to the manufacturer's specifications. One of the modern and most debated approaches to improving dentin adhesion is the use of the ethanol wet-bonding protocol. This shear bond strength test provides quantitative data for the objective evaluation of the effectiveness of conventional and ethanol-based adhesive fixation protocols. In our study, a simplified ethanol bonding technique was applied, consisting of the application of 95% ethanol for 30 seconds in combination with water and Optibond FL primer, which is considerably easier to implement in clinical dentistry. The study included 40 second and third molars extracted from both jaws for orthodontic or surgical reasons, of approximately similar dimensions, without carious lesions, cracks, restorations, or structural defects. Following extraction, the teeth were stored in 2% chlorhexidine solution for no longer than one month; the solution was replaced every 14 days to avoid contamination. The occlusal surface of each molar was sectioned at the equator using diamond disks under water and air cooling, followed by finishing with a turbine handpiece (Dentsply Sirona, Germany) and fine-grit burs (Meisinger, Germany) under water and air cooling. After sectioning, each tooth was mounted in a block of self-curing acrylic resin so that the surface intended for bonding with the test materials remained free and accessible for treatment. The teeth were randomly assigned to four groups. A ceramic fragment with a 3×3 mm base made of Vita Mark II was adhesively bonded to the dentin of each tooth using Duolink dual-cure composite cement (Bisco, USA). Adhesive protocol No. 1 was applied in groups 1 and 3, and adhesive protocol No. 2 in groups 2 and 4. Another distinctive feature of this study was the use of a protocol for indirect ceramic restoration fixation involving co-polymerization, whereas in other studies the adhesive was polymerized separately prior to independent polymerization of the composite block.

A characteristic feature of this method is the considerable stress transmitted to the dental hard tissues and the ceramic block, which often results in cohesive fractures of the specimens. Such outcomes were observed in the 3rd and 4th groups, where 6 and 5 specimens, respectively, fractured cohesively within the ceramic layer. This indicates that the strength of the adhesive joint exceeded that of the ceramic itself and could hypothetically have been even greater than the measured values. The 24-hour results for groups 1 and 2 showed mean bond strength values of 18.40

MPa (standard deviation 4.75) and 14.27 MPa (standard deviation 4.0), respectively. The standard Optibond FL protocol demonstrated higher values, though the difference remained within the margin of error. After 72 hours, specimens from groups 3 and 4 showed mean values of 20.21 MPa and 24.87 MPa, respectively; the ethanol-based Optibond FL protocol demonstrated a statistically significant improvement. Results reported by Shan Shan Duan et al. (2019) in a comparable shear bond strength study also showed a significant increase in adhesion strength in the ethanol bonding groups. The difference in bond strength between the 24- and 72-hour intervals can be explained by the findings of Jang Y. et al. (2021): the degree of conversion of a light-cured resin polymerized for 20 seconds through a 4 mm ceramic block is lower after 24 hours compared with that of a dual-cure material that was not exposed to light polymerization. Therefore, waiting more than 24 hours before testing could have allowed further polymer chain growth and improved adhesive conversion [11,13–15]. The difference between the mean values of the ethanol protocol at 24 and 72 hours was statistically significant with high probability (t -test = 0.0006183). The high variance (53.227035) in the ethanol protocol group tested at 72 hours indicates considerable variability of the parameters. Nevertheless, the lowest bond strength value in this group (12.41 MPa) exceeded the corresponding minimum values in the other groups.

Based on the tensile bond strength results, it was concluded that the ethanol-based adhesive protocol provides superior adhesion strength (Fig. 3).

Analysis of the adhesive interface morphology demonstrated uniform and deep penetration of adhesive tags into dentinal tubules, regardless of the type of adhesive protocol; however, the morphological pattern may vary considerably depending on the sectioning angle relative to the spatial orientation of the tubules [14,16,17].

Experimental studies of adhesion were conducted according to the criteria modified by Nathaniel C. Lawson et al. (2020). These and most other clinical studies on adhesion were carried out for direct composite restorations, whose rate of change and degradation is several times higher than that of ceramic restorations. Nathaniel C. Lawson et al. (2020) reported 19 cases of marginal defects, 6 cases of marginal discoloration, 3 cases of secondary caries, and 3 cases of debonding among 126 treated teeth one year after restoration. Most of the unsatisfactory results were observed in groups using self-etch adhesive systems [18].

In the case of more reliable ceramic restorations combined with a fourth-generation adhesive system, the results included 2 cases of marginal defects and 8

cases of superficial marginal discoloration. No statistically significant difference was observed between groups 1 and 2.

The analysis of these findings indicates that the clinical evaluation method demonstrates low sensitivity for investigating specific aspects of adhesive system performance. Several authors have confirmed this conclusion in their studies. For more precise clinical assessment of the effect of the ethanol bonding protocol, new methods of clinical evaluation are required, whereas laboratory methods consistently confirm higher bond strength when it is used.

DISCUSSION

Both domestic and international research groups have conducted a considerable number of studies on the use of air-abrasion and hydrofluoric acid etching. Authors such as Goro Nishigawa et al. (2019), Alireza Keshvad et al. (2019), Michele Carrabba et al. (2021), and Ilknur Caglar et al. (2018) reported positive effects of sandblasting and etching on adhesive bond strength [14,16,18,19]. However, in most of these studies, one type of air-abrasion protocol was applied that is rarely used in actual clinical practice—sandblasting for 10–30 seconds (Goro Nishigawa et al., 2019; Michele Carrabba et al., 2020).

Moreover, in the majority of cases, the studies focused on evaluating the direct effect of sandblasting on bond strength without analyzing the morphology and microroughness of the surface (Goro Nishigawa et al., 2021) [18,20].

SEM analysis in our study confirmed earlier findings regarding the numerical values of microroughness by showing no morphological differences between specimens subjected to different sandblasting protocols. This contrasts with the results of Uwalaka C.O. et al. (2022) and other investigations that assessed the effect of air-abrasion [18,20,21]. It should be noted, however, that the sandblasting duration in those studies (10–30 s) differed substantially from that applied in our study (5 s).

The obtained results may be applied in clinical dentistry to justify the use of short-term air-abrasion for cleaning contaminated ceramic surfaces without causing surface damage or compromising surface quality.

In the field of adhesive systems, fourth-generation adhesives remain the “gold standard.” Publications by David Pashley et al. (2020), Shan Shan Duan et al. (2021), and Muhammet Kerim Ayar et al. (2023) support the use of the ethanol bonding protocol to improve the quality of dentin adhesion.

The original ethanol bonding technique involved sequential dentin saturation with 50%, 70%, 80%, and

95% ethanol for 30 seconds each, lasting a total of 3–4 minutes, as applied in the studies by C. Yesilyurt et al. (2018). Li F. et al. (2019) reported enhanced bonding of commercial adhesive systems to dentin when dentin was saturated with 100% ethanol for 1 minute, similar to the method proposed by F.T. Sadek et al. (2018), which, however, represents a clinically challenging protocol [2,8,16,22]. The effectiveness of the ethanol protocol remains inconclusive: most authors studying this issue suggest that it should be considered primarily as an *in vitro* method and emphasize the lack of sufficient evidence supporting its *in vivo* effectiveness (Muhammet Kerim Ayar et al., 2020). In our study, adhesive shear bond strength was evaluated using the Macro Shear Bond Strength Test (SBS), comparable to the methodologies applied by Shan Duan et al. (2021), Yesilyurt et al. (2019), and Li F. et al. (2019).

For more accurate data, the Macro Tensile Bond Strength Test (TBS) may be employed, in which the force is applied perpendicularly to the adhesive interface and cohesive specimen fractures occur less frequently, allowing for more reliable results [18,21–23].

Today, the literature describes a large number of different methods that increase the adhesion of zirconia to luting cements, which indicates the relevance of this problem. Based on the analysis of available sources, the following methods were identified as enhancing zirconia–cement adhesion: grinding with diamond burs, sandblasting, acid etching, laser surface treatment, electrical discharge machining (EDM), tribochemical silica coating (TBS method), selective infiltration etching (SIE), nano-alumina coating, zirconia powder fusion sputtering (FS), MDP-containing primers, MDP-free primers, and universal adhesive systems [7,8,14,20–23].

Sandblasting involves treating the material surface with particles (most commonly aluminum oxide) propelled by a high-speed source. Particles of various diameters, ranging from 25 to 150 μm , may be used. The recommended particle size depends on the type of material, particularly its translucency, in order to achieve greater surface relief while minimizing damage. Other parameters—including air pressure, particle shape, distance and angle of impact, and treatment duration—also influence the final outcome. Sandblasting removes surface contaminants, increases bonding surface area and roughness, and improves wettability [16,18,20–23]. However, this method also has drawbacks, including the potential formation of defects and microcracks, as well as excessive tetragonal-to-monoclinic phase transformation, which reduces the mechanical properties of the final restoration [23–25].

Zirconium dioxide is generally considered a

polycrystalline ceramic resistant to acid exposure at room temperature for short, clinically relevant periods. Nevertheless, several attempts have been made to modify etching conditions, and recent studies have demonstrated improved effectiveness of this method [8,12,18,22]. Various highly concentrated acids—such as nitric, hydrofluoric, and hydrochloric acid, as well as their mixtures—can be used for etching. Additional components, including alcohol, hydrogen peroxide, or ferric chloride, may be added to the solution. Different temperature regimes and etching times may also be employed. Under properly selected conditions, this method enables the creation of a uniform, homogeneous, and rough surface on the fitting surface of the restoration. Furthermore, the absence of direct physical impact helps avoid microdamage to the material and unwanted phase transformations [20,22].

Despite the numerous proposed methods and their combinations, no universally accepted adhesion protocol has yet been established that ensures consistent and stable long-term results. None of the reviewed methods can be unequivocally recommended as the most effective, accessible, and non-destructive for clinical practice. However, based on comparative evaluation, certain methods appear more favorable and may be considered suitable for broader clinical application.

Thus, additional research is required to evaluate the long-term outcomes of adhesion and to develop standardized protocols for zirconia surface pretreatment.

CONCLUSIONS

Comparative analysis of the results of aeroabrasive treatment of the ceramic surface in different modes showed the absence of significant differences in its microroughness. Such treatment can be used for surface decontamination. Dynamic or static etching for 30–60 s does not have a significant effect on the microroughness of Vita Mark II ceramics when etched with 4.5% hydrofluoric acid and does not differ from the results obtained after aeroabrasive treatment. At the same time, the effect of dynamic etching with 9% hydrofluoric acid on the ceramic surface leads to an increase in the average microroughness parameters Ra and Rz by 1.58 μm compared to static etching, increasing the area of the adhesive surface and improving the quality of adhesive fixation. SEM study of the ceramic surface showed an increase in the microroughness relief with increasing exposure time and concentration of the etching gel. Dynamic etching causes visualized morphological changes in the microstructure. In vitro adhesion strength analysis demonstrated maximum adhesion strength in the

alcohol adhesive protocol group after 72 hours and no increase in adhesive bond strength in the standard protocol group at the same time. SEM morphology analysis of the adhesive interface showed high-quality and deep dentin hybridization, no visible differences between the standard and alcohol protocol groups.

Thus, our study allows us to answer questions in clinical dentistry that are currently under active discussion

in the dental community, namely: the possibility and safety of using sandblasting to clean ceramics, the role of dynamic etching at the stage of preparing ceramics for fixation, and the effectiveness of using the alcohol adhesive protocol. The results obtained, based on the principles of evidence-based medicine, can be used by dental professionals to improve the quality of treatment of patients with hard dental tissue defects.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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Evaluation of serum Neopterin levels in patients with type 2 diabetes mellitus and hypothyroidism

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ABSTRACT

Aim: This study aims to investigate the variation in serum Neopterin levels between adult individuals (T2DM with hypothyroidism and without hypothyroidism).

Materials and Methods: Ninety participants were divided into three groups: 30 patients with T2DM and hypothyroidism, 30 patients with T2DM and without hypothyroidism and 30 Control patients. They were obtained from national diabetes center, Mustansiriyah University.

Results: The current findings have found that patients in Group I (T2DM with hypothyroidism) showed significantly raised neopterin levels (614.87 ± 166.27 pg/ml) as compared to Group II (T2DM without hypothyroidism) which is (335.27 ± 173.34 pg/ml) and healthy control group (267.29 ± 254.4 pg/ml). The level of neopterin in T2DM with hypothyroidism patients, and T2DM without hypothyroidism patients have a significant ($P < 0.05$) positive correlation coefficient with BMI, HbA1c, TC, TG, AIP and TSH. Furthermore, a high significant ($P < 0.01$) positive correlation with FBS, HOMA-IR and LDL-C was also found.

Conclusions: The elevated levels of neopterin in type 2 diabetic patients with hypothyroidism and without hypothyroidism play as a regulator of energy consumption, leading to the hypothesis that thyroid gland disease may influence its level. In addition, the potential for use as a diagnostic marker for diabetics with thyroid disease due to the occurrence of complications of diabetes, including cardiovascular diseases.

KEY WORDS: hypothyroidism, insulin resistance, lipid profile, Neopterin, T2DM

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INTRODUCTION

Diabetes mellitus (DM) ranks as one of the most widespread metabolic disorders worldwide, characterized by persistent hyperglycemia and disturbances in the metabolism of different nutritional molecules [1]. Insulin metabolism is impaired in diabetes mellitus, which results in a wide range of clinical symptoms. A metabolically hypoactive state is a hallmark of hypothyroidism, a condition in which thyroid hormones are insufficient for a variety of reasons [2]. Thyroid hormones and insulin, which are linked to diabetes mellitus, work together to control the body's metabolism in a complex dance [3]. Females with diabetes are more likely to have thyroid issues [4]. Diabetic patients with longstanding hyperlipidemia, obesity, and anemia are at greater chance of having underlying hypothyroidism [5]. Similar to hypothyroidism affects the emergence of diabetes problems, there is typically a substantial clinical association between these two common endocrine disorders [6]. Neopterin (NP) is a biomarker of immune system activation that is produced by activated macrophages

and monocytes, which causes its concentration to rise in various inflammatory conditions [7]. When T-lymphocytes identify the pathogen and damaged tissue, they release interferon-gamma (INF- γ), which activates guanosine triphosphate cyclohydrolase I (GCH I). This produces 7,8-dihydroneopterin, which under oxidative stress breaks down into neopterin, a small molecule also called a pteridine molecule [8]. Since NP is a byproduct of 7,8-dihydroneopterin's oxidation, its concentration indicates the degree of oxidative stress that occurs during inflammation. In addition to the extracellular marker of activated immune cells, it also induces anti-oxidant and anti-inflammatory conditions, that act as mediators and modulators of inflammation [9]. It offers a possible instrument for assessing the emergence of various inflammatory conditions [10]. It has been found that the serum concentration of NP is strongly associated with the parameters of glucose metabolism and is closely related to glucose intolerance. In addition, NP is described as a sign of diabetes progression and its associated consequences [11].

AIM

This study aims to investigate the variation in serum Neopterin levels between adult individuals (T2DM with hypothyroidism and without hypothyroidism).

MATERIAL AND METHODS

Between December and the end of March 2024, 90 participants from the National Diabetes Centre at Mustansiriya University took part in the study. They were further divided into three groups: Group G1 included 30 patients with type 2 diabetes mellitus (T2DM) and hypothyroidism. Group G2 comprised of 30 patients with only T2DM, and Group 3 (G3) comprised additional 30 healthy control subjects. Informed verbal consent was obtained from all participants after explaining the study objectives and ethical considerations. All participants underwent a detailed clinical evaluation to exclude other comorbid conditions. Demographic data; age, sex, weight, height, and BMI were documented. Fasting blood sugar (FBS) was measured using an enzymatic colorimetric method with aid of automated biochemical analyzer (Cobas c 111 analyzer, Roche Diagnostics). Total cholesterol (TC), triglycerides (TG), HDL-C, LDL-C, HbA1c have been measured using immunoturbidimetric method. Serum insulin levels were measured using chemiluminescent immunoassay. Insulin resistance was calculated by using the formula Homeostatic Model Assessment of Insulin Resistance; that is, $[\text{Fasting Insulin } (\mu\text{U/mL}) \times \text{Fasting Glucose } (\text{mg/dL}) / 405]$. The Atherogenic Index of Plasma (AIP) was calculated as the logarithm of $(\text{TG}/\text{HDL-C})$. Thyroid function tests total triiodothyronine (T3), total thyroxine (T4), free thyroxine (FT4), and thyroid-stimulating hormone (TSH) were measured in miniVIDAS immunoassay system (BioMérieux, France) which works on ELFA principles. Neopterin levels were measured by ELISA through available commercial kit (Cosiabio, Cat. No. E09144h).

Excluding criteria: It did not include patients with hyperthyroidism, type 1 diabetes mellitus, pregnancy, current insulin therapy, or any other systemic disease.

Including criteria: The study included adults aged 35 to 60 years with T2DM for more than 5 years.

ETHICAL APPROVAL

The study procedures were conducted in accordance with the ethical standards outlined in the Declaration of Helsinki (protocol number 148# in 2025).

STATISTICAL ANALYSIS

All data were reported as mean $\pm$ SD. LSD (Least Significant Difference) for ANOVA was used for the statistical

analysis, with $p < 0.05$ serving as the minimum level of significance. Version 22 of the SPSS software (SPSS, Chicago, IL, USA) is utilized.

RESULTS

Table 1 presents the anthropometric measurements of the three groups in this study: group I, T2DM with hypothyroidism; group II, T2DM without hypothyroidism; and the control group. The mean ages of the participants for all groups were not significantly different ($p = 0.124$), suggesting a comparable distribution of ages. Although group I had a slightly higher mean weight than group II and the control group (83.13 ± 9.64 vs. 79.53 ± 8.52 vs. 73.17 ± 6.43 kg), it was bordering on being statistically significant ($p = 0.05$). No significant difference in height was observed among the groups ($p = 0.103$). The body mass index was maximum in group I (33.21 ± 3.44 kg/m²) than in group II (28.41 ± 2.22 kg/m²) and control group (24.33 ± 3.25 kg/m²), with a borderline significant difference ($p = 0.05$). It may indicate that patients having both T2DM and hypothyroidism would have an increased tendency toward higher BMI values as an additive metabolic burden from hypothyroidism.

Table 2 exhibits the clinical markers for the three study groups: group I (T2DM with hypothyroidism), group II (T2DM without hypothyroidism), and healthy controls. Most markers varied significantly between the groups ($p < 0.05$). FBS, HbA1c, insulin levels, and HOMA-IR were all raised in both diabetic groups versus controls; however, values were highest in group I indicating more severe insulin resistance and glycaemic derangement among T2DM patients if associated with hypothyroidism. Lipid profile parameters of total cholesterol, triglycerides, LDL-C and AIP were also significantly raised in group I as compared to group II and control implying a more atherogenic lipid profile in patients when hypothyroidism coexists. The highest level of HDL-C was seen in the control. Regarding thyroid hormones, Group I was found to have significantly lower levels of T3 and T4 along with raised TSH levels as compared to Groups II and controls.

The current findings have reported that patients in group I (T2DM with hypothyroidism) showed significantly raised neopterin levels (614.87 ± 166.27 pg/ml) as compared to group II (T2DM without hypothyroidism) which is (335.27 ± 173.34 pg/ml) and healthy control group (267.29 ± 254.4 pg/ml), as illustrated in table 3.

Correlation analysis shows that neopterin levels positively correlate with metabolic parameters in both group I (T2DM with hypothyroidism) and group II (T2DM without hypothyroidism). In group I, it is correlated strongly with LDL-C ($r = 0.687$, $p < 0.01$), HOMA-IR ($r = 0.655$, $p < 0.01$), and FBS ($r = 0.502$, $p < 0.01$). This may indicate a link between

Table 1. Anthropometric measurements among groups of study

Variables	Mean±SD			p-value
	Group I (n=30)	Group II (n=30)	Control (n=30)	
Age (years)	48.53 ± 3.57	47.1 ± 3.34	44.4 ± 3.12	0.124
Weight(kg)	83.13 ± 9.64	79.53 ± 8.52	73.17 ± 6.43	0.05
High (cm)	169.67 ± 5.48	168.8 ± 8.62	169 ± 0.08	0.103
BMI (kg/m ²)	33.21 ± 3.44	28.41 ± 2.22	24.33 ± 3.25	0.05

Source: Own material

Table 2. Clinical markers among the three study groups

Markers	Mean±SD			
	Group I (n=30)	Group II (n=30)	Control (n=30)	
FBS (mg/dl)	142.9 ± 25.1 ^A	145.67 ± 58.7 ^A	86.9 ± 8.48 ^B	
HbA1c %	9.89 ± 1.02 ^A	8.27 ± 1.85 ^A	5.31 ± 0.21 ^B	
Insulin level (U/l)	18.59 ± 4.38 ^A	16.87 ± 7.08 ^A	11.71 ± 5.51 ^B	
HOMA-IR	7.22 ± 1.32 ^A	6.25 ± 1.52 ^A	2.00 ± 0.84 ^A	
TC (mg/dl)	299.97 ± 40.7 ^A	262.83 ± 18.3 ^B	159 ± 29.2 ^C	
TG (mg/dl)	263.13 ± 42.9 ^A	203.73 ± 103.7 ^B	112.33 ± 21.21 ^C	
HDL-C (mg/dl)	47.93 ± 6.48	45.13 ± 3.15	50.93 ± 1.41	
LDL-C (mg/dl)	119.41 ± 9.68 ^A	98.25 ± 7.93 ^A	91.43 ± 7.21 ^C	
AIP (log TG/HDL-C)	4.52 ± 0.79 ^A	3.00 ± 0.85 ^B	2.02 ± 0.19 ^C	
T3 (nmol/l)	0.64 ± 0.19 ^A	1.84 ± 0.55 ^B	1.11 ± 0.28 ^B	
T4 (nmol/l)	49.77 ± 6.72 ^A	79.03 ± 13.82 ^B	78.2 ± 4.95 ^B	
TSH (IU/L)	18.83 ± 5.77 ^A	4.25 ± 1.07 ^B	53.35 ± 0.99 ^C	

^A, ^B, ^C: different letters refer to statistically significant difference at p<0.05

Source: Own material

Table 3. Differences in neopterin levels among study groups

	Mean±SD			p-value
	Group I (n=30)	Group II (n=30)	Control (n=30)	
Neopterin (pg/ml)	614.87 ± 166.27	335.27 ± 173.34	267.29 ± 254.4	0.001

Source: Own material

neopterin and insulin resistance or dyslipidemia when hypothyroidism coexists. It is also correlated moderately with BMI, TG, AIP, and TSH. In Group II it is also correlated significantly with LDL-C (r=0.621), FBS (r=0.586), and HOMA-IR; (r=0.547). However, correlation with HDL-C was negative and not significant (Table 4).

The analysis of the ROC curve of neopterin shows that area under the curve (AUC) equals 0.98 and hence gives diagnostic accuracy of the most excellent level (Fig.1., Table 5). The cut-off value for neopterin was set at 311.1 pg/ml, giving sensitivity=100%, specificity=87%, and statistically significant (p-value p=0.000) with a confidence interval of 95% running between 0.958 and 1.0. It means results having been attained suggested neopterin to possess very strong discriminative abilities in identifying those T2DM patients who have hypothyroidism.

The ROC curve analysis for neopterin as a predictive marker in T2DM patients without hypothyroidism indicates fairly moderate diagnostics. It is shown that the area under the curve is 0.698. The optimal cut-off value was identified as 405.5 pg/ml, sensitivity was 60% and with a specificity of 100%. These findings are summarized in figure 2 and they imply that though neopterin has limited sensitivity it remains highly specific in detecting T2DM cases without coexisting hypothyroidism (Fig. 2., Table 6).

DISCUSSION

Endocrine dysfunction is the root cause of both diabetes and thyroid disease, and both conditions have been shown to influence each other [12]. Patients with thyroid disorders have a higher prevalence of diabetes mellitus. This trend may be explained by increased medical sur-

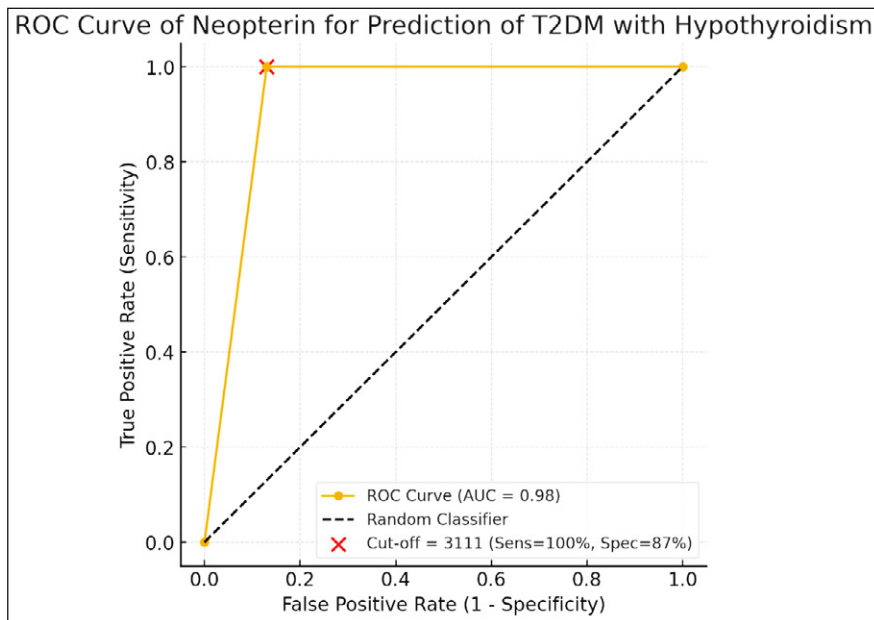


Fig. 1. ROC curve analysis of neopterin for the prediction of T2DM with hypothyroidism
Source: Own material

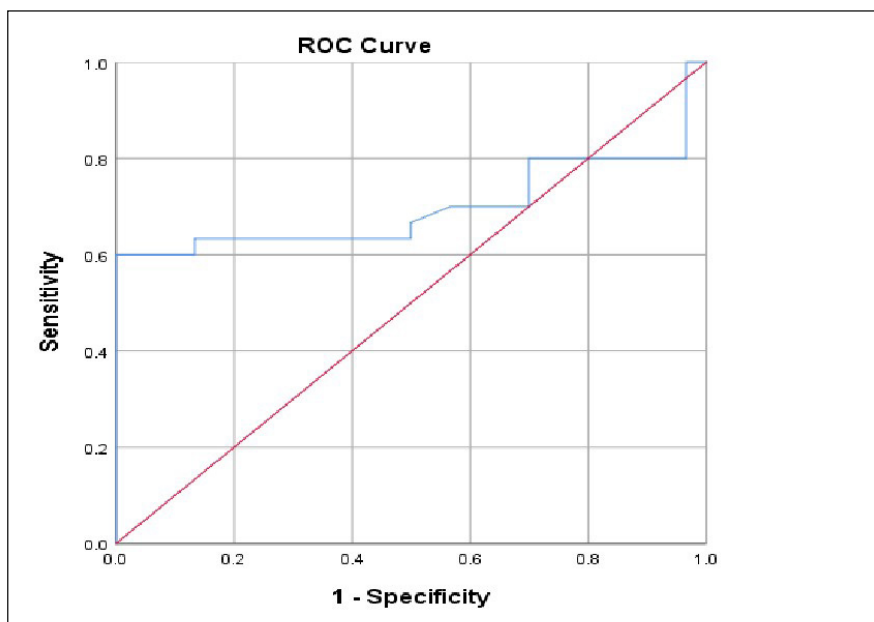


Fig. 2. ROC curve analysis of neopterin for the prediction of T2DM without hypothyroidism
Source: Own material

veillance of these patients, but there are also possible pathophysiological mechanisms that could explain why thyroid disorders develop in DM patients and why people with thyroid disorders are more likely to develop DM themselves [13]. Thyroid hormone (TH) increases gastrointestinal motility, which improves the absorption of glucose [14]. High levels of GLUT2 in the liver increase glucose output by the liver. Thus, it triggers endogenous glucose production, including gluconeogenesis and glycogenolysis [15]. Overactivated metabolic pathways cause hyperinsulinemia and impaired glucose tolerance, which also lowers insulin sensitivity within peripheral tissues [14]. Thyroid hormones act directly by enhancing the secretion of insulin by β -cells of the pancreas and α -cell-mediated glucagon release. At the same time, it promotes the breakdown of fat in adipose tissue; this increases the amount of free fatty acids in circulation

that further support gluconeogenesis in the liver [16]. The amount of cellular immunological activity can be determined by looking at the production of neopterin. In patients with thyroid disorders, neopterin level measurement may provide offer prognostic and predictive information [17]. The migration of mononuclear cells in thyroid disorders is regulated by adhesion molecules and inflammatory cytokines [18]. The production of cytokines and autoantibodies by B cells and extracellular matrix leads to the expansion of T-cells. It is known that neopterin is a biochemical indicator of activation of cellular immunity, since neopterin, a particular indicator of monocyte activity, and other cytokines make monocytes in healthy people inactive [19]. Significantly higher levels of neopterin were observed among the of patients with T2DM and hypothyroidism, patients with T2DM without hypothyroidism, and the control group;

Table 4. Correlation coefficient for the correlation between neopterin and other biomarkers

Variables	Pearson's Correlation Coefficient (r)	
	Group I	Group II
BMI	0.357*	0.371*
FBS	0.502**	0.586**
Insulin level	0.037	0.119
HOMA-IR	0.655**	0.547**
HbA1c %	0.359*	0.339*
TC	0.308*	0.388*
TG	0.369*	0.363*
HDL-C	0.029	-0.148
LDL-C	0.687**	0.621**
AIP	0.350*	0.317*
T ₃	0.124	0.185
T ₄	0.182	0.146
TSH	0.399*	0.234

*: significant correlation at $p < 0.05$; **: high significant correlation at $p < 0.01$

Source: Own material

Table 5. ROC curve analysis of neopterin for the prediction of T2DM with Hypothyroidism

AUC	Cutt-off	SE	p-value	95% CI		Sensitivity	Specificity
				Lower bound	Upper bound		
0.98	311.1	0.12	0.000	0.958	1.0	100	87

Source: Own material

Table 6. ROC curve analysis of neopterin for the prediction T2DM without hypothyroidism patients

AUC	Cutt-off	SE	p-value	95% CI		Sensitivity	Specificity
				Lower bound	Upper bound		
0.698	405.5	0.076	0.008	0.549	0.847	60	100

Source: Own material

similar results were observed by previous articles. [20, 21]. Elevated levels of neopterin are a consequence of the autoimmune response characterized by a considerable infiltration of macrophages in the thyroid gland. This infiltration results in a marked production of neopterin, which in turn elevates its concentration in the peripheral blood. It has been suggested that the interplay between neopterin levels and the titer of TSH-receptor stimulatory antibodies may serve as an indicator of autoimmune activity that is specific to the thyroid [21]. Previous studies have also reported a positive associations between neopterin and BMI [22-24]. They postulated that in obese people with thyroid disorders, insulin resistance is caused by inflammation of the adipose tissue mediated by an activated immune system, rather than just an increase in adipose tissue mass, which directly results in attenuation of insulin action [25]. The neopterin level has a positive correlation coefficient with HbA1c ($p < 0.05$) as well as with FBS and HOMA- ($p < 0.01$) in T2DM with hypothyroidism patients, as well as T2DM without

hypothyroidism patients, which is consistent with the results of several study [26]. In the development of type 2 diabetes, serum neopterin seems to be linked to both insulin resistance and decreased insulin production. Elevated blood glucose levels may cause the pancreatic beta cells to become more stimulated. In hypothyroidism, there is an increase in the amount of insulin secreted in response to a glucose stimulation. Hypothyroidism also slows the breakdown of insulin. This may be the cause of elevated HOMA-IR index and elevated serum insulin levels in hypothyroid people [27]. Furthermore, the positive correlation between neopterin level and TC, TG and AIP (at $p < 0.05$), as well as the high positive correlation between neopterin level and LDL-C ($P < 0.01$) in patients with T2DM and hypothyroidism, as well as in patients with T2DM without hypothyroidism, and this is agreement with several study [28-30]. All of the aforementioned alterations add up to higher levels of total cholesterol, triglycerides, and LDL, which is a risk agent for the occurrence of "nonalcoholic fatty liver disease"

(NAFLD). Another factor that links hypothyroidism to NAFLD is hepatic insulin resistance, which is exacerbated by increased triglyceride accumulation in the liver [31]. Furthermore, lipoprotein lipase enzyme activity is reduced in overt hypothyroidism, which prevents the clearance of TG-rich lipoproteins and raises serum TG levels. Neopterin and AIP showed a strong positive connection in patients with type 2 diabetes who also had hypothyroidism. It has already been shown that the primary atherogenic component of LDL, serum LDL level, has a substantial correlation with AIP. However, it has been demonstrated that thyroid conditions are linked to highly atherogenic pattern B of LDL-C subfractions. According to reports, AIP is a better predictor of cardiovascular danger than a traditional lipid profile and offers predictive value beyond that of personal lipids as a marker of lipoprotein particle size [32]. Lipid profiles are greatly impacted by hypothyroidism, which raises the risk of cardiovascular disease. Thyroid hormone is essential for controlling lipid metabolism and cholesterol levels. Serum levels of LDL-c, TC and may be TG rise with hypothyroidism. Atherosclerosis and other cardiovascular diseases are facilitated by this changed lipid profile [33]. Neopterin levels have a positive correlation coefficient with TSH in hypothyroidism patents with T2DM patients ($p < 0.05$), and this is in agreement with other study [34]. It can be believed that many internal cascade reactions may be triggered with

TSH inhibition in thyroid illnesses and this might depress neopterin release by the hypothalamus pituitary axis [35]. Total neopterin and HDLC have an inverse relationship; this is because HDLC helps to transfer extra cholesterol to the liver for excretion after removing it from peripheral tissue [36]. HDL's anti-inflammatory and antioxidant properties are among its many uses. In peripheral tissue, cholesterol builds up and leads to atherosclerosis and inflammation if the function is compromised [37, 38]. ROC curve analysis of neopterin as a prognostic marker in patients with type 2 diabetes mellitus without hypothyroidism shows a rather moderate diagnostic value. It was reported that neopterin, a key molecule taking part in organization of immune response, energy expenditure and the continued rise levels of neopterin are considered a risk indicator for cardiovascular disease and its complications.

CONCLUSIONS

The elevated levels of neopterin in type 2 diabetic patients with hypothyroidism and without hypothyroidism play as a regulator of energy consumption, leading to the hypothesis that thyroid gland disease may influence its level. In addition, the potential for use as a diagnostic marker for diabetics with thyroid disease due to the occurrence of complications of diabetes, including cardiovascular diseases.

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CONFLICT OF INTETEST

The Authors declare no conflict of interest

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High-energy Nd: YAG laser therapy for papulopustular rosacea: Quantitative assessment with digital image processing

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ABSTRACT

Aim: To provide a quantitative assessment of the clinical effectiveness of the high-energy Nd:YAG laser therapy versus traditional pharmacotherapy in patients with papulopustular rosacea.

Materials and Methods: The study included 40 patients with clinically diagnosed papulopustular rosacea. Participants were divided into two subgroups: one received standard pharmacotherapy with systemic retinoids, and the other underwent fractional Nd:YAG 1064 nm laser treatment. Clinical outcomes were assessed using digital image analysis (ImageJ software), with erythema quantified in pixel units and relative facial area involvement. Statistical evaluation was performed with Student's t-test, considering $p < 0.05$ as significant.

Results: Both treatment modalities demonstrated clinical improvement; however, outcomes were more pronounced in the laser-treated group. Traditional pharmacotherapy achieved a 22% reduction in erythema area, while Nd:YAG laser therapy resulted in a 79% reduction. The proportion of erythematous facial area decreased from 24.4% to 16.7% in the pharmacotherapy group and from 27.6% to 5.7% in the laser group. No systemic or local adverse effects were reported.

Conclusions: Nd:YAG FRAC3® laser therapy demonstrated superior efficacy over pharmacotherapy in reducing erythema and inflammatory manifestations in papulopustular rosacea. These findings support its role as an effective and safe alternative or adjunct to standard pharmacological management.

KEY WORDS: papulopustular rosacea; Nd:YAG 1064 nm laser; erythema; digital image analysis; retinoids; laser therapy

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INTRODUCTION

Papulopustular rosacea (PPR) is a chronic inflammatory skin disorder marked by persistent facial erythema with papules and pustules, but without comedones as in acne vulgaris [1]. It usually develops between 30 and 50 years of age, is more common in women, and carries significant psychosocial impact [2, 3].

The pathogenesis involves dysregulated innate immunity, neurovascular reactivity, and alterations of the skin microbiome [3]. Overexpression of cathelicidin peptides, increased Demodex folliculorum density, and environmental triggers such as UV exposure and stress contribute to recurrent inflammation.

Standard therapy includes topical agents (ivermectin, azelaic acid, metronidazole), systemic antibiotics, and isotretinoin in resistant cases. Newer approaches—such as microencapsulated benzoyl peroxide, biologics targeting interleukin pathways, and Nd:YAG laser therapy—offer promising alternatives [4].

Despite therapeutic advances, PPR remains a relapsing condition that requires optimized, mechanism-based management strategies [5, 6].

AIM

The aim of the study is to evaluate the comparative effectiveness of standard pharmacotherapy and high-energy Nd:YAG 1064 nm (FRAC3 mode) laser therapy in patients with papulopustular rosacea, with objective assessment of erythema dynamics using digital image analysis.

MATERIALS AND METHODS

The clinical investigation was conducted on patients diagnosed with papulopustular rosacea (PPR). A total of 40 individuals were selected following comprehensive clinical, laboratory, and instrumental evaluations aimed at confirming the diagnosis and ruling out comorbidities or contraindications to systemic and laser-based therapies. All participants provided written informed consent regarding both treatment procedures and photographic documentation. The study cohort was balanced for age and sex distribution.

Inclusion in the PPR group required the presence of persistent centrofacial erythema accompanied by

Table 1. Baseline demographic and clinical characteristics of the study cohorts

Indicator, Unit of Measurement	Groups		
	PPR	PPR-Nd	P _{PPR-PPR-Nd}
Number, n	20	20	p > 0.05
Age, years	37±5.1	38±4.8	p > 0.05
Gender, males, n (%)	3 (15.0)	4 (20.0)	p > 0.05
TC, mmol/L	4.1 ± 0.82	4.1 ± 0.76	p > 0.05
LDL-C, mmol/L	2.36 ± 0.65	2.38 ± 0.60	p > 0.05
TG, mmol/L	3.85 ± 0.26	3.45 ± 0.24	p > 0.05
BMI, kg/m ²	22.5 ± 4.0	23.0 ± 4.21	p > 0.05
ESR, mm/hour	7.67 ± 3.2	5.8 ± 4.62	p > 0.05
D-dimer, ng/L	0.27 ± 0.12	0.26 ± 0.15	p > 0.05
SBP, mmHg	126.2±5.3	130.3±7.4	p > 0.05
HbA1c, %	4.5±0.62	5.0±0.68	p > 0.05
Glucose, mmol/L	4.8±0.87	5.0±0.82	p > 0.05
Hemoglobin, g/L	149.1±7.89	154.3±9.62	p > 0.05
Erythrocytes, 10 ¹² /L	5.0±0.83	4.9±0.92	p > 0.05

Source: compiled by the authors of this study

Table 2. Comparative analysis of objective digital erythema parameters across patient groups

Groups	PPR	PPR-Nd	P
S, Pixels	96622.78 ± 83805.56	101022.2 ± 6295.77	p > 0.05
S_percentage_of_affected_area, %	24.44 ± 20.33	27.68 ± 12.25	p > 0.05

Source: compiled by the authors of this study

Table 3. Variation in mean erythema surface area before and after one month of treatment in different study arms

Groups	S1, Pixels	S2, Pixels	ΔS, %	p
PPR	96622.78 ± 83805.56	65500.11 ± 6968.87	22.22	p < 0.05
PPR-Nd	101022.2 ± 6295.77	20944.4 ± 7556.78	79.10	p < 0.05

Source: compiled by the authors of this study

Table 4. Ratio of erythematous area to the overall facial surface: mean values across treatment groups

Groups	S_selected_area, Pixels	S1_percentage_of_affected_area, %	S2_percentage_of_affected_area, %	p
PPR	354268.22 ± 167526.69	24.44 ± 20.33	16.7 ± 10.2	p<0.05
PPR-Nd	386856.5 ± 124369.96	27.68 ± 12.25	5.72 ± 5.37	p<0.05

Source: compiled by the authors of this study

inflammatory papules, pustules, and localized edema. These features occurred against the background of vascular alterations characteristic of rosacea, differentiating the condition from acne vulgaris by the absence of comedones. Patients also reported subjective symptoms such as burning or stinging sensations. The diagnosis was established by a board-certified dermatologist based on standard clinical criteria.

Participants were stratified into two therapeutic subgroups:

Conventional pharmacological therapy (PPR group). Oral isotretinoin (Roaccutane, F. Hoffmann-La Roche, Switzerland) administered at 0.5–1.0 mg/kg body

weight once daily. Dosage adjustments were made according to body weight and tolerability. Female participants of reproductive age were enrolled under the mandatory pregnancy prevention program.

Laser therapy (PPR-Nd group): High-energy Nd:YAG laser treatment (Fotona Dualis SP II, Slovenia) performed in fractional FRAC3 mode.

Objective assessment of treatment efficacy was performed through standardized digital photography under constant lighting and exposure conditions [7]. Image analysis was carried out using ImageJ software (version 1.54k, NIH, USA). Regions of interest (ROIs) were selected from affected facial zones. The erythema



Fig. 1. Digitalized representation of the erythematous zone in a patient with papulopustular rosacea

Picture taken by the authors

index was quantified by measuring the proportion and mean intensity of red pixels per square area (PPI – pixels per inch). Comparative analysis was conducted using pre-treatment and one-month post-treatment images.

Additional indices, including the red-to-green channel intensity ratio (R/G ratio), were calculated to provide a relative measure of vascular inflammation. Baseline laboratory tests were performed for all participants to exclude systemic comorbidities. These included complete blood count, lipid profile, fasting glucose, HbA1c, and D-dimer assays. Results were within reference ranges, confirming the localized inflammatory nature of the disease.

RESULTS

The assessment of therapeutic response in PPR was carried out by objective digital analysis of erythema severity [8]. Quantification was based on the measurement of red channel intensity within predetermined

regions of interest (ROI) from standardized clinical photographs. A decline in red pixel intensity following treatment was interpreted as attenuation of the underlying inflammatory process.

To evaluate dynamic changes, a delta-percentage method was applied using the following equation:

$$\Delta = \left| \frac{X_{fin} - X_{init}}{X_{init}} \right| * 100$$

where X_{fin} represents the post-treatment value and X_{init} is the baseline value.

Relative reduction of the erythematous surface area was determined by the formula:

$$\Delta S = \frac{S_1^{\square} - S_2}{S_1^{\square}} * 100\%$$

with S_1 denoting the area of erythema prior to intervention and S_2 the corresponding area after intervention.



Fig. 2. Sequential visual documentation illustrating changes in erythema extent in a PPR patient during therapy
Picture taken by the authors



Fig. 3. Quantitative digital assessment of erythema dynamics in a PPR patient, based on pixel analysis
Picture taken by the authors

Beyond mean values, additional insight was obtained by generating histograms of red channel distribution within selected ROIs. Therapeutic improvement was reflected by a leftward shift of the dominant peak toward lower intensity values, consistent with reduced erythema severity. Heat-map visualization, produced by applying LUT filters such as “Red Hot” in ImageJ, provided qualitative confirmation of the observed changes.

As a complementary index, the Red-to-Green ratio (R/G ratio) was computed to characterize the predominance of redness relative to the baseline skin tone. A decline in this parameter following treatment was indicative of progressive normalization of the cutaneous

condition. All quantitative data are presented as mean $\pm$ standard deviation ($M \pm SD$).

According to patient histories and clinical examination (Table 1), the first noticeable signs of papulopustular rosacea typically began as persistent erythema on the central face, most often on the cheeks, which subsequently extended to the upper and lower thirds of the facial region.

Unlike the erythematotelangiectatic form, the papulopustular subtype was consistently accompanied by inflammatory papules, pustules, and variable edema of the affected skin. In 83.3% of patients, the lesions recurred repeatedly, while one-third (33.3%) demon-

strated clear tissue infiltration. A smaller proportion (16.6%) also reported subjective symptoms such as itching, burning, or discomfort. The representative clinical appearance of these patients is illustrated in Fig. 1.

Quantitative image analysis confirmed the more pronounced inflammatory profile of PPR. The baseline erythematous surface area in patients from the PPR and PPR-Nd groups measured $96,622.78 \pm 83,805.56$ pixels and $101,022.2 \pm 62,957.7$ pixels, respectively (Table 2). An additional confirmatory parameter, the relative proportion of erythematous involvement to the total facial surface area (*S_percentage_of_affected_area*), averaged $24.44\% \pm 20.33$ in the PPR group and $27.68\% \pm 12.25$ in the PPR-Nd group. These values demonstrate the predominance of diffuse erythema combined with inflammatory elements in papulopustular rosacea.

In line with the study protocol, patients were allocated to different therapeutic strategies:

PPR group (pharmacotherapy): Received oral isotretinoin (Roaccutane, F. Hoffmann-La Roche, Switzerland) at a dosage of 0.5–1.0 mg/kg daily. Dosing was adjusted based on body weight and tolerability. In all patients, treatment was well tolerated, with no systemic or local adverse reactions and no cases of hypersensitivity to the active substance or excipients.

PPR-Nd group (laser therapy): Treated with Nd:YAG laser in FRAC3 fractional mode (1064 nm), applying both focal and diffuse regimens. Cooling was provided via Zimmer CRY06 or ice packs to minimize thermal discomfort.

Since erythema is the primary clinical hallmark of rosacea but lacks standardized clinical grading, digital image analysis was employed to objectively monitor treatment response. Standardized photographs were processed in ImageJ, allowing pixel-based quantification of erythema intensity and surface distribution (Fig. 2, Fig. 3).

After one month of treatment, significant improvement was documented in both subgroups, though the magnitude of response differed. In the pharmacotherapy group, the mean erythematous area decreased by 22.22%, reflecting moderate but measurable improvement. In contrast, the laser-treated group (PPR-Nd) exhibited a far greater therapeutic effect, with an average reduction of 79.1% in erythematous surface area (Table 3).

To correct for potential anthropometric bias, an additional parameter was analyzed: the ratio of erythematous surface area to the total facial area. Both therapeutic modalities demonstrated statistically significant reductions (Table 4). In the PPR group, the ratio decreased from $24.44\% \pm 20.33$ at baseline to $16.7\% \pm 10.2$ post-treatment. In the PPR-Nd group, the same pa-

rameter declined from $27.68\% \pm 12.25$ to $5.72\% \pm 5.37$.

Collectively, these findings demonstrate that although conventional pharmacological therapy achieves partial improvement, high-energy Nd:YAG FRAC3 laser therapy produces a substantially more pronounced and rapid reduction in erythema and inflammatory lesions. Thus, Nd:YAG laser treatment may serve as a highly effective alternative or adjunct to systemic pharmacotherapy in patients with papulopustular rosacea.

DISCUSSION

Papulopustular rosacea (PPR) represents a chronic inflammatory phenotype of rosacea characterized by persistent erythema, papules, pustules, and localized edema. Conventional systemic therapy, such as isotretinoin or tetracycline derivatives, primarily targets sebaceous activity and inflammatory pathways, yet often provides only partial and delayed reduction of vascular symptoms [6]. In the present study, isotretinoin treatment produced a modest decrease in erythematous surface area (22%), confirming its limited efficacy against the persistent vascular component of PPR.

By contrast, Nd:YAG FRAC3 laser therapy achieved a substantial improvement, with an average erythema reduction of nearly 80%. The superior efficacy of Nd:YAG can be attributed to its deeper dermal penetration (1064 nm) and selective absorption by hemoglobin, allowing targeted coagulation of dilated vessels and attenuation of perivascular inflammation [9]. This dual effect not only diminishes background erythema but also facilitates regression of papules and pustules, aligning with prior reports that highlight the advantages of vascular laser therapy over pharmacologic interventions for inflammatory rosacea [10].

The strengths of this study include the use of digital imaging and quantitative pixel-based analysis, which minimized subjectivity and provided reproducible measures of erythema intensity and area. However, limitations should be acknowledged: the modest sample size and relatively short follow-up do not allow conclusions regarding long-term efficacy or recurrence.

Taken together, these findings support Nd:YAG FRAC3 laser therapy as a highly effective treatment for papulopustular rosacea, offering greater clinical benefit than systemic isotretinoin and representing a valuable adjunct or alternative within a comprehensive therapeutic strategy [10].

CONCLUSIONS

1. The application of digital image processing with ImageJ proved to be a valid and reproducible

method for objective quantification of erythema in papulopustular rosacea.

2. Systemic isotretinoin therapy resulted in a moderate improvement with a 22.2% reduction of erythematous area, while high-energy Nd:YAG 1064

nm FRAC3 laser therapy demonstrated superior efficacy, achieving a 79.1% reduction of erythema and marked regression of inflammatory lesions, confirming its role as an effective alternative or adjunct to pharmacotherapy.

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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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Modulation of renal FOXO3 gene expression by Urolithin A in a rat model of renal ischemia-reperfusion injury

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ABSTRACT

Aim: This work uses a male Wistar Albino rat model of experimentally induced renal ischemia-reperfusion injury (IRI) to examine the nephroprotective potential of Urolithin A.

Materials and Methods: Twenty-eight rats (N=7) were randomly assigned to four groups: DMSO pre-injection as a vehicle, bilateral renal IRI for 30 minutes followed by two hours of reperfusion as a control, sham (laparotomy without IRI), and treatment (Urolithin A pre-injection for three days). ELISA and histological analysis were used to assess biomarkers of kidney injury, oxidative stress, inflammation, and apoptosis.

Results: While GSH levels were enhanced, Urolithin A therapy primarily decreased TNF- α , IL-1 β , MDA, Caspase-3, and KIM-1 levels. Furthermore, the group treated with Urolithin A showed a substantial downregulation of FOXO3 expression. Histopathological results verified that the therapy group had less kidney damage.

Conclusions: These findings suggest that Urolithin A produces nephroprotective effects against IRI via modulating oxidative stress, inflammation, and apoptosis, particularly through the control of FOXO3. This study suggests that Urolithin A is a good treatment candidate for renal IRI control.

KEY WORDS: Urolithin A, IRI, IL-1 β , TNF- α , KIM-1, FOXO3

Wiad Lek. 2025;78(12):2648-2660. doi: 10.36740/WLek/214626 DOI

ABBREVIATIONS

UA: urolithin A

I/R: ischemia reperfusion),

KIM-1: kidney injury molecule 1

TNF- α : tumor necrosis factor alpha

IL-1 β : interleukin -1 beta

FOXO3: Forkhead Box O3

DMSO: dimethylsulfoxide

INTRODUCTION

One of the main causes of acute kidney injury (AKI) is renal ischemia-reperfusion damage (IRI), and the inflammatory response has a significant impact on the pathophysiology of this condition [1]. Reactive oxygen species (ROS) overproduction is the primary cause of ischemia-reperfusion injury after reoxygenation, which can lead to a variety of cellular consequences, including necrosis, apoptosis, and the release of injury-associated proteins that trigger the inflammatory response [2–3]. Two main mechanisms mediate apoptosis: the extrinsic pathway, which is initiated by death receptors belonging to the tumour necrosis factor (TNF) receptor

family, and the intrinsic pathway, which is principally controlled by mitochondrial signals [4]. The dynamic process of ischemia-reperfusion-induced kidney injury is marked by complex interactions between oxidative stress, lipid peroxidation, and inflammatory mediators, all of which exacerbate tissue damage [5]. Because it phosphorylates FOXO3, a transcription factor that controls apoptosis, the oxidative stress response, and cell cycle progression, the PI3K/AKT signaling pathway is essential to this process [6]. ROS overproduction is a sign of oxidative stress, which damages renal cells by causing lipid peroxidation and depleting vital antioxidants like glutathione (GSH). As a byproduct of lipid peroxidation, the buildup of malondialdehyde (MDA) serves as a biomarker for oxidative stress and is linked to the degree of kidney damage. These reactive oxygen species have the potential to cause apoptosis, energy imbalances, and mitochondrial malfunction [7]. Furthermore, the inflammatory response is exacerbated by inflammatory cytokines including interleukin-1 beta (IL-1 β) and tumour necrosis factor-alpha (TNF- α), which are important mediators in the development of acute kidney injury (AKI). Biomarkers such as kidney injury

molecule (KIM-1), which provide insight into the degree of ischemia-reperfusion injury, are commonly used to assess renal function. Multifunctional compounds, particularly those with anti-inflammatory and antioxidant properties, are thought to be highly effective in reducing kidney damage. Their capacity to alter intracellular pathways linked to the dynamics of oxidative stress is primarily responsible for their protective role [8–10]. Antioxidant therapy has also shown promise in shielding cells from oxidative damage brought on by infrared radiation. These substances may provide protective advantages by restoring compromised intracellular functions associated with oxidative stress [11]. Prophylactic measures can lessen the impact of IRI, which often causes inflammation and may exacerbate kidney impairment. The pathophysiology of renal disorders is significantly influenced by the main pro-inflammatory cytokines, specifically interleukin-1 beta (IL-1 β), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF- α). In a rat model of ischemia-reperfusion injury, this study aimed to assess the renal protective effectiveness of UA through the analysis of multiple biomarkers, such as Kidney Injury Molecule-1 (KIM-1), IL-1 β , TNF- α , GSH, malondialdehyde (MDA), Caspase-3, Foxo3 gene expression, and histopathological analysis.

AIM

This work uses a male Wistar Albino rat model of experimentally induced renal ischemia-reperfusion injury (IRI) to examine the nephroprotective potential of Urolithin A.

MATERIALS AND METHODS

PREPARATION OF ANIMALS

In this study, 28 adult male Wistar Albino rats weighing ([20–22] weeks, 300 $\pm$ 50 g). The animals were kept under standard laboratory conditions, which comprised a 12-hour light/dark cycle, unfettered access to food and water, a controlled temperature of 25°C, and a relative humidity of 60–65%. Every step involved in the care and experimentation of animals is carried out in compliance with accepted ethical standards for the use of animals in research. The rats were kept in the University of Kufa's College of Sciences' animal facility. The sources of the experimental materials were Macrogen (Korea), Sun-long Technology Lab (China), and Med-Chem-Express.

ETHICAL STATEMENT

This study was carried out at the University of Kufa's Middle Euphrates Unit for Cancer Research, Faculty

of Medicine, and Department of Pharmacology and Toxicology, Faculty of Pharmacy. Ethical approval was granted by the Central Bioethics Committee at the University of Kufa.

All procedures involving the handling and experimentation on rats, as well as the conducted tests, were carried out in compliance with the applicable guidelines and regulations for the ethical use of animals, Kufa University (20545 in 29/8/2024). The animals were housed in the animal facility at the College of Sciences, University of Kufa.

UA

UA is a secondary metabolite of ellagic acid, a polyphenolic antioxidant, exhibiting antiproliferative, anti-inflammatory, and antioxidant properties [13]. The pomegranate is a significant source of ellagitannins, a category of natural polyphenols that are converted to Urolithin by gut microbiota [14]. Walnuts and raspberries are abundant in ellagitannins [15]. The pure powder of UA was gutted from med-Chem-express, USA Company. Formal Name: 3,8-dihydroxy-6H-dibenzo[b,d]pyran-6-one, Chemical Name: UA, Molecular Formula: C₁₃H₈O₄, CAS Number: 1143-70-0, Molecular Weight: 228.2, Physical Description: A crystalline solid, Color: Yellow, Solubility: In DMSO: 30 mg/ml. DMSO was considered the standard vehicle for preparation this drug before use. The dose of drug that used was 10 mg/kg of rat weight intraperitoneally [16].

EXPERIMENTAL DESIGN

Four groups of seven Wistar Albino rats each were randomly assigned to this experiment, and each group was given a different set of treatment procedures. The ischemia-reperfusion injury (IRI) technique was not performed on the sham group, which served as the negative control. By clamping the renal pedicles for 30 minutes, the control group which served as the positive control—was subjected to bilateral renal ischaemia. For two days in a row before sacrifice, the vehicle group received intraperitoneal injections of DMSO once *per* 24 hours. On the third day, they also received a dose an hour before the induction of ischaemia. This group also underwent 30 minutes of renal ischemia followed by two hours of reperfusion for two days prior to sacrifice, the UA-treated group received an intraperitoneal injection of 10 mg/kg of UA once daily, with an additional dosage administered an hour before ischaemia was produced on the third day. These injections were given at the same intervals as the vehicle group.

RENAL ISCHEMIA REPERFUSION INJURY RAT MODEL

Rats were kept on a heat plate at 37°C after being sedated with intraperitoneal ketamine (100 mg/kg) and xylazine (10 mg/kg). To reveal the renal pedicles, a midline abdominal incision was done following shaving and antiseptic washing. Clamping both renal pedicles for 30 minutes caused bilateral renal ischemia. For hydration, one milliliter of warm, sterile saline was injected intraperitoneally. Clamps were taken off for reperfusion after ischemia, and the incision was sutured and covered with sterile gauze soaked in saline to avoid dehydration. Following bilateral nephrectomy, the kidneys were rinsed with ice-cold phosphate-buffered saline (PBS) to remove residual blood. The rats were then euthanized via cardiac puncture [17]. The left kidney was sectioned sagittal into two halves: one half was immediately stored at -80 °C for subsequent bimolecular analysis, while the other half was fixed in 10% formalin and later embedded in paraffin for histopathological examination.

PREPARATION OF TISSUE FOR MEASUREMENT OF IL-1B, TNF-A, GSH, MDA, CASPASE-3 AND KIM-1 BY ELISA

After completing the surgical procedure, the left kidney was carefully removed from the sacrificed rat and rinsed thoroughly with chilled normal saline to clear any remaining blood. The kidney was then bisected, and one half was processed for biochemical analysis. This tissue was homogenized in a 1:10 (w/v) ratio of phosphate-buffered saline supplemented with 1% Triton X-100 and a protease inhibitor cocktail, using a high-intensity ultrasonic liquid processor [18]. The prepared homogenate was subjected to centrifugation at 17,530 ×g for 20 minutes at a temperature of 4°C. The supernatant was collected to quantify interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), GSH, malondialdehyde (MDA), KIM-1, and Caspase-3 using the enzyme-linked immunosorbent assay (ELISA) technique, conducted with a Bio-Elisa Reader (Bio-Tek Instruments, USA).

KITS AND REAGENTS

Human IL-1β (Thermo Fisher/Invitrogen, cat. KAC1211), human TNF-α (Abcam, cat. ab46087), total GSH (Elabscience, cat. E-EL-0026), MDA (competitive ELISA; ELK Biotech, cat. ELK8658), human KIM-1 (Elabscience, cat. E-EL-H6029), and human caspase-3 (Elabscience, cat. E-EL-H0017) were procured via regional distributors servicing Iraq and stored at 2-8 °C. All kits were re-

search-use only. Substrates (TMB), stop solution, wash buffers, and plate sealers were those supplied with each kit.

TISSUE SAMPLING FOR HISTOPATHOLOGY ANALYSIS AND DAMAGE SCORES

After preserving a portion of the left kidney in 10% neutral buffered formalin (NBF), tissues were processed by standard methods (graded alcohol dehydration, xylene clearing, and paraffin embedding). Sections (4–5 μm) were cut and stained with hematoxylin and eosin (H&E). Microscopic examination was performed by a pathologist blinded to group allocation, and semi-quantitative scoring was performed by a second investigator, also blinded. Tubular injury was assessed at the corticomedullary junction in 10 non-overlapping fields per section at 40x total magnification. Injury was scored on a 0–4 scale adapted from established IRI scoring systems [20–21]: 0 = normal histology; 1 = focal injury in <10% of tubules (loss of brush border, epithelial swelling/blebbing); 2 = injury in 10–25% of tubules; 3 = injury in 26–50% of tubules with occasional casts; 4 = injury in >50% of tubules with widespread casts and frank necrosis. Each animal's score was the mean of all fields examined. Additional qualitative features (e.g., tubular epithelial enlargement, vacuolar degeneration, necrotic tubules, and cast formation) were documented as supportive findings [19].

ASSESSMENT OF TISSUE FOXO3 GENE EXPRESSION BY RT-QPCR

1. Total RNA extraction. Total RNA was isolated from ~30 mg renal cortex using [Easy-spin™ (DNA-free) Total RNA Extraction Kit; Manufacturer, Country; Cat. No. XXXX] following the manufacturer's protocol (including on-column DNase treatment). RNA quantity and purity were assessed by spectrophotometry (A260/A280 and A260/A230), and integrity was verified by agarose gel electrophoresis.
2. cDNA synthesis. [X μg] of RNA per sample was reverse-transcribed using [AddScript (or equivalent) cDNA Synthesis Kit; Manufacturer, Country; Cat. No. XXXX] in a [final volume X μL] with random hexamers/oligo(dT)/mixed primers [choose] according to the manufacturer's instructions ([temperature/time profile]). No-RT controls (minus reverse transcriptase) were included to exclude genomic DNA amplification
3. Primers and amplicons. Primers for Foxo3 (target) and [reference gene; e.g., Gapdh or Actb] were

Table 1. Primers used in this study

Host		Gene		5'-3'	Product (bp)	Accession number	Reference
Rattus	—	FOXO-3	F	AAAGGGGAAATGGGCAAAGC	83	XM_032888496.1	[22-23]
			R	GGCTGAGAGCAGATTGGCA			
Rattus	—	GAPDH	F	ATGACTCTACCCACGGCAAG	89	NM_017008	
			R	CTGGAAGATGGTGATGGGTT			

Source: Own materials

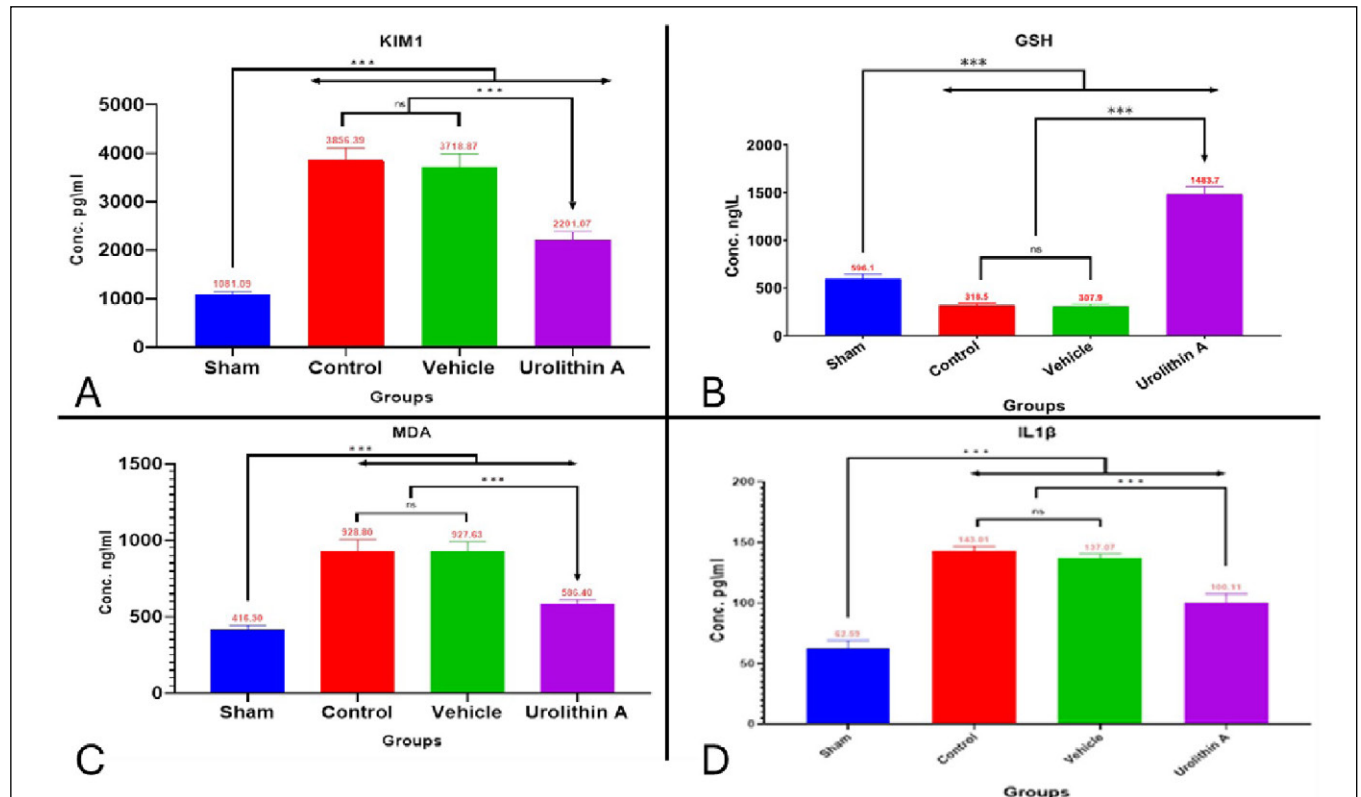


Fig. 1. Statistical of KIM1, GSH, MDA, and IL-1β; **A:** Statistical analysis of KIM-1 conc. (pg/mL) in renal tissues was made across the four experimental groups, each consisting of seven rats. The results showed a highly significant difference between the sham group and both the vehicle and control groups ($***P < 0.001$). Similarly, the UA-treated group showed a statistically significant decrease in KIM-1 levels when compared to the vehicle and control groups ($***P < 0.001$), **B:** The mean conc. of GSH in renal tissues, measured in ng/L, were statistically analyzed among the four experimental groups, each comprising seven rats. The sham group showed significantly higher GSH levels compared to the vehicle and control groups ($***P < 0.001$). Likewise, the UA-treated group exhibited a marked increase in GSH concentrations relative to the vehicle and control groups ($***P < 0.001$), showing a strong antioxidative effect, **C:** The statistical evaluation of malondialdehyde (MDA) conc., expressed as mean values in ng/mL, was conducted across the four experimental groups, each consisting of seven rats. The sham group showed significantly low MDA levels compared to both the vehicle and control groups ($***P < 0.001$). Similarly, rats treated with UA exhibited a notable reduction in MDA conc. relative to the vehicle and control groups ($***P < 0.001$), indicating a strong attenuation of oxidative stress, **D:** The mean concentrations of interleukin-1 beta (IL-1β) in renal tissues, measured in pg/mL, were statistically analyzed at the conclusion of the study across four animal groups, each containing seven rats. A highly significant increase in IL-1β levels was observed in both the vehicle and control groups compared to the sham group ($***P < 0.001$). Conversely, treatment with Urolithin A resulted in a significant reduction in IL-1β concentrations compared to the vehicle and control groups ($***P < 0.001$), demonstrating its anti-inflammatory potential.

Source: Own materials

synthesized by [Vendor, Country; Cat./Order No. XXXX]. Amplicon lengths were [X] bp (Foxo3) and [X] bp (reference). Primer specificity was confirmed by melt-curve analysis and a single band on gel. Primer efficiencies (standard curve, $R^2 \geq 0.99$) were [90–110]%. Full sequences, amplicon sizes, accession numbers, and efficiencies are provided in Table 1.

4. Primers Used in this Study [1, 2]Stefanie</author><author>Wolloscheck, Tanja</author><author>Hölter, Philip</author><author>Wengert, Alexander</author><author>Grether, Markus</author><author>S-ticht, Carsten</author><author>Weyer, Veronika</author><author>Wolfrum, Uwe</author><author>Spesert, Rainer%J Journal of neurochemistry</author></authors></contributors><titles><title>Transcriptional

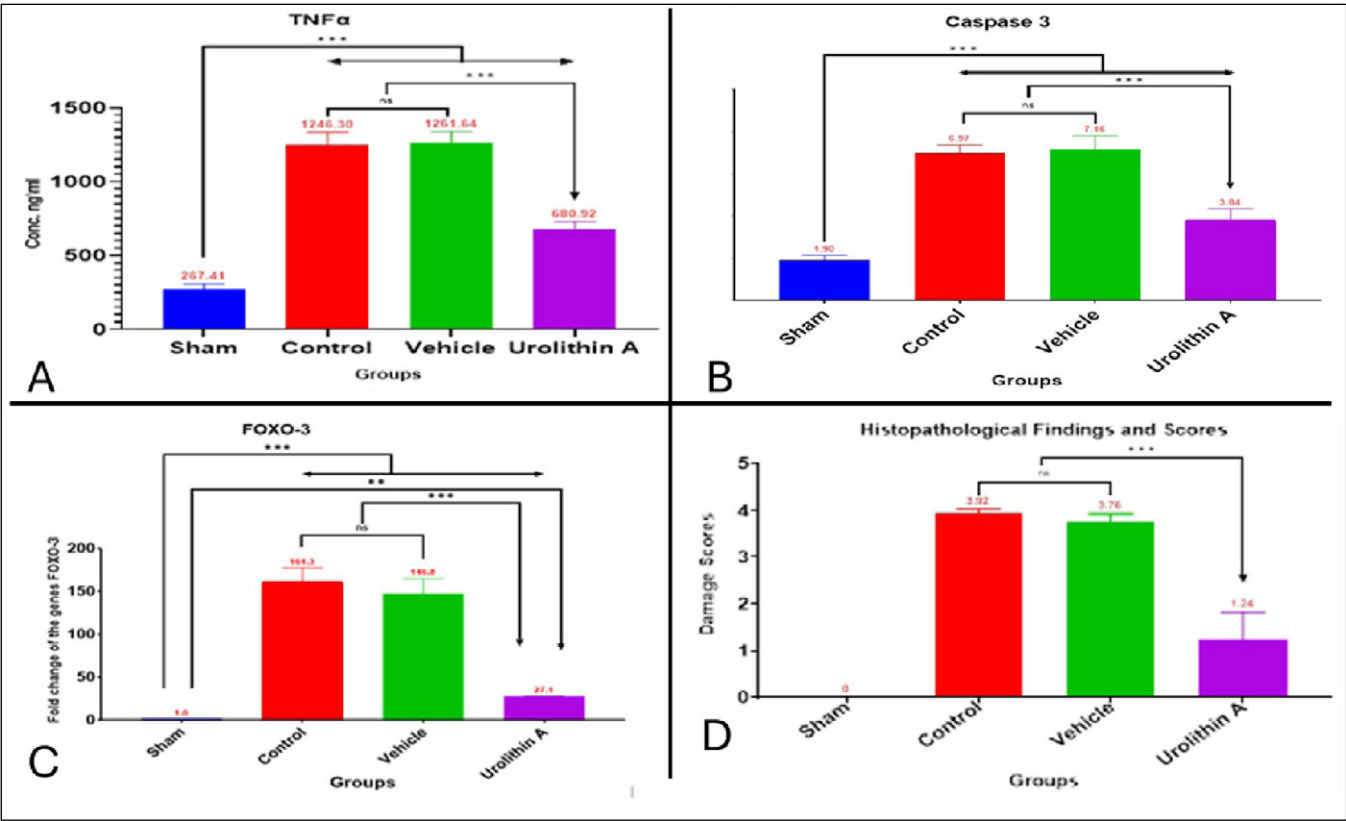


Fig. 2. Statistical of TNF α , caspase-3, Mean of fold change of FOXO3, and Score severity mean of renal tissue histopathology Statistical comparisons of TNF- α , Caspase-3, FOXO3 gene expression, and renal histopathological scores among experimental groups; **A:** Mean concentrations of TNF- α (ng/L) in renal tissues across the four study groups at the end of the experiment (n = 7 per group). Significant differences were observed between the sham group and both the vehicle and control groups (***P < 0.001), as well as between the UA-treated group and the vehicle and control groups (***P < 0.001); **B:** Mean Caspase-3 levels (ng/L) in renal tissues among the four experimental groups (n = 7 each). Both the sham and UA groups showed significantly lower levels compared to the vehicle and control groups (***P < 0.001); **C:** Fold change in FOXO3 gene expression in renal tissue across the four groups at the study endpoint (n = 7 per group). Significant downregulation was observed in the UA and sham groups compared to vehicle and control groups (***P < 0.001); **D:** Mean histopathological severity scores of renal tissue in the four groups (n = 7 each). Both the sham and UA-treated groups exhibited significantly reduced scores relative to the vehicle and control groups (***P < 0.001).
Source: Own materials

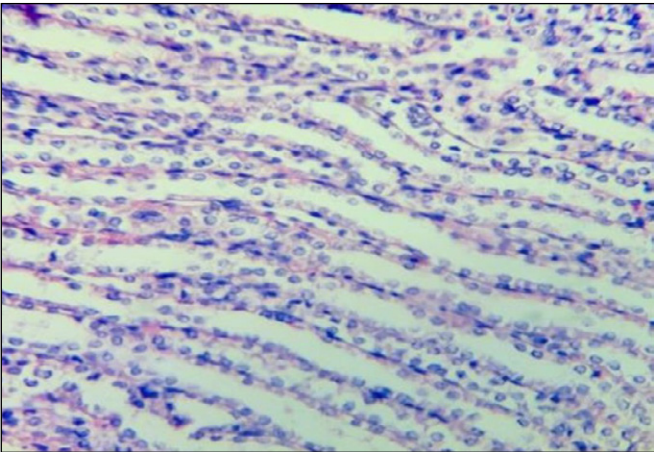


Fig 3. Sham group, a microscopic cross section of left kidney represented normal tissues histology, normal renal tubules, and normal cell size and there are no cast formation, cells edema or loss of brush boarder. Sham group. H & E stain $\times 400$.The mean of the severity score was zero (score severity mean = 0 and represent 0% of damage renal tubules)
Source: Own materials

analysis of rat photoreceptor cells reveals daily regulation of genes important for visual signaling and light damage susceptibility</titles><pages>757-769</pages><volume>124</volume><number>6</number><dates><year>2013</year></dates><isbn>0022-3042</isbn><urls></urls></record></Cite></EndNote>

5. Protocol of GoTaq[®] RT-qPCR System for Real-Time qPCR (Gene expression assay).

STATISTICAL ANALYSIS

Statistical analysis was conducted using GraphPad Prism and SPSS version 28.0 for Windows. Results were presented as mean $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) was employed to compare differences among the groups, followed by Bonferroni post-hoc tests for pairwise comparisons. For evaluating histopathological changes in kidney tissue,

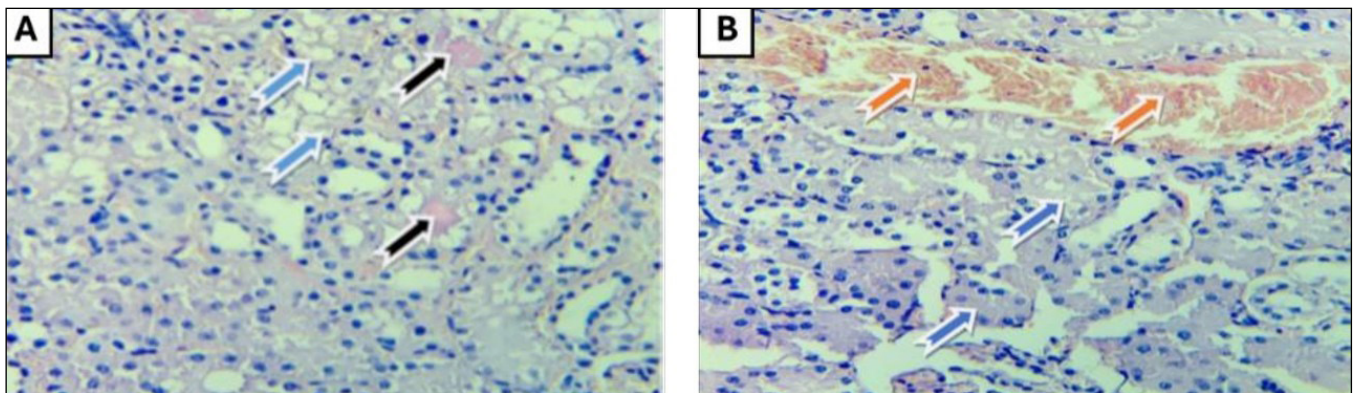


Fig. 4. (A) Control group. Rat kidney, renal tubules with score 4 damage involving 100% of the examined tubules. Cytoplasmic vacuoles (blue arrows) and eosinophilic cast (black arrows). H&E. X400, figure (B) Control group. Rat kidney, renal tubules with score 4 damage involving 100% of the examined tubules. Vascular congestion (orange arrows) and cytoplasmic eosinophilia (blue arrows). H&E. X400

Source: Own materials

the Kruskal-Wallis test was applied to determine the significance of differences in the overall severity scores across groups. A p-value of ≤ 0.001 was considered to indicate a statistically significant difference.

RESULTS

EFFECTS OF UA ON-KIDNEY FUNCTION PARAMETERS (KIM-1)

The renal tissue level of (KIM-1) in control and vehicle groups were significantly ($p < 0.001$) higher than that in the sham group, On the other hand, the level in the UA treated group is significantly ($p < 0.001$) lower than that in the control group, figure (1A).

EFFECT OF UA ON THE OXIDATIVE STRESS MARKER GSH

The mean GSH levels were significantly lower in the Control and control vehicle group compared to the UA treated groups ($P < 0.001$) while slightly elevation when compare with sham group, figure (1B).

EFFECT OF UA ON THE OXIDATIVE STRESS MARKER MALONDIALDEHYDE (MDA)

The renal tissue level of MDA in control and vehicle groups were significantly ($p < 0.001$) higher than that in the sham group, On the other hand, their levels in the UA were significantly ($p < 0.001$) lower than that in the control group, figure (1C).

EFFECT OF UA ON INFLAMMATORY MARKER INTERLEUKIN -1 BETA

There was a significant ($P < 0.001$) difference in IL-1 β levels among the studied groups. The sham group had the lowest

levels than all other groups ($P < 0.001$ for all comparisons). Conversely, IL-1 β levels were significantly ($P < 0.001$) lower in the UA treated group compared with control groups, figure (1D).

EFFECT OF UROLITHIN A ON THE INFLAMMATORY MARKER TUMOR NECROSIS FACTOR-ALPHA (TNF-A)

The renal tissue level of (TNF- α) in control and vehicle groups were significantly ($p < 0.001$) higher than that in the sham group. On the other hand, their levels in the UA were significantly ($p < 0.001$) lower than that in the control group, figure (2A).

EFFECTS OF UROLITHIN A ON THE MARKER OF APOPTOSIS, CASPASE3

The renal tissue level of Caspase3 in control and vehicle groups were significantly $p < 0.001$ higher than that in the sham group, On the other hand, their levels in the UA were significantly ($p < 0.001$) lower than that in the control group, figure (2B).

THE EFFECT OF UROLITHIN A ON THE INTRACELLULAR SIGNALING (FOXO3) AFTER IRI BY PCR TECHNIQUE

The renal tissue levels of FOXO3 expression in control and vehicle groups were significantly ($p < 0.001$) higher than that in the sham group, Meanwhile, the expression level of FOXO3 in the UA treated group is significantly ($p < 0.001$) lower than that in the control group, figure (2-C).

HISTOPATHOLOGY FINDINGS

Results of the histopathological examination of renal tissues from the four study groups are shown in figures (2D, 3, 4A-B, 5-6)

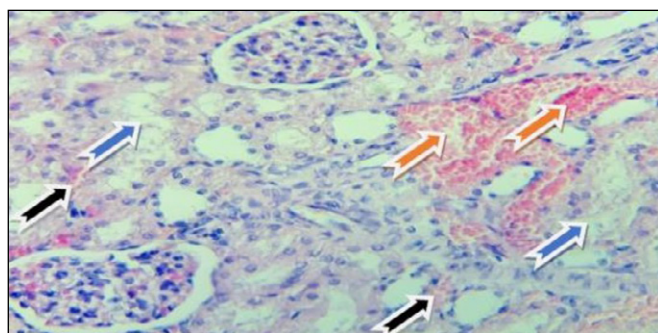


Fig. 5. Vehicle treated group. Rat kidney, renal tubules with score 4 damage involving 95% of the examined tubules. Cytoplasmic swelling and increased cytoplasmic eosinophilia (black arrows), cytoplasmic vacuoles (blue arrows), hemorrhage (orange arrows). H&E. X400

Source: Own materials

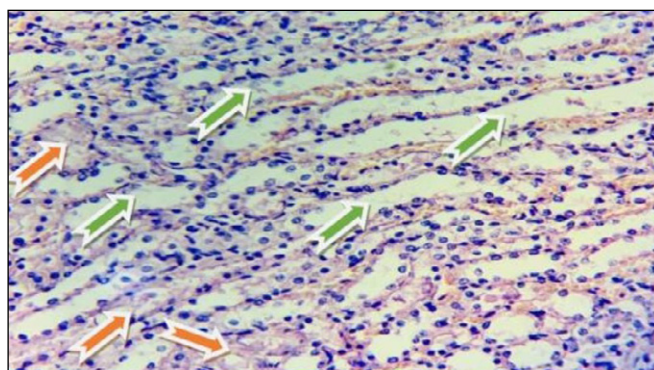


Fig. 6. UA treatment group. Rat kidney with score 1 damage involving 25% of the examined tubules, Damaged tubules (orange arrows) and normal tubules (green arrows). H&E. X400

Source: Own materials

DISCUSSION

Acute kidney injury (AKI), a syndrome commonly associated with substantial morbidity and mortality, is largely caused by renal ischemia-reperfusion injury (IRI). The overproduction of ROS is a key contributor to the development of this injury. ROS worsen the condition by causing inflammatory responses, encouraging the release of vasoconstrictive substances, and causing renal tubular cells to undergo both necrosis and apoptosis [24–25]. As a result, treatment approaches that lower ROS levels have attracted a lot of attention from researchers. By evaluating important biomarkers, this study assessed UA's possible nephroprotective benefits in IRI. KIM-1, a sensitive measure of kidney injury, was markedly decreased by UA therapy, indicating a preventive effect. Additionally, UA showed anti-inflammatory properties by reducing levels of Tumour Necrosis Factor-alpha (TNF- α) and Interleukin-1 beta (IL-1 β), which in turn decreased inflammatory responses. Malondialdehyde (MDA) and other indicators of oxidative stress were reduced by UA, while GSH and other antioxidant defenses were strengthened. The study also showed that UA altered the expression of FOXO3, indicating its involvement in cellular survival pathways, and decreased the apoptotic marker Caspase-3, indicating anti-apoptotic effects. These results demonstrate that UA has the potential to reduce IRI-induced kidney damage via anti-inflammatory, anti-apoptotic, and antioxidant mechanisms, making it a promising therapeutic option for the treatment of AKI.

EFFECTS OF IRI AND UA ON KIM-1

Notably, the UA-treated groups in this experiment showed significantly $P \leq 0.001$ lower levels of KIM-1 than the vehicle and control groups. On the other hand, compared to the sham group, the control and vehicle

groups showed a significant rise in KIM-1 levels in renal ischemic tissues. These outcomes are consistent with research by [26], which demonstrated that renal tissue expresses KIM-1, which is markedly upregulated in response to tubular injury. Due to its excellent sensitivity and specificity in identifying kidney injury, KIM-1 has been acknowledged and authorised by the U.S. Food and Drug Administration (FDA) as a trustworthy biomarker for assessing nephrotoxicity. Previous studies have shown that KIM-1 expression occurs on the apical surface of renal proximal tubule epithelial cells in response to ischaemia and toxic damage [27–28]. A possible nephroprotective effect of UA in IRI is suggested by the decreased KIM-1 levels seen in the treatment groups in this investigation. The effect of UA on KIM-1 levels in relation to IRI has not been examined in any prior research. This agent's anti-inflammatory and antioxidant qualities are probably responsible for the decrease in KIM-1 levels seen in this investigation. These results are consistent with the findings of [29], who showed that pioglitazone has an antioxidant effect by lowering oxidative stress, inflammation, and renal tubular damage by targeting multiple cellular pathways, such as TNF- α , NF- κ B signaling, KIM-1, and NGAL pathways.

EFFECTS OF IRI AND UA ON-TUMOR NECROSIS FACTOR-ALPHA (TNF-A)

The findings of this study demonstrated that administering UA prior to the onset of ischemia led to a significant reduction ($P \leq 0.001$) in TNF- α levels within renal tissues subjected to ischemia, when compared to the ischemia-reperfusion (IR) control and vehicle-treated groups. Previous experimental research has reported elevated TNF- α concentrations following 30 minutes of renal ischemia and a subsequent 2-hour reperfusion period in rats. This increase has been associated with

marked alterations in endothelial function, with the rise in TNF- α contributing to the observed endothelial dysfunction [30]. Another study in a rat model showed that the level of the TNF- α gene was highly elevated in injured kidney tissues in rats that are undergone nephrectomy of the right kidney and ischemia in the left kidney for 45 min then reperfusion [31]. On the other hand, growing evidence suggests that UA possesses diverse and potent pharmacological properties, including antioxidant, anti-inflammatory, and anticancer effects. Studies have shown that UA can effectively suppress the activation of NF- κ B triggered by pro-inflammatory cytokines such as IL-1 β and TNF- α , thereby exerting anti-inflammatory effects in colon fibroblast cells [32]. These observations are in agreement with the results of the present study.

EFFECTS OF IRI AND UA ON INTERLEUKIN-1B (IL-1B)

The results in the present study, indicated that the control and vehicle group, which underwent IRI without any therapeutic intervention, exhibited significantly $P \leq 0.001$ elevated levels of IL-1 β . These results are consistent with the previous experimental studies that shown a substantial increase in serum creatinine, Urea, IL-1 β and caspase-3 levels after 30 min of ischemia followed by 2hrs of reperfusion in rats [33]. Conversely, the groups treated with UA demonstrated significantly $P \leq 0.001$ lower levels of IL-1 β compared to both the control and vehicle groups. The observed decrease suggests that UA might have anti-inflammatory properties, possibly by inhibiting the production of IL-1 β . Previous findings agreed with our, demonstrating that UA attenuated the pro-inflammatory factors production (IL-6, IL-1 β and others) in vitro studies [34]. By lowering IL-1 β levels, UA may lessen the inflammatory response linked to IRI and prevent additional renal damage.

EFFECTS OF IRI AND UA ON OXIDATIVE STRESS MARKERS MALONDIALDEHYDE (MDA)

The control and vehicle group, which received IRI without therapeutic intervention, exhibited significantly greater levels of MDA ($P \leq 0.001$) compared to the sham group, as indicated by the results of this experimental rat model study. This rise reflects the oxidative stress induced by ischemia damage. These findings correspond with those reported by [35], indicating that heightened levels of ROS, diminished antioxidant defenses, reduced superoxide dismutase (SOD) activity, and increased malondialdehyde (MDA) collectively exacerbate oxidative stress and tissue damage in renal IRI. The MDA concentrations in the

UA-treated groups were considerably lower ($P < 0.001$) compared to the control and vehicle groups, suggesting a beneficial antioxidant activity. The decrease in MDA levels suggests that UA may possess antioxidant properties, possibly via processes that include the neutralization of free radicals or the augmentation of intrinsic antioxidant defenses. These findings align with several research that validate the antioxidant properties of UA. A study indicated that UA alleviates oxidative stress and inflammation in HepG2 cells, a model for hepatocellular carcinoma. UA diminished ROS production by approximately 50%, augmented antioxidant defenses (superoxide dismutase and glutathione peroxidase), and lowered lipid peroxidation (malondialdehyde levels). The findings indicate that UA safeguards against oxidative and inflammatory damage, which are principal factors in the progression of liver cancer. Additional investigations demonstrated the protective effect of UA against oxidative stress in rats experiencing DOX-induced hepatotoxicity. DOX treatment substantially elevated tissue malondialdehyde (MDA) levels, indicating increased lipid peroxidation, whereas UR mitigated this increase and dramatically enhanced antioxidant enzyme activity.

EFFECTS OF IRI AND UA ON ANTIOXIDANT MARKER GSH

GSH is a critical endogenous antioxidant that plays a vital role in protecting cells from oxidative stress by neutralizing free radicals and reactive oxygen species. Previously found data revealed that the level of kidney glutathione in untreated IRI rats was significantly $P \leq 0.001$ lower than that of control rats [37]. Other studies confirmed that the level of GSH significantly decreased, which was accompanied with a significant increase in MDA level of kidney tissue following I/R [38]. Our investigation revealed markedly reduced GSH levels in the control group exposed to IRI, but UA pretreatment resulted in a substantial elevation of GSH levels relative to the control and vehicle groups. This indicates that enhancing GSH production or reducing oxidative stress augments renal antioxidant capability. Cásedas et al. (2020) indicate that these findings align with previous research demonstrating UA's in vitro antioxidant activity, encompassing its antiproliferative and protective effects on HepG2 hepatic cancer cells [39]. The potential antioxidative and neuroprotective properties of UA have been concurrently examined utilizing the Neuro-2a neuroblastoma cell line in mice. The findings indicated that UA exhibited many significant cytoprotective effects alongside robust antioxidant activity, as demonstrated by its results in the ORAC assay (13.1 μ mol TE/mg). UA significantly enhances mitochondrial performance in cells exposed to hydrogen

peroxide-induced oxidative stress (H_2O_2), suggesting a protective role in maintaining cellular energy metabolism. Moreover, it markedly reduced lipid peroxidation, an essential indicator of membrane damage generated by ROS. Moreover, UA enhanced the activity of critical antioxidant enzymes essential for neutralizing ROS and preserving cellular redox equilibrium, including glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx). Significantly, it was discovered that UA might enhance the antioxidant defense system by upregulating the expression of peroxiredoxins 1 and 3 (Prx1 and Prx3), enzymes that mitigate peroxides and protect cells from oxidative harm, in a dose-dependent manner.

EFFECT OF IRI AND UA ON APOPTOTIC MARKER (CASPASE3)

This experimental study confirmed a significant $P \leq 0.001$ increase in Caspase3 levels in renal tissue of both the I/R control and vehicle groups relative to the sham group following IRI. These findings supported earlier research by [18] who found that the level of Caspase3 was increased after 30 minutes of ischemia followed by 2 hours of reperfusion in rats. Another studies in a rat model appeared that the level of Caspase3 was highly elevated in injured kidney tissues in rats that undergone ischemia for 45 minutes then reperfusion for 24 hours and ischemia for 60 minutes then reperfusion for 2 hours, respectively [40-41]. In contrast, this study revealed that the pretreatment with UA before ischemia induction can significantly $P < 0.001$ down-regulate the expression of caspase-3 in injured renal tissues in comparison with those in both control and vehicle groups. The results of the present study align with the findings of [42], which demonstrated that UA significantly enhanced cell viability and reduced intracellular ROS levels in a dose-dependent fashion in SK-N-MC cells. Additionally, UA was shown to lower the Bax/Bcl-2 ratio and suppress the expression of key apoptotic markers, including cytochrome c, cleaved caspase-9, cleaved caspase-3, and cleaved PARP. This reduction in Caspase-3 levels indicates UA may exert anti-apoptotic effects, potentially through mechanisms that involve the inhibition of pro-apoptotic signaling pathways or the enhancement of cell survival pathways. By decreasing the levels of Caspase-3.

EFFECT OF IRI AND UA ON THE MOLECULAR PROTEINS FOXO3

In the present study we found that the renal tissues from the control and vehicle groups, which underwent

IRI without any treatment, exhibited significantly $P \leq 0.001$ higher levels of FOXO3 expression compared to the sham group. In contrast, the groups treated with UA exhibited a significant reduction ($P \leq 0.001$) in FOXO3 gene expression within renal tissues. FOXO3 is a transcription factor broadly expressed across various tissues in the body and plays a critical role in regulating cellular homeostasis, including responses to oxidative stress and apoptosis. Its activity is tightly controlled by the PI3K/AKT signaling pathway; when phosphorylated by this pathway, FOXO3 becomes inactivated and is retained in the cytoplasm. Upon dephosphorylation, however, it translocate into the nucleus, where it functions as a transcriptional regulator of genes involved in cell survival, oxidative defense, and apoptosis. The observed downregulation of FOXO3 in the UA-treated groups may reflect modulation of this pathway, possibly contributing to reduce apoptotic signaling and enhanced cellular protection in renal tissues [43] several studies have demonstrated that antioxidants can activate the PI3K/AKT pathway, leading to enhanced phosphorylation of AKT at specific sites such as Ser473 and Thr308. This activation results in downstream effects that promote cell survival, reduce apoptosis, and enhance cellular antioxidant capacity. Meng et al., 2021, demonstrated that the activation of the PI3K/AKT signaling pathway plays a critical role in reducing renal cell apoptosis, which is a significant factor in the development of AKI after AOLT. Specifically, the nuclear FoxO3-mediated transcription of pro-apoptotic genes Bim and FasL was implicated in renal cell apoptosis, and the activation of PI3K/AKT was shown to counteract this effect. Also [44] showed that Oleanolic Acid has been shown to reduce oxidative stress, which is a significant contributor to renal injury during ischemia and reperfusion. By activating the PI3K/AKT pathway, OA may enhance the expression of antioxidant enzymes and reduce the production of ROS, thereby protecting renal cells from oxidative damage. Another study reported by [45] Previous research found that UA enhances cardiac function in myocardial ischemia/reperfusion (I/R) injury by activating the PI3K/AKT signaling pathway. UA treatment increased phosphorylation of PI3K and AKT, an effect blocked by the PI3K inhibitor LY294002, suggesting its anti-apoptotic role in cardiomyocytes during hypoxia/reoxygenation injury. These findings align with our study, which explored UA's Reno protective effects in AKI caused by renal IRI. Notably, UA had not been previously investigated in this model. Our results demonstrated significant kidney protection, highlighting UA's potential as a novel therapeutic agent for ischemia-reperfusion-induced renal injury.

EFFECT OF IRI ON KIDNEY PARENCHYMA

Histological analysis revealed an increase in tissue damage after IRI, as shown by the presence of Cytoplasmic swelling and increased cytoplasmic eosinophilia, vacuolization, dilated renal tubules, glomerular alterations and hemorrhage, as well as a dilatation of the Bowman's capsule and a lack of brush boundaries. These changes had been in agreement with some other studies [46-49] that showed the same histopathological changes.

EFFECT OF UA ON KIDNEY PARENCHYMA

This investigation revealed that UA pretreatment significantly reduced kidney injury severity ($P < 0.001$) in comparison to the control and vehicle groups. Histological research revealed modest kidney damage in the UA-treated group, while the control and vehicle groups displayed severe harm. These findings align with previous research on UA's protective properties against cisplatin-induced nephrotoxicity, demonstrating that UA maintained renal architecture and markedly reduced tubular injury. The efficacy of UA as a therapeutic agent for renal preservation during chemotherapy and ischemia-reperfusion injury was further substantiated by quantitative histological assessments. These findings underscore UA's capacity to mitigate cisplatin-induced nephrotoxicity, which is crucial for enhancing patient safety during oncological therapies [50]. Another study cited in [51] demonstrated that UA has considerable protective benefits against kidney damage generated by D-galactose in aging rats. The model group exhibited indications of apoptosis, including tubular ne-

crosis and basement membrane thickening, alongside significant glomerular and tubulointerstitial damage. The high-dose UA (150 mg/kg) restored these changes, restoring renal morphology to values nearly equivalent to those of the control group. The efficacy in mitigating renal failure induced by aging and oxidative stress was evidenced by histological scoring, which revealed decreased inflammatory cell infiltration and necrosis in the treated animals. These results indicate that UA holds potential as a therapeutic agent for preserving kidney health in age-related conditions. The [52] indicated that UA exhibited dose-dependent protective effects against DOX-induced hepatic injury in rats. Histopathological enhancements including decreased necrosis, inflammation, and vacuolization, alongside near-normal liver architecture at elevated doses (5 mg/kg). These observations underscore UA's potential as a therapeutic drug for maintaining hepatic tissue integrity under toxic stress.

CONCLUSIONS

Together, these findings support UA's potential as a therapeutic agent for the treatment of AKI. Its numerous protective benefits are highlighted by its capacity to lower kidney injury indicators, inhibit inflammation, boost antioxidant defenses, and alter apoptotic pathways. These findings' translational significance emphasizes the necessity of additional research to prove UA as a workable medical treatment, which will ultimately enhance patient outcomes in critical care and nephrology settings.

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AVAILABILITY OF DATA AND MATERIALS

All data are available upon request

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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Intima-media thickness, telomere length and neuropsychological status: is there any connection?

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ABSTRACT

Aim: To determine the relationship between telomere length and telomerase activity and indicators of oxidative stress in patients with CA and T2DM.

Materials and Methods: to investigate relationship between telomere length and cognitive function and identify predictors of cognitive impairment in patients with cerebral atherosclerosis and type 2 diabetes mellitus. The MMSE and MoCA scales, which are widely used in most modern epidemiological and clinical studies, were used for screening and assessment of cognitive disorders.

Results: Patients were divided into 2 groups: I – with scores on MMSE scale < 26 (moderate cognitive deficit, 26 people), II – with scores on MMSE scale ≥ 26 (mild cognitive deficit, 135 people). As a result of analysis, it was found that length of telomeres was statistically significantly shorter in patients of group I, the patients studied less, they had more pronounced situational anxiety, more pronounced intima-media thickness in both carotid arteries, and according to MoCA scale, they had pronounced impairment of cognitive functions ($p < 0.05$). Group II patients had longer telomeres, studied longer, and had high personal anxiety.

Conclusions: patients with cerebral atherosclerosis and type 2 diabetes mellitus with mild cognitive impairment have longer telomeres, high personal anxiety, and a higher level of education.

KEY WORDS: telomerase activity, cerebral atherosclerosis, MMSE, type 2 diabetes mellitus, cognitive function

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INTRODUCTION

Impairment of cognitive functions is one of the most significant characteristics of cerebrovascular pathology, the frequency of which increases with age and with the development of type 2 diabetes mellitus (T2DM). Age is extremely important in determining the functional state of the body and its response to environmental influences. The study of cellular and molecular changes in the blood that coordinate the development of premature cellular aging will make it possible to establish biomarkers associated with this process [1-7]. The study of telomere length, telomerase activity and the genes that regulate these indicators (TERT, TERF1, TERF2) is an important link in the study of biological aging. A number of previous studies have revealed a connection between stress and shortening of telomere regions, a relationship between telomere length and the development of cardiovascular pathology and dementia [5, 6, 9]. On the other hand, shorter telomere length is observed in patients with a combination of prediabetes/diabetes and atherosclerotic diseases compared to cases of isolated diabetes or cardiovascular disease [10, 11]. Olivieri F, et al. showed that patients with diabetes who had

a myocardial infarction had shorter telomere lengths compared to those who had diabetes but did not have a history of myocardial infarction [12-17]. Thus, telomere length can be considered as a biomarker of aging or stress. With age, cognitive functions decline and telomere length shortens. Cognitive deficits accompany the aging process and may be related to telomere length. S. E. Harris et al. found an inverse relationship between the verbal test and telomere length [8]. However, no significant correlations were observed with tests of nonverbal reasoning, verbal declarative memory, and dementia screening in patients aged 79 years. Contrasting with this is the study by Valdes et al., where, in a study of 382 female patients with a wide age range (mean age 50 years, range 19 to 78 years), a correlation was found between telomere length and cognitive functions: four out of six cognitive tests, except recognition images and spatial memory tasks [17]. These studies demonstrate that telomere length is associated with cognitive function and may be a biomarker of cognitive aging in patients before the onset of dementia.

There are a large number of various tests for the psychometric diagnosis of disorders of cognitive functions.

The MMSE technique is widely used in most modern epidemiological and clinical studies for screening and assessment of cognitive disorders, which confirms its validity and suitability. At the same time, the question of the role of telomere length in the progression of cognitive functions in elderly people with cerebral atherosclerosis (CA) and T2DM remains relevant.

AIM

The aim of our study is to determine the relationship between telomere length and telomerase activity and indicators of oxidative stress in patients with CA and T2DM.

MATERIALS AND METHODS

The comprehensive clinical and instrumental study involved 161 patients with the grade of 1–3 CA. The diagnosis “Cerebral Atherosclerosis” (CA) has been formulated in accordance with the classification of atherosclerosis of World Health Organization since 2015 and confirmed by laboratory and instrumental research (Doppler ultrasonography of cerebral arteries, brain magnetic resonance imaging (MRI)).

STUDY DESIGN

Simple, prospective, non-randomized, with sequential inclusion of patients.

The study did not include patients with all forms of atrial fibrillation, uncorrectable blood pressure (BP) >160/90 mm Hg. Art., other rhythm disturbances requiring antiarrhythmic therapy, a decrease in EF <40% assessed by two-dimensional echocardiography (Echocardiography), severe heart failure, renal and hepatic impairment, drug or alcohol dependence, and those who had been suffering from acute inflammatory diseases during previous month. The patients who underwent revascularization as a result of unstable angina or myocardial infarction or rheumatic heart diseases did not participate in the study as well.

All patients underwent generally accepted clinical, laboratory (general blood and urine analysis, determination of lipid profile, creatinine, urea, glucose, aspartate aminotransferase, alanine aminotransferase, bilirubin) and instrumental examination (transthoracic echocardiography, electrocardiography, ECG, transcranial Doppler ultrasound and brain MRI).

ETHICS

The study protocol was approved by the ethics commit-

tees of the Institute of Endocrinology and Metabolism and the Institute of Gerontology NAMS of Ukraine. All participants gave the written informed consent. The Declaration of Helsinki (2000) and the applicable national standards regarding their participation in research were taken into account.

COLLECTION AND STORAGE OF BLOOD SAMPLES

Blood samples were taken in the vacutainers containing EDTA. Within 30 minutes after blood sampling, peripheral blood mononuclear cells were isolated on the gradient (1.077 g / cm³). After isolation, the cells were frozen and stored in liquid nitrogen at -196°C. DNA was isolated from thawed cells with the use of the phenol-chloroform purification method [15]. The purity, concentration, and integrity of DNA were checked with the use of spectrophotometry and agarose gel electrophoresis.

MEASUREMENT OF TELOMERE LENGTH (TL)

Relative telomere length (RTL) was measured with the use of monochrome multiplex quantitative polymerase chain reaction (MMQPCR) [4]. The PCR reaction mixture was prepared by using the commercial Luna® Universal qPCR and RT-qPCR reagent kit (New England Biolabs) supplemented with betaine (Sigma-Aldrich) to the final concentration of 1M. For MMQPCR the pair of *telg* and *telc* telomeric primers (the final concentrations of 450 nmol of each) was combined with the pair of *albu* and *albd* primers (the final concentrations of 250 nmol of each) in a master mix. The list of primers used for the MMQPCR is given below in the Table 1.

The thermal cycling profile was as follows: 95°C for 15 minutes; 2 cycles: 94°C – 15 s and 49°C – 15 s; 32 cycles: 94°C – 15 s, 62°C – 10 s, 74°C – 15 s with signal acquisition, 84°C – 10 s, 88°C – 15 s with signal acquisition. The calibration curve was built on the points of four concentrations of reference DNA (in duplicate), which span a range of 27-fold serial dilutions.

All DNA samples were analyzed in triplicates. The amplification curves were generated by the Opticon Monitor 3 software. For this, after thermal cycling and initial data collection, by using the Opticon Monitor 3 software the two standard curves were constructed for each formulation: for the telomeric signal and for the signal of the single copy albumin gene. The RTLs were expressed as the T / S ratio, where T is the quantity of telomeric DNA, and S is the quantity of albumin DNA.

MEASUREMENT OF TELOMERASE ACTIVITY

Telomerase activity was determined by using a tandem repeat amplification protocol with real-time detection (TRAP) [1]. The peripheral blood mononuclear cells and the HEK293 cells (positive control) were

Table 1. List of the primers used for quantitative polymerase chain reaction in real time (MMQPCR)

Primer name	Primer nucleotide sequence
TS	5'-AATCCGTCGAGCAGAGTT-3'
ACX	5'-GCGCGGCTTACCCTTACCCTTACCCTAACC-3'
telg	5'-ACACTAAGGTTTGGGTTTGGGTTTG GGTGGGTTAGTGT-3'
telc	5'-TGTTAGGTATCC CTATCCCTATCCCTATCCCTATCCCTAACA-3'
albu	5'-CGGCGGCGGGCGGCGGGCTGGGCGGAA ATGCTGCACAGAATCCTTG-3'

Source: compiled by the authors of this study

treated with the Invitrogen NP-40 lysis buffer (50 mmol Tris, pH 7.4, 250 mmol NaCl, 5 mmol EDTA, 50 mmol NaF, 1 mmol Na_3VO_4 , 1% Nonidet™ P40 (NP40) 0.02% NaN_3) with 1 mmol PMSF (Sigma-Aldrich) and 10 $\mu\text{l}/\text{ml}$ (v/v) solution with a protease inhibitor (Sigma-Aldrich) on ice for 30 minutes. Subsequent centrifugation was carried out at 16400g for 20 min at +4°C. 180 μl of the supernatant was transferred into a fresh tube. Protein concentration was measured using a Pierce™ BCA Protein Analysis Kit (Thermo Scientific) according to the manufacturer's protocol.

The reaction mixture for TRAP was prepared on the basis of Luna Universal qPCR and RT-qPCR (New England Biolabs) with the addition of EGTA to the final concentration of 5 mM. The final concentrations of primers were 400 nM of ACX and 400 nM of TS. The 2 μl of the lysate were added to 23 μl of the TRAP mixture and incubated for 30 min at 30°C. Then, real time qPCR was performed under the following conditions: 95°C for 1 min; 40 cycles: from 95°C – 15 s, 60°C – 1 min and signal acquisition. The PCR products were quantified using Chromo4 (Bio-Rad) and analyzed with the use of the Opticon Monitor v3.1 software. The HEK293 cells were used to generate the standard curve set on the points of the five dilutions.

MEASUREMENT OF THE ACTIVITY OF CATALASE, SUPEROXIDE DISMUTASE, GLUTATHIONE AND OTHER MARKERS OF OXIDATIVE STRESS

To determine the activity of blood catalase, the blood hemolysate that was obtained by osmotic hemolysis of whole blood with distilled water and single freezing cycle followed by centrifugation was used. The diluted blood hemolysate was incubated with a hydrogen peroxide solution and the catalase activity was determined spectrophotometrically for the amount of the reaction product of the residual hydrogen peroxide with ammonium molybdate. To create the calibration graph, the solution of commercially available catalase was used (Sigma, C9322). The results are expressed in the units of enzyme activity per 1 ml of blood.

The activity of superoxide dismutase (SOD, EC 1.1.15.1.) in the plasma of blood was determined by an indirect spectrophotometric method based on the reaction of superoxide-dependent oxidation of quercetin, in an alkaline environment, in the presence of tetramethylethylenediamine. The reaction was accompanied by discoloration of the working solution in the transmission region with the maximum at 406 nm. The enzyme superoxide dismutase intercepts superoxide radicals and inhibits the oxidation of quercetin. At the incubation time of 20 minutes, the degree of inhibition is strictly quantitatively dependent on the concentration of SOD. The enzyme content in the biological material is calculated using a calibration graph obtained on the basis of measuring the activity of commercially available SOD (Sigma, S9697). The SOD activity was expressed in the units of activity based on the 1 ml of plasma.

The concentration of TBA-active products was measured using the reaction of heating malondialdehyde (MDA) with 2-thiobarbituric acid (TBA) in an acidic medium to form the colored trimethine complex with the maximum of fluorescent radiation at $\mu = 530$ nm under conditions of the light excitation from $\mu = 484$ nm. The blood plasma was incubated in TBA in the presence of trichloroacetic acid with heating. After cooling the samples, TBA-active products were extracted with n-butanol. The fluorescence intensity was measured on a spectrofluorometer. The concentration of TBA-active products (MDA) was calculated according to the calibration curve created using commercial MDA (Sigma, 63287) and expressed as μM per liter. Plasma glutathione (GSH) in was determined by a spectrofluorometric method using orthophthalic aldehyde that results in the formation of highly fluorescent products from GSH, which are excited by the radiation at 350 nm and have the distinct fluorescence peak at 420 nm [16]. The concentration of GSH has been calculated according to the calibration curve created using commercial GSH (Sinbias, Ukraine) and expressed in the units of micromoles per liter. The fluorescence intensity of the glycated proteins in blood plasma was measured by the excitation of 370 nm and emission of 440 nm using

the Varioscan spectrofluorometer, and expressed in the arbitrary units of glycated protein micromoles per liter. Non-enzymatically glycated protein – BSA-glucose was prepared to create the calibration curve. The mixture of BSA and d-glucose in phosphate buffer was incubated at 37°C for 6 weeks.

BASIC MEASUREMENT

Systolic blood pressure (systolic blood pressure) and diastolic blood pressure (diastolic blood pressure) (mmHg) were measured twice using the standard sphygmomanometer in a sitting position after at least 10 minutes of rest. Plasma glucose levels were determined by the standard glucose oxidase method.

NEUROPSYCHOLOGICAL TESTING

Cognitive impairment was assessed using the Mini-mental State Exam (MMSE) scale and the Montreal Cognitive Assessment Scale (MoCA) [17]. Orientation in time and place, concentration of attention and calculation, perception, memory, and language functions were assessed using the 6-item MMSE scale. Absence of cognitive deficit corresponded to 30 points, mild cognitive impairment – 27-26 points, moderate cognitive impairment – 25-24 points, mild dementia – 23-20 points, moderate dementia – 19-11 points, severe dementia – 10 points and less.

Attention and concentration, executive functions, memory, language, visual-constructive skills, abstract thinking, calculation and orientation were evaluated with the help of MoSA. The duration of the MoSA test was 10 minutes, demonstrating cognitive damage was estimated at 26 points or more.

Detection of psychoemotional disorders was carried out using the Spielberger-Khanin anxiety scale. According to the Spielberger-Khanin scale, low anxiety was defined as having 30 points, moderate – 31-45, high – 46 points and more.

HRV was studied on a Schiller AT-10 plus device (Switzerland) using statistical analysis of the time domain and spectral analysis of a short (five-minute) sequence of R-R electrocardiographic intervals at rest. The following parameters of the time analysis were determined: standard deviation (SDNN, ms), standard deviation of the differences in the duration of adjacent R-R intervals (RMSSD, ms). When performing spectral analysis, the following were determined: the total power of the heart rhythm spectrum (tP, ms²), power in the range of 0.00–0.04 Hz (VLF, ms²), 0.04–0.15 Hz (LF, ms²), 0, 15–0.4 Hz (HF, ms²) and lf / hf ratio. The spectral components LF and HF were analyzed both in

absolute values and in the normalized units (i.e.) derived from them, which were automatically calculated by the formulas: $LF_{norm} = LF / (tP - VLF) \cdot 100\%$ and $HF_{norm} = HF / (tP - VLF) \cdot 100\%$. The spectrum structure was also determined as a percentage of the components: % VLF, % LF, % HF.

STATISTICAL ANALYSIS

In order to provide the results in the case of quantitative variables, the average value of the indicator and its standard deviation ($\pm$ SD) for the case of the normal distribution law were calculated. Meantime the median value of the indicator (Me) and the values of the first (QI) and third (QIII) quartile for the case of the distribution law different from the normal were calculated. The distribution was checked for normality using the Shapiro-Wilk test. To represent the qualitative features, their frequency (%) was calculated. When comparing the quantitative indicators in the two groups, the t-criterion (in the case of the normal distribution law), the Mann-Whitney criterion (in the case of the distribution law other than the normal) were used. When comparing quality indicators the Fisher's exact test was used.

The method of building and analyzing logistic regression models was used to analyze the relationship between the T/S indicator (dependent variable) and factor characteristics. The odds ratio (OR) and its 95% confidence interval (95% CI) were calculated to assess the degree of association between dependent and independent variables [1]. To analyze the adequacy of regression models, ROC analysis [1] was performed, and the area under the operating characteristics curve (AUC) was calculated. The threshold of significance in all cases was set at $p < 0.05$.

The statistical analysis was performed with the use of the MedCalc v. 18.10 (MedCalc Software Inc., Broekstraat, Belgium, 1993–2018).

RESULTS

Patients were divided into 2 groups: I – with scores on the MMSE scale < 26 (moderate cognitive deficit, 26 people), II – with scores on the MMSE scale ≥ 26 (mild cognitive deficit, 135 people) (Table 2).

As a result of the analysis, it was found that the telomere length was statistically significantly shorter in patients of group I, the patients studied less, they had more pronounced situational anxiety, more pronounced thickening of the intima-media complex in both carotid arteries, and according to the MoCA scale, they had a pronounced impairment of cognitive functions ($p < 0.05$). Group II patients had longer

Table 2. Clinical-instrumental and laboratory characteristics of patients

Indicator	I group		II group		p
	Me	Q1;Q3	Me	Q1;Q3	
Patients with a stroke, (%)	69.2		42.2		0.04
Patients with a T2DM, %	42.9		44.5		0.16
Fasting blood glucose, mmol/l	4.95±1.86		4.87±1.74		0.76
Glycosylated Hemoglobin (HbA1c), %	6.38±0.76		6.43±0.57		0.82
Duration T2DM, years	4.80±1.78		4.90±2.10		0.80
Catalase	503.92	439.28; 599.68	549.41	446.46; 661.93	0.21
Glutathione (GSH), mkM/l	3.45	3.39; 3.63	3.49	3.38; 3.60	0.85
SOD	8.51	8.15; 8.87	8.43	7.85; 9.23	0.79
Thiobarbituroreactive Substances (TBARs), mcM/l	17.81	16.91; 21.33	17.54	16.03; 19.11	0.35
Glycation End Products (AGE)	27.78	24.54; 33.09	31.66	26.48; 36.45	0.15
TTelomerase activity	2.6	1.96; 3.44	3.2	2.34; 4.29	0.32
Telomere length	2.34	1.91; 2.49	2.74	2.17; 3.58	0.03
Stady, years	15.5	12.00; 16.00	16	15.00; 18.00	0.01
MoCA, points	22	17.00; 22.00	27	25.00; 28.00	0.01
Situational anxiety, points	45	37.00; 45.00	36	32.00; 42.00	0.03
Personal anxiety, points	45	36.50; 46.50	47	42.00; 51.00	0.03
Age, years	65.5	61.00; 79.00	63	59.00; 70.75	0.14
LDL, mmol/l	2.73	2.17; 4.02	2.98	2.73; 4.32	0.14
CIMT right, cm	1	1.00; 1.10	1	0.96; 1.10	0.03
CIMT left, cm	1	1.00; 1.20	1	1.00; 1.10	0.03
Distance per day, km	2	1.00; 3.00	2	1.00; 3.00	0.09
Time watching TV, hour	3	2.00; 5.50	3	2.00; 5.00	0.78
Index HRV	6.50	5.00; 10.00	8	6.00; 9.00	0.17

To present the results in the case of quantitative variables, the mean value of the indicator and its standard deviation ($\pm$ SD) were calculated in the case of a normal distribution law, or the median value of the indicator (Me) and the value of the first (QI) and third (QIII) quartiles in the case of a distribution law that was different from normal $p < 0.05$ - statistically significant differences; CIMT – carotid intima-media thickness, HRV index - heart rate variability index, EDV - end-diastolic volume, EF - ejection fraction, LVMM - left ventricular myocardial mass, E/A and E/E' - indicators of left ventricular diastolic function

Source: compiled by the authors of this study

Table 3. Multivariate analysis of indicators

Independent variables	Regression coefficient, $b \pm m$	The level of significance of the difference of the coefficient from 0, p^*	OR (95% CI)
Years of study	0.31±0.15	0.03	1.37 (1.02–1.84)
IMT	–8.77±3.41	0.01	0.0002 (0.001–0.12)
Telomere length	1.33±0.62	0.03	1.78 (1.13–12.65)

* $p < 0.05$ - statistically significant differences

Source: compiled by the authors of this study

telomeres, studied longer, and had high personal anxiety. Both groups were comparable in terms of age, sex, and telomerase activity ($p < 0.05$).

The method of constructing logistic regression models was used to identify factors affecting cognitive functions. The following categories were used when

building the models: MMSE < 26 (moderate cognitive deficit, 26 patients) – case ($Y=1$), MMSE ≥ 26 (mild cognitive deficit, 161 patients) – non-case ($Y=0$)

It is important to take into account the fact that it is the combination of risk factors, and not each individual risk factor separately, that can lead to telomere shortening

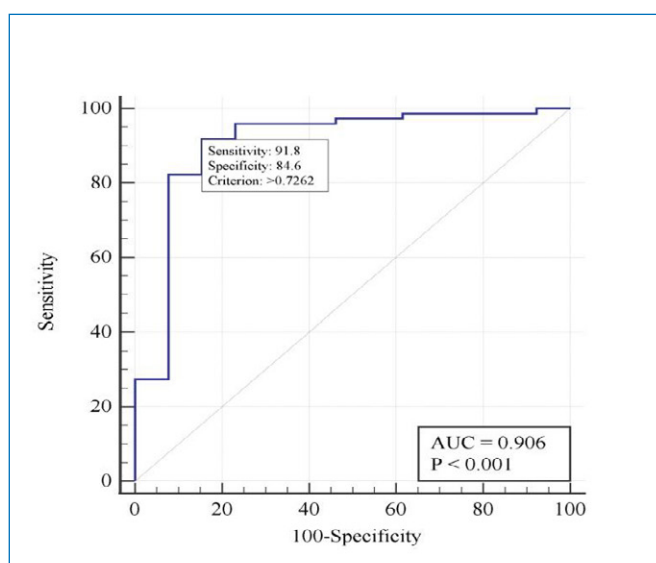


Fig. 1. ROC curve of the 3-factor model for predicting the risk of cognitive decline AUC = 0.91 (95% CI 0.82-0.96)

Picture taken by the authors

[14]. The method of stepwise inclusion/exclusion of features was used to select a set of significant risk factors (Stepwise at the exclusion threshold $p > 0.15$ and the inclusion threshold $p < 0.03$). A multivariate logistic regression model was built on the selected significant risk factors, which included the indicators presented in Table 3.

A good agreement of the selected factor characteristics with the risk of deterioration of cognitive functions was revealed - AUC = 0.91 (CI 0.82 - 0.96) (Fig. 1), which may indicate the completeness of the model and predictors of deterioration of cognitive functions considered in this study. All indicators presented in table 3 are significant in the general multifactor model.

DISCUSSION

The question of the relationship between the carotid intima-media thickness (CIMT) and the biology of telomeres is currently not fully resolved. Some researchers believe that there is no connection between the manifestations of subclinical atherosclerosis and the length of telomeres, emphasizing that shorter telomeres are not associated with atherosclerosis [3, 5]. On the other hand, there are a number of works in which the opposite results were obtained [14, 15]. It can be assumed that the relationship between the length of telomeres and the development of cardiovascular diseases (CVD) can be mediated by the influence of so-called modulating factors that determine the severity of oxidative stress and chronic inflammation. Accelerated shortening of telomeres can lead to aging of the endothelium and the occurrence of atherosclerosis with subsequent development of CVD.

In the literature, there is a rather limited number of works devoted to the study of the connections between cognitive and emotional mental processes with DNA sections that do not encode the amino acid sequence of peptide and protein molecules, but actively participate in the adaptation of the organism to external environmental conditions, aging and determining life expectancy. Telomeres are non-coding regions of DNA. Telomeres are the terminal structures of eukaryotic chromosomes that protect linear chromosomes from degradation and fusion, are involved in the process of cell division and in maintaining the stability of the genome. The telomere consists of repeated double-stranded sections of DNA (5' TTAGGGn-3') and a single-stranded 3' protruding section - the G chain, which, intertwined with the double-stranded section, forms a t loop. The length of a human telomere is 250-1500 repeats of the TTAGGG sequence [11]. As a result of each cell division, telomeres are shortened, which is associated with incomplete replication of chromosome ends [16]. The process of telomere shortening is limited by the so-called Hayflick limit, the limit of the number of divisions of human somatic cells, which die after approximately 50 divisions and show signs of aging when approaching this limit. The shortening of telomere length with age underlies the mechanism of replicative senescence. Maintenance of a certain length of telomeres occurs due to the activity of the enzyme - telomerase, which forms a single functional telomere-telomerase system with telomeres. It is assumed that the mechanism of functioning of the telomere-telomerase system and the gene expression of physiologically active substances associated with this system are at the basis of mental activity processes.

A study of the age-related deterioration of cognitive functions associated with a short leukocyte telomere length in healthy men aged 65-91 showed that it is combined with a change in a number of peripheral blood parameters: a decrease in the concentration of albumin, the level of beta-amyloid, an increase in the content of glucose, CRP and pro-inflammatory IL-6, cystatin C, which is a predictor of chronic renal failure and CVD. The authors suggested that the common cause of shortening of telomeres and deterioration of cognitive functions is oxidative stress associated with mitochondrial dysfunction [9].

Increased inflammatory activity accelerates the shortening of telomeres both due to the activation of cell reproduction and the release of reactive oxygen species [9, 11]. The relationship between telomerase activity and chronic inflammation is ambiguous. Early chronic inflammation through different signaling

pathways (involving NF- κ B, protein kinase C, or Akt kinase) via phosphorylation or transcription of hTERT is able to activate telomerase, which likely compensates for accelerated telomere shortening. However, in the late stages of delayed inflammation, telomerase activity decreases, which leads to telomere shortening [15]. In our study, T2DM was not associated with telomere length and telomerase activity. This is consistent with the results of M. Sampson et al. no connection was found between shortening the length of lymphocyte telomeres and indicators of carbohydrate metabolism. Perhaps this can be explained by the short duration of diabetes and the absence of long-term severe hyperglycemia, since it is long-term hyperglycemia that is associated with the development of severe and persistent oxidative stress. It is important to emphasize that, according to our data, the level of oxidative stress, chronic inflammation and telomerase activity in patients with T2DM did not differ significantly. It can be assumed that with a short duration of T2DM, the genetically determined long telomere length protects patients from the harmful effects of oxidative stress and chronic inflammation, ensuring better

and faster recovery of damaged tissues. In our study, we established: a negative relationship of cognitive decline with the size of the IMT of carotid arteries and positive relationships with the length of telomeres and the duration of education regardless of the presence of T2DM.

CONCLUSIONS

1. Patients with cerebral atherosclerosis and T2DM with moderate cognitive impairment are characterized by short telomeres, more pronounced situational anxiety and thickening of the IMT of the carotid arteries.
2. Patients with cerebral atherosclerosis and T2DM with mild cognitive impairment have longer telomeres, high personal anxiety and a higher level of education.
3. For patients with CA of various stages with T2DM, predictors of cognitive impairment are the length of telomeres, the thickness of the IMT of the common carotid arteries and the duration of training AUC = 0.91 (95% CI 0.82 - 0.96).

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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Effect of some immunological markers on the level of anti-Müllerian hormone (AMH) in women infected with *Toxoplasma gondii*: further investigations

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ABSTRACT

Aim: To explore the effects of *T. gondii* on immune levels and try to find out correlations that maybe manifest later on women who have had the infection and to assess the direct relationship between the infection and a rise in the value of anti-Müllerian hormone (AMH). Specifically, we examined the relationship between AMH and IL-1 β , IL-6, and TNF- α .

Materials and Methods: Blood samples were collected from two identical groups of 80 (40 healthy control and 40 infected with *T. gondii*). The participants were women at an age ranging from 20 to 25 years; Group 1, healthy women who were not infected with *Toxoplasma* comprised the control series; Group 2, women who were *Toxoplasma*-infected and who visited medical facilities. *Toxoplasma* infection was diagnosed using the Latex Agglutination Test (LAT). Serum human interleukins (IL-1 β and IL-6), tumor necrosis factor alpha (TNF- α) and anti-Müllerian hormone (AMH) were detected by Enzyme Linked Immunosorbent Assay (ELISA) method.

Results: Compared with the control group, infected women had significantly higher levels of human IL-1 β , IL-6, and tumor necrosis factor alpha (TNF- α). AMH concentrations correlated significantly in a positive manner with human IL-1 β , IL-6, and tumor necrosis factor alpha (TNF- α).

Conclusions: Three immune parameters (IL-1 β , IL-6, and TNF- α) had elevated circulating levels, which implied the strong association between AMH and these immune variables.

KEY WORDS: toxoplasmosis, IL-1 β , IL-6, tumor necrosis factor alpha (TNF- α), anti-Müllerian hormone

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INTRODUCTION

Toxoplasma gondii (*T. gondii*) is a parasite belonging to the group of inner parasites of cats, for adults are the intermediate host. Symptoms in most instances, patients are often asymptomatic [1]. The parasite *T. gondii* frequently invades the central nervous systems of reptiles, birds and mammals [2], furthermore, the disease may extend to the vaginal tract and skeletal muscles [3-4]. This parasite has a potential to be deadly, as it may lead to acquired toxoplasmosis in organ recipient patients [5]. It also causes congenital toxoplasmosis in the offspring of some domestic and wild animals, such as goats, sheep and pigs [6]. This disease has now been thought to occur in about a third of the population in the whole world. *Toxoplasma gondii* (the etiological agent of toxoplasmosis), a protist most commonly found in those that are also organisms from Apicomplexa, is ubiquitous and well-known [7-8]. It has almost unique ability to behave as a parasite of mammal cells including human and farm animals as well as birds and various marines. Tomasina and colleagues extensive-

ly discussed the pathogenesis of *T. gondii* [9]. The basic mechanism of pathogenesis is that in order to complete it's indefinitely multiplying and disseminating stage within an isolated infected cell, the parasite needs to make the lysis of cells. However, it is precisely this capacity to change from a fast-dividing, disease-causing form (the tachyzoite) to a slow-dividing 'encysted'/latently-infected mode (the bradyzoite) that allows this cunning and canny organism to coexist in relative equilibrium with its host without actually doing it in. Acute toxoplasmosis is caused by the former, but chronic toxoplasmosis can be caused by the latter. In farm animals, especially sheep, toxoplasmosis costs millions of dollars in lost production. Similarly, it has been demonstrated that toxoplasmosis has a significant impact on threatened wild animal populations, with catastrophic ecological consequences and effects of particular severity in marine life [10]. Eating undercooked meat contaminated with bradyzoites that are encysted in the brain or skeletal muscle is a common method by which this parasite is passed to humans and other carnivores [11]. When a naive

host contracts the disease while pregnant, it may also be passed on to the fetus. Besides that, unintentional contact with infective oocysts produced by feline species in their droppings could bring about transmission. Human infertility is the most common clinical manifestation of acute toxoplasmosis. Chronically infected immunocompromised patients may have other clinical manifestations including oral, cervical, or uterine sores, bleeding abnormalities [12], and still other symptoms; ocular hypertension is one such clinical manifestation in a renal transplant recipient diagnosed with molluscum contagiosum [13]. Ophthalmic and neurological complications also occur in persons with chronic toxoplasmosis and HIV. Those with normal immune systems who are congenitally infected or become chronically infected are also at risk of developing ocular toxoplasmosis [14]. In the small intestine, *T. gondii* further develops into rapidly multiplying tachyzoites [15]. Two components of the immune response elicited by this approach anchor the parasite's life cycle. *T. gondii* replicates within immune cells it invades. Following infection and dissemination, the parasite extravasates into a new host where it invades the brain, muscles and other organs [16]. The majority of tachyzoites are cleared in the course of a chronic infection and the parasites differentiate and express a bradyzoite transcriptional profile [17]. In order to survive as the parasite pass through the intestines of the next host a cyst wall is produced that is sugar-based [18]. Therefore, the window of transmission is limited, as the parasite kills the host before this transformation takes place in the absence of a strong immune response. Tumor necrosis factor alpha (TNF- α), interleukin 1 (IL-1 β) and interleukin-6 (IL-6) are key molecules in the onset and progression in innate as well as in adaptive immune response in cells such as endothelium or epithelial cells [19-20]. Inflammasomes also facilitate immunity against microbes, multimolecular interactions, and a variety of cytokines [21]. These kinds of cytokines are secreted by the cells of the innate immune system, e.g. monocytes, dendritic cells, and macrophages [22-23]. Anti-Müllerian hormone (AMH) is an ovarian hormone produced in growing follicles recruited from the primordial follicle pool but have not undergone dominance selection [24]. In view of its ability to reflect ovarian follicular pool volume in a woman of reproductive age, it is considered to be a reliable marker of ovarian reserve. In contrast to other hormonal indexes such as serum FSH, there is little intra- and intermenstrual cycle variability proposed for AMH [25]. In the male fetus, the physiologic degeneration of the Mullerian ducts is induced by AMH. According to Cedars and colleagues AMH can be used as a therapeutic option for screening reduced ovarian reserve [26]. This article following our published work that dealt with AMH level's possible relationship with specific immune markers (IL-2, IL-10, and IL-12) [27].

AIM

The aim of the present study is to explore the effects of *T. gondii* on immune levels and try to find out correlations that maybe manifest later on women who have had the infection and to assess the direct relationship between the infection and a rise in the value of anti-Müllerian hormone (AMH) and to evaluate its putative correlation with other immune markers. Specifically, we examined the relationship between AMH and IL-1 β , IL-6, and TNF- α .

MATERIALS AND METHODS

PARTICIPANTS, ETHICAL APPROVAL AND SCOPE OF THE STUDY

Eighty venous blood samples were obtained from women (20 to 25 years). Women were assigned into two groups (40 women per group; healthy control group and infected group). Women with toxoplasma involved in this study are those who visited used medical care services. Exclusion Criteria: Women with PCOS, autoimmune diseases, hormonal therapies, pregnant or recently pregnant women, women with ovarian pathologies, ovarian malignancies, chronic systemic illnesses, immunosuppressant drugs or corticosteroids or substance abuse.

The study was performed during the period 15th of January -23rd of April 2023. An ethical approval was obtained from the ethical committee in the College of Pharmacy/ University of Alkafeel (protocol number 544, 25/11/2022).

ASSESSMENT OF THE STUDY PARAMETERS

Toxoplasma infection was determined through LAT and AMH as well as inflammatory markers were measured using ELISA. Blood samples were collected in sterile plain tubes. After a 5-minute centrifugation of the blood at 3000 rpm, the serum was transferred into plastic numbered containers and stored at -20°C until several immunological and serological investigations could be performed.

STATISTICAL ANALYSIS

Data are presented as mean $\pm$ standard error of mean (SEM). GraphPad Prism 9.3.1 was used in the analysis and presentation of data. The number of participant (n) is mentioned in the legend of each figure. Student's t-test was performed to compare between healthy controls and infected women. A p-value < 0.05 was considered significant.

RESULTS

This study adds a piece of information supporting our previous published data. Both papers deal with the impact

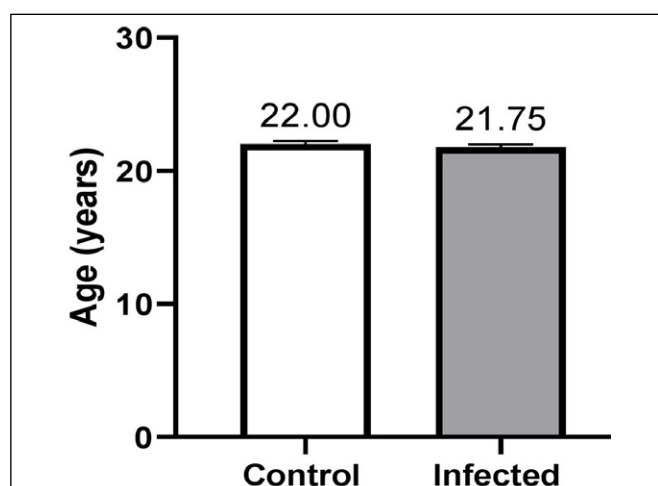


Fig. 1. Age-related distribution of participants in control and infected group, no significant difference was detected ($n=40 \pm \text{SEM}$, t-test)
Source: Own materials

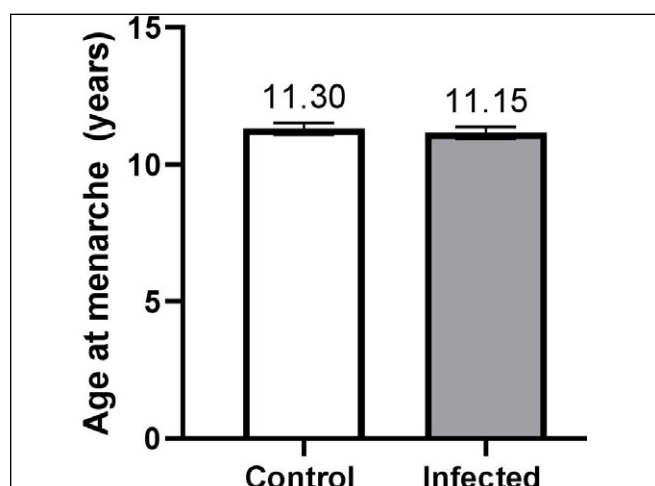


Fig. 3. Age of participants at menarche in control and infected group, no significant difference was detected ($n=40 \pm \text{SEM}$, t-test)
Source: Own materials

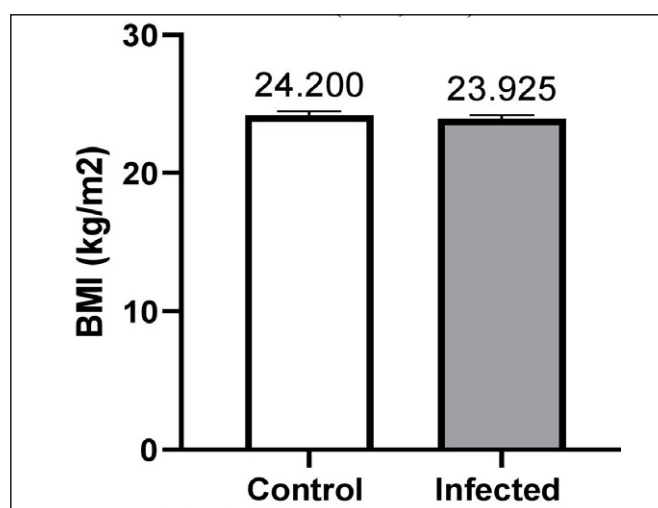


Fig. 2. Body mass index of participants in control and infected group, no significant difference was detected ($n=40 \pm \text{SEM}$, t-test)
Source: Own materials

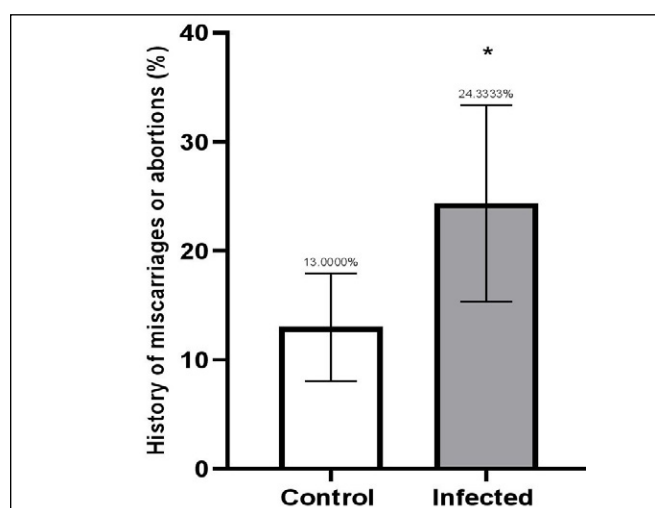


Fig. 4. History of miscarriages or abortions among married women in healthy control and infected women ($n=21$ and $22 \pm \text{SEM}$, respectively, * = significantly different $p < 0.05$ when compared to control, Chi-square test)
Source: Own materials

of *Toxoplasma gondii* infection on a host of inflammatory biomarkers and their association with Anti-Müllerian Hormone (AMH) in women. Similar findings were found that increased AMH is correlated with an increase in the risk of one or more reproductive morbidities. Both studies test the theory that an immune response following *T. gondii* infection changes the levels of AMH, which could be indicative for a possible risk of reproductive disturbances at any later instance in time. This study represents, therefore, also a strong progression and validation of those previous investigations in which is now demonstrating that by using unrelated group of topically relevant proinflammatory markers, the linkage with observed AMH association may be confirmed together with direct clinical evidence for adverse reproductive outcome (history of miscarriage/abortion). Identification also included in this study emphasized the pre-eminence of the higher frequency of miscar-

riages/abortions really reinforcing the original idea, that both infection and its associated inflammatory/endocrine profile were involved in adverse reproductive outcomes.

AGE-RELATED DISTRIBUTION OF PARTICIPANTS

As it is mentioned above, participants involved in this study aged 20-25 years. However, the age made no significant difference between healthy and women infected with *T. gondii* as mentioned in figure (1).

BODY MASS INDEX OF PARTICIPANTS

Body mass index showed no significant difference between healthy and women infected with *T. gondii* as mentioned in figure (2).

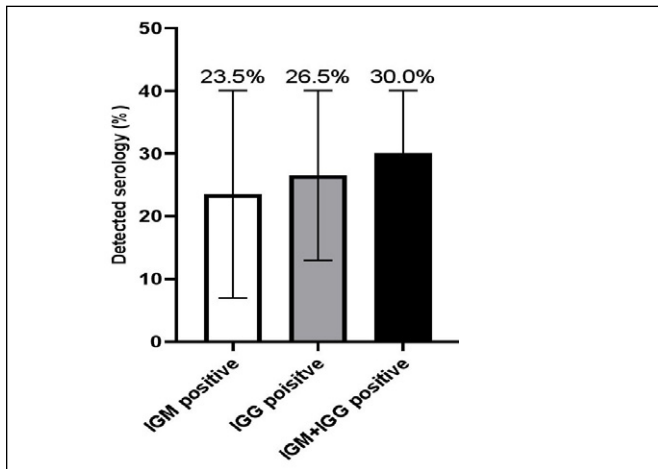


Fig. 5. Serological characterization of antigens against *Toxoplasma* in infected women. It shows that 23.5% of infected women showed serum IGM immunoglobulin, 26.5% of them showed serum IGG immunoglobulin and 30% of women in disease group showed both IGM and IGG immunoglobulin (n=40±SEM).

Source: Own materials

AGE AT MENARCHE

There was no significant difference between control and infected women regarding the age of participants at menarche as mentioned in figure (3).

HISTORY OF MISCARRIAGES OR ABORTIONS AMONG MARRIED WOMEN

Our results showed that 13% of married women in the healthy control group subjected to miscarriages or abortion while it accounted to 24.3% in infected women representing a significant difference between these two groups as shown in figure (4).

SEROLOGICAL TEST FOR TOXOPLASMOSIS

It was found that 23.5% of infected women showed serum IGM immunoglobulin while 26.5% of them showed serum IGG immunoglobulin. On the other hand, 30% of women in disease group showed both IGM and IGG immunoglobulin as shown in figure (5).

SERUM LEVEL OF IL-1B

It was found that the serum level of IL-1 β in infected women is significantly higher than its level in healthy control group as shown in figure (6).

SERUM LEVEL OF IL-6

It was found that the serum level of IL-6 in infected women is significantly higher than its level in healthy control group as shown in figure (7).

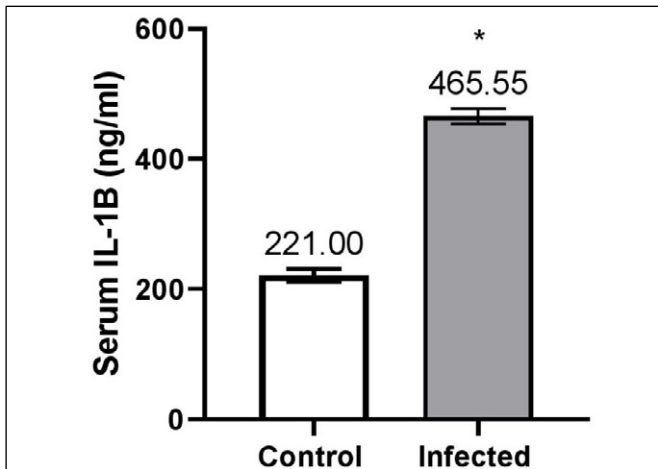


Fig. 6. Serum level of IL-1 β in healthy control and infected women (n=40±SEM, *= significantly different (p<0.05) when compared to control, t-test)

Source: Own materials

SERUM LEVEL OF TNF-A

A higher level of serum TNF-α was reported in infected women compared to healthy control group as shown in figure (8).

SERUM LEVEL OF AMH

A higher level of serum AMH was reported in infected women compared to healthy control group as shown in figure (9).

DISCUSSION

This study documented higher serum levels of IL-1, IL-6 and TNF-α in the infected group with a high AMH levels as compared to the control group. These results may be due to a function of those inflammatory markers involved in host's resistance to *T. gondii* infection, since we have shown that *T. gondii* infection promotes IL-2 release which could be due to the activation of primary human monocytes [28]. This increase might occur because CD4 and CD8 T lymphocyte secrete IFN-gamma spontaneously; as well as the natural killer cells secrete IFN- γ when the infection is starting to encounter dendritic cells and neutrophil leukocyte early in the production of IL-1 [29]. As a consequence, it stimulates natural killer cells to produce interferon (IFN-γ) and is produced in high concentration by T-lymphocyte during chronic infection of toxoplasmosis for prevention of reactive parasite's tissue cyst, and for stimulation of macrophages to which they provide antigen presentation and increase the effectiveness of lysosomes in macrophage cell [30]. In other words, it stimulates the effectiveness of natural killer cells. In a previous study, we reported a relationship between elevated level of human cytokines and elevated level

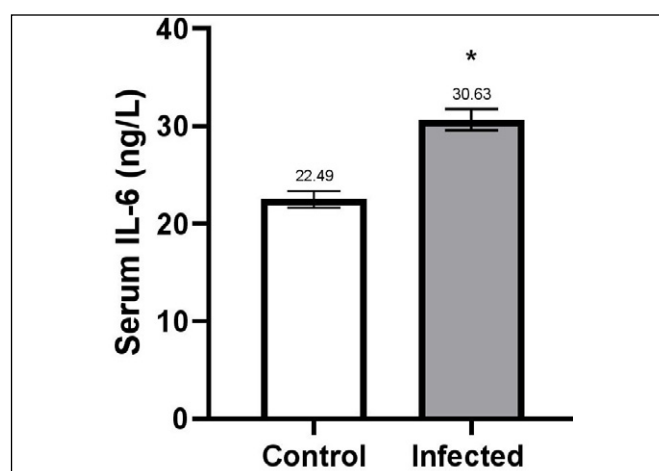


Fig. 7. Serum level of IL-6 in healthy control and infected women ($n=40 \pm \text{SEM}$, * = significantly different ($p < 0.05$) when compared to control, t-test)

Source: Own materials

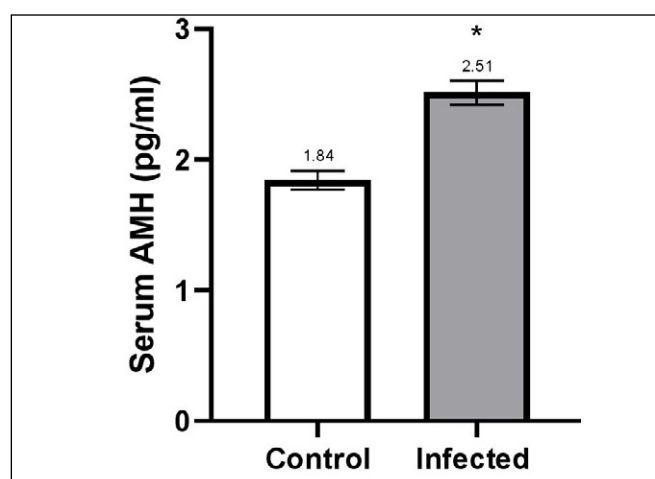


Fig. 9. Serum level of AMH in healthy control and infected women ($n=40 \pm \text{SEM}$, * = significantly different $p < 0.05$ when compared to control, t-test)

Source: Own materials

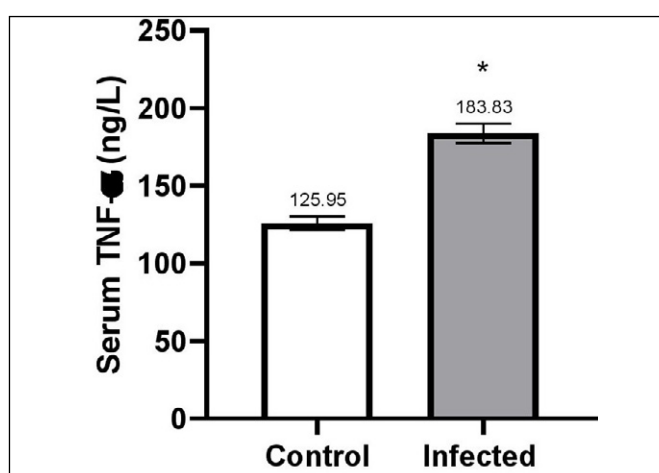


Fig. 8. Serum level of TNF-α in healthy control and infected women ($n=40 \pm \text{SEM}$, * = significantly different ($p < 0.05$) when compared to control, t-test)

Source: Own materials

of AMH in *T. gondii* infection [27]. Elevated level of cytokines in women infected with *T. gondii* was also reported in another study [31]. Higher level of sex hormone has been reported in infected women compared to control whether concurrent increase or decrease [32–33] findings that support the hypothesis that there is a relationship between elevated level of AMH and increased production of inflammatory markers. High levels of immune markers can be linked to the fact that aborted women inflammatory response was increasing and that the neutrophils and lymphocytes were attracted to the endometrium. Zicari and colleagues reported that both neutrophils and lymphocytes in the endometrium can have a pivotal role in the phenomenon of protease induced neurogenic inflammation leading to labor or abortions [34], fur-

thermore, Madhappan and colleagues also hypothesized that the levels interleukins (particularly IL-8) in fetal tissue samples obtained from miscarriage cases were considerably above the inflammatory threshold than from elective abortion women [35], however, the findings obtained by Koumantaki and co-workers contradicted with those belong to Madhappan's team which showed that women who underwent spontaneous abortion had much lower plasma IL-8 levels compared with women carrying normal babies [36]. In addition to that, another research showed that serum levels in women prospectively include with miscarriage women and normal pregnancy women detected no difference [37]. This inconsistency may reflect the amount of data collected or may also be related to the genetic and ethnic variation in the study samples. In addition, the low level of IL-1 may be due to an increased release induced by macrophage, neutrophil, epithelial, and by endothelial cells. As a result, inflammatory components are recruited to the inflamed area. However, Saud and colleagues reported that the concentration of IL-1 was 19.06 ± 1.28 (pg/ml) in control versus 28.80 ± 1.20 (pg/ml) in infected women which still higher regardless of significance [38], it seems more than one factor can influence the serum level of immune markers. Several studies have investigated the relationship between inflammatory markers and AMH. For example, in women with regular menses, Schon and colleagues found that CRP and IL-6 are associated with levels of AMH [39]. In another work, however, where the correlation between serum IL-6 and IL-21 levels and AMH was further studied, it was found that IL-6 and IL-2 levels were negatively correlated with AMH indicating that their level was

higher, and the lower the level of AMH, the worse ovarian reserve function. Such contradictory findings could be related to the diseases itself since Sun and colleagues evaluated the level of inflammatory markers in women with premature ovarian failure not with *T. gondii* infection [40]. It seems that toxoplasma infection participated in the stimulation of immune system including the release of immune markers. The influence of *T. gondii* infection on mast cells was also studied. EunAh and colleagues focused on the generation of pro-inflammatory cytokines (TNF- α , IL-4), chemokines (CXCL8, MCP-1) and NO by mast cells following stimulation with soluble lysate of *T. gondii* tachyzoites [41]. They used RT-PCR and ELISA to measure the production of CXCL8 (IL-8), MCP-1, TNF- α , IL-4. For CXCR-1 and CXCR-2 detection, western blot analysis was carried out. They demonstrated that *T. gondii* lysates induced mast cells of releasing CXCL8, MCP-1, TNF- α , IL-4 and generating NO. This indicates that mast cells are critical to inflammatory responses to *T. gondii*. The results of the current study demonstrate no difference in mean age, body mass index, and age of menarche among control and infection women; however, differences were found in some reproductive and immune parameters. Miscarriages/abortions in married *T. gondii* infected women were significantly higher suggesting a potential deleterious reproductive effect due to the infection. In addition, infected females also presented increased plasma levels of pro-inflammatory cytokines IL-1 β , IL-6 and TNF- α , indicating the presence of an immune response. The serum levels of Anti-Müllerian Hormone (AMH) were also notably higher in the infected compared to the uninfected group, which could be indicative

of disturbed ovarian reserve or immune-mediated endocrine regulation. Taken together, these data highlight the potential role of *T. gondii* in the reproductive health of women and the importance of immune-inflammatory markers in *T. gondii*-induced reproductive dysfunction. Further studies may need to focus on prospective studies to establish causal relationships between *T. gondii* infection, immune parameters, and reproductive outcomes over time. In addition, mechanistic Investigations may be required to explore the molecular and cellular mechanisms by which *T. gondii* influences reproductive hormones, ovarian reserve markers like AMH, and immune responses.

CONCLUSIONS

Three immune parameters (IL-1 β , IL-6 and TNF- α) were circulating with increased concentrations in addition to the already reported raised pro-inflammatory markers, again suggesting a tight link between AMH levels and these cytokines. The study confirms the main assumption that immune response post *T. gondii* infection modifies Anti-Müllerian Hormone (AMH) secretion. Through establishing a confirmed link between AMH and another distinct but important set of proinflammatory markers, the current investigation strongly validates the identified linkage for AMH. Importantly, the finding of more miscarriages/abortions in the infected group is a direct clinical proof of an unfavorable reproductive outcome. This result complements the previous assumption that both infection and its related inflammatory/endocrine profile contribute to the etiopathogenesis of the observed reproductive disorders, predicting potential future morbidities.

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Hormonal and vitamin D metabolic profiles in reproductive-age women with abnormal uterine bleeding and associated extragenital conditions

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ABSTRACT

Aim: To assess hormonal status and vitamin D metabolic features in women of reproductive age presenting with abnormal uterine bleeding and associated extragenital conditions.

Materials and Methods: The study was conducted at the clinical base of the Department of Obstetrics, Gynecology, Pediatric Gynecology, and Medical Genetics of Kharkiv National Medical University (KhNMU). A total of 110 women of reproductive age were examined and divided into two clinical groups. The first group (main group, MG) included 80 patients diagnosed with abnormal uterine bleeding (AUB) in combination with extragenital conditions. The second group (control group, CG) consisted of 30 somatically healthy women who presented for routine preventive examinations. Hormonal status was assessed by measuring serum concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone (PRG), estradiol (E2), and vitamin D using enzyme-linked immunosorbent assay (ELISA) methods. Statistical analysis was performed using the general-purpose software package Statistica 6.0.

Results: The average age of the participants was 31.5 ± 13.5 years. Among women in the main group, the most frequently observed extragenital conditions were diseases of the endocrine, circulatory, and digestive systems. Analysis of serum hormone levels revealed a decrease in FSH (1.84-fold), LH (1.64-fold), and PRG (2.6-fold) (KWT, $p < 0.01$). Conversely, E2 levels were elevated by 3.52-fold in the MG (KWT, $p < 0.01$). Serum vitamin D levels in the MG were reduced by 2.25-fold compared to the CG (KWT, $p < 0.01$). A statistically significant ($p < 0.05$) positive correlation was found between serum E2 and vitamin D levels ($r = 0.61$).

Conclusions: The study revealed distinct features of the hormonal profile and vitamin D metabolism in women of reproductive age with abnormal uterine bleeding and concomitant extragenital conditions. Specifically, this patient population demonstrated decreased serum levels of LH, FSH, PRG, and vitamin D, accompanied by hyperestrogenemia. A statistically significant correlation was observed between serum E2 and vitamin D levels.

KEY WORDS: Abnormal uterine bleeding, reproductive age, extragenital conditions, hormonal profile, vitamin D metabolism

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INTRODUCTION

Bleeding in obstetrics and gynecology is one of the most pressing problems faced by doctors worldwide [1]. Abnormal uterine bleeding (AUB) is the most common type of bleeding in gynecology [2, 3].

AUB affects up to one third of women of reproductive age and is the most debilitating symptom [4]. According to Sun Y et al., 2018 [5], this disorder of the reproductive age is the fourth most common reason for seeking gynecological services [6]. While AUB rarely poses a direct threat to life, it often leads to a substantial decline in quality of life [4].

Among the many causes of AUB, extragenital conditions (ECs) play a certain role. This is due to the characteristics of the endometrium, which depend on changes in the general hormonal background of patients. And the functional state of some organs and systems has a

certain influence on the characteristics of the hormonal background [7, 8]. Thus, AUB can be caused by thyroid dysfunction, uncontrolled diabetes, metabolic disorders, changes in blood clotting (most often von Willebrand disease), various infectious diseases (influenza, pneumonia, Lyme disease, etc.), taking medications (corticosteroids, antipsychotics or antiepileptic drugs), climate change, eating habits, psychotraumatic situations, etc. [9,10]. Given the various functions of vitamin D in the body, increasing attention is paid to its use in medical practice [11,12].

Vitamin D₃ has been shown to be produced under the influence of ultraviolet radiation or obtained through dietary intake. Deficiency of this vitamin is a cause of various diseases in people of all ages. In recent years, scientists have reconsidered vitamin D as a hormone with numerous biological properties

and health benefits for patients. Historically, vitamin D was believed to primarily maintain skeletal health and regulate calcium and phosphorus balance in the body. However, recent scientific studies show a much broader spectrum of action for this vitamin [13]. It is essential for extrarenal tissues, prenatal health, brain function, immunity, pregnancy, cancer prevention, and cardiovascular health [14]. Recently, in addition to its crucial role in bone health and immune function, vitamin D has attracted increased attention for its possible influence on many processes related to women's reproductive health. Studies by Dragomir R. et al. (2024) demonstrate the role of vitamin D in maintaining adequate hormonal status, fertility, and pregnancy outcomes [15].

Research into the role of vitamin D deficiency in the development of menstrual disorders in women has recently gained wide interest. Studies have shown that taking high doses of vitamin D can lead to the restoration of the menstrual cycle (MC). Vitamin D receptors are expressed in the ovaries, placenta, and uterus. In animal experiments, dietary vitamin D deficiency reduced fertility by 45–70%, and the inability to convert vitamin D to its active form disrupted the menstrual cycle, affecting follicular development and causing anovulatory cycles. Vitamin D deficiency is a risk factor for polycystic ovary syndrome (PCOS). In women with PCOS, vitamin D supplementation can normalize the menstrual cycle and improve ovarian folliculogenesis [16, 17]. However, there are still few studies on the effects of vitamin D on menstrual function in women with AUB.

Wu Z et al. (2018) conducted a study concluding that menstrual pain is associated with low serum vitamin D concentrations [18]. Similarly, Rahnamaei FA et al. (2021) found that weekly high-dose vitamin D supplementation can reduce menstrual pain [19].

Amzajerdi A et al. (2023) demonstrated that high-dose vitamin D not only decreases pain intensity but also reduces the need for nonsteroidal anti-inflammatory drugs in women with primary dysmenorrhea and vitamin D deficiency. Consequently, they recommended including vitamin D in the treatment regimen. The authors also emphasized the need for additional clinical trials to further confirm the effectiveness of vitamin D in reducing menstrual blood loss [20].

Therefore, according to Fedosiuk KV (2022) [21], the adjunctive use of vitamin D represents a rational approach in gynecological practice and contributes to improving women's quality of life. Despite recommendations for its widespread use, it is crucial to be aware of the potential side effects of vitamin D and to administer it under close medical supervision [22].

AIM

To assess hormonal status and vitamin D metabolic features in women of reproductive age presenting with abnormal uterine bleeding and associated extragenital conditions.

MATERIALS AND METHODS

The study was conducted at the Department of Obstetrics, Gynecology, Pediatric Gynecology and Medical Genetics of KhNMU (clinical base Municipal Non-profit Enterprise "Professor O. I. Meshchaninov City Clinical Hospital of Emergency and Intensive Medical Care" of Kharkiv City Council). The study included 110 women of reproductive age, divided into two clinical groups. The first group (main group, MG) comprised 80 patients with AUB accompanied by extragenital conditions (ECs). The second group (control group, CG) consisted of 30 somatically healthy women who attended scheduled preventive examinations.

Women in the MG received inpatient treatment in the gynecological department of the medical institution. All participants provided written informed consent for the use of their data in scientific research, in accordance with the ethical principles outlined in the latest edition of the Declaration of Helsinki by the World Medical Association.

Exclusion criteria included pregnancy (as a potential cause of uterine bleeding), decompensated somatic diseases, cancer, and ongoing hormone therapy at the time of examination. All women underwent thorough evaluation using comprehensive clinical and laboratory methods.

In assessing the hormonal status of women in both the MG and CG, serum concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone (PRG), and estradiol (E2) were measured by enzyme-linked immunosorbent assay (ELISA) using standard test kits from Biointernational, France.

Serum 25-hydroxyvitamin D (25-OH)D, vitamin D) was determined by competitive ELISA with a luminescent reagent (Siemens Healthcare Diagnostics, Germany) on an Atellica IM 1600 analyzer (Germany).

Statistical data processing was performed using the general-purpose software package Statistica 6.0, applying contingency tables and Pearson's chi-square (χ^2) test.

FRAMEWORK

The study was conducted within the framework of the research project "Optimization of Clinical, Diagnostic, and Therapeutic Approaches to Managing Gynecologi-

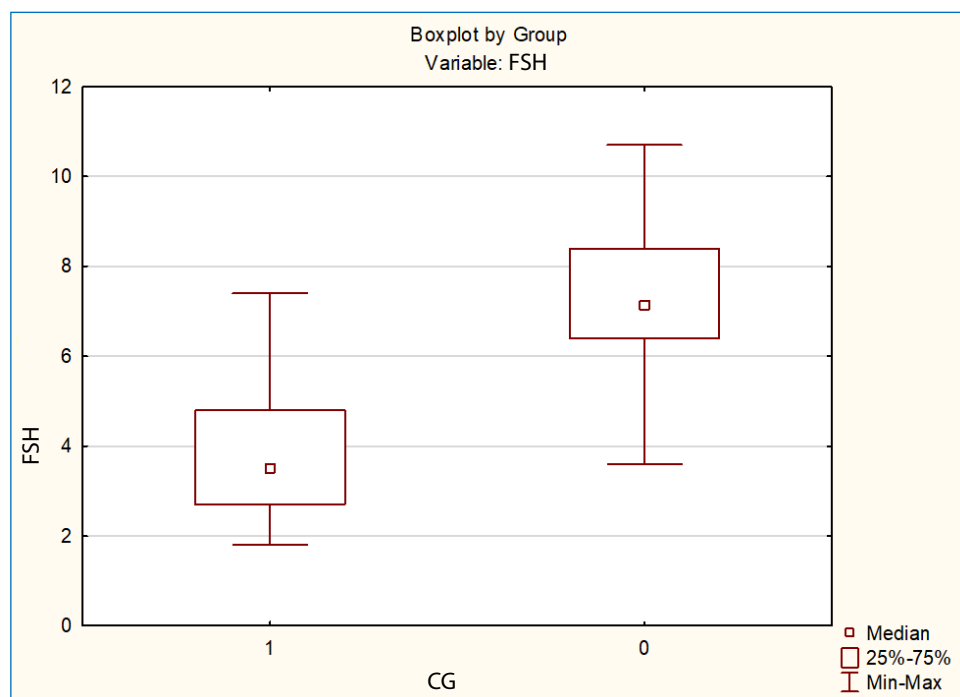


Fig. 1. Serum FSH levels in women from the MG and Contr.G (mIU/mL), 1 – MG, 0 – Contr.G

Picture taken by the authors

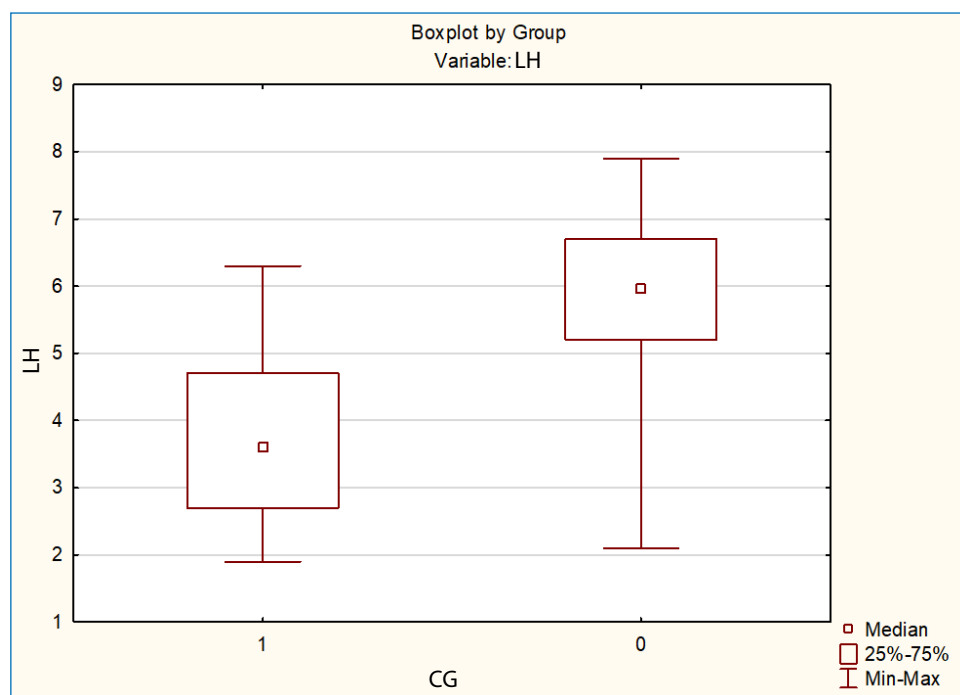


Fig. 2. Serum LH levels in women from the MG and Contr.G (mIU/mL), 1 – MG, 0 – Contr.G

Picture taken by the authors

cal Patients Considering Age and the Presence of Extra-genital Conditions", registration number 0122U000257.

RESULTS

The study examined women with a mean age of 31.5 ± 13.5 years. The most common ECs identified were diseases of the endocrine, circulatory, and digestive systems. Within these, endocrine disorders predominated, with obesity accounting for 75% and thyroid disorders for 17.5%. Digestive system diseases, primarily chronic gastritis, were present in 16.25% of cases, while cardio-

vascular conditions such as hypertensive disease were found in 15%. Among the patients with AUB, 41.25% had two or more concurrent somatic diseases. Specifically, 9 patients (11.25%) presented with both hypertensive disease and obesity; 4 patients (5%) had obesity combined with varicose veins; 9 patients (11.25%) had neurocirculatory dystonia alongside chronic gastritis; 8 patients (10%) suffered from obesity and type II diabetes mellitus; and 3 patients (3.75%) exhibited chronic gastritis with obesity.

Discriminant analysis of the variables, conducted using the Kruskal–Wallis rank test (KWT), demonstrated

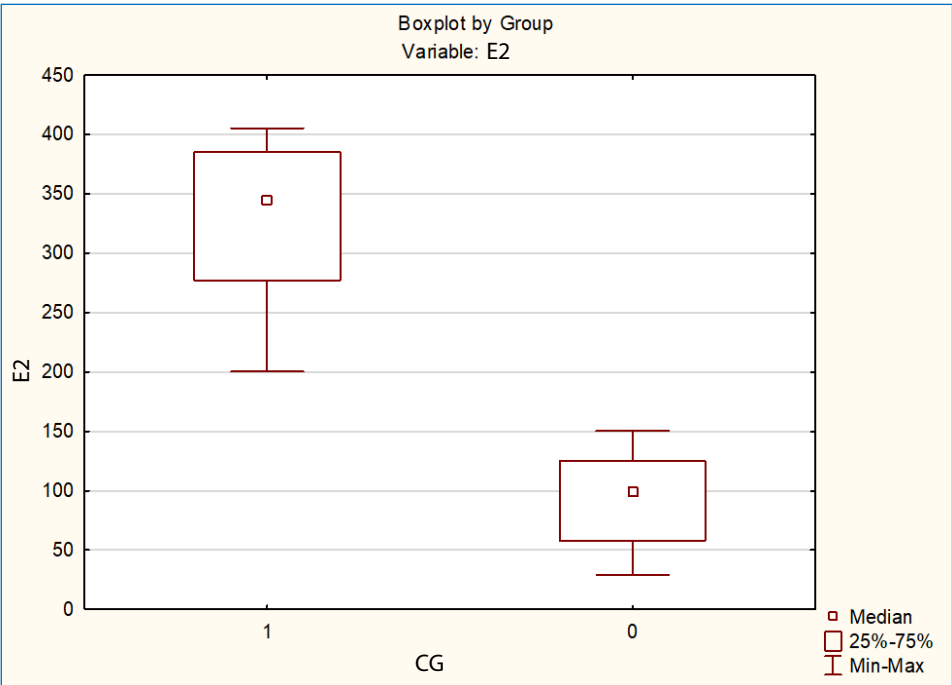


Fig. 3. Serum E2 levels in women from the MG and Contr.G (pg/mL), 1 – MG, 0 – Contr.G
Picture taken by the authors

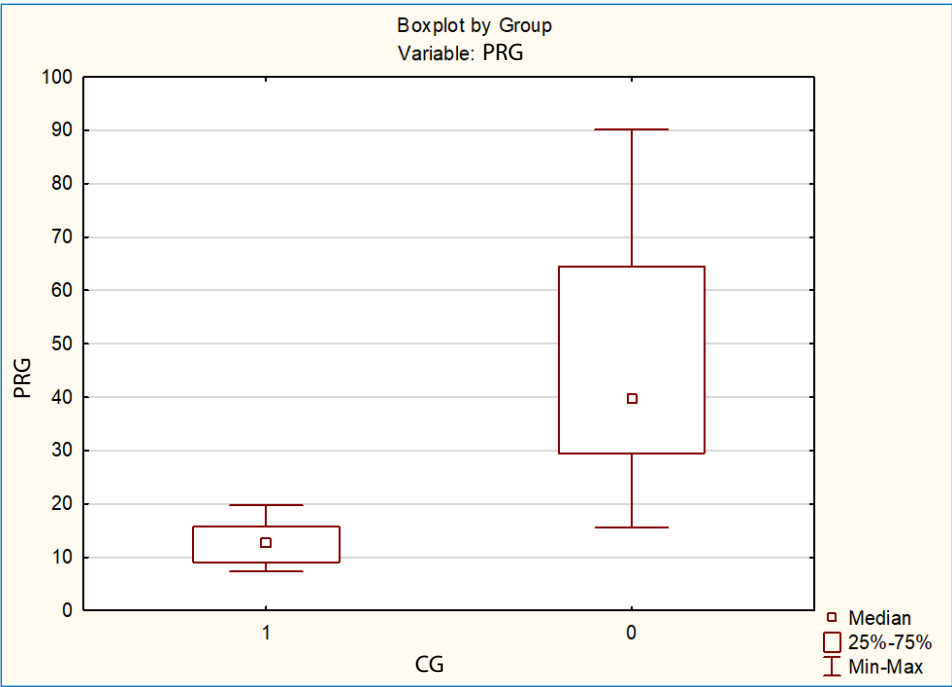


Fig. 4. Serum PRG levels in women from the MG and Contr.G (nmol/L), 1 – MG, 0 – Contr.G
Picture taken by the authors

a statistically significant dependence of all measured parameters on the group. This finding supports the relevance and potential informativeness of comparing these indicators across the groups.

In patients from the MG, the interquartile range of serum FSH levels was 1.97 ÷ 7.59, with a median value of 3.89. In the CG, the interquartile range was 3.60 ÷ 10.70, with a median value of 7.15 (Fig. 1).

Data on serum LH levels in women from the MG and CG are presented in Fig. 2.

In patients from the MG, the interquartile range of serum LH levels was 1.99 ÷ 6.30, with a median value of 3.62. In the CG, the interquartile range was 2.10 ÷

8.94, with a median value of 6.05.

Data on serum E2 levels in women from the MG and CG are presented in Fig. 3.

In patients from the MG, the interquartile range of serum E2 levels was 200.07 ÷ 410.15, with a median value of 349.10. In the CG, the interquartile range was 28.90 ÷ 150.30, with a median value of 99.10.

Data on serum PRG levels in women from the MG and CG are presented in Fig. 4.

In patients from the MG, the interquartile range of serum PRG levels was 8.99 ÷ 19.98, with a median value of 14.82. In the CG, the interquartile range was 15.60 ÷ 90.10, with a median value of 39.75.

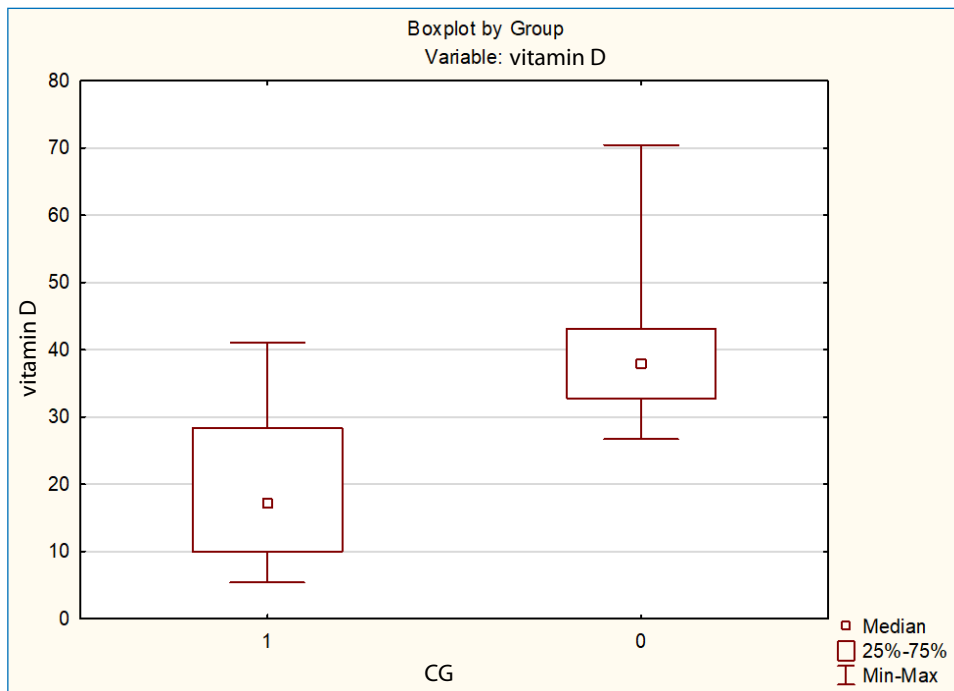


Fig. 5. Serum vitamin D levels in women from the MG and Contr.G (ng/ml), 1 – MG, 0 – Contr.G

Picture taken by the authors

Data on serum vitamin D levels in women from the MG and CG are presented in Fig. 5.

In patients from the MG, the interquartile range of serum vitamin D levels was $6.15 \div 40.78$, with a median value of 16.80. In the CG, the interquartile range was $26.70 \div 70.40$, with a median value of 37.85.

DISCUSSION

The obtained data show that the mean values, medians, and interquartile ranges of serum FSH, LH, PRG, and vitamin D levels were generally lower in MG patients compared to those in the CG.

Effective diagnosis of conditions causing AUB is based on the systematic evaluation of all potential contributing factors. Diagnostic methods used in the examination of such patients typically include the assessment of hormone concentrations such as E2, PRG, FSH, LH, among others. The need for these tests is individualized and primarily determined by the specific clinical status of the patient.

Laboratory findings demonstrated decreased serum concentrations of the following hormones in MG patients compared to CG: FSH by 1.84 times (Fig. 1), (KWT, $p < 0.01$); LH by 1.67 times (Fig. 2), (KWT, $p < 0.01$); PRG by 2.68 times (Fig. 4), (KWT, $p < 0.01$). Serum vitamin D levels in the MG were also reduced by 2.25 times compared to the CG (Fig. 5), (KWT, $p < 0.01$).

Serum E2 levels in women from the MG were significantly higher than those in the CG, with elevated mean values, medians, and interquartile ranges. Specifically, serum E2 levels in the MG were 3.52 times higher compared to the CG (Fig. 3), (KWT, $p < 0.01$).

All women in the MG exhibited hyperestrogenism. Both absolute and relative hyperestrogenism are commonly associated with the development of non-atypical endometrial hyperplasia. Several mechanisms may contribute to this condition. A decrease in serum sex hormone-binding globulin (SHBG) leads to increased levels of free estrogens in plasma. In metabolic disorders such as hyperinsulinemia, insulin resistance, and obesity, E2 is synthesized in adipose tissue due to reduced SHBG production by the liver. This hormonal background is particularly unfavorable for sufficient PRG synthesis during the second phase of the menstrual cycle [23].

In total, 60 women (75%) from the MG were overweight or obese, which indicates a high prevalence of metabolic disturbances. These findings confirm the statistically significant increase in serum E2 levels in MG patients (KWT, $p < 0.01$).

PRG also plays a key role in endometrial disorders in reproductive-age women. Understanding its role is essential in addressing clinical challenges related to the treatment and prevention of hyperplastic and proliferative disorders such as endometriosis and uterine fibroids. Elevated E2 levels alone may not support adequate PRG synthesis in the luteal phase.

Moreover, PRG deficiency can impair estradiol synthesis. E2 regulates endometrial proliferation, including the receptor-mediated actions of PRG [24]. Inadequate activation of PRG receptors often results from insufficient estrogen levels. Without the balanced action of both estrogen and progesterone, physiological endometrial transformation cannot occur. Even when PRG levels are adequate, low E2 concentrations or altered receptor sensitivity may lead to morphological immaturity of the

endometrium. Therefore, not only hormone levels but also the integrity of all mechanisms involved in their biological activity are essential for reproductive health [25].

Over the past decade, a growing number of researchers have investigated the functions of vitamin D, its effects on the functioning of various organs and systems, and its role in the development of pathological processes [26].

MC disorders among women of reproductive age remain a significant societal issue, while vitamin D deficiency is highly prevalent. Singh V et al. (2021), in their study, identified that optimal serum levels of vitamin D play a role in folliculogenesis, normal ovulatory function, and menstrual regularity. The study aimed to explore the potential association between vitamin D deficiency and MC disturbances. Given that vitamin D is inexpensive, widely accessible, and has minimal side effects, the authors suggested it may be recommended for women with menstrual irregularities [27].

In a separate study, Jukic AMZ et al. (2018) concluded that vitamin D levels may influence MC length through their association with a prolonged follicular phase due to delayed ovulation [17].

More recently, Muthukumaran D et al. (2025) emphasized the essential role of vitamin D in maintaining menstrual health and its broad impact on women's reproductive well-being. Summarizing their findings, the authors highlighted a complex relationship between vitamin D levels and MC regulation, hormonal balance, and reproductive function. In particular, vitamin D deficiency is common and may exacerbate menstrual disorders such as dysmenorrhea and menorrhagia [28].

The study revealed a statistically significant correlation ($p < 0.05$) between serum levels of E2 and vitamin

D in the examined groups of patients ($r = 0.61$).

In the MG, hyperestrogenism was accompanied by a deficiency in the luteal (second) phase of the menstrual cycle. The observed correlation between E2 and vitamin D levels indicates metabolic disturbances in these women, with 75% of MG patients suffering from obesity, which contributes to additional estrogen synthesis in adipose tissue alongside vitamin D deficiency in serum

CONCLUSIONS

This study revealed distinct features of the hormonal profile and vitamin D metabolism in women of reproductive age with abnormal uterine bleeding and concomitant extragenital conditions. Notably, these patients exhibited decreased levels of LH, FSH, PRG, and vitamin D amid hyperestrogenemia. Furthermore, a correlation was found between E2 and vitamin D levels in the blood serum.

PROSPECTS FOR DEVELOPMENT

Effective identification of the causes of AUB relies on a systematic investigation of all potential etiological factors. Diagnostic methods used during the examination of patients with AUB include, in particular, the assessment of hormone levels such as E2, PRG, FSH, LH, as well as vitamin D. The necessity for these tests is determined individually, primarily depending on the specific condition of each patient. All of the above will contribute in the future to optimizing reproductive system function and reducing the risk of emergency complications in this group of women.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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***In silico* study, design, synthesis, and evaluation of anti-neoplastic activity of hybrid natural antioxidants as histone deacetylase inhibitors**

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ABSTRACT

Aim: To design and synthesize hybridized natural antioxidants by following the pharmacophore of the reference Histone Deacetylase (HDAC) Inhibitors with potential to enhance pharmacokinetic parameters and investigate anti-neoplastic activity as a trial to replace the known HDAC inhibitor that suffers from life-threatening adverse effects and poor pharmacokinetics related to hydroxamate functionality.

Materials and Methods: Designing of new natural hybridized molecules, HDAC inhibitor consisting of cap, linker & zinc binding domain. The cap involved (natural antioxidants Thymol, Eugenol, vanillin, and Umbelliferon) linked to the bioactive molecule succinate via an ester, and on the other hand, succinate acts as a zinc zinc-binding group by anion-free carboxylate. Molecular docking analysis, absorption, distribution, metabolism and excretion (ADME) pharmacokinetic prediction, and analysis of the designated new antioxidants hybrid molecules. In addition, organic synthesis, chemical identification, and biological assessment for their antineoplastic activity.

Results: The results of the molecular docking and SWISS ADME for four designated molecules are potentially as HDAC6 inhibitors with chemical synthesis for four molecules and identified by Fourier-Transform Infrared spectroscopy (FTIR), Proton Nuclear Magnetic Resonance spectroscopy (¹HNMR), Carbon-13 Nuclear Magnetic Resonance (¹³C NMR), Mass, and some physiochemical properties. Anti-neoplastic activity assay in MDA-MB-231 3-(4,5-di methyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay in breast cancer refers to the designated hybrid molecule-1 as significantly cytotoxic, but molecules-2, molecule-3, and molecule-4 are less significant.

Conclusions: The designated hybrid molecules, especially molecule-1(Thymol-based HDAC inhibitor), exhibit exceptional *in silico* selectivity for HDAC6, the safety of molecule-1, and superior computational pharmacokinetics parameters, making it a building block of a new generation of HDAC inhibitors. The new hybridized molecules of natural origin showed potential anti-neoplastic activities with improved pharmacokinetic parameters compared to SAHA and Tucidinostat.

KEY WORDS: natural hybrid, molecular docking, bioactive molecules, HDAC inhibitor

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INTRODUCTION

Histone Deacetylase (HDAC) inhibitors are a promising backbone for the drug design of cancer, mainly due to their epigenetic modulation in cancer and neurodegenerative and chronic autoimmune diseases. Cardio toxicity mainly QT prolongation, Haematological toxicity like thrombocytopenia, neurotoxicity involving cognitive impairment and peripheral neuropathy. Suberoylanilide hydroxamic acid (SAHA) and belinostat are hydroxamate-based HDAC inhibitors limited clinically due to major adverse effects associated with their drug resistance, poor pharmacokinetics, and lack of selectivity. The leading cause of death worldwide is cancer [1]. A 9.6 million People died in 2018 due to cancer, although extensive cancer drug research and treatment [2]. The growing incidence and prevalence of cancer in

the world and an emergent resistance to most drug of cancer drugs associated with the complicated adverse effects related to a lack of selectivity to cancer cells, all these factors are driving force to focus on natural antioxidants and making them as nucleus in design of cancer drug. Antioxidants are functioning in counteracting free radicals and oxidative stress, as well as in fighting cancer both prevention and treatment significant attention for their activity as scaffold nucleus in drug design [3]. Resveratrol and Curcumin are marked antioxidants that can efficiently modify HDAC activity, HDACs [4]. Chemical modification of natural antioxidant molecules via semi-synthetic derivatives have enhanced antiproliferative potential, pharmacokinetic properties and reduced toxicity [5]. Thymol induces oxidative stress-associated mitochondrial damage and both intrinsic and

extrinsic apoptosis in cancer cells. It also suppresses the proliferation of cancer cells. Thymol anticancer effectiveness in several studies may be attributed to its powerful antioxidant activity, cytoprotective action, anti-apoptotic capability, marked anti-inflammatory/immunomodulatory action, and significant genomic protection from cytotoxic agents to the normal cells [6].

Thymol-Derived Hybrid Molecules Demonstrating Anticancer Efficacy: The investigation of novel therapies employing plant-derived molecules, due to their biocompatibility and non-toxic effects on healthy cells. Therefore, employing them in the production of hybrid therapeutic molecules can mitigate their limitations and represent a viable approach for designing drugs. A variety of Thymol-based hybrid molecules was assessed for their anti-tumour activity against four distinct cancer cell lines ex. MCF-7 & MDA-MB-231. Many of the synthesized compounds exhibited acceptable cytotoxic activity relative to the reference drugs [7].

Eugenol is an aromatic phenolic antioxidant derived from *Syzygium aromaticum*, possessing promising pharmacological activities including anticancer, antiviral, anti-diabetic, anti-parasitic, anti-inflammatory, and antibacterial activity. This attractive natural molecule inhibits the enzyme cyclooxygenase II (COX-2), lipoxygenase enzyme -5 (5-LOX) and DNA synthesis, increases reactive oxygen species (ROS) production, arrests the cellular cycle, and induces programmed cell death. Numerous semisynthetic pharmaceutical molecules based on Eugenol in their nucleus in recent years, exhibiting enhanced bioactivities and a wide range of mechanisms of action. Eugenol has been investigated for its safety and flexibility in drug design [8].

Vanillin has several pharmacological activities, such as anti-neoplastic activity, antidiabetic characteristics, marked antioxidant activity, antibacterial activity, anti-inflammatory characteristics, cardioprotective agent, and diuretic action. Molecular hybridization strategy is employed for the design of novel drug compounds derived from vanillin [9].

Umbelliferon: promising anticancer benefits in preclinical phases, but their poor solubility and bioavailability limit their application in cancer therapy. A Coumarins derivative is found in Rutaceae and Apiaceae plants. It can reduce cancer cell proliferation and confer considerable cytotoxicity. Furthermore, Coumarins, based on a 2H-1-benzopyran-2-one structure, demonstrate a wide range of therapeutic activities, encompassing anticancer activity. Antiviral, antibacterial activity, anticoagulant ability, blood pressure-lowering action, antioxidant, anti-inflammatory, and neuroprotective capacity, figure (1) [10].

The neglect of toxicity of Umbelliferon from oral administration of within the therapeutic range of

200 mg per kilogram of body weight renders it a compelling framework for the synthesis of active core 7-hydroxy Coumarins-derived molecules in drugs discovery as an innovative platform a multifunctional scaffold for the synthesis of various physiologically active molecules. Umbelliferon is receiving significant attention for its anticancer activities. Preclinical outcomes suggest potential usefulness in the future the therapy of multiple solid tumours, including mouth epithelial cells carcinoma, colorectal carcinoma, cutaneous carcinoma, prostate cancer, breast cancer, and cancer of urinary bladder, as well as malignancies of central nervous system. Umbelliferon, regardless of the type of cancer, activates pathways that facilitate the arrest of cell cycles, apoptotic cascade or restrict both of migration and invasion malignant cells [11].

Succinic acid has selective anti-neoplastic activities via inducing a programmed cell death in cancer cells and its normal metabolic activity in non-cancer cells. The pathway Tricarboxylic Acid (TCA) cycle and its enzymatic constituents depend mainly on succinic acid [12]. Furthermore, succinate-based drugs are used to establish it is potential therapeutic anti-cancer activity and selective capacity of the therapeutic characteristics, as well as improving the pharmacokinetic parameters [13].

The Thymol, Eugenol, vanillin, and Umbelliferon are selected to design hybrid molecules to act as HDAC inhibitors by following the structural pharmacophore of SAHA, figure (2).

Rationale for design

1. Pharmaceutical design of future HDAC inhibitor generation, either to enhance the poor pharmacokinetic characteristics of new classes of HDAC inhibitors¹⁴ or secondly, the efficacy and specificity of the HDAC inhibitors must be improved [15].
2. Hybrid molecules with Anticancer Activity Based on Antioxidants (Thymol, Eugenol, vanillin and Umbelliferon): within limits Lipinski's criteria [7].
3. In oncology, succinic acid has great potential due to its ability to kill cancer cells without harming healthy cells. Succinic acid has been chosen in designated of hybrid molecules.
4. Computational modeling speeds up drug discovery, making it more affordable and faster. To assure the safety and efficacy of possible hypothetical anticancer drugs, computational studies must be validated by experimental testing [16].
5. In designing of Captopril as Angiotensin converting enzyme inhibitor (ACEI) limited it is therapeutic usefulness by adverse effects dry cough (accumulation of bradykinin), skin rash and dysgeusia related to it is sulfhydryl functionality leading cause to the re-introduce of carboxylate as zinc binding group

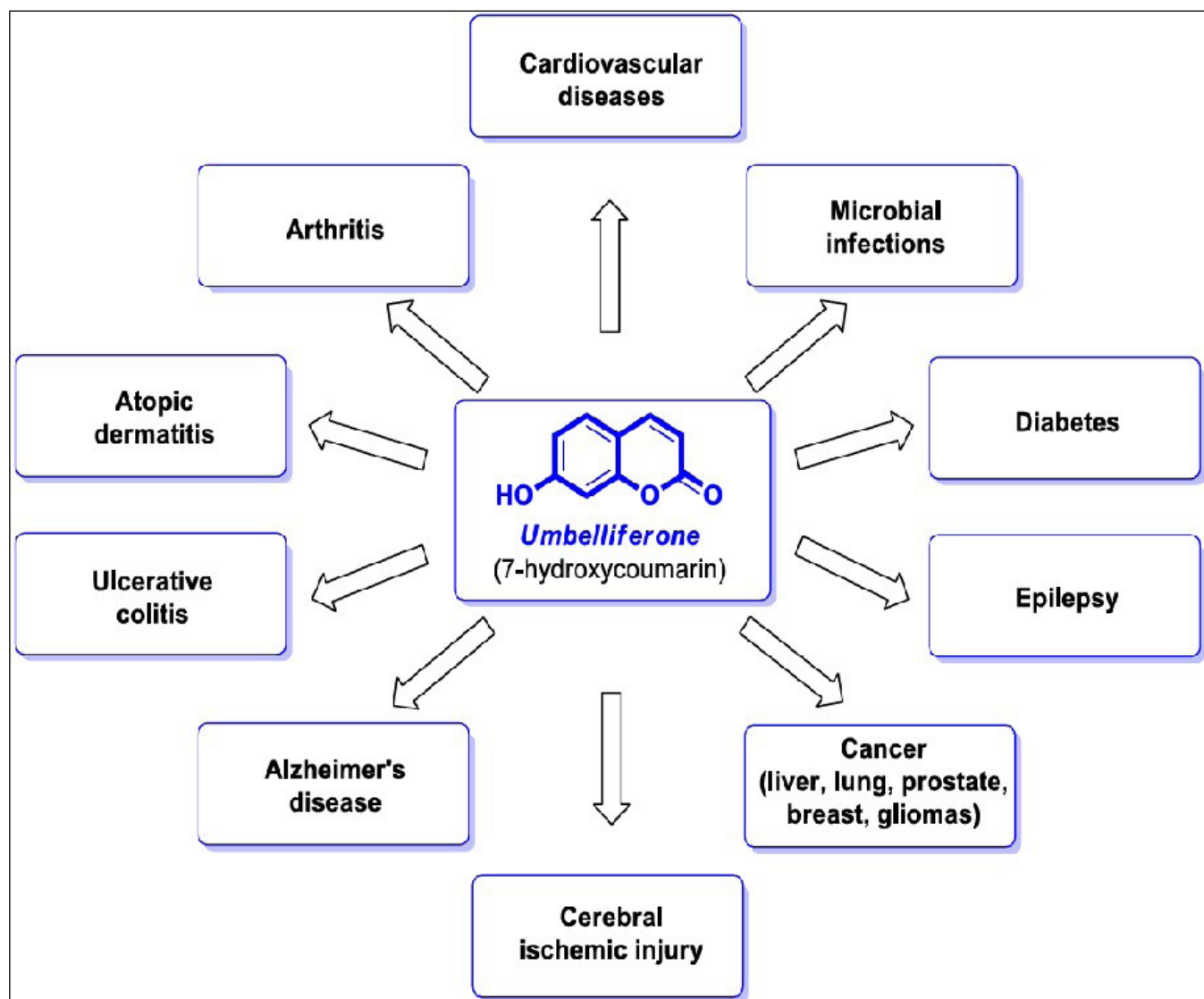


Fig. 1. Umbelliferone in drug design [11]

Source: Own materials

instead of -SH Group in the design of inhibitor as in enalapril and lisinopril [17].

6. HDACs perform many physiological functions, with their dysregulation frequently associated with human diseases, especially cancer [18].
7. Consequently, enhancing HDAC selectivity to reduce off-target toxicity [19].

AIM

The aim of this study is to design and synthesize hybridized natural molecules considering pharmacophore reference HDAC inhibitor data with potential pharmacodynamics and pharmacokinetic candidates and investigate anti-neoplastic activity as a trial to replace the known HDAC inhibitor that suffers from

life-threatening adverse effects and poor pharmacokinetics.

MATERIALS AND METHODS

CHEMICAL SYNTHESIS

Thymol, Eugenol, vanillin, and Umbelliferone were purchased from China; the source of the triethylamine was BLD Pharm, China. The solvent used was HPLC grade and without further purification. Thin-layer chromatography (TLC) on Merck silica gel 60F25 reaction progression monitoring under ultraviolet (UV) light via solvent system mobile phase Ethylacetate3: Hexane7 ratio. Identification of the functional Group of compounds by the Fourier-Transform Infrared spectroscopy (FTIR) spectropho-

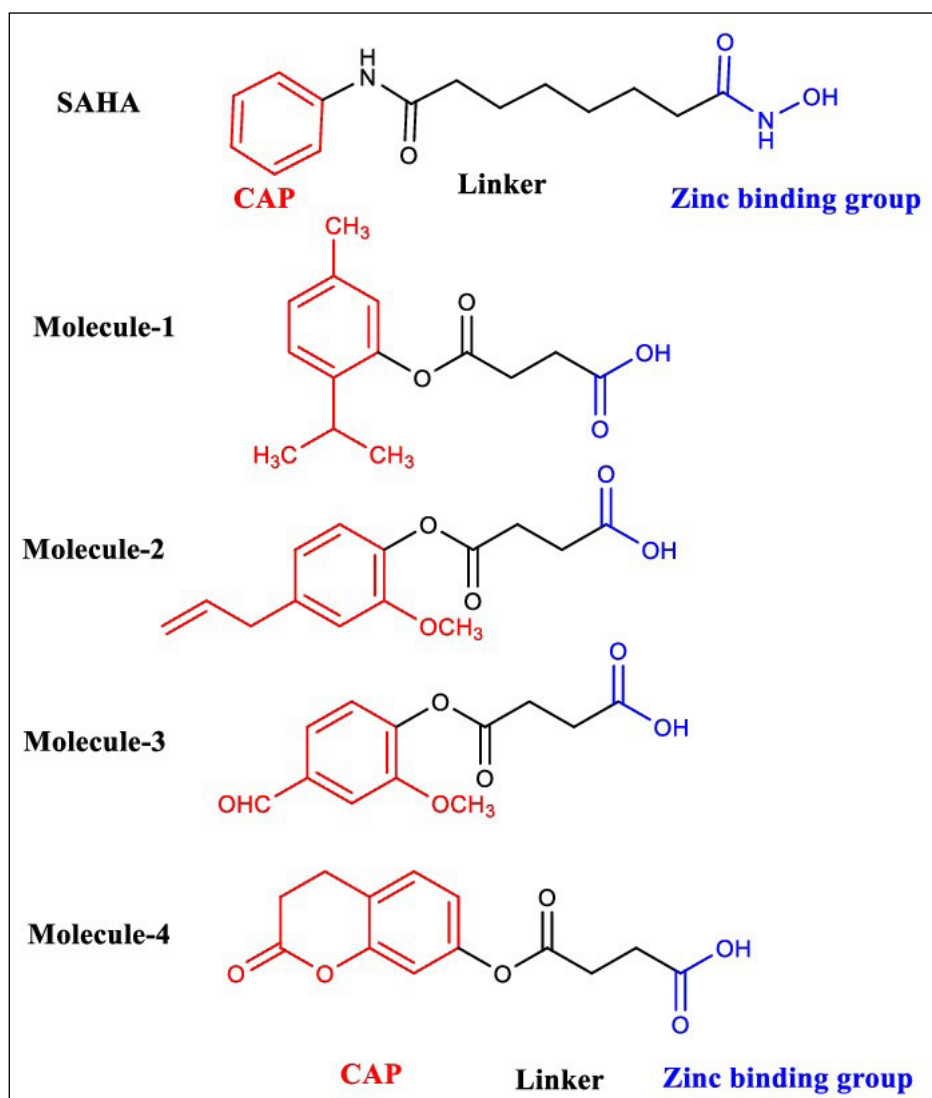


Fig. 2. Designated hybrid molecules compared to the SAHA pharmacophore
Source: Own materials

tometer in University of Kufa, Faculty of Pharmacy, which were recorded on FTIR spectrophotometer, Shimadzu, Japan,₂ (Proton Nuclear Magnetic Resonance spectroscopy (¹H NMR) spectroscopy ¹H-NMR (300 MHz) (Bruker Avance II),₃ [Carbon-13 Nuclear Magnetic Resonance (¹³C NMR) spectroscopy ¹³C NMR (75 MHz) (Bruker Avance II)].

COMPOUND -1

Thymol: (0.75 gm., 5 mmol) was dissolved in 20 ml of dry chloroform, then triethylamine (0.7 ml, 5 mmol), 4-Dimethylaminopyridine (DMAP) (0.1gm) was added dropwise. The resulting mixture had been stirred at room temperature for 10 minutes and then succinic anhydride (0.5 gm., 5 mmol) was poured. The reaction mixture was stirred at room temperature for a period of 24 hours at 25°C. After finishing the reaction, the solvent was evaporated under declined pressure to get the final product as colourless oily substances, furthermore, dissolved in dry chloroform again and re-distilled. Re-crystallized using petroleum ether 85%.

FT-IR (cm⁻¹): 1749 (ester group), 3500 (-OH carboxylic acid), 1143 (ether group), 3062 (C-H aromatic group), 1570 (C=C bending aromatic). ¹H NMR (300 MHz, Dimethyl sulfoxide (DMSO)-d₆) δ 9.48 (s, ¹H, -COOH), 6.98-6.54 (m, 3H, Ar-H), 2.37(s, 3H, -O-CH₃), 2.97 (m, 2H, CO-CH₂-), 2.46 (m, 2H, CH₂-COOH). ¹³C NMR (75 MHz, DMSO-d₆) δ 175.38 carboxylic carbon, 174 (carbonyl carbon), 154.70, 135.69, 131.60, 126.02, 119.99 (aromatic carbon), 23.05, 21.12 (-CH₃), 26.48 (Carbonyl-CH₂-), 31.25 (-CH₂-Carboxyl) [20].

COMPOUND-2

Eugenol: (0.82 gm., 5 mmol) density 1.066 gm./cm³ which corresponds to 0.76 ml liquid was dissolved in 20 ml of dry chloroform. Then, triethylamine (0.7 ml, 5 mmol), DMAP (0.1 gm.) was added dropwise. The mixture had been stirred at room temperature for 10 min and then succinic anhydride (0.5 gm., 5 mmol) was poured. The reaction mixture was stirred at room temperature for 48 hours at 25°C. After finishing the reaction, the solvent was evaporated under

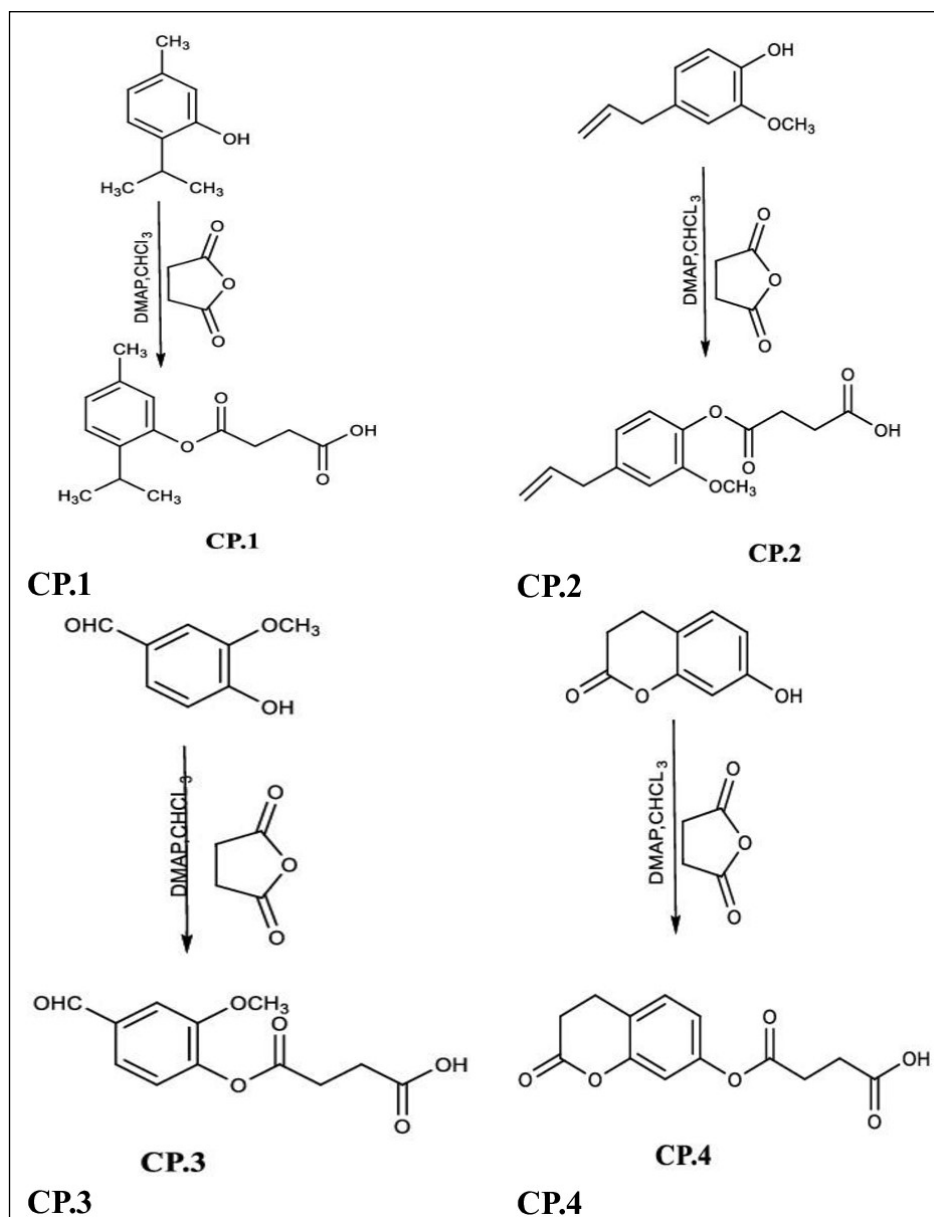


Fig. 3. Chemical synthesis scheme
Source: Own materials

lower pressure to get the final product as colourless oily substances; furthermore, dissolved in dry chloroform again and re-distilled. Re-crystallized using petroleum ether 89%. FT-IR (cm^{-1}): 1720 (ester group), 3431 ($-\text{OH}$ carboxylic acid), 1163 (ether group), 3070 (C-H aromatic group), 1516 ($\text{C}=\text{C}$ bending aromatic). ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 10.56 (s, 1H, $-\text{COOH}$), 7.01-6.01 (m, 3H, Ar-H), 5.98(=CH2) 5.09.37 (s, 3H, $-\text{O}-\text{CH}_3$), 2.91 (m, 2H, $\text{CO}-\text{CH}_2-$), 2.44 (m, 2H, CH_2-COOH). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 175.27 carboxylic carbon, 172.80 (carbonyl carbon), 155.40, 147.93, 145.22, 138.63, 120.96, 115.83, 112.99 (aromatic carbon), 55.94 ($-\text{OCH}_3$), 29.84 ($\text{Carbonyl}-\text{CH}_2-$), 29.57 ($-\text{CH}_2-\text{Carboxyl}$).

COMPOUND-3

Vanillin: (0.76 gm., 5 mmol) was synthesized in the same procedure as Compound 2, to get the final product as red

oily substance. Furthermore, dissolve in dry chloroform again and redistill. Re-crystallized using petroleum ether 74%. FT-IR (cm^{-1}): 1720 (ester group), 3433 ($-\text{OH}$ carboxylic acid), 1163 (ether group), 3020 (C-H aromatic group), 1516 ($\text{C}=\text{C}$ bending aromatic). ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 11.88 (s, 1H, $-\text{COOH}$), 9.89 (s, 1H) ($-\text{CHO}$), 7.03.98-6.56 (m, 3H, Ar-H), 3.75 (s, 3H, $-\text{O}-\text{CH}_3$), 2.93 (m, 2H, $\text{CO}-\text{CH}_2-$), 2.44 (m, 2H, CH_2-COOH). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 191.38 ($-\text{CHO}$), 175.33 (carboxylic carbon), 172.86 (carbonyl carbon), 154.02, 148.72, 128.66, 127.71, 126.64, 115.88 (aromatic carbon), 55.94 ($-\text{OCH}_3$), 29.59 ($\text{Carbonyl}-\text{CH}_2-$), 29.88 ($-\text{CH}_2-\text{Carboxyl}$).

COMPOUND-4

Umbelliferon: (0.81 gm., 5 mmol) was synthesized in the same procedure as Compound 2, to get the final product as a yellow oily substance. Furthermore, dissolve in dry chloroform again

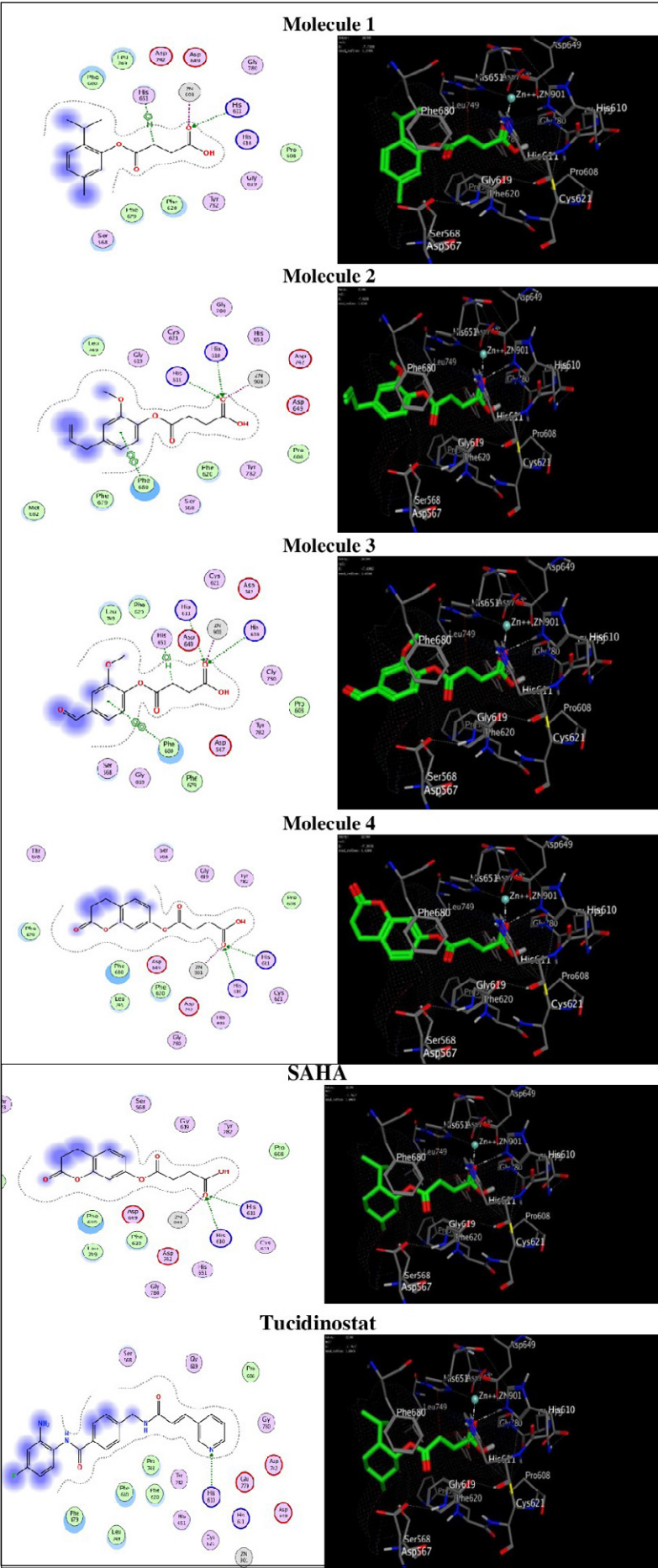


Fig. 4. 2-D and 3-D pose of the four designated hybrid molecules in the active site of PDB 5EDU HDAC6, and two references, ASHA and Tucidinostat
Source: Own materials

Table 1. S-score & RMSD value of all the molecules are listed

mseq	S-score	RMSD_refine
Molecule 1	-7.73931	1.238553
	-7.72422	1.145665
	-7.53628	0.962826
	-7.52902	1.148029
	-6.8746	1.708983
Molecule 2	-7.62932	1.624763
	-7.54466	1.542013
	-7.44754	1.247373
	-7.31624	0.713143
	-7.07375	1.551248
Molecule 3	-7.49815	1.659777
	-7.30356	1.556132
	-7.25448	1.176839
	-7.20749	1.192163
	-6.90244	2.53427
Molecule 4	-7.41243	2.453249
	-7.30557	1.416625
	-7.215	1.468781
	-7.04577	1.428322
	-7.00273	2.629645
SAHA	-8.84678	1.968135
	-8.83205	1.627789
	-8.63910	2.2169015
	-8.49357	1.8273996
	-8.45259	2.5052261
Tucidinostat	-7.02955	1.8229722
	-6.95895	1.7007053
	-6.84878	2.3356028
	-6.83148	2.2330139
	-6.78206	2.1497548

Source: Own materials

and redistill. Re-crystallized using petroleum ether 88%. FT-IR (cm⁻¹): 1724 (ester group), 3439 (-OH carboxylic acid), 1170 (ether group), 3070 (C-H aromatic group), 1510 (C=C bending aromatic). ¹H NMR (300 MHz, DMSO-d₆) δ 11.97 (s, 1H, -COOH), 7.95-6.66 (m, 3H, Ar-H), 6.64 (cyclic carbon proton), 2.91 (m, CO-CH₂-), 2.45 (m, 2H, CH₂-COOH). ¹³C NMR (75 MHz, DMSO-d₆) δ 175.41 carboxylic carbon, 172.84 (carbonyl carbon), 162.30 (cyclic carbon), 156.02, 155.37, 145.02, 130.09, 127.71, 115.00 (aromatic carbon), 29.65 (Carbonyl-CH₂-), 29.98 (-CH₂-Carboxyl), the chemical synthesis scheme is shown in figure (3).

BIOLOGICAL EVALUATION

In Sharif University of Technology, MDA-MB-231 (cell line for human breast cancer) was purchased from the

Iranian National Bank for Cell of (Pasteur Institute, Iran). Cells were developed in RPMI-1640 medium (Gibco) with standard supplement 10% phosphate-buffered saline (PBS) (Gibco) with effective antibiotics 100 U/ml and 100 µg/ml of penicillin and streptomycin, respectively. Cells were maintained C under humidified air containing 5% CO₂ at 37°C and were passed on trypsin/EDTA (Gibco) and phosphate-buffered saline combination (PBS) solution.

RESULTS AND DISCUSSION

MOLECULAR DOCKING OUTCOMES

The docking score (S) and root-mean-square deviation (RMSD) value of all the molecules are listed and given in table (1).

Table 2. Pharmacokinetic parameters of designated molecules and two references

Molecule	Molecule 1	Molecule 2	Molecule 3	Molecule 4	Molecule 5 (SAHA)	Molecule 6 (Tucidinostat)
Molecular weight (MW)	250.29	264.27	252.22	264.23	264.32	390.41
#Rotatable bonds	6	8	7	5	10	8
#Hydrogen-bond acceptors	4	5	6	6	3	4
#Hydrogen-bond donors	1	1	1	1	3	3
Molecular Refractivity (MR)	68.87	69.92	61.21	63.68	73.33	110.01
Topological Polar Surface Area (TPSA)	63.6	72.83	89.9	89.9	78.43	97.11
Implicit log P (iLOGP)	2.29	2.46	1.68	1.66	1.84	2.51
eXtended LOGP version 3 (XLOGP3)	2.6	2.15	0.43	0.83	1.86	2.3
Gastrointestinal absorption	High	High	High	High	High	High
Blood–Brain Barrier (BBB) permeant	Yes	Yes	No	No	No	No
P-glycoprotein (Pgp) substrate	No	No	No	No	No	Yes
CYP1A2 inhibitor	No	Yes	No	No	No	Yes
CYP2C19 inhibitor	No	No	No	No	No	Yes
CYP2C9 inhibitor	No	No	No	No	No	Yes
CYP2D6 inhibitor	No	No	No	No	No	Yes
CYP3A4 inhibitor	No	No	No	No	No	Yes
Lipinski #violations	0	0	0	0	0	0
Ghose #violations	0	0	0	0	0	0
Veber #violations	0	0	0	0	0	0
Egan #violations	0	0	0	0	0	0
Muegge #violations	0	0	0	0	0	0
Bioavailability Score	0.85	0.85	0.56	0.56	0.55	0.55
PAINS #alerts	0	0	0	0	0	0
Brenk #alerts	1	2	2	1	2	2
Leadlikeness #violations	0	1	0	0	1	2

Source: Own materials

A Two-dimension (2-D) and three-dimension 3-D pose for the four designated hybrid molecules in the active site of Protein Data Bank (PDB) 5EDU HDAC6, and two references, SAHA and Tucidinostat are given in figure (4), while Pharmacokinetic parameters of designated molecules and two references is given in table (2).

ACCORDING TO THE KINETICS RESULTS, WE ANALYSED THE DATA OBTAINED IN TWO WAYS: Firstly: the BOILED-Egg, figure (5). The plotting of WLOGP versus TPSA, arranges the position of any molecule in the white area, referring to the ability of the

drug molecule to undergo passive absorption from the gastrointestinal tract, while the yellow area refers to the ability of the drug molecule to brain. The blue color point indicates the drug molecule is a suitable substrate for Pgp -glycoprotein, while the red point color refers to the drug molecule free from efflux by Pgp –glyco-protein [21]. Neither Tucidinostat nor SAHA crosses the references cross blood-brain barrier. Only designated hybridized molecule-1(Thymol-based HDAC inhibitor) and (Eugenol-based inhibitor) cross the blood–brain barrier (BBB) permeant and reach to the brain.

The Biological assay (MTT) of antineoplastic activity, where IC50% for M1 (37.95), M2 (93.72), M3 (540.81) and M4 (115.98) are shown in figure (6).

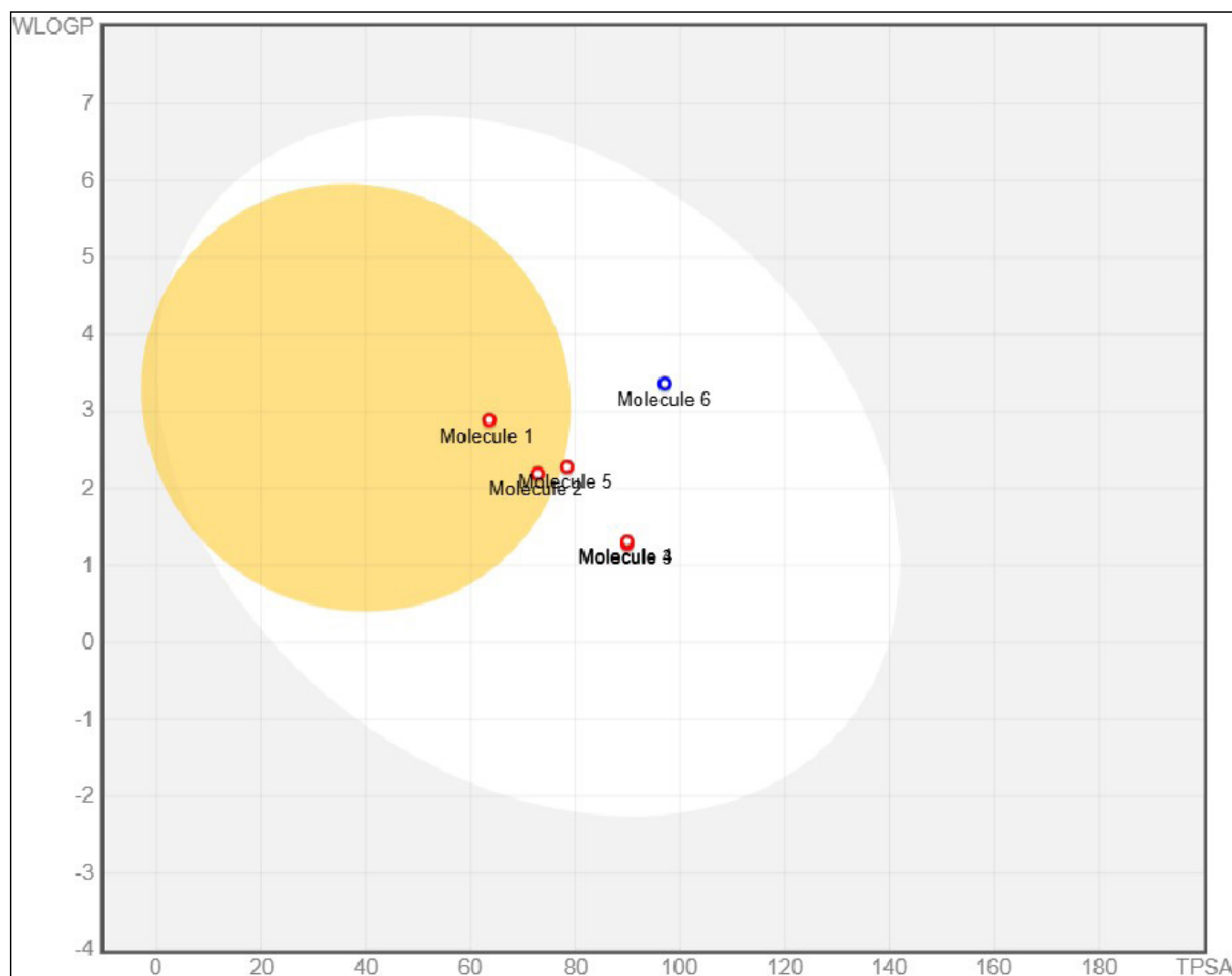


Fig. 5. BOILED-Egg -SWISS-ADME for four designated hybrid molecules and two references, where M1 = Thymol-based inhibitor M2=Eugenol-based inhibitor, M3 = based inhibitor, M4 = Umbelliferon-based inhibitor, M5=SAHA reference-1 and M6=Tucidinostat -reference-2

Source: Own materials

FLUORESCENT ASSAY

The concentrations for Thymol-based inhibitor, Eugenol-based inhibitor, Vanillin-based inhibitor, and Umbelliferon-based inhibitor are shown in figure (7)

SAHA is the FDA-approved pan HDAC inhibitor associated with cardiotoxicity QT prolongation, cardiac arrhythmia, haematological toxicity including thrombocytopenia, severe anaemia, hepatotoxicity and neuron toxicity including impaired cognitive function, seizure, and peripheral neuropathy, leading to the limitation in clinical use and reduced patient compliance. Furthermore, the poor pharmacokinetic characteristics of SAHA. The major adverse effects of SAHA and its poor pharmacokinetic profile are caused by the hydroxamate functionality. By applying pharmaceutical isosteric to design new molecules with an isosteric entity of hydroxamate to it is carboxylate that resembles in steric and electronic environments and

has zinc chelation activity [22]. Valproic acid is an HDAC synthetic-based carboxylate inhibitor, but also associated with adverse effects, but acceptable pharmacokinetics parameters, so drug designing of natural molecules (Thymol, Eugenol, vanillin and Umbelliferon) with succinate to yield natural hybrid molecules with structural similarity to HDAC inhibitors. The computer aid drug design involve molecular docking program (MOE), in-silico pharmacokinetics analysis, protein data bank sources are the leading way to design new potential chemical molecules and their drug likeness candidate by verification with two the references drug, our designated molecules exhibited comparable inhibitory activity relative to the (hydroxamate based inhibitor) SAHA and (benzamine based inhibitor) Tucidinostat as references drugs. The design of specific hybridized molecules to establish the specific requirements of HDAC enzyme inhibition.

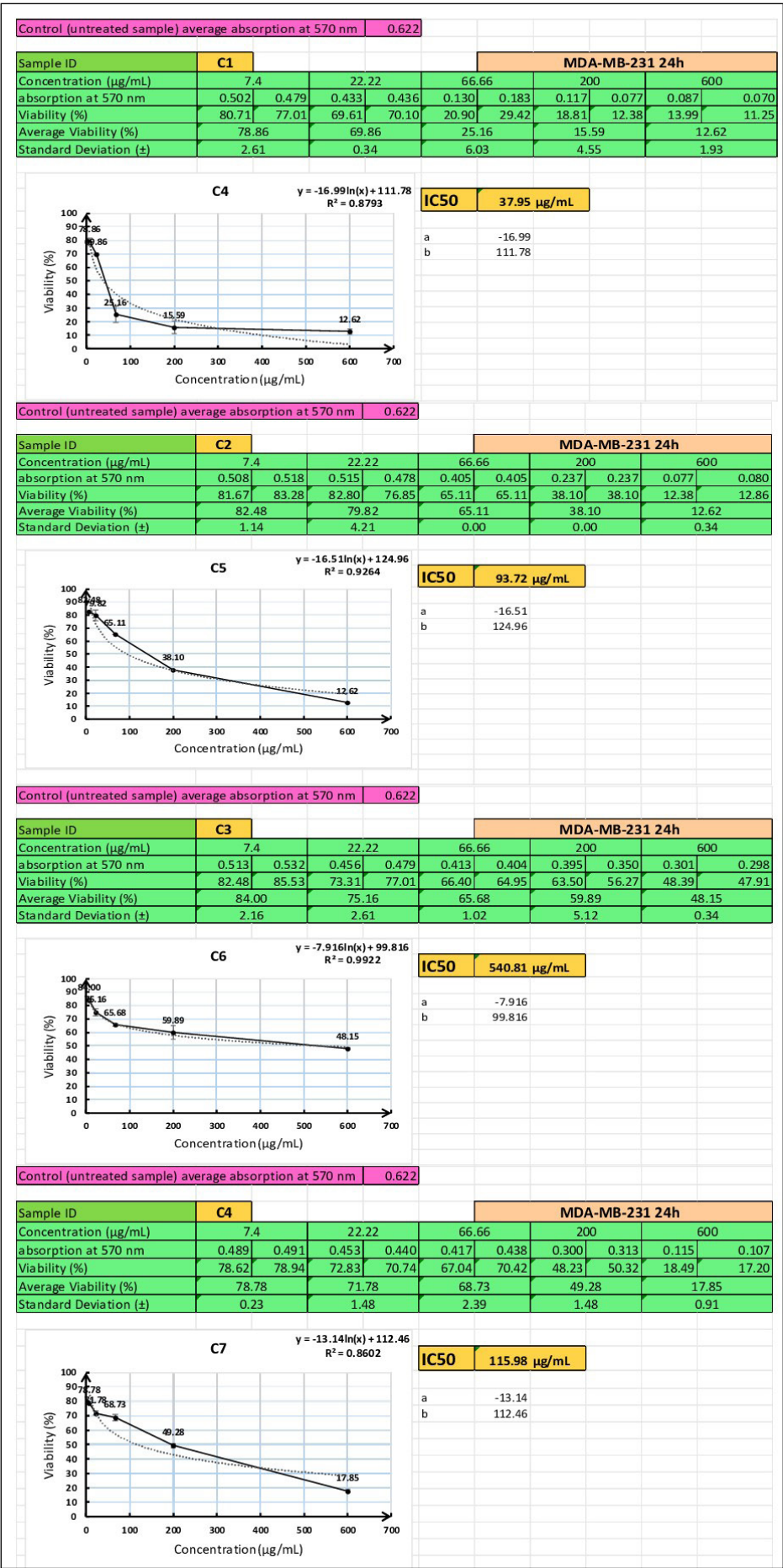


Fig. 6. Biological assay (MTT) of anti-neoplastic activity. Where IC50% for M1 (37.95), M2 (93.72), M3 (540.81) and M4 (115.98)
M1=Thymol-based inhibitor, M2=Eugenol-based inhibitor, M3=Vanillin-based inhibitor and M4=Umbelliferon-based inhibitor
Source: Own materials

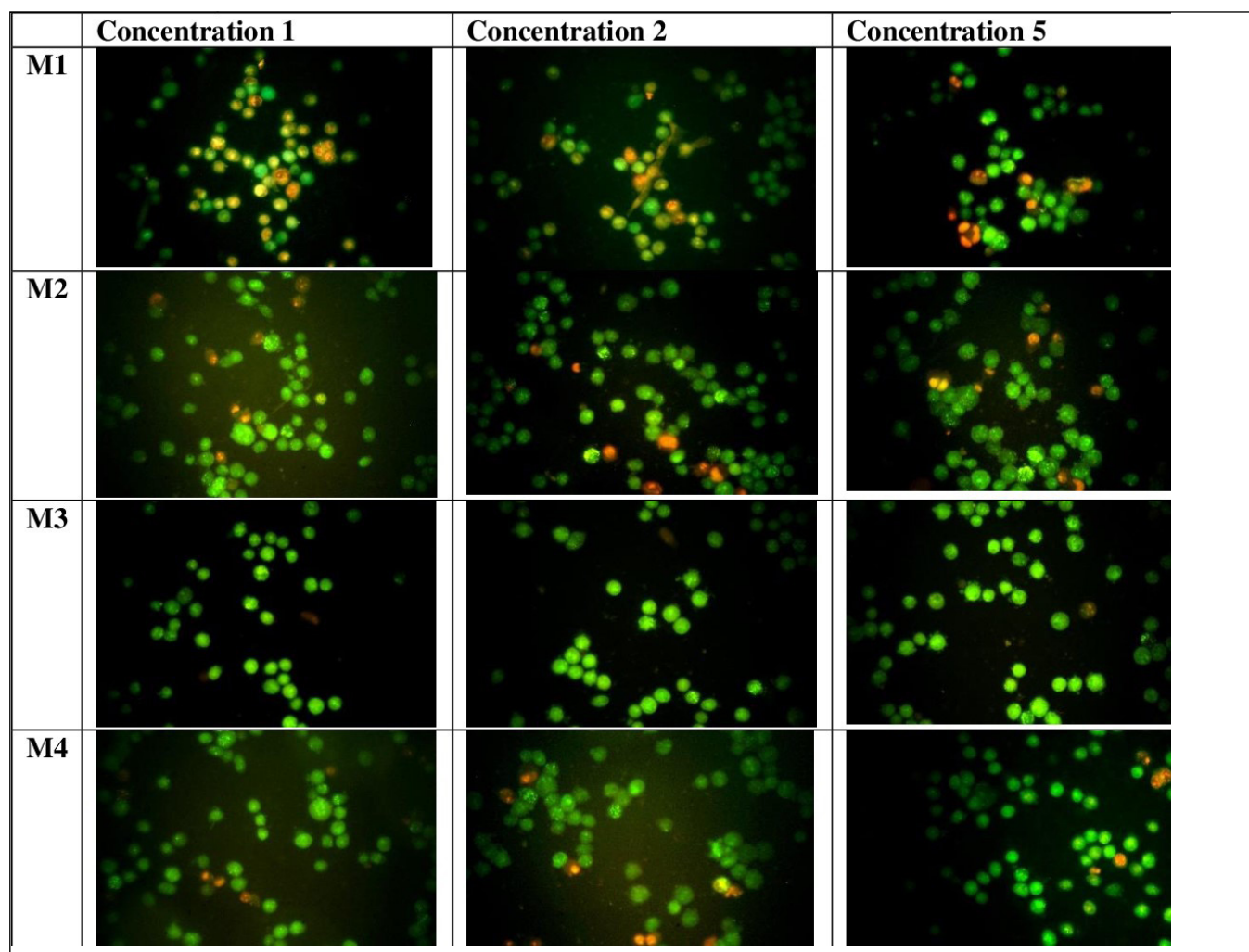


Fig. 7. concentrations for Thymol-based inhibitor, Eugenol-based inhibitor, Vanillin-based inhibitor, and Umbelliferon-based inhibitor
Source: Own materials

Analysis of molecular docking of active site of HDAC6 (5EDU) PDB shows score (S-value), molecule-1(Thymol-based HDAC inhibitor) exhibited (-7.7) the lowest S-value, establishing it as the most effective ligand among our selected candidates for inhibiting HDAC6 activity & than the two references, SAHA (-8.8) &Tucidinostat (-7.029), regarding all acceptable RMSD values below 2. S-score & RMSD value of all the molecules. The binding of hybrid designated molecules and two references to HDAC6 (5EDU). Molecule-1 exhibited the highest binding affinity among the proposed inhibitors analysed, based on Lipinski's rule of five and the BOILED-egg technique, yet it was not considered a therapeutic drug according to Lipinski's criterion and the BOILED-egg approach.

CONCLUSIONS

Our study elucidates, in comparison to two of the references, firstly the hydroxamate-based inhibitor SAHA and

secondly the benzamide-based inhibitor Tucidinostat, the binding characteristics of established designated hybrid molecules as HDAC6 inhibitors. The molecular docking analysis refers to the hybridized molecules exhibiting zinc binding activity at the active site of the enzyme HDAC6. Designated hybridized molecules have an exceptional zinc-binding capacity as to that of the SAHA HDAC inhibitor. The *in silico* ADME pharmacokinetics of molecules (1, 2, 3 and 4) exhibit good dynamics scores, and the computational kinetic parameters are superior to the reference molecules (SAHA and Tucidinostat). In summary, we introduced a new class of HDAC6 inhibitors. The compounds exhibit exceptional selectivity for HDAC6, especially Molecule-1 Thymol-based HDAC inhibitor, which is superior in drug-receptor binding (S score in molecular docking and no substrate to Pgp, a Pgp-glycoprotein with computationally validated crossing of the blood brain barrier. As a result, molecule-1 (Thymol-based HDAC inhibitor) has a higher cytotoxic effect than other designated hybrid molecules. The biosafety

of molecule-1 and excellent *in silico* pharmacokinetics parameters make it a building unit of the new generation of HDAC6 inhibitors. The new hybridized molecules of

natural origin showed potential anti-neoplastic activities with improved pharmacokinetic parameters compared to SAHA and Tucidinostat.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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Comparison of early and late results of open and laparoscopic retropubic prostatectomy in surgical treatment of benign prostatic hyperplasia

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ABSTRACT

Aim: To compare and evaluate the early and late results of open and laparoscopic retropubic prostatectomy in the surgical treatment of large benign prostatic hyperplasia (more than 80 ml).

Materials and Methods: There were 50 one-stage laparoscopic extraperitoneal retropubic prostatectomies and 120 one-stage open retropubic prostatectomies performed in patients with benign prostatic hyperplasia in the urology department of the Vinnytsia Regional Hospital named after M. I. Pirogov in the period from 2021 to 2023. Indications for surgery in patients were: acute (including repeated) retention of urine; pronounced symptoms of the disease due to the lack of effect from conservative therapy; repeated hematuria, which is caused by BPH; bladder stones; recurrent infections of the urinary tract (cystitis, pyelonephritis) due to BPH. Patients with and suspected prostate cancer were excluded from the study. All patients were surveyed to determine IPSS indices and quality of life (QOL) of the International Prostate Symptoms Questionnaire, digital rectal examination, laboratory tests.

Results: The average time of laparoscopic prostatectomy - 120.3 ± 11.7 minutes, open - 81.1 ± 17.4 ; average intraoperative blood loss - 118.7 ± 33.6 ml and 520.5 ± 67.4 ml, postoperative bed-day 6.1 ± 1.1 and 9.8 ± 2.9 , respectively. Transfusion of blood components - in 9 (7.5%) patients after open retropubic prostatectomy, never after laparoscopic. Urological complications after laparoscopic prostatectomy - in 2% of patients, open - 19.2%, complications according to Clavien - Dindo - 4% and 33.3%, respectively. Laparoscopic and open retropubic prostatectomies allow equally effective restoration of the art of urination after surgery late results of treatment and postoperative complications were less (2.5% versus 8%) after laparoscopic retropubic prostatectomy.

Conclusions: Laparoscopic and open retropubic prostatectomy are effective operations for the treatment of patients with benign prostatic hyperplasia of large size (more than 80 ml) with satisfactory early and late results, as well as restoration of urination. In the hands of an experienced laparoscopic surgeon, laparoscopic retropubic prostatectomy can achieve better treatment results, than open retropubic prostatectomy. This makes it possible to recommend laparoscopic retropubic prostatectomy in the practice of urologists of urological clinics of Ukraine where laparoscopic equipment are present.

KEY WORDS: benign prostatic hyperplasia, open and laparoscopic retropubic prostatectomy, early and late results

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INTRODUCTION

Benign prostatic hyperplasia (BPH) is one of the most common diseases in men after 60 years old, half of them have symptoms of frequent and difficult urination about 30-50% of such patients seek a surgical help [1, 2]. According to the recommendations of the European Association of Urologists (EAU, 2023) [3] for large BPH (more than 80 ml), open prostatectomy of first choice and can be performed with transvesical or retropubic (transcapsular) approach. Open prostatectomies are traumatic interventions for patients and accompanied a significant percentage of intra- and postoperative bleeding from the prostate bed and need blood transfusions and repeated operations; significant pain syndrome in the postoperative wound; a large number of infectious complications with the possibility of suppu-

ration of the postoperative wound; a long postoperative bed-day and a long rehabilitation period [1, 2, 4-7].

To reduce and avoid the above-mentioned complications of open prostatectomies, laparoscopic prostatectomy and later laparoscopic robot-assisted prostatectomy were introduced into the practice of urologists [1, 2]. Due to the significant cost of equipment for performing laparoscopic robot-assisted prostatectomy and the cost of consumables for its performance, laparoscopic prostatectomy is a more common surgical intervention for large BPH [8, 9]. Laparoscopic prostatectomy for BPH is performed via transperitoneal or extraperitoneal access. Most urologists (80-90%) prefer laparoscopic extraperitoneal prostatectomy to avoid urine entering into abdominal cavity, abdominal organ damage, and adhesion disease [2, 10, 11]. Laparoscopic prostatecto-

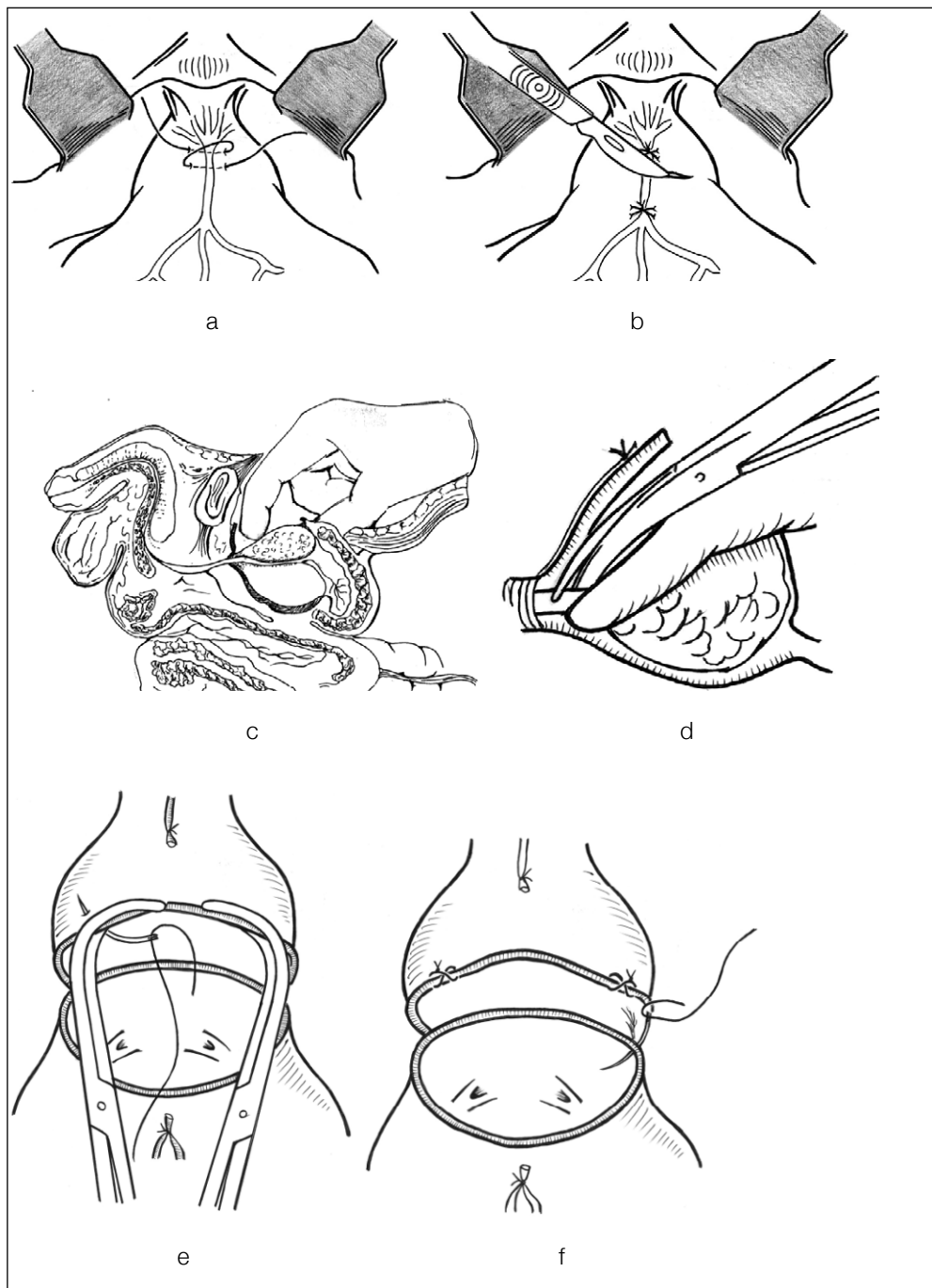


Fig. 1 (a-i). The technique of one-stage open retropubic prostatectomy with trigonization of the lower semicircle of the bladder neck

Picture taken by the authors

mies, like open prostatectomies, are performed through a transvesical or retropubic (transcapsular) approach. We prefer retropubic access as less traumatic, since there is no trauma to the bladder wall and its drainage and urination is restored faster and the postoperative bed-day is reduced [1, 12, 13]. In Ukraine, there are no data on the comparison of the results of open and laparoscopic prostatectomy in the surgical treatment of benign prostatic hyperplasia. We were the first in Ukraine to describe the experience of performing first 30, and then 50 one-stage laparoscopic extraperitoneal retropubic (transcapsular) prostatectomies in patients with BPH [12, 13].

AIM

To compare and evaluate the early and late results of open and laparoscopic extraperitoneal retropubic prostatectomy in the surgical treatment of large benign prostatic hyperplasia (more than 80 ml).

MATERIALS AND METHODS

There were 50 one-stage laparoscopic extraperitoneal retropubic prostatectomies and 120 one-stage open retropubic prostatectomies performed in patients with benign prostatic hyperplasia in the urology department of the Vinnytsia Regional Hospital named after M. I.

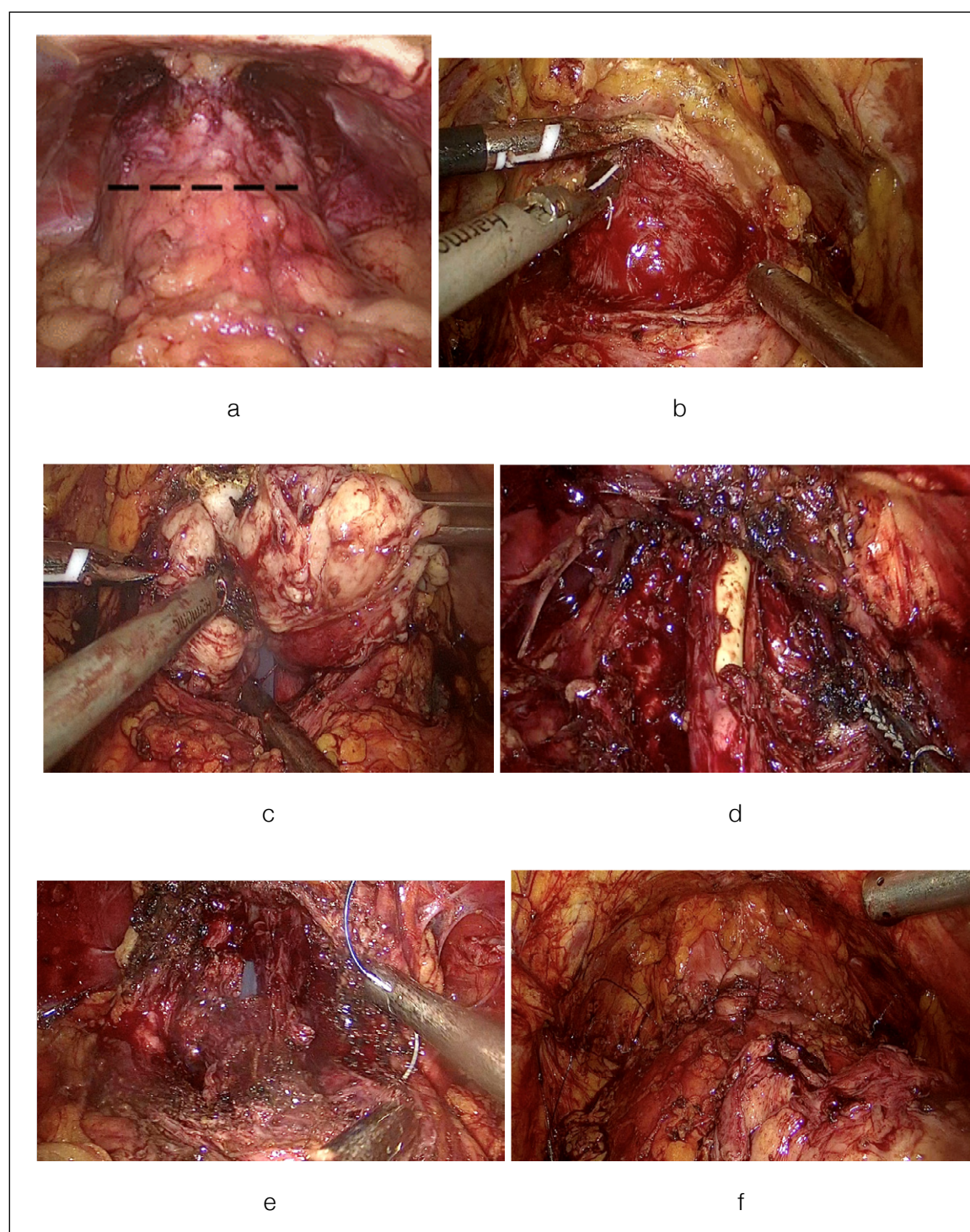


Fig. 2 (a-h). Technique of one-stage laparoscopic retropubic prostatectomy (in 10 (20%) patients it was possible to preserve the prostatic part of the urethra)

Picture taken by the authors

Pirogov in the period from 2021 to 2023. Indications for surgery in patients were: acute (including repeated) retention of urine; pronounced symptoms of the disease due to the lack of effect from conservative therapy; repeated hematuria, which is caused by BPH; bladder stones; recurrent infections of the urinary tract (cystitis, pyelonephritis) due to BPH. Patients with and

suspected prostate cancer were excluded from the study. All patients were surveyed to determine IPSS indices and quality of life (QOL) of the International Prostate Symptoms Questionnaire, digital rectal examination, laboratory tests (general blood and urine analysis, urine culture, blood urea and blood creatinine, determination of the level of prostate-specific antigen

Table 1. Results of examination and treatment of patients who underwent simultaneous laparoscopic and open retropubic prostatectomy

Indexes	Laparoscopic retropubic prostatectomy (n=50)	Open retropubic prostatectomy (n=120)
Average age of patients	68.2 ± 2.4	68.0 ± 7.2
Average body mass index (kg/m ²)	28.5 ± 1.5	28.8 ± 3.5
Average prostate volume (cm ³)	111.5 ± 17.4	94.4 ± 42.3*
Average PSA (prostate specific antigen)	4.9 ± 1.2	4.1 ± 1.3
The average index of the ASA scale	1.8 ± 0.2	1.8 ± 0.3
Patients with a urethral catheter	17 (34%)	44 (36.7%)
Average IPSS index before surgery in patients with preserved urination	25.3 ± 3.3	25.4 ± 2.1
Average IPSS index after surgery	5.3 ± 0.6	5.8 ± 0.9
Average QOL index before surgery	5.1 ± 0.7	5.0 ± 0.6
Average QOL index after surgery	2.0 ± 0.5	2.1 ± 0.6
The maximum flow rate before surgery (Q _{max}), ml/s	7.4 ± 1.2	7.3 ± 0.8
The maximum flow rate after surgery (Q _{max}), ml/s	27.4 ± 1.5	26.1 ± 1.8
Bladder stone(s).	2 (2%)	13 (10.8%)*
Average operation duration (min.)	120.3 ± 11.7	81.1 ± 17.4*
Average intraoperative blood loss (ml)	118.7 ± 33.6	520.5 ± 67.4*
The number of administered narcotic painkillers after surgery (ampoules)	1.2 ± 0.1	4.2 ± 0.2*
Duration of bladder irrigation after surgery (hours)	28.7 ± 3.6	74.7 ± 13.6*
Average postoperative bed day	6.1 ± 1.1	9.8 ± 2.9*

Note: * - p < 0.05

Source: compiled by the authors of this study

Table 2. Urological complications in patients after laparoscopic and open retropubic prostatectomy

Complications	Number of cases	
	Laparoscopic retropubic prostatectomy (n=50)	Open retropubic prostatectomy (n=120)
Bleeding after surgery, which required transfusion of blood components	-	9 (7.5 %)*
Discharge of urine through the suprapubic wound after removal of the urethral catheter	-	1 (0.8 %)
Funiculitis, epididymorchitis	-	1 (0.8 %)
Acute or exacerbation of chronic pyelonephritis	-	1 (0.8 %)
Hyperthermia after removal of urethral drainage (due to prostatic venous reflux)	1 (2%)	7 (5.8%)
Stress urine incontinence	-	2 (1.7%)
Acute retention of urine	-	1 (0.8%)
Paravesical hematoma	-	1 (0.8%)
In total	1 (2%)	23 (19.2%)*

Note: * - p < 0.05

Source: compiled by the authors of this study

in blood serum - PSA), uroflowmetry in patients with preserved urination, ultrasound examination of the kidneys and bladder with the determination of residual urine, ultrasound transrectal examination of the prostate to determine the volume of the prostate, if necessary, MRI of the prostate and cystoscopy (to rule out cancer of prostate and bladder tumors). In addition, an examination of the cardiopulmonary system was

performed (ultrasound examination of the heart, electrocardiography with the consultation of a cardiologist) and ultrasound examination of the veins of the lower extremities to rule out thrombus formation. Preoperative assessment of the physical condition of patients was performed according to the classification system of the American Society of Anesthesiologists (ASA) [1, 2]. Body mass index was determined for all patients.

Table 3. Non-urolological complications in patients after laparoscopic and open retropubic prostatectomy

Early non-urolological complications	Patients after one-stage prostatectomy	
	Laparoscopic retropubic prostatectomy (n=50)	Open retropubic prostatectomy (n=120)
Thromboembolism of small branches of the pulmonary artery	-	2 (1.7%)
Hypertensive crisis	1 (2%)	2 (1.7%)
Acute coronary syndrome	-	1 (0.8%)
Acute psychosis	-	1 (0.8%)
Stomach dyskinesia with pain syndrome	-	1 (0.8%)
Exacerbation of gouty arthritis of the knee joint	-	1 (0.8%)
In total	1 (2%)	8 (6.7%)*

Note: * - $p < 0.05$

Source: compiled by the authors of this study

Table 4. Clavien-Dindo classification of surgical complications after laparoscopic and open retropubic prostatectomy

Degree of complication	Complications (number of patients in absolute values and percentages) in patients after laparoscopic retropubic prostatectomy (n=50)	Complications (number of patients in absolute values and percentages) in patients after open retropubic prostatectomy (n=120)	Treatment of complications
I	Hyperthermia after removal of urethral drainage(1 - 2%)	Hyperthermia after removal of urethral drainage(7 - 5.8%)	Re-inserting of the Foley catheter, antibacterial, antipyretic, anti-inflammatory therapy
	-	Urine excretion through the suprapubic wound (1 - 0.8%)	Repeated inserting of the Foley catheter, antibacterial therapy
	-	Acute retention of urine (1 - 0.8%)	Repeated installation of the Foley catheter, antibacterial therapy
	-	Paravesical hematoma (1 - 0.8%)	Dressings using local antiseptic solutions
	-	Stress urinary incontinence "d" (2 - 1.7%)	Kegel exercises, duloxetine
	Hypertensive crisis (1 - 2%)	Hypertensive crisis (2 - 1.7%)	Hypotensive therapy
	-	Stomach dyskinesia with pain syndrome (1 - 0.8%)	Fibrogastroduodenoscopy, spasmolytic, analgesic therapy
	-	Acute or exacerbation of chronic pyelonephritis (1 - 0.8%)	Antibacterial, anti-inflammatory, antipyretic, detoxification therapy
	-	Acute psychosis (1-1.71%)	Antipsychotic drugs
	-	Exacerbation of gouty arthritis of the knee joint (1-1.7%)	Anti-inflammatory, antipyretic, antibacterial therapy
II	-	Bleeding from the bed of the prostate (9 - 7.5%)	Hemostatic therapy, transfusion of blood components
	-	Postoperative anemia (4 - 3.3%)	Transfusion of blood components
IIIa	-	-	-
IIIB	-	-	-
IVa	-	Postoperative hypotension (6 - 5%)	Treatment in the intensive care department
	-	Acute coronary syndrome (1 - 0.8%)	Treatment in the intensive care department
	-	Thromboembolism of small branches of the pulmonary artery (2 - 1.7%)	Treatment in the intensive care department
IVb	-	-	-
V	-	-	-
In total	2 (4%)	40 (33.3%)*	

Note: * - $p < 0.05$

Source: compiled by the authors of this study

Table 5. Determination of the average maximum flow rate (Qmax, ml/s) in patients after laparoscopic and open retropubic prostatectomies

Type of intervention, uroflowmetry index	Upon discharge from the hospital	1 month after surgery	3 months after surgery
Laparoscopic retropubic prostatectomy (Qmax, ml/s)	(n=40) 27.4 ± 1.3	(n=30) 28.3 ± 1.4	(n=20) 27.1 ± 1.5
Open retropubic prostatectomy (Qmax, ml/s)	(n=100) 26.1 ± 1.7	(n=70) 27.6 ± 1.5	(n=50) 27.0 ± 1.6

Source: compiled by the authors of this study

Table 6. Complications after laparoscopic and open retropubic prostatectomies

Complications	Number of complications	
	Laparoscopic retropubic prostatectomy abs. (%) (n=40)	Open retropubic prostatectomy abs. (%) (n=100)
Bladder neck stricture	-	1 (1%)
Urethral stricture	-	1 (1%)
Acute epididymorchitis	1 (2,5%)	3 (3%)
Bladder stone	-	1 (1%)
Exacerbation of chronic pyelonephritis	-	1 (1%)
Stress urine incontinence	-	1 (1%)
In total	1 (2,5%)	8 (8%) *

Note: * - $p < 0.05$

Source: compiled by the authors of this study

Uroflowmetry before surgery (with preserved urination) and after surgery (at discharge, after 1 and 3 months) with determination of the maximum flow rate (Qmax, ml/s) was performed on the domestic uroflowmeter "Potik - K" (Dnipro) [14].

The technique of one-stage open retropubic prostatectomy is presented on Fig. 1, and the technique of laparoscopic extraperitoneal retropubic prostatectomy - Fig. 2. Laparoscopic extraperitoneal retropubic prostatectomy was performed under intubation anesthesia, open retropubic - spinal anesthesia. Transverse or longitudinal skin incisions were used for open prostatectomy. The capsule of the prostate in both interventions was dissected with a transverse incision. Intraoperative blood loss was determined by the weight method [1, 2]. Assessment of intra- and postoperative complications after surgery was performed according to Clavien-Dindo classification [15]. Open retropubic prostatectomies were performed by 5 urologists with the highest category and surgical experience of at least 20 years. Laparoscopic retropubic prostatectomies were performed by one surgeon (Moraru-Burlesku R.P.).

Statistical processing of the obtained data was carried out using methods of variational statistics. The significance of the difference was determined using Student's t-test and Mann-Whitney's U-test. The integrated system STATISTICA (USA) was used for statistical calculations.

ETHICS

This work complies with the principles of the Declaration of Helsinki.

RESULTS

The results of examination and treatment of patients who underwent one-stage laparoscopic and open retropubic prostatectomy are presented in Table 1. According to Table 1, the mean age of the patients, body mass index, ASA score, IPSS score, QoL, Qmax before and after surgery, as well as the number of patients with urinary retention and an indwelling urethral catheter in both groups, were statistically similar.

The mean duration of laparoscopic prostatectomy in our patients (120.3 ± 11.7 minutes) corresponded to data reported in the literature. McCullough T.S. [6] performed 96 retropubic prostatectomies with a mean operative time of 95.1 ± 6.0 minutes. Suceken F.Y. [7] reported a mean operative time of 152.1 ± 42.6 minutes in 35 patients undergoing laparoscopic retropubic prostatectomy. Autorino R. et al. [10], after performing 843 procedures, reported a mean time of 95 minutes.

A comparison of laparoscopic versus open prostatectomy in BPH shows an advantage of laparoscopic intervention over the open approach. The latter authors reported an average blood loss of 280 ml during laparoscopic prostatectomy; McCullough reported 350

ml, and Suceken — 80 ml [6, 7]. In our patients, this parameter was 118.7 ± 33.6 ml.

Urological complications in patients after laparoscopic and open retropubic prostatectomy are presented in Table 2. According to Table 2, the rate of blood transfusions after open prostatectomy was 7.5%, while no transfusions were performed after laparoscopic prostatectomy. After laparoscopic prostatectomy, the overall complication rate was 2%, compared with 9.2% after open surgery. There were no cases of intraoperative bleeding, repeated operations due to bleeding from the prostate bed, and no deaths in both groups of patients. Urethritis and suppuration of the postoperative wound were also absent in both groups of patients.

Non-urological complications in patients after laparoscopic and open retropubic prostatectomy are presented in Table 3. Cases of hospital-acquired pneumonia were not observed in both groups of patients.

Classification of surgical complications after laparoscopic and open retropubic prostatectomy according to Clavien-Dindo is presented in Table 4. According to Table 4, the number of in-hospital complications classified according to Clavien-Dindo was higher in the group that underwent open prostatectomy (33.3% vs. 4%).

The mean operative time was longer in the group that underwent laparoscopic extraperitoneal prostatectomy, whereas intraoperative blood loss was greater after open prostatectomy.

According to Table 5, both open and extraperitoneal prostatectomy were equally effective in restoring normal urination after the procedure.

According to Table 6, in the long-term postoperative period, the number of complications was higher after open prostatectomy (8%) compared with 2.5% after robotic surgery.

DISCUSSION

The percentage of complications after open retropubic prostatectomy in our patients corresponded to and did not exceed the percentage of complications in various urological clinics, according to the literature [4-6, 10, 16, 17].

The postoperative bed-day after laparoscopic retropubic prostatectomy was 6.1 ± 1.1 days, open retropubic prostatectomy - 9.8 ± 2.9 . Thus, laparoscopic retropubic prostatectomy, when compared with open retropubic prostatectomy, is characterised by many significant advantages: absence of bleeding from the prostate bed and hemotransfusions, less use of narcotic drugs in the postoperative period, a short period of bladder irrigation after surgery and a short postoperative bed-day, fewer urological and non-urological complications despite the longer duration of the operation. This is

due to minimal invasiveness of the operation, excellent visualization of the operative field with step-by-step hemostasis, preservation (partial) of the prostatic urethra and preservation of an intact bladder neck.

To determine and compare the restoration of urination after laparoscopic and open retropubic prostatectomies, we conducted urodynamic studies of patients on the domestic uroflowmeter "Potik - K" (Dnipro) with the determination of the maximum flow rate (Q_{max} , ml/s) on the day of discharge from the department, through 1 and 3 months (table 5). Not all patients in the hospital were able to perform uroflowmetry due to various factors, as well as in the late period.

According to Table 5, the average maximum flow rate after both types of interventions was statistically the same, slightly increasing 1 month after surgery. Thus, laparoscopic and open retropubic prostatectomies make it possible to equally effectively restore the urination in patients after surgery.

Observations (repeated examination and inpatient treatment, telephone survey) of the patients were carried out during the first 2 years after the operations in order to identify late complications of both surgical interventions. Complications of laparoscopic and open retropubic prostatectomies are presented in Table 6. Not all patients could be re-examined and interviewed due to various factors.

One patient underwent TUR of the bladder neck after open retropubic prostatectomy due to bladder neck stricture. Acute epididymo-orchitis occurred in patients of both groups who did not undergo bilateral scrotal vasoresections. Antibacterial, anti-inflammatory and anti-edema therapy was carried out. The condition of the patients improved and we never had to operate them. A small bladder stone (up to 1 cm) occurred in one patient after open retropubic prostatectomy, it was successfully crushed using transurethral contact ultrasound cystolithotripsy. A patient with exacerbation of chronic pyelonephritis after open prostatectomy underwent repeated inpatient treatment using antibacterial, anti-inflammatory and detoxification therapy. Stress urinary incontinence, which occurred in 1 patient after open retropubic prostatectomy, significantly decreased after a course of conservative therapy. He was recommended to continue Kegel pelvic floor muscle training, duloxetine and sibutin, use male urological pads and urinary devices. Thus, when analysing the late results of treatment of patients with benign prostatic hyperplasia, fewer postoperative complications were noted after laparoscopic retropubic prostatectomy.

CONCLUSIONS

Laparoscopic and open retropubic prostatectomy are effective operations for the treatment of patients with

benign prostatic hyperplasia of large size (more than 80 ml) with satisfactory early and late results, as well as restoration of urination. In the hands of an experienced laparoscopic surgeon, laparoscopic retropubic prostatectomy can achieve better treatment results with fewer

complications than open retropubic prostatectomy. This makes it possible to recommend laparoscopic retropubic prostatectomy in the practice of urologists of urological clinics of Ukraine where laparoscopic equipment are present.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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Influence of glycemic control in sleep status in diabetes mellitus patients type 2 and its related with SNPs of SLC47A2: Intron variant

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ABSTRACT

Aim: The relationship between diabetes mellitus (DM) and lifestyle quality become important in diabetes research in last year. The present study aims to study the influence of metformin response in sleep in diabetes mellitus patients type 2.

Materials and Methods: A cross sectional study was designed to achieve study goal, glycemic parameters included fasting blood glucose (FBG), glycated protein (HbA1c%), insulin (IN), insulin resistance (HOMA-IR) and insulin sensitivity (IS). PCR sequencing was used to detect *SLC47A2* intronic variants and its related with glycemic control and sleep status.

Results: Among the study population, about 26.3% achieved well glycemic control, 30% were moderately controlled, and 43.8% were poorly controlled. Sleep quality assessment showed that the majority of participants in all glycemic groups experienced intermediate sleep. The prevalence of insomnia increased with worsening glycemic control, from 4.8% in the well-controlled group to 17.1% in poorly controlled participants, in non- statistically significant ($p = 0.722$). Biochemical parameters confirmed significant differences in fasting blood glucose and HbA1c across the three glycemic categories ($p < 0.001$), insulin, HOMA-IR, and insulin sensitivity did not differ significantly. Multiple regression analyses indicated that none of the biochemical predictors significantly explained sleep in any group ($p > 0.05$), in poorly controlled patients, non-significant opposing trends were observed for insulin and insulin resistance, sociodemographic factors included supplement use, education level, and employment were associated with better sleep among poorly controlled patients. Genetic analysis of two intronic variants in the *SLC47A2* gene (g.19716681G>C and rs1597652185) revealed no significant associations with glycemic control or sleep, though both showed similar distribution patterns across groups. Statistical analysis didn't find significant association between either variant and glycemic or sleep status ($p > 0.05$).

Conclusions: Poor glycemic control was common and associated with higher insomnia prevalence. While demographic and clinical factors showed no clear links with glycemic control or sleep, supplement use emerged as a protective factor. FBG and HbA1c strongly differentiated control groups, but other biomarkers and *SLC47A2* variants were not predictive. Findings suggest that combining metabolic management with supportive measures like supplementation may improve sleep and outcomes in type 2 diabetes..

KEY WORDS: glycemic control, sleep status, diabetes mellitus, type 2, glycemic control status

INTRODUCTION

A global health disorder in the world is Diabetes mellitus (DM) among population in last decades and this cause about 5 million deaths every year consequences by complications, diabetes mellitus type 2 (DM2) is a disease related to lifestyle and other factors like glyce-

mic control by food intake and treatment, more than 422 million of adults are living with DM worldwide, projected to reach about 642 million by 2040 [1]. The diabetes burden is mostly impacted resource-limited countries where screening and access to medication and care are not readily available [2].

The major public health among DM patients is Poor and inadequate glycemic control and it is a significant risk factor of disease progression and development, that can markedly raise healthcare costs and life expectancy and quality reduction [3]. Glycemic control is considered the most effective means of preventing complications in DM [4]. However, a small percentage of DM cases maintain the level of blood sugar low than 7% glycated hemoglobin, while 53–70% of have uncontrolled [5].

Some reports found that good glycemic control might be implicated to access and availability to better knowledge level, primary care and best lifestyle [6, 7]. Furthermore, other investigations demonstrated that glycemic control is correlated with some factors like age, sex, disease duration, treatment type, body mass index (BMI), FBG, education, job type, comorbidities, self-care system, and psychosocial health [8-11].

Sleep duration and quality Optimizing is a way of glycemic control improving in DM2 [12]. Since sleep is related to some changes in hormone status which change glucose metabolism, it is important to assess the correlation between sleep duration with glycemic control [13].

AIM

The present article aims to study the influence of metformin response in sleep in diabetes mellitus patients type 2.

MATERIALS AND METHODS

Study subjects and design of study: a cross sectional study was conducted in diabetes mellitus center in Al-Saader hospital city during (February to may/2024), about 80 DM type 2 cases were enrolled in this study, all patients under metformin drug (1000 mg/ day) only. Each patients was diagnosed as type 2 DM using clinical and biomarker which treated by metformin drug (1000 mg/ day) for at least 3 month.

ETHICAL APPROVAL

Ethical approval: this study was conducted according to ethical approval of environment and health ministry in Iraq on (20-9-2023), written consent was optioned from each case to contributed in this research.

SAMPLE COLLECTION

About 3 ml of blood was drained from Venus by disposable siring, about one ml put in EDTA tube and other quantity was using to serum extraction by gel tube, serum then transfer to store at -20 °C.

GENETIC STUDY

DNA was isolated from whole blood using extraction kit, the target SNPs of SLC47A2: Intron Variant sequence was amplified by specific primers and TM 58°C, the products were sent to macrogene company (Korea) for sequencing, data were analyzed using MEGA11.

DATA COLLECTION:

Data was collected from patients by questionnaire which included (name, age, weight, length, sex, duration of disease, education, family history, job, sleep period and supplement uptake (including vitamins and minerals).

Exclusion criteria: the following criteria was excluded from this study included (obesity patients, cancer patients, viral infection patients, patients with other type of diabetic medications, diabetes complications and patients refused to contribute in this research).

Glycemic parameters test: Fasting blood glucose (FBG) and HbA1c% were estimated by routine lab work, insulin level was estimated using BT lab detection kit (E0010Hu), HOMA-IR and insulin sensitivity according to Minh et al., [14].

DATA ANALYSIS

The study subjects were classified to three categories of glycemic control included (<7 is good control, 7-8 is intermediate control and > 8 is poor control group) according to [15]. The sleep status also classified to three groups according to Seow et al., [16] included well sleep, intermediate sleep and insomnia. Results were represented as mean±SD for continuous data while percentage was used in categorical data. ANOVA one way and indpendant sample t test were used to compare among study groups, chi square, a linear regression to estimate the relationship between glycemic parameters and sleep in glycemic control groups, all analyses use $p < 0.05$.

RESULTS

The distribution of study subjects showed that only 26.3% of cases achieved well glycemic control, while 30% were in the moderately controlled group and the largest proportion, 43.8%, fell into the poor control group. This indicates that inadequate glycemic control was common among the study population.

When comparing sleep status across glycemic groups, the majority of cases in all categories were classified as having intermediate sleep quality. In the well-controlled group, 66.7% recorded intermediate sleep, whileist 28.6%

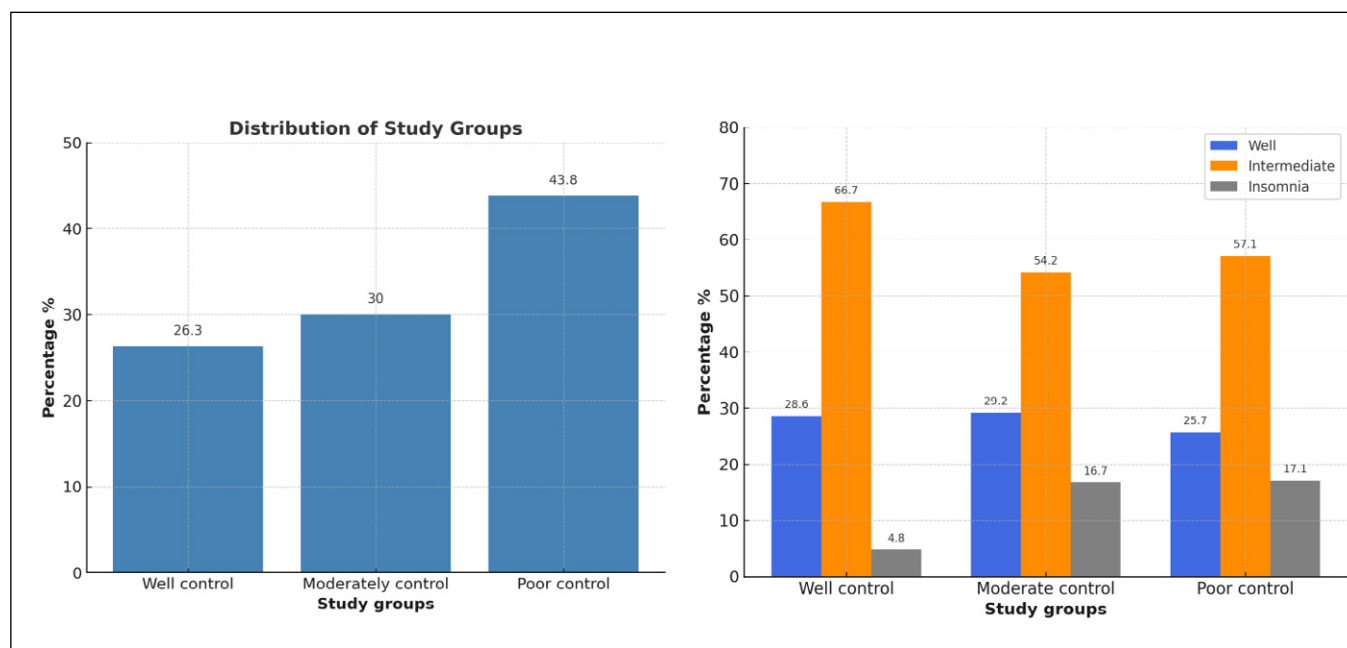


Fig. 1. Classification of Diabetes Mellitus (DM2) patients and sleep status based on glycemic control groups

Source: Own materials

had good sleep and low proportion 4.8% suffered from insomnia. In the moderately controlled group, 54.2% recorded intermediate sleep, 29.2% good sleep, and 16.7% insomnia. Among poorly controlled participants, 57.1% had intermediate sleep, 25.7% good sleep, and 17.1% insomnia, all changes were non-significant association ($p=0.722$) (Fig. 1).

These findings suggest that insomnia prevalence increased with worsening glycemic control (rising from 4.8% in well-controlled to 17.1% in poorly controlled participants). At the same time, the proportion of individuals with good sleep was lower in the poor control group compared with well and moderately controlled groups. Overall, the results indicate that poorer glycemic control is associated with greater sleep disturbance, although intermediate sleep problems were common across all glycemic categories (Fig. 1).

The comparison of baseline characteristics among glycemic control groups demonstrate no statistically significant differences in age, BMI, or disease duration ($p > 0.05$). Similarly, educational level, family history of diabetes, sex distribution, and job status did not differ significantly between groups. Although not significant, a greater proportion of poorly controlled patients had only primary school education (80%) compared with well-controlled patients (61.9%).

Supplement use was only variable reporting a statistically significant difference was ($p = 0.048$). A higher proportion of patients in the well-controlled group reported supplement intake (23.8%) compared with those in the moderate (4.8%) and poor control groups (8.6%). This suggests a possible beneficial role of supplementation in maintaining better glycemic control.

Overall, the results indicate that demographic and socio-clinical factors such as age, sex, education, occu-

pation, or family history were not strongly associated with glycemic control. However, supplement intake appeared to have a positive association with glycemic outcomes, and poor glycemic control tended to coincide with a higher prevalence of sleep disturbance.

The comparison of study variables across the three glycemic control groups is clarified in Table (1). As expected, FBG and HbA1c% showed highly significant differences among the groups ($p = 0.000$). Patients in the poor control group had markedly higher FBG (293.02 ± 87.69 mg/dL) compared with the moderate (209.9 ± 49.04 mg/dL) and well control groups (139.04 ± 45.11 mg/dL). In same manner, HbA1c% values elevate progressively from the well-controlled group to the moderate group and were highest in the poor control group in Table (2).

In contrast, IN did not differ significantly across groups (6.32 ± 4.93 , 10.32 ± 13.04 , and 7.34 ± 8.43 mIU/L, $p = 0.513$). Likewise, HOMA-IR values were higher in the moderate (92.20 ± 110.71) and poor control groups (94.40 ± 116.80) compared to the well control group (39.58 ± 31.39), but the difference did not reach statistical significance ($p = 0.185$), likely due to large variability. IS also showed no significant variation between groups ($p = 0.472$).

When cases were compared according to sleep status groups (good, intermediate, insomnia), no statistically significant differences were found across biochemical parameters ($p > 0.05$). Age, BMI, and disease duration were comparable between groups, though cases with insomnia tended to have slightly longer disease duration (9.27 ± 4.83 years) than those with good (6.65 ± 4.84 years) or intermediate sleep (6.11 ± 5.19 years). FBG and

Table 1. Social-demographic distribution of study subjects according to glycemic control group

Study variables	Well control	Moderate control	Poor control	P
Age (year)	49.38±8.85	52.25±	53.02±9.233	0.590
BMI (kg/M2)	28.17±3.92	27.50±2.95	26.76±4.478	0.402
Duration	5.17±5.28	7.68±5.21	6.93±5.980	0.787
Education				
Primary school	13 (61.9)	17 (70.8)	28 (80.0)	0.5205
High school	5 (23.8)	5 (20.8)	6 (17.1)	
Undergraduate	3 (14.3)	2 (8.3)	1 (2.9)	
Family history				
Yes	13 (61.9)	16 (66.7)	26 (74.3)	0.604
No	8 (38.1)	8 (33.3)	9 (25.7)	
Sex				
Male	7 (33.3)	8 (33.3)	16 (45.7)	0.529
Female	14 (66.7)	16 (66.7)	19 (54.3)	
Sleep period				
Well	6 (28.6)	7 (29.2)	9 (25.7)	0.722
Intermediate	14 (66.7)	13 (54.2)	20 (57.1)	
Insomnia	1 (4.8)	4 (16.7)	6 (17.1)	
Supplement				
Yes	5 (23.8)	1 (4.8)	2 (8.6)	0.048
No	16 (76.2)	23 (95.2)	33 (91.4)	
Job				
Yes	10 (47.6)	8 (33.3)	13 (37.1)	0.597
no	11 (52.4)	16 (66.7)	22 (62.9)	

Source: Own materials

Table 2. Glycemic parameters and sleep period mean differences in the study groups.

Study variables	Well control	Moderate control	Poor control	P
FBG mg/dL	139.04±45.11	209.9±49.04	293.02±87.69	0.000
HbA1c%	5.96±0.52	7.90±0.724	11.43±1.797	0.000
IN mIU/L	6.32±4.93	10.32±13.04	7.34±8.43	0.513
HOMA-IR	39.58±31.39	92.20±110.71	94.40±116.8	0.185
IS	1.12±0.413	1.20±0.46	1.06±0.46	0.472
Sleep period (hours)	6.09±1.37	6.00±1.44	5.80±1.47	0.741

Source: Own materials

HbA1c% were non-significant highest in the insomnia group (256.9 ± 87.74 mg/dL and $9.93 \pm 2.56\%$), Insulin and HOMA-IR levels varied widely across groups; patients with insomnia showed lower insulin (3.67 ± 1.57 μ IU/mL) and lower HOMA-IR (41.81 ± 20.92) compared to the other groups, though again without statistical significance. Insulin sensitivity (IS) was slightly decrease in the insomnia group (0.92 ± 0.27) compared to those with better sleep, but the trend was not significant.

In other words, the results suggest that while cases with insomnia showed a tendency toward poorer glycemic control (higher HbA1c and FBG, lower insulin sensitivity) and longer disease duration, these differences were non statistical significance (Table 3).

Multiple regression analysis was performed separately for the well, moderate, and poor glycemic control groups, with

sleep as the dependent variable. Across all three groups, none of the biochemical predictors were statistically significant ($p > 0.05$). In the well-controlled group, IN showed the highest standardized coefficient ($\beta = 1.154$, $t = 0.988$, $p = 0.339$), suggesting a possible positive influence on sleep, although the effect was not significant. Other predictors, including FBG and HbA1c, demonstrated weak and non-significant effects.

In the moderately controlled group, all predictors had small standardized coefficients with no significant differences to sleep. HbA1c ($\beta = -0.119$, $p = 0.659$) and IN ($\beta = -0.126$, $p = 0.968$) indicated weak negative associations, while insulin resistance ($\beta = 0.207$, $p = 0.948$) showed a negligible positive association.

In the poor-control group, only the model constant was statistically significant ($t = 2.315$, $p = 0.028$). While none of the biochemical variables independently pre-

Table 3. Glycemic parameters mean differences according to sleep status in the study groups

Study variables	Good sleep	Intermediate sleep	Insomnia	P
Age (year)	49.36±8.12137	52.76±10.05682	52.81±8.400	0.351
BMI (Kg/m ²)	26.47±3.70	27.87±3.89	26.94±4.49	0.366
Duration (year)	6.65±4.84	6.11±5.19	9.27±4.83	0.182
FBG	206.00±88.214	231.00±95.416	256.90±87.74	0.312
HbA1c%	8.857±2.795	8.746±2.61	9.933±2.56	0.410
IN	8.92±8.923	8.53±10.51	3.67±1.57	0.266
HOMA-IR	79.29±80.770	88.17±118.92	41.81±20.92	0.398
IS	1.17±0.48	1.14±0.46	0.92±0.27	0.295

Source: Own materials

Table 4. Multiple regression analysis of predictors of sleep across glycemic control groups

Variable	Well Control β	t	Sig.	Moderate Control β	t	Sig.	Poor Control β	t	Sig.
Constant	–	1.260	0.227	–	1.574	0.133	–	2.315	0.028
FBG	-0.216	-0.437	0.668	0.021	0.059	0.953	-0.087	-0.356	0.724
HbA1c	0.166	0.603	0.555	-0.119	-0.448	0.659	0.115	0.632	0.532
IN	1.154	0.988	0.339	-0.126	-0.041	0.968	0.909	1.002	0.325
IS	-0.690	-1.387	0.186	-0.075	-0.149	0.883	0.228	0.758	0.454
IR	-0.411	-0.383	0.707	0.207	0.066	0.948	-0.901		

Source: Own materials

dicted sleep, IN ($\beta = 0.909$, $p = 0.325$) and IR ($\beta = -0.901$, $p = 0.284$) showed relatively stronger but opposing trends, suggesting potential metabolic imbalance in this subgroup (Table 4).

Comparison across groups found that the well-controlled group display slightly stronger insulin-related effects, the moderate group displayed the weakest associations overall, and the poor-control group demonstrate more pronounced but non-significant trends for IN and HOMA-IR

These outputs indicate that glycemic biomarkers alone do not significantly predict sleep in any control category. Sleep quality in DM2 may instead be influenced by a combination factors including disease duration, psychological stress and lifestyle. Although IN and HOMA-IR trends in the poor-control group may point to underlying biological relationships, further studies with others factors are needed to clarify these associations.

In the well and moderate glycemic control groups, no significant changes were found in sleep across supplement use, education level, job status, family history, and sex. This suggest that in patients with better glycemic regulation, sociodemographic variables did not appear to have a measurable impact on sleep quality.

By contrast, in the poor control group, some socio-demographic factors effect significantly in the sleep. Supplement use was associated with higher sleep, indicating a potential benefit of supplementation in cases with poor glycemic control. Education level also influenced sleep quality, with high school graduates

reporting better sleep compared with those with primary or undergraduate education. Job status was another significant factor, as employed individuals had higher sleep compared to unemployed individuals. Sex differences were not significant but showed a trend toward higher sleep in males compared to females. Family history did not show any significant relationship with sleep in any of the groups (Table 5).

Taken together, these findings suggest that sociodemographic factors exert a stronger influence on sleep quality among DM2 with poor glycemic control, while their impact is negligible in those with well or moderately controlled diabetes. This finding highlights the complex interaction between metabolic control and sociodemographic determinants in shaping sleep outcomes.

GENETIC STUDY OF SLC47A2: INTRON VARIANT

In this study, intronic variants of SLC47A2 gene were analyzed to investigate their potential association with glycemic control and sleep status in DM cases, two SNPs were identified: a novel variation g19716681G>C and rs1597652185, statistical analysis revealed non-significant association between either variants and glycemic or sleep quality (Tables 5 and 6), interestingly, both variants exhibited similar frequency across the study variables (Fig. 2), suggesting shared distribution pattern within the population samples.

Table 5 Mean $\pm$ SD sleep scores by sociodemographic variables across glycemic control groups

Variable	Well (Mean $\pm$ SD)	Moderate (Mean $\pm$ SD)	Poor (Mean $\pm$ SD)
Supplement (No)	6.13 $\pm$ 1.41	6.00 $\pm$ 1.48	5.59 $\pm$ 1.36
Supplement (Yes)	6.00 $\pm$ 1.41	6.00 $\pm$ 0.09	8.00 $\pm$ 0.00
P	0.864	0.950	0.005*
Education (Primary)	6.31 $\pm$ 1.25	6.24 $\pm$ 1.52	5.50 $\pm$ 1.43
Education (High school)	6.00 $\pm$ 1.73	5.40 $\pm$ 0.89	7.17 $\pm$ 0.98
Education (Undergrad.)	5.33 $\pm$ 1.53	5.50 $\pm$ 2.12	6.00 $\pm$.
P	0.558	0.480	0.036*
Job (No)	6.18 $\pm$ 1.25	5.75 $\pm$ 1.39	5.32 $\pm$ 1.29
Job (Yes)	6.00 $\pm$ 1.56	6.50 $\pm$ 1.51	6.62 $\pm$ 1.45
P	0.771	0.239	0.010*
Family history (No)	6.13 $\pm$ 1.46	5.88 $\pm$ 1.13	5.33 $\pm$ 1.22
Family history (Yes)	6.08 $\pm$ 1.38	6.06 $\pm$ 1.61	5.96 $\pm$ 1.54
P	0.940	0.772	0.276
Sex (Male)	6.29 $\pm$ 1.80	6.13 $\pm$ 1.46	6.25 $\pm$ 1.53
Sex (Female)	6.00 $\pm$ 1.18	5.94 $\pm$ 1.48	5.42 $\pm$ 1.35
P	0.665	0.772	0.097

Source: Own materials

Table 6. The genotype distribution of g19716681G>C and rs1597652185 according to glycemic control

SNPs	Well control		Moderate control		Poor control		p
	GG	GC	GG	GC	GG	GC	
g19716681G>C	7	6	3	2	4	7	0.588051 ^{NS}
rs1597652185	7	6	3	3	4	7	0.588051 ^{NS}

Source: Own materials

Table 7. The genotype distribution of g19716681G>C and rs1597652185 according to sleep status.

SNPs	Good sleep		Intermediate sleep		Insomnia		p
	GG	GC	GG	GC	GG	GC	
g19716681G>C	4	2	9	9	1	2	0.3261 ^{NS}
rs1597652185	4	2	9	9	1	2	0.3261 ^{NS}

Source: Own materials

Table 6 shows the distribution of genotype of the two SLC47A2 intronic variants -g.19716681G>C and rs1597652185 - based on glycemic control status in DM cases. For the g.19716681G>C variant, the GG genotype was found in 7, 3, and 4 cases across the well-controlled, moderately controlled, and poorly controlled groups, respectively, while the GC genotype found in 6, 2, and 7 cases in the same respective groups. A similar distribution pattern was observed for rs1597652185, with the GG genotype recorded in 7, 3, and 4 individuals, and the GC genotype in 6, 3, and 7 individuals across the glycemic control groups, the statistical analysis showed non-significant association, these findings referred that the intronic SNPs g.19716681G>C and rs1597652185 do not significantly influence glycemic control in the studied DM cases

Table 7 clarified the genotypes distribution of SLC47A2 intronic variants - g.19716681G>C and rs1597652185—according to sleep status. For the g.19716681G>C variant, the GG genotype was observed in 4 cases with good sleep, 9 with intermediate sleep, and 1 with insomnia. The GC genotype was detected in 2, 9, and 2 cases, respectively, across the same sleep categories. A nearly identical distribution was observed for rs1597652185. Statistical analysis found a p-value of 0.3261 for both variants, indicating no significant association between genotype and sleep status. These findings suggest that the g.19716681G>C and rs1597652185 variants of the SLC47A2 gene are not significantly related to sleep quality among the cases.

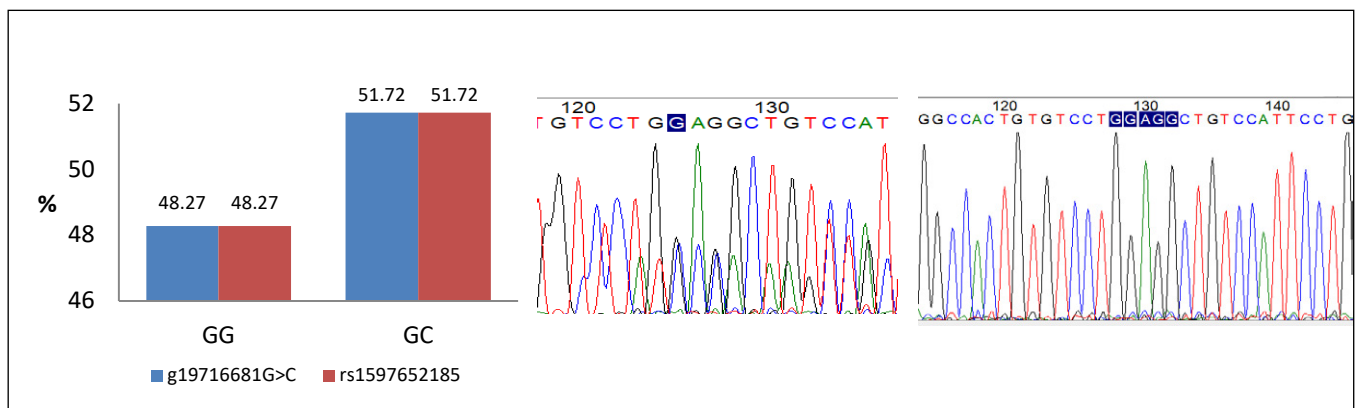


Fig. 2. The genotypes distribution of g19716681G>C and rs1597652185 with histogram in study cases

Source: Own materials

DISCUSSION

This study as suggested to investigate the problem of insomnia accompanying most of DM 2 patients, the results showed that HbA1c% which depended in study group classification has significant effect in sleep, evidences explored that good glycemic control achievement among DM2 cases is a paramount essential in delaying and/or early onset of complications preventing that related to morbidity and mortality elevation, high percentage of study subjects enrolled in poor glycemic control and this dis agree with general coverage in worldwide that found about 50% achieved good glycemic control [17], in sub-Saharan Africa region reports have demonstrated that majority (74%) of DM2 have poor glycemic control [18]. Other investigations agree with this results, [19] indicated that the prevalence of poor glycemic control observed was significantly high. The explanation in the poor glycemic control prevalence among DM2 have been reported in many countries and this perhaps belong to different factors, like health systems improvement including DM2 care and knowledge among patients and population about diseases like DM2 and how to improvement blood sugar levels control, also the Fragile health systems most common factors which effect in the diabetes care [20, 21]. The irregular follow up of patients, bad lifestyle, low exercise activity and genetic factors are implicated in glycemic control [22-23].

Sleep status is a factor that was significantly associated with poor glycemic control which based on the HbA1c% level in present work, results showed significant association with insomnia (<4 h). this result agree with report of [24, 25], in Brazil and Japan, in contrast, long sleep period significant association with poor glycemic control have been reported in Japan, and China who clarified that good sleep helps to have good glycemic control during 6-8 h [26, 27].

Generally, the pattern of sleep has a major modulatory impact on metabolism of glucose and energy uptake that have effects on the good glycemic control mainte-

nance in DM2 cases, moreover, the high poor glycemic control prevalence with sleep deprivation and/or poor sleep quality can be belong to appetite up-regulation, and impairment of glucose metabolism [27], in addition to cortisol that causes elevation in plasma glucose and high insulin resistance and disturbance in melatonin hormone level which may affected by insulin [28, 29].

Regarding to others factors which depended in this study, the supplement uptake by DM2 cases showed that in the well and moderate glycemic control groups, sleep quality did not differ between supplement users and non-users ($p > 0.8$). However, in the poor control group, supplement users showed significantly better sleep ($p = 0.005$). This align with study that certain supplements - particularly magnesium - improve sleep duration and reduce insomnia severity in type 2 diabetes patients [30, 31]. Magnesium promotes melatonin production and regulates cortisol and GABA activity, thereby enhancing relaxation and sleep stability [32].

Regarding to Education Level no significant differences were observed in the well and moderate groups while significantly among poorly controlled patients, with high school graduates reporting better sleep. Lower education has been linked to reduced diabetes self-management capacity and increased sleep disturbance [33].

Job status did not affect sleep in well or moderate groups, but in the poor-control group, employed individuals had significantly better sleep ($p = 0.010$). Employment provides structure, social stability, and financial security, which are known to support healthier sleep patterns and diabetes outcomes. Family history had no significant impact on sleep in any group ($p > 0.27$), suggesting that hereditary predisposition alone does not directly influence sleep quality. Sex differences in sleep were non-significant in all group which partially dis agree with [34].

This study detected the association between two intronic variants of the SLC47A2 gene g.19716681G>C and

rs1597652185 with glycemic control and sleep quality in DM2 cases, The finding revealed no significant results between either variant and glycemic markers or sleep quality. Both SNPs found similar genotype frequencies in study groups in non-significant p-values for both glycemic control ($p = 0.588$) and sleep status ($p = 0.326$), these suggesting that these intronic variants may not have a functional impact in the studied groups.

The *SLC47A2* gene encodes Multidrug And Toxin Extrusion Protein 2-K (MATE2-K), a renal transporter that has important role in endogenous metabolites and drugs excretion, like metformin (first-line antidiabetic agent). some reports have explored the pharmacogenetic role of *SLC47A2* variations, in the context of metformin pharmacokinetics and therapeutic efficacy. As well as, Becker et al. [35] found that common variants in *SLC47A2* can influence renal clearance of metformin, potentially affecting glycemic response. Moreover, [36] demonstrated that certain *SLC47A2* polymorphisms were correlated with variations in metformin efficacy among Chinese patients with T2DM.

However, most studies have highlighted on exonic or regulatory variants with known functional consequences, while the clinical significance of intronic variants remains less understood. Intronic SNPs may influence gene expression by impact on splicing or regulatory elements, but not all intronic changes exert measurable phenotypic effects. The absence of association in the current study may indicate that the investigated variants do not affect *SLC47A2* expression or function, or that their effect is too subtle to be detected in this sample size.

In addition, the disturbances in sleep are commonly recorded in DM2 cases and have been linked to poor glycemic control, insulin resistance, and cardiovascular complications [37, 38]. Despite of some genetic reports

have studied clock genes or neurotransmitter-related pathways in relation to sleep regulation and DM, there is limited studies linking drug transporter genes such as *SLC47A2* to sleep status. Therefore, this findings of no significant association between these variants and sleep patterns are consistent with the current lack of mechanistic or clinical evidence supporting such a relationship.

CONCLUSIONS

This study demonstrated that poor glycemic control was common among the study population, with nearly half of the patients falling into the poorly controlled group. While sleep disturbances, particularly intermediate sleep quality, were prevalent across all glycemic categories, the prevalence of insomnia increased with worsening glycemic status. Although most demographic and clinical factors such as age, sex, BMI, education, occupation, and family history were not significantly associated with glycemic control or sleep, supplement intake emerged as a significant factor, being more frequent among well-controlled patients and associated with better sleep quality in poorly controlled individuals.

Regression analyses suggested that glycemic biomarkers alone were not sufficient to predict sleep quality. Finally, genetic analysis of *SLC47A2* intronic variants revealed no association with glycemic status or sleep.

Overall, these findings highlight the interplay between glycemic regulation, sleep quality, and lifestyle factors, suggesting that interventions targeting metabolic control alongside supportive measures such as supplementation may improve sleep and health outcomes in patients with type 2 diabetes.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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Psychological aspects of the quality of life of men with hypoactive sexual desire disorder

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ABSTRACT

Aim: To analyze the psychological aspects of the quality of life of men with hypoactive sexual desire disorder.

Materials and Methods: An observed case-control study was conducted. The main group consisted of 61 men with hypoactive sexual desire disorder, the control group consisted of 200 practically healthy men. Standard questionnaires were used to assess quality of life and psychological characteristics: Quality of Life Enjoyment and Satisfaction Questionnaire – Short Form (Q-LES-Q-SF), Oxford Happiness Inventory (OHI), Hospital Anxiety and Depression Scale (HADS).

Results: It was found that hypoactive sexual desire disorder in men is accompanied by a significant decrease in the quality of life (by 37.5%), the main components of which are: dissatisfaction with sexual drive, interest and/or performance (98.4% of respondents), mood (55.7 %), leisure time activities (44.4%) and general physical health (41.0%). Men with hypoactive sexual desire disorder are characterized by an extremely low level of the integral happiness index (13.8% [6.9-20.7] of the maximum possible 100% according to the OHI scale) against the background of 100% prevalence of clinically expressed depression and significant prevalence of subclinical (32.8%) and clinically expressed (55.7%) anxiety. It was displayed a strong negative Correlation between the integral happiness index and the level of anxiety ($r_s = -0.89$) and a moderate negative Correlation – between the integral happiness index and the level of depression ($r_s = -0.43$).

Conclusions: In the management of hypoactive sexual desire disorder a mandatory component should be psychological therapy aimed at depression and anxiety correcting and the quality of life improving.

KEY WORDS: male hypoactive sexual desire disorder, quality of life, anxiety, depression, happiness index, medical care management

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INTRODUCTION

Libido is one of the basic instincts that people need to reproduce, and satisfying their sexual desires is an important aspect of their quality of life.

Libido varies greatly from person to person and can temporarily decrease due to various psychological conditions such as fatigue, anxiety, etc. However, a consistently low libido already belongs to the components of male sexual dysfunction. According to the 4th International Consultation on Sexual Medicine (ICSM), Hypoactive Sexual Desire Disorder (HSDD) is defined as a “persistent or recurrent deficiency or absence of sexual or erotic thoughts or fantasies and desire for sexual activity (clinical principle)”. The ICSM consensus statement resulted in a detailed assessment of the International Classification of Diseases, 10th Edition (ICD-10) and the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) [1].

According to ICD-10 this disease has code F 52.0 as a primary problem, not a secondary one, for example, against the background of erectile dysfunction [2].

In the DSM-5, male HSDD is defined as “the persistent or recurrent deficiency (or absence) of sexual or erotic thoughts or fantasies and desire for sexual activity”. The decision about the level of deficiency or absence is determined by the clinician, taking into account factors that can affect sexual activity, such as: age; the general and sociocultural context of a man’s life; persistence of symptoms for at least six months that cause mild, moderate, or severe distress. HSDD can arise from the beginning of sexual life or after relatively normal sexual functioning; can be situational – limited to a certain type of stimulation, in certain situations and with certain partners, as well as generalized – not limited to a certain type of stimulation, in certain situations and with certain partners. Therefore, to establish a diagnosis of HSDD, a mandatory condition is the presence of distress caused by a weakened sexual drive, since there are men who are satisfied with such a condition [3].

A decrease in sexual desire is more characteristic of women, but it also occurs in men. According to various scientific data the prevalence rates of HSDD in the male

population in the world ranges from 3 to 28%, with an average of 15.8% [4-7]. In particular, the DSM-5 indicates that approximately 6% of men aged 18 to 24 and almost half (41%) aged 66 to 74 have problems with sexual desire, so in today's aging society it may be one of the important problems that worsen the quality of life of elderly couples. It is also noted that the prevalence of HSDD among men aged 40-80 varies in different cultures from 12.5% in Northern Europe to 28% in Southeast Asia [3].

All scientists agree that sex drive gradually declines with age, and is a well-known symptom of late-onset hypogonadism syndrome, caused by declining testosterone levels with age [8]. However, HSDD is also described in young men (18-29 years old) with prevalence rates from 6 to 19% [9-11].

Core etiological factors of HSDD are changes among the main three components of sexual drive, namely: psychological, cultural, and biological. Usually, these components are intertwined in clinical practice [12].

The psychological aspects of low libido are based on negative obsessions, such as: lack of erotic thoughts, preoccupation with erections, and limited attitudes towards sexuality. Unattractiveness of a partner, long-term relationships, conflicts in relationships, stress at work, anxiety, depression, age, can be provoking factors of HSDD [13-16].

Among the biological components of low libido in men, the most significant are: androgen deficiency, hyperprolactinemia, stroke, epilepsy, cardiovascular failure, coronary heart disease, chronic prostatitis/chronic pelvic pain syndrome, lower urinary tract symptoms, bodybuilding. Also, other sexual disorders, for example erectile dysfunction and premature ejaculation, can be accompanied by low sexual desire [7-8, 17].

Management of low sexual desire is based on etiological factors, and therefore it is advisable to combine psychotherapy and pharmacotherapy. It has been proven that psychological methods focused on cognitive and behavioral strategies, based on the needs of the couple, including age, are effective in the case of HSDD. Hormone replacement therapy is used for low testosterone levels, and dopamine agonists are used for hyperprolactinemia. In addition, it is necessary to correct endocrine disorders in diabetes, hyper- or hypothyroidism [18-20].

Despite the fact that enough scientific sources have been devoted to the study of HSDD, comprehensive researches of the psychological consequences of reduced libido are still lacking.

AIM

The purpose of this study was to analyze the psychological aspects of the quality of life of men with hypoactive sexual desire disorder.

MATERIALS AND METHODS

An observed case-control study was carried out during 2023-2024, at three private-funded health care out-patient facilities of the Ivano-Frankivsk region of Ukraine.

Sexual desire was assessed on the basis of the unified questionnaire - International index of erectile function (IIEF) [21] in the domain "Sexual desire" (with a score of less than 6 on questions 11, 12). Out of 402 men with sexual dysfunctions, 61 were diagnosed with «hypoactive sexual desire disorder».

The assessment of quality of life (QoL) was carried out on the basis of a short standardized questionnaire Q-LES-Q-SF (Quality of Life Enjoyment and Satisfaction Questionnaire – Short Form) [22], as well as the Oxford Happiness Inventory (OHI).

The Q-LES-Q-SF consists of 14 main questions about satisfaction with various aspects of quality of life and two additional questions about satisfaction with treatment outcomes and life in general. For each of the questions, the respondents chose one of the answers: very bad, bad, satisfactory, good, very good, which corresponded to points from 1 to 5. For each respondent, his individual quality of life integral index was also calculated for the main 14 questions as a percentage of the maximum score (70) and its percent decrease from the maximum possible 100%.

The OHI contains 29 questions, each of which has four answers, corresponding to points from 0 to 3. Accordingly, an individual integral happiness index was calculated for each respondent as a percentage of the maximum possible 87 points. At values of 0-20%, the index was evaluated as low, 21-40% - below average, 41-60% - average, 61-80% - above average, and 81-100% - high.

The HADS (Hospital Anxiety and Depression Scale) [23] was used for screening detection of anxiety and depression, which consists of 14 questions, the unpaired ones of which are designed to assess anxiety, and the paired ones – depression. There are four answers to each question, corresponding to points from 0 to 3. According to the corresponding sum of points (for paired and unpaired questions), the level of anxiety and depression was determined for each respondent: 0-7 points – normal, 8-10 points – subclinical, and more than 11 points – clinically expressed anxiety and depression.

The median age of the examinees was 33 [26.0-40.0] years. Accordingly, the largest shares of patients were under 30 years old (44.3%) and 30-39 years old (31.1%). The rest – equally represented each of the following age groups - 8.2% of people aged 40-49, 50-59 and over 60 years.

The individual data obtained were compared with the indicators of 200 practically healthy men who,

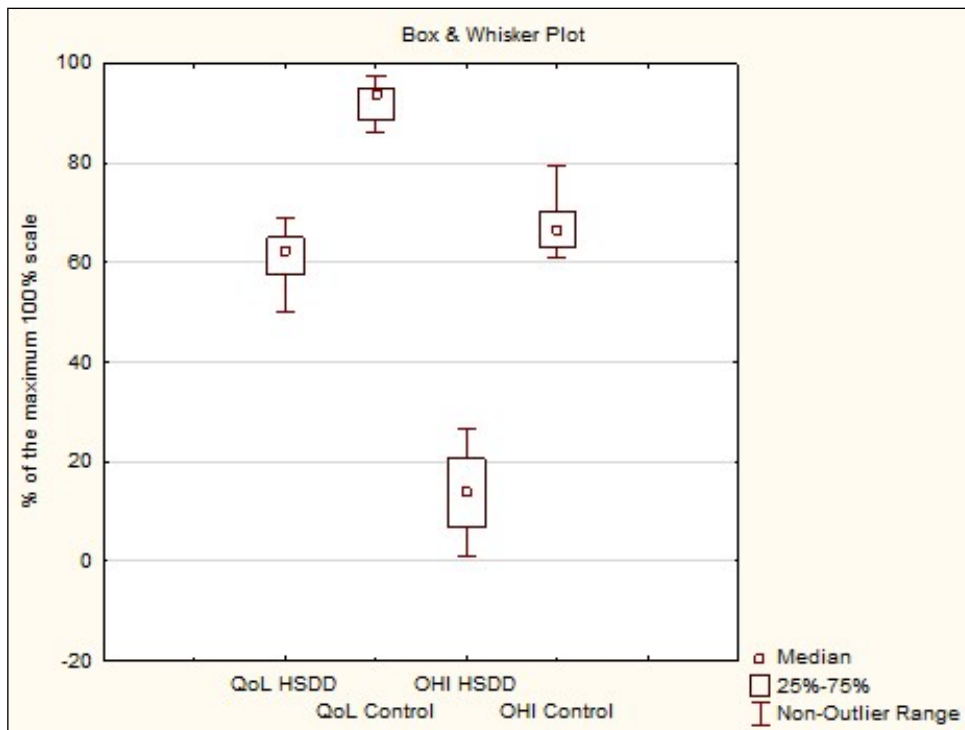


Fig.1. Quality of life integral indices and integral happiness indices in the comparison groups
Picture taken by the authors

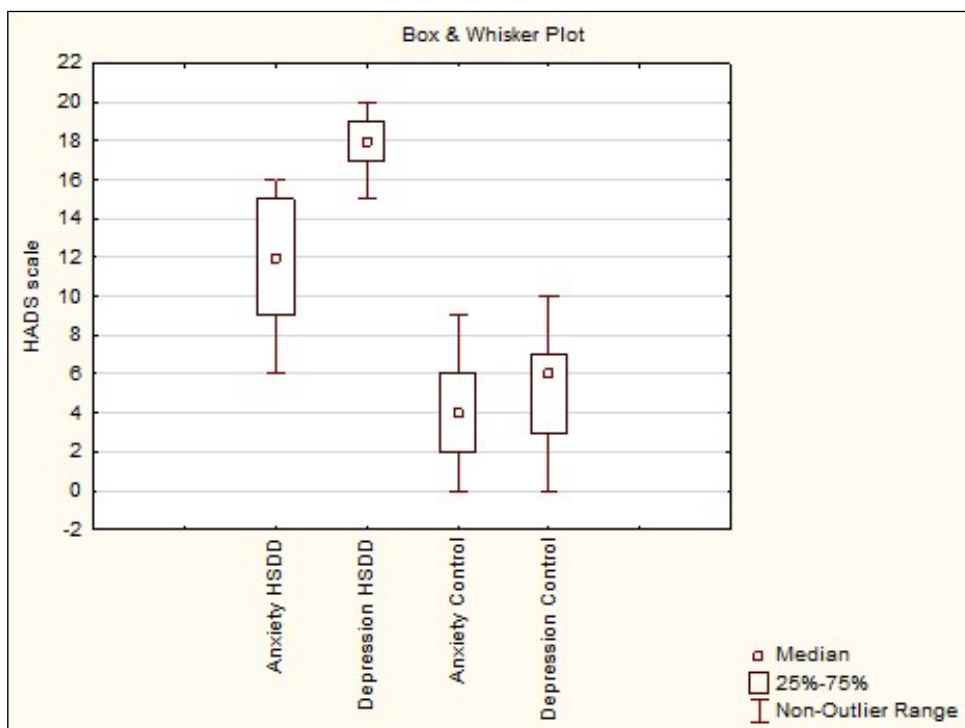


Fig. 2. Levels of anxiety and depression according to the HADS scale in comparison groups
Picture taken by the authors

according to the results of their examination, did not have sexual dysfunctions. The median age of the control group did not differ from the same age in the main group ($p > 0.05$) and was 33.0 [27.0-42.0] years.

The research design and programs were reviewed and approved by the Ivano-Frankivsk National Medical University Ethics Committee (protocol № 133/23 dated March 29, 2023).

All statistical calculations were performed with the help of built-in Microsoft Excel data analysis license

packages and the Statistic 10.0 program.

Quantitative data obtained in the study (age, scores of answers to individual questions and questionnaires in general, individual integral indicators of quality of life and indices of happiness) were first checked for the type of their distribution according to the Shapiro-Wilk's W test. Since most of them did not correspond to the law of normal distribution, the median (Me) and inter-quartile range (25%-75%) were chosen to represent the measure of central tendency, and non-parametric the

Mann-Whitney U test was used to assess the reliability of the data between the main and control groups.

Accordingly, the relationships between the indicators were evaluated based on the calculation of the non-parametric Spearman's rank correlation coefficient (r_s).

Statistical processing of categorical (qualitative) data was carried out by calculating the prevalence rates of answers per 100 r, and respondents the reliability of their differences in comparing groups was assessed using the Pearson chi-square (χ^2) test.

RESULTS

It was found that the median of the quality-of-life integral index of respondents with HSDD is significantly lower than in the control group: 62.5% [57.5-65.0] of the maximum possible 100% versus 93.6% [88.6-95.0], respectively ($p < 0.001$). That is present decrease of QoL in men with HSDD was -37.5%, while in the control group the quality of life was reduced by 6.4%.

The study of individual components of QoL showed that the main reasons for its deterioration in the respondents of the main group were quite predictably dissatisfaction with sexual drive, interest and/or performance (98.4% of respondents), combined with negative self-assessments of mood (55.7%), quality of leisure time activities (44.3%) and general physical health (41.0%), while dissatisfaction with other aspects of QoL (financial situation, work, relationships with people, etc.) ranged from 6.6-18.0%.

Such an obvious predominance of psychological aspects of QoL in respondents with HSDD may explain the generally low integral happiness index OHI among them (Fig. 1).

Thus, the median of the integral happiness indices in men with HSDD was only 13.8% [6.9-20.7] of the maximum possible 100%, which is assessed as a low level according to the criteria for interpreting the results of the OHI questionnaire. It should be noted that in the control group this index was significantly higher ($p < 0.001$), but far from high, and corresponded to an above average level - 66.7% [63.2-70.1].

The prevalence rates of an anxiety and depression were much higher in the group of men with HSDD than in the control group ($p < 0.001$). Thus, only 11.5% of the respondents of the main group corresponded to the norm according to the "Anxiety" subscale of HADS, 32.8% had subclinical anxiety and more than half (55.7%) had clinically expressed anxiety. As for depression, all 100% of the respondents from the main group had clinically expressed one. The majority of respondents from the control group had the norm according to the HADS criteria and none of them had

clinical of anxiety and depression, but 6.5% of them had subclinical anxiety and almost every fifth (21.0%) – subclinical depression.

Accordingly, the median of scores on the "Depression" subscale in the HSDD group were higher than on the "Anxiety" subscale: 18.0 [17.0-19.0] versus 12.0 [9.0-15.0] points (Fig. 2). In the control group, similar indices were 6.0 [3.0-7.0] and 4.0 [2.0-6.0] points ($p < 0.001$).

The correlation analysis displayed that in men with HSDD there is a negative strong Correlation ($r_s = -0.89$, $p < 0.05$) between the integral happiness index and the level of anxiety (Fig. 3) and a moderate negative Correlation – between the integral happiness index and the level of depression ($r_s = -0.43$, $p < 0.05$) (Fig. 4).

DISCUSSION

The hypoactive sexual desire disorder is more often studied in the female population, since this pathology is more common among them [24-25]. While among men, scientists pay more attention to other forms of sexual disorders that are more common among them, such as erectile dysfunction and premature ejaculation. [26]. At the same time, HSDD is also occur in men and is accompanied, as research data show, by low sexual well-being, the sexual distress growth, and the deterioration of interpersonal relationships due to the decrease of the quality of sexual life [27-29].

Usually, scientists note a decline in sexuality in men with age [30], but they emphasize that HSDD also occurs among young people [11]. In particular, our study included 61 male persons with HSDD, whose median age was 33 [26,0-40,0] years, which highlights of the problem relevance, because they are mostly young workable people. Nevertheless, the data obtained in our study showed a significant decrease in the quality of life in men with HSDD – by 37.5%, mainly due to the psychological components of the QoL. In our opinion, this can serve as an explanation for the critically low level of the integral happiness indices OHI, the median of which in the observed men with HSDD was only 13.8% [6.9-20.7] of the maximum possible 100%. However, in the control group, this index corresponded to an above-average level - 66.7% [63.2-70.1]. In our opinion, this situation is largely explained by the fact that the study was conducted during a full-scale war in Ukraine, which has a negative impact on the mental health of all population.

It should be noted that the scientists pay considerable attention to such psychological companions of HSDD as anxiety and depression, which can be both predictors of the occurrence and manifestations of reduced sexual desire. [12, 14-17]. In our study, it was shown that depression prevails

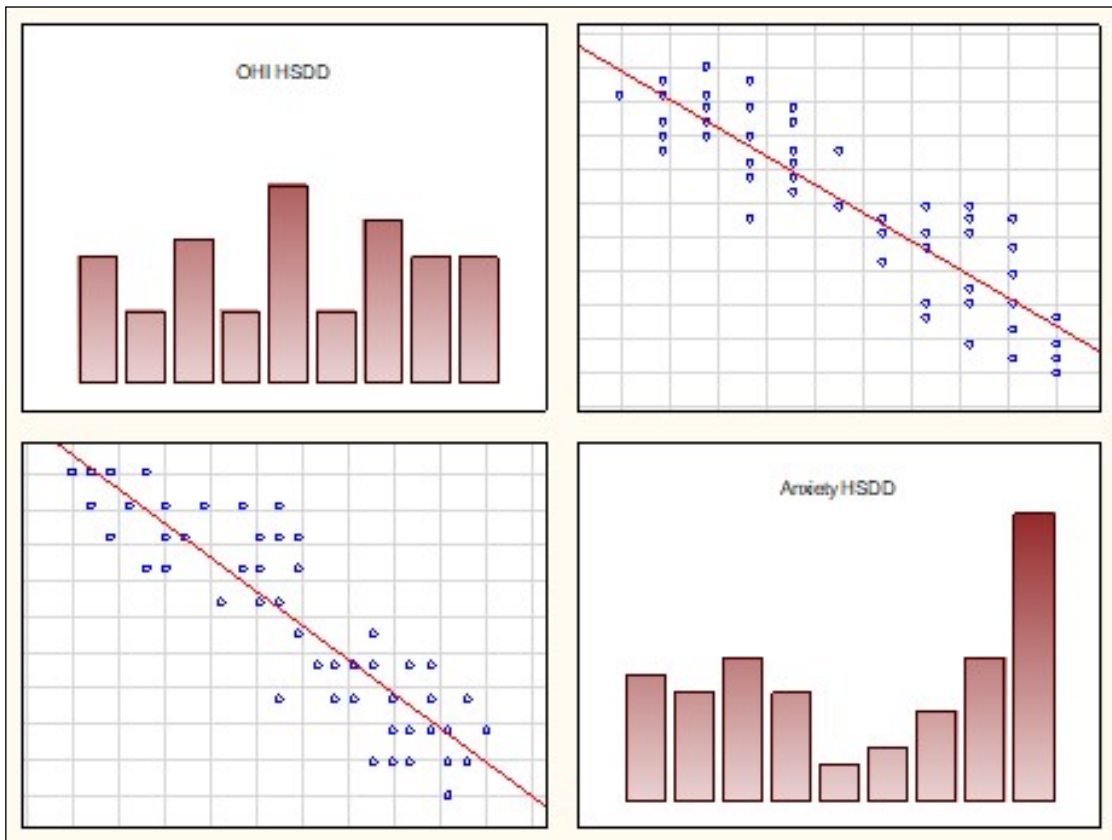


Fig. 3. Correlation between the integral happiness index and the level of anxiety among men with hypoactive sexual desire disorder
Picture taken by the authors

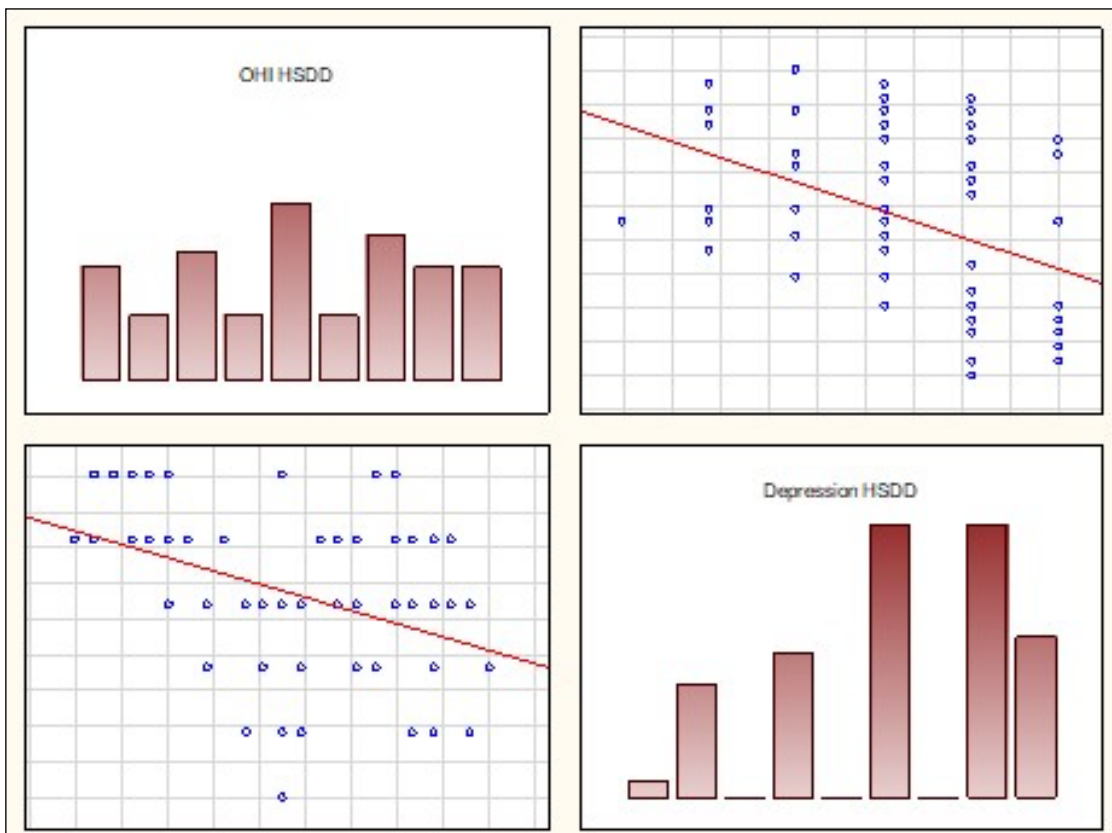


Fig. 4. Correlation between the integral happiness index and the level of depression among men with hypoactive sexual desire disorder
Picture taken by the authors

in men with HSDD - 100% of them had clinically advanced form according to the HADS scale. The same level of anxiety was found in 55.7% of respondents in the main group, and another 32.8% had subclinical manifestations of it.

The correlation analysis displayed that in men with HSDD there is a negative strong Correlation ($r_s = -0.89$) between the integral happiness index and the level of anxiety and a moderate negative Correlation – between the integral happiness index and the level of depression ($r_s -0.43$). That is, with the development of anxiety and depression, the feeling of happiness decreases.

LIMITATIONS

The study was conducted during the full-scale aggression of the Russian Federation, which could deepen psychological reactions and be an additional factor in the decrease in sexual activity due to the generally negative impact of the war on the mental health of the whole population.

The research was conducted in private-owned health-care facilities. After all, currently there are practically no publicly-owned health care facilities in Ukraine where patients can receive sexological care, which, as it is known, has a negative impact on its financial accessibility. Obviously, this was the main reason why the sample included mostly young working-age people, although HSDD are more common among older men. On the other hand, this may be a reflection of the higher need

of young men to seek medical care when the quality of their sexual life decreases, while older people tend to consider a decrease in libido as a normal option.

CONCLUSIONS

It was found that hypoactive sexual desire disorder in men is accompanied by a significant decrease in the quality of life (by 37.5%), the main components of which are: dissatisfaction with sexual drive, interest and/or performance (98.4% of respondents), mood (55.7 %), leisure time activities (44.4%) and general physical health (41.0%).

It is shown that men with hypoactive sexual desire disorder are characterized by an extremely low level of the integral happiness index (13.8% [6.9-20.7] of the maximum possible 100% according to the OHI scale) against the background of 100% prevalence of clinically expressed depression and significant prevalence of sub-clinical (32.8%) and clinically expressed (55.7%) anxiety.

It was displayed a strong negative Correlation between the integral happiness index and the level of anxiety ($r_s = -0.89$) and a moderate negative Correlation – between the integral happiness index and the level of depression ($r_s = -0.43$).

In the management of hypoactive sexual desire disorder a mandatory component should be psychological therapy aimed at depression and anxiety correcting and the quality of life improving.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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Evaluation of the nephroprotective effect of Dibenzazepin γ -secretase inhibitor on renal ischemia reperfusion injury in male rats

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ABSTRACT

Aim: This study was performed to investigate the Dibenzazepin potential nephroprotective effect on bilateral renal ischemia/reperfusion injury in model of male rats.

Materials and Methods: Male rats (number 40) were classified into four groups' n=10: first sham, second control, third DMSO, and fourth DBZ, the sham group had a median laparotomy under anaesthesia without induction of ischemia/reperfusion; the control group underwent clamping for thirty minutes on the bilateral renal artery, after that two hours of reperfusion; the vehicle group received DMSO one hour before induction of ischemia; and the DBZ group received 2 mg/kg of DBZ one hour before ischemia. Biochemical parameters (Kidney injury molecules KIM1, IL-1 β , TNF- α , F2-isoprostane, GSH, and caspase-3) were assessed by ELISA technique. Furthermore, histological changes were investigated, and the Notch signalling pathway was evaluated using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR).

Results: IRI caused a significant increase in renal tissues of kidney injury molecules (KIM1), IL-1 β , TNF- α , F2-isoprostane, and caspase-3) with significant decreased the level of GSH, DBZ pre-treated mitigated these effects by significant enhancing antioxidant markers and decreased inflammatory and apoptotic markers. Improving histological results and significant decrease the Notch1 and Jagged-1 gene expression in kidney tissues after renal ischemia/reperfusion damage.

Conclusions: DBZ (γ -secretase inhibitor) has considerable nephroprotective benefits in renal IRI *via* inhibiting the Notch pathway, anti-apoptotic, antioxidant, and anti-inflammatory effect.

KEY WORDS: renal IRI, DBZ, inflammation, oxidative stress, Notch1, Jagged-1, apoptosis, nephroprotection

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INTRODUCTION

Kidney ischemia-reperfusion (IR) damage present when there is a brief decrease in blood flow to the kidneys, which is return comes after that. Cellular stress is the result of oxygen and nutrient depletion caused by this disruption in blood flow. Restored of blood flow caused triggered of burst from ROS. This increases inflammation and oxidative stress, which cause damage of renal tubules and lead to acute kidney injury (AKI). Significant alterations take place in the mitochondria under certain circumstances, such as decreased mitochondrial energy, excessive mitochondrial fragmentation, and increased formation of (ROS) within the mitochondria [1]. The following of ischemic insult, a significant decrease in mitochondrial adenosine triphosphate generation, consequent opening of mitochondrial permeability transition pore and membrane potential mPTP [2]. Proximal tubule cells' ATP levels drop to a trough in a minutes after ischemia is induced [3]. Moreover, this

fall in the levels of ATP might limit Na⁺/K⁺-ATPase activity, resulting in the build-up of intracellular Na⁺ and the subsequent influx of Ca²⁺, which leads to mitochondrial aggregation. After reperfusion, significant enhancements in reactive oxygen species and calcium ions levels inside mitochondria might cause dysfunction of mitochondrial [3-4]. Reactive oxygen species such as the hydroxyl radical interact with DNA cause damage the ribose-phosphate backbone. Additionally, reactive oxygen species' interactions with lipid bilayers remove hydrogen atoms from unsaturated fatty acids which bound to phospholipid; this process is called lipid peroxidation which lead to reduce the integrity and functional of cellular membranes [5-6]. Consequently, that cause the mPTP to release cytochrome C, starting a caspase cascade and beginning the mitochondrial death pathway [7-8]. Notch signalling pathway; Mammals contain Notch ligands include Jagged-1, Jagged-2, Delta-like 1, Delta-like 3, and Delta-like 4, as well as 4

Notch receptors (from Notch one to 4). The receptor and its ligands are represent transmembrane proteins with many extracellular domains [9]. Ligand interaction induces a modulation in the receptor which cause stimulating two successive proteolytic processes. The extracellular area is shedding as a result of the primary cleavage, that is achieved by metalloproteases belonging to the ADAM own family. The γ -secretase complex catalyses the second one cleavage, which occurs within the transmembrane domain. Following that, the Notch intracellular domain (NICD) is cleaved and travels to the nucleus to form a transactivation complex [10]. This technique removes co-repressing complexes via co-activators, leading in the transcription of Notch target genes which compose two families of transcriptional factors include hairy/enhancer-of-split linked with YRP-Wmotif (Hey) (HEY1 and HEY2), and hairy-enhancer of split (Hes) (HES-1 and HES-5) [11]. Moreover, at the time of ischemia reperfusion the activation of Notch pathway by ROS lead to activate metalloproteases (ADAM17) so indirectly trigger the discharge NICD, NICD translocate to the nucleuse and activate transcription of its target gens which cause the formation of fibrosis [12]. Diben-zazepin (DBZ) is a γ -secretase preventing the activation of all Notch signalling pathway by inhibit the Notch receptors [13]. Several γ -secretase inhibitors exhibit anti-proliferative and anti-inflammatory effects [14-15]. DBZ contributes to protecting mechanisms through its anti-inflammatory and antioxidative consequences, restoring regular levels of GSH, CAT, MDA, and iNOS to mitigate oxidative damage. Additionally, DBZ exhibits anti-tumour activity towards various types of cancer cells, beside it action on Notch pathway [16-17].

AIM

This study was performed to investigate the Diben-zazepin potential nephroprotective effect on bilateral renal ischemia/reperfusion injury in model of male rats.

MATERIALS AND METHODS

PREPARATION OF ANIMALS

We got 40 male Wistar albino rats aged 8 to 12 weeks from the University of Kufa's Faculty of Science, weighing between 200 and 300 g each. The rats lived at the Faculty of Science's animal facility at Kufa University. The animals were kept in cages with a 12h light/dark cycle, a temperature of $22 \pm 2^\circ\text{C}$, and humidity levels ranging from sixty to sixty five percent. The rats fed a standard diet of commercial regular chow pellets and tab water. The Institutional Animal Care were used in

all experimental protocols and Use Committee (IACUC) at the University of Kufa.

STUDY DESIGN

Forty Wistar albino male rats were randomly divided to four groups of ten each: sham, control, DMSO, and DBZ. The sham group had a median laparotomy under anaesthesia with no ischemia or reperfusion in the kidneys. While the IRI group (control group) submitted to clamping in bilateral renal artery for thirty minutes, after that two hours of reperfusion, the vehicle group received the DMSO 1 hour intraperitoneally one hour before the induction of ischemia [18-19]. In the DBZ group, 2 mg/kg DBZ was given intraperitoneally one hour before the start of I/R [20] and for anaesthesia during the surgery, the rats received IP injection of 10 mg per kg of xylazine and 100 mg per kg of ketamine [21].

ETHICAL CONSIDERATIONS

All animals that involved in this study deal with care, also in handling, treatment; feeding, scarification, and surgery operation criteria according to Institutional Animal Care and Use Committee (IACUC) at Kufa University by taking the approval after complete the required experiences or instructions (approval number 20546 in 29/8/2024).

SAMPLING TECHNIQUES

TISSUE SAMPLING FOR BIOCHEMICAL ANALYSIS

The tissues of kidney were kept at -80°C until homogenized using a high-intensity ultrasound liquid processor in 1:10 W/V phosphate buffered saline containing 1% Triton X-100 and a protease inhibitor cocktail. The homogenate samples were centrifuged at 4°C for 15 minutes at $17,550 \times g$ [22-23], then we tested the supernatants for kidney damage molecules (KIM, TNF- α , IL-1 β , F2-isoprostane, GSH, and caspase-3).

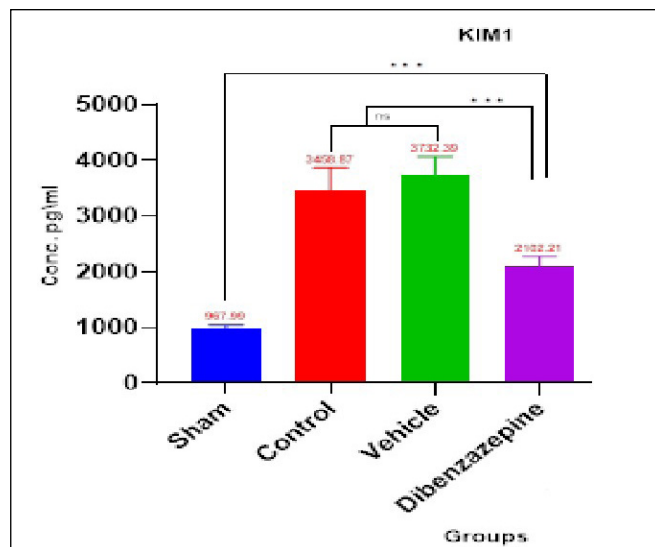
TISSUE SAMPLING FOR HISTOPATHOLOGY

The tissue of kidney slice was fixed in ten percent formaldehyde, then dehydrated with series of alcohol, rinsed with xylene, and embedded in paraffin. Kidney tissues were paraffin-fixed and cut into 5-m-thick slices. The sections were then stained with hematoxylin and eosin. Damaged cells were identified and scored in five non-overlapping pictures. The scoring system

Table 1: Primers of gene expression experiment

Host	Gene		5'-3'	Product (bp)	Accession number	Reference
Rattus	Jagged1	F	AACTGGTACCGGTGCCAA	190	XM_032904296.1	[24]
		R	TGATGCAAGATCTCCCTGAAAC			
Rattus	Notch1	F	CACCCATGACCACTACCCAGTT	186	XM_032903023.1	[24]
		R	CCTCGGACCAATCAGAGATGTT			
Rattus	GAPDH	F	ATGACTCTACCCACGGCAAG	89	NM_017008	[25]

Source: Own materials

**Fig. 1.** Impact of DBZ on KIM1 levels following renal IR, tissue KIMI (pg/ml) among groups

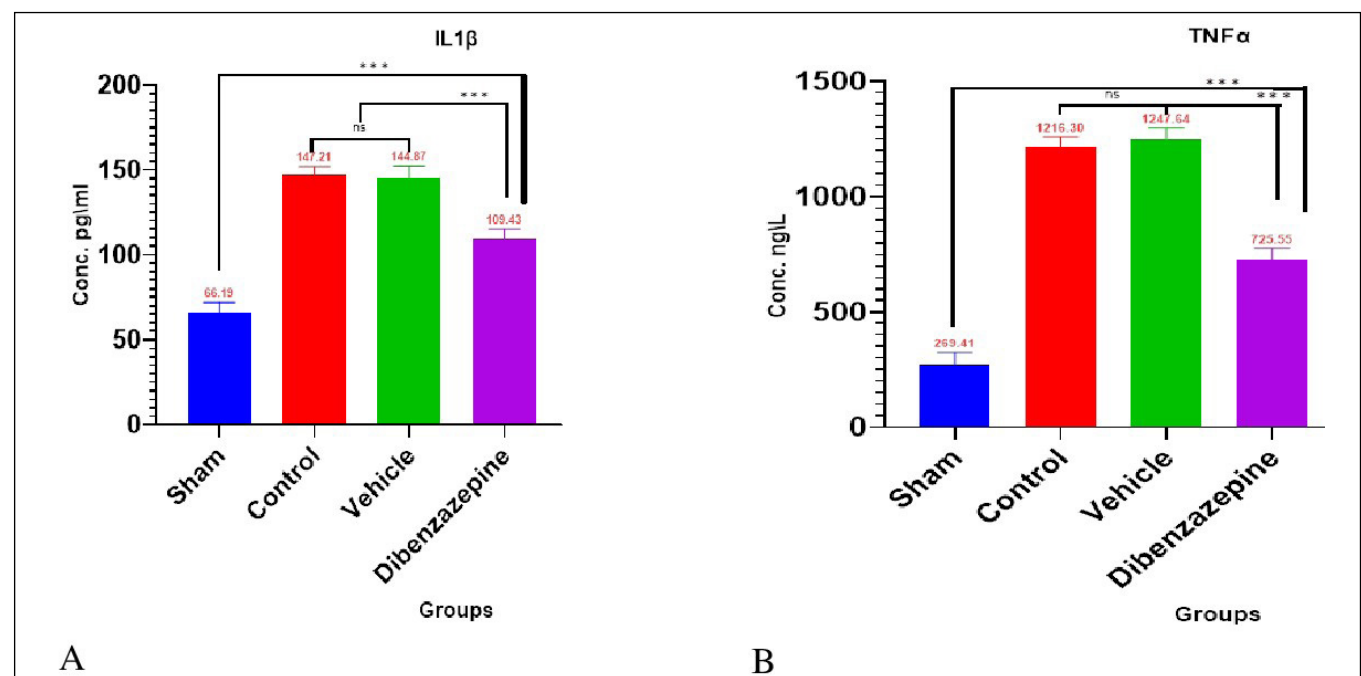
Mean ± SD, n=10; ***P≤0.001 vs. sham; ***P≤0.001 vs. control/vehicle

Source: Own materials

used five scores: 0 for normal kidney tissue, 1 for kidney damage area less than 25%, 2 for kidney damage area between 25% and 50%, 3 for kidney damage area 50%-75%, and 4 for kidney damage area higher than 75%.

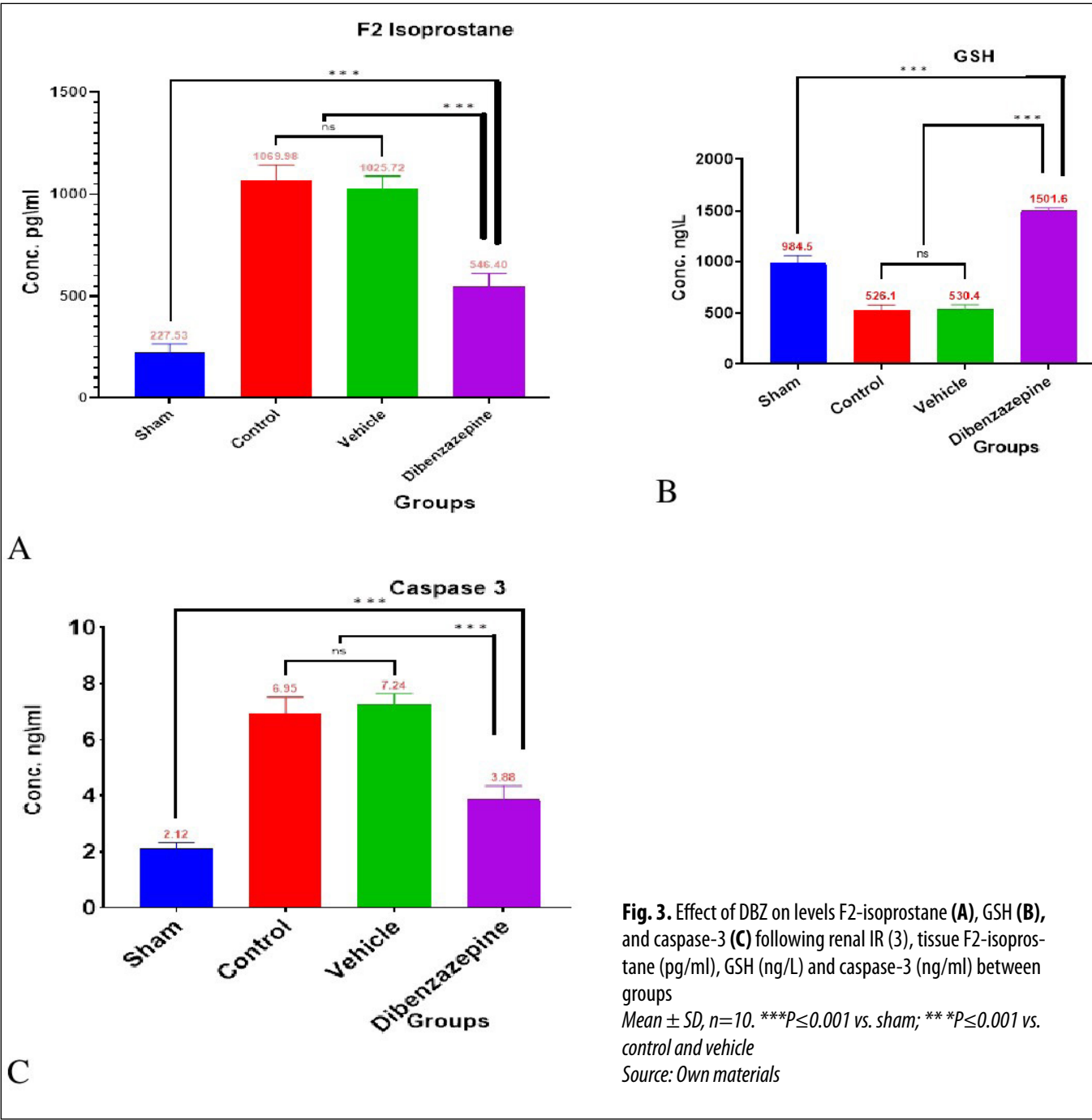
ANALYSIS OF NOTCH SIGNALLING PATHWAY BY QRT-PCR

Notch-1 and Jagged-1 expression levels in the kidney were measured using qRT-PCR. For complete RNA extraction, we consumed around 50-100 mg of kidney tissue precisely following the Easy-spin™ (DNA free) total RNA extraction Kit (Intron/Korea) adhere to the manufacturer's protocol, cDNA was created using reverse transcription using Add Script cDNA Synthesis Kit following the manufacturer's protocol. PCR was performed according to the instruction of GoTaq® RT-qPCR System. The sequences of the primer show in table 1 for specific gene amplification. The 2- $\Delta\Delta C_t$ technique

**Fig 2.** Effect of DBZ on TNF- α and IL-1 β levels after renal IR, including tissue TNF- α (ng/L) and IL-1 β (pg/ml) between groups, n = 10; mean ± SD.

***P≤0.001 vs. sham ***P≤0.001 compared control/vehicle

Source: Own materials



was used to determine fold changes in gene expression. Gene expression is quantified as a relative fold change to GAPDH, an internal control reference gene.

INVESTIGATIONS

BIOCHEMICAL MARKERS ANALYSIS

By using Elisa kits obtained from SunLong Biotech Co.LTD, China, measured the levels of [KIM1 (Catalogue Number: SL0433Ra), IL-1 β (Catalogue Number: SL0402Ra), and TNF- α (Catalogue Number: SL0722Ra), F2-isoprostane (Catalogue Number: SLD2059Ra), GSH

(Catalogue Number: SL1093Ra), and caspase-3 (Catalogue Number: SL1366Ra)].

STATISTICAL ANALYSIS

Statistical analysis for this study was done by using Graph Pad Prism version 8 software. After testing the normal distribution of data .The one-way ANOVA test used to parametric variables and selected Post Hoc. Test using Bonferroni method to make multiple comparisons between groups. Statistical significance in all the tests was considered when P $\leq$ 0.001. All data are expressed as mean $\pm$ SD.

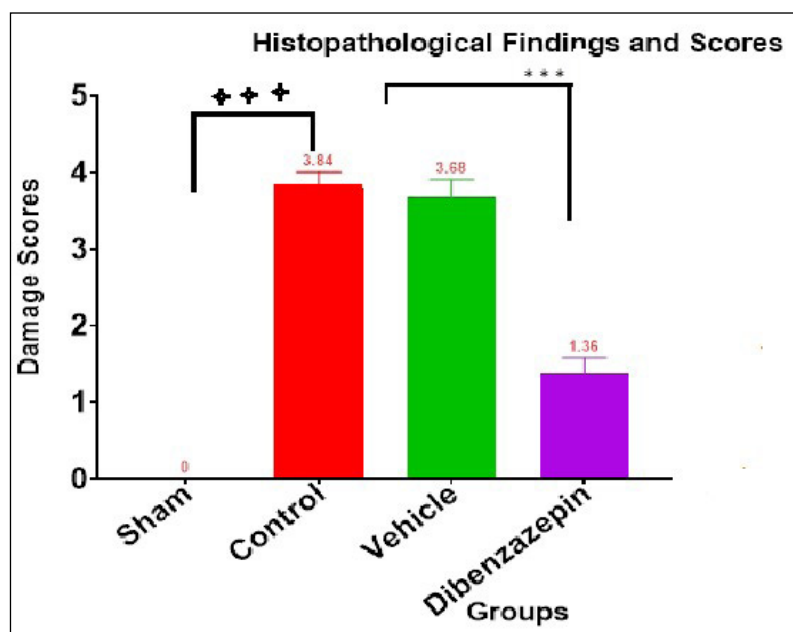


Fig. 4. Mean of Histopathological kidney injury score
 +++ $p < 0.001$ vs. sham group
 *** $p < 0.001$ vs. control & vehicle groups, the Kruskal-Wallis test was used for analysis, data are expressed as mean $\pm$ SD
 Source: Own materials

RESULTS

The ischemia lasted 30 minutes, followed by two hours of reperfusion. Prior to one hour of ischemia, the rats were given DMSO, DBZ (2 mg/kg), or left untreated (Sham and control groups). Several biochemical indications were employed to determine the severity of renal damage.

IMPACT ON KIDNEY INJURY MOLECULE (KIM1)

Following renal I/R, the IRI (control) group had a significantly higher tissue level of KIM1 than the Sham group. The amount of KIM1 in tissues dropped considerably following DBZ therapy (Fig. 1).

IMPACT ON KIDNEY TISSUE INFLAMMATORY MARKERS

The IRI group showed significantly higher levels of IL-1 β and TNF- α in their tissues than the sham group. The DBZ group dramatically reduced tissue inflammatory parameters (TNF- α and IL-1 β) (Fig. 2).

IMPACT ON OXIDATIVE AND APOPTOTIC MARKERS

F2-isoprostane, and caspase-3 levels in rat renal tissue were significantly more in IRI group than in the Sham group while GSH level in rat renal tissue were significantly lower in the IRI group than in the Sham group. DBZ therapy significantly enhanced antioxidant marker (GSH) levels in renal tissue while significantly decreasing apoptotic marker (Caspase-3) levels and oxidative marker F2-isoprostane in comparison to the control (IRI) group (Fig. 3A-C).

HISTOPATHOLOGICAL FINDINGS

Sham group reported much less damage as compared with other groups. DBZ group exhibited much lower damage than the control and DMSO groups, suggesting a nephroprotective effect (Fig. 4).

The control group showed a marked abnormality in renal structure with severe renal changing of tissue architecture including tubular increased cytoplasmic eosinophilia, cellular swelling, degeneration of tubular epithelium, vascular congestion, eosinophilic cast and cytoplasmic vacuoles (score = 4 and represent > 75% of damage). The group received DMSO had similar pathology to the IRI group. The DBZ group which observed moderate changes in renal architecture (score = 2), figure (5). The Sham group had have a normal histological appearance without morphological change. The control group had severe damage in renal tubules, characterized by Cytoplasmic swelling and increased cytoplasmic eosinophilia, cytoplasmic vacuoles, and eosinophilic cast. The DMSO group had the same histopathological changes to control group. DBZ pre-treated group the renal tubules damage with score 2 characterized by involving 30% of the examined damaged tubules (Fig. 5A-F).

IMPACT ON NOTCH1 AND JAGGED1 mRNA EXPRESSION

The renal tissue levels of Notch1 and Jagged-1 expression in vehicle and control groups were significantly ($p < 0.001$) more than that in the sham group, while the vehicle group was no insignificant difference than control group. On the other hand, the level in the Dibenazepin was significantly lower ($p < 0.001$)

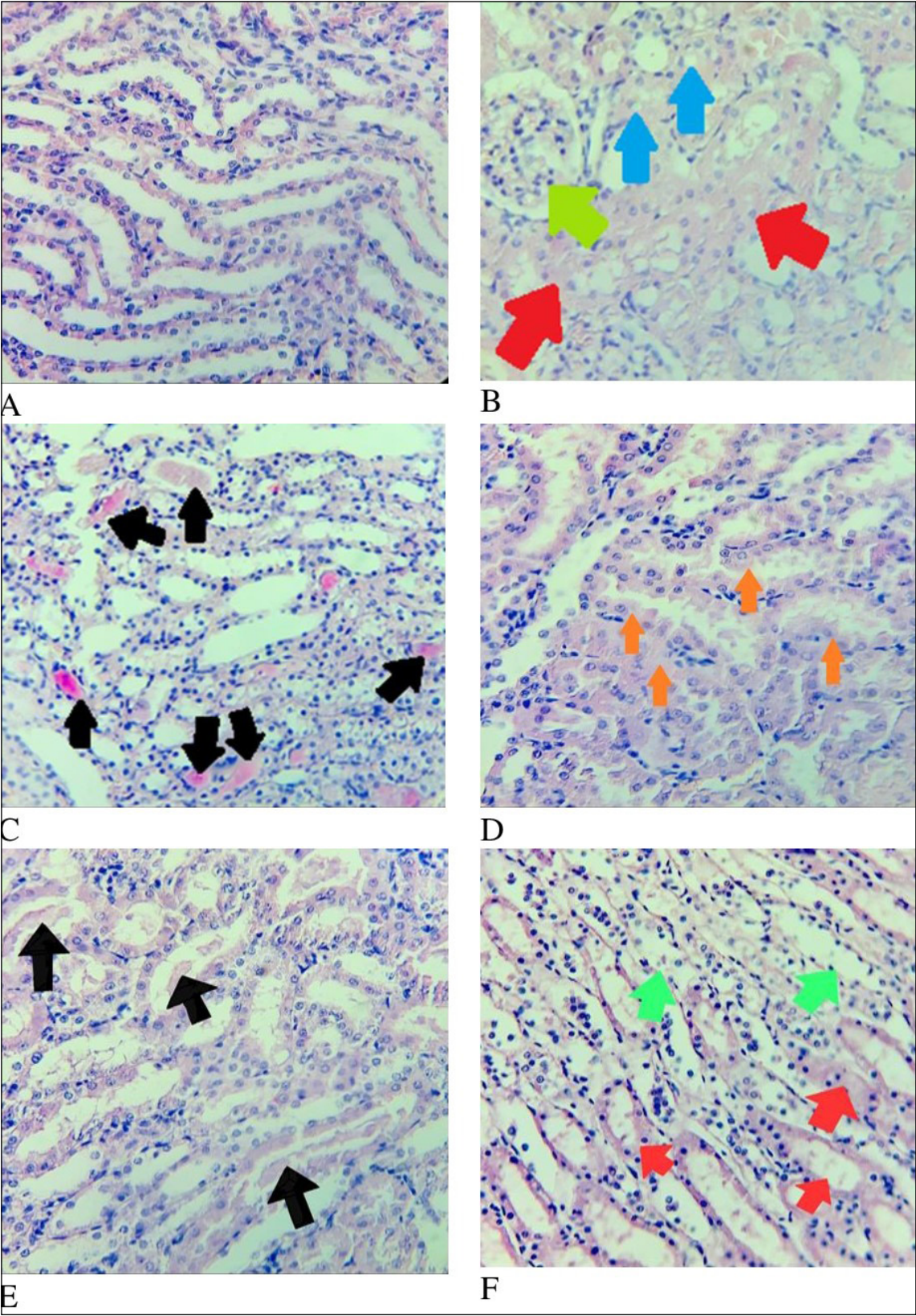


Fig. 5. Histopathological analysis of renal tissues, **(A)** Sham group has normal renal tubules, **(B) & (C)** Control group, renal tubules with score 4 damage, cytoplasmic swelling and increased cytoplasmic eosinophilia (red arrows), cytoplasmic vacuoles (blue arrows), eosinophilic cast (black arrows), normal glomerulus (green arrow); **(D) & (E)** Vehicle group, renal tubules with score 4 damage, Cytoplasmic swelling and increased cytoplasmic eosinophilia (red arrows), eosinophilic cast (black arrows), vascular congestion (orange arrow); **(F)** DBZ group renal tubules with score 2 damage, damaged tubules (red arrows), normal tubules (green arrows), H&E. X400

Source: Own materials

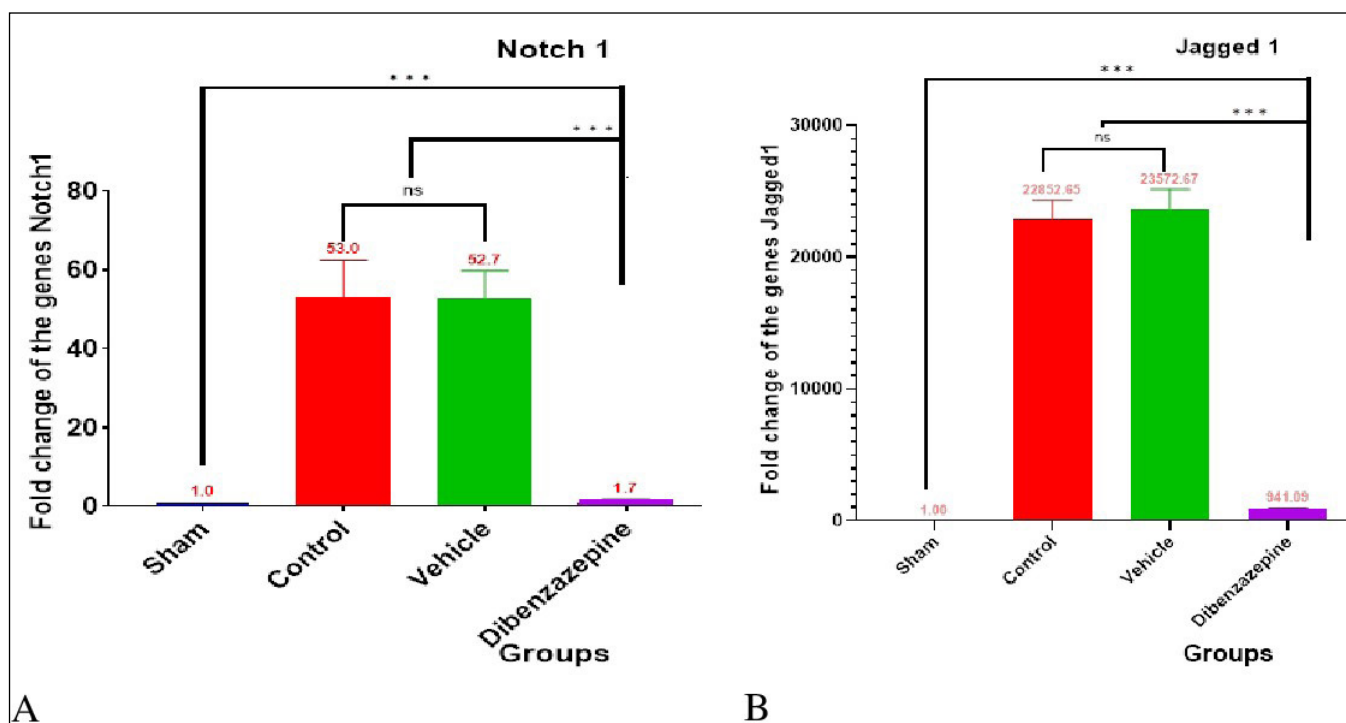


Fig. 6. (A) The bar graph Notch gene expression, (B) The bar graph Jagged-1 gene expression, all data were expressed as mean $\pm$ SD (n = 10).

Sham group vs. vehicle & control groups

***P < 0.001; DBZ treated group vs. vehicle & control groups, ***P < 0.001

Source: Own materials

than that in the sham, control, and vehicle groups (Fig. 6A-B).

DISCUSSION

Ischemia is a rapid temporary reduction in blood supply to the organ and subsequent reoxygenation which accompanied by a strong oxidative stress and inflammatory response to hypoxia and reperfusion lead to disrupts organ function [26] due to exacerbation in tubular and glomerular damage, and producing a strong inflammatory response, called "reperfusion injury" [1]. This research aims to explain the nephroprotective impact of DBZ in the kidneys through decrease inflammation, oxidative stress, and apoptotic pathway. The current investigation found that renal IRI raised tissue levels of KIM1, IL-1 β , TNF- α , F2-isoprostane, and caspase-3 with decreased the level of GSH in kidneys of rats. Interestingly, pre-treated with DBZ reduced KIM1 levels, inflammatory markers (IL-1 β , TNF- α), oxidative stress marker (F2-isoprostane) and apoptotic marker (Caspase-3) with increased antioxidant (GSH). The study by [27] revealed that the increase the level KIM-1 in the proximal tubule in early damage of kidney have advantage as a sensitive, specific and ideal biomarker marker for early diagnosis of kidney injury including acute kidney injury (AKI) and as a predictor of prognosis. The findings suggest that

Dibenazepin has a protective impact on kidney function parameters (KIM) following renal IRI. The important parameters for detection of inflammation in RIRI are (IL-1 β and TNF- α) [16] demonstrate that the elevation of the inflammatory cytokines (IL-1 β and TNF- α) as a result of hypoxia due to decreased in renal supply which cause infiltrate of different inflammatory cells into the injured tissue. The decrease in the levels of inflammatory factors explained by [28-17] said that cisplatin causes nephrotoxicity in rats by activating NF κ B, which leads to increased production of proinflammatory cytokines such IL-1 β and TNF α . Pre-treatment with dibenzazepine reduces nuclear translocation of NF- κ B and significantly suppresses production of inflammatory markers TNF α and IL-1 β . The imbalance in antioxidant agents and the amount of ROS production causes the accumulation of ROS, which may cause cellular damage. This process is known as oxidative stress and plays a key role in various cellular signalling pathways by destroying lipids, proteins, and DNA, resulting in cell death [29]. During ischemia, there is a significant reduction in oxygen supply, which causes a decrease cellular ATP synthesis and lactate build-up, resulting in acidosis. As a result, Na $^{+}$ /K $^{+}$ ATPases, Na $^{+}$ /H $^{+}$, and Ca $^{2+}$ -ATPase pumps become dysfunctional, leading in the build-up of sodium, hydrogen, and calcium in the cytoplasm and consequent hyper-osmolality, an increase in water transport across

cell membranes, and cellular swelling. Furthermore, the overproduction of ROS during ischemia/reperfusion through production of several enzymes able to decrease molecular oxygen producing all of which contribute to oxidative stress, which plays a significant role in organ damage following I/R by triggering apoptosis and lipid peroxidation [30]. The nephroprotective effects of pre-treated DBZ in cisplatin-induced kidney damage in rats was achieved via significantly lowered oxidative stress, inflammation, and apoptotic indicators, as well as the Notch signalling pathway these findings reflect that the Notch pathway can play important role in the pathogenesis of cisplatin nephrotoxicity [28]. Another study by [31] have demonstrated that during IRI, the inflammation and tubular cell injury can trigger by burst of (ROS) ,therefore, reducing oxidative stress, can prevent damage in IRI by assessed F2-isoprostane. The current study showed significant decreased in Glutathione (GSH) in renal tissue of the control group and vehicle group in comparison with the sham group after renal IRI, this result agreement with [32] which demonstrated that GSH levels significantly reduced in RIRI and vehicle groups when compared with the sham group in response to renal ischemia reperfusion, suggesting that this results due to deterioration in the ability of kidney to function as an antioxidant. The [33] shown that when renal IRI occurs, the mitochondria of kidney cells are damaged lead to reduce the activities of GSH and SOD lead to unable to remove ROS and aggravating kidney damage. The pre-treated group with DBZ significantly increased the level of GSH and that consist with research by [34-28] which shown that the group which pre-treatment with DBZ in rats induced nephrotoxicity by cisplatin-injected cause improved the oxidative stress by significantly raising catalase and GSH. The significant increase of Caspase-3 in current study is in agreement with [35] shown that after thirty min of renal ischemia after that two hours of reperfusion in rats model the significant increase in Caspase-3level with significant decrease in Bcl-2 which explain the results that apoptosis is a conclusive process in renal IRI and the stimulation of caspase-3 have important role for programmed cell death because the equilibrium between antiapoptotic (Bcl-2) proteins and pro-apoptotic (Bax) is essential for the survival of cells, the disruption

of this equilibrium in IRI through down regulating Bcl-2 levels and up regulating Bax expression, promoting apoptosis. The significant decreased in Caspase-3level in DBZ pre-treated group shown the nephroprotective effect of DBZ after renal IRI. Furthermore, histological examinations confirmed DBZ's kidney-protective properties. These examination showed a substantial reduction in renal tissue damage, including decrease in damage of renal tubules, infiltration of inflammatory cells, in rats treated with DBZ compared to control and vehicle groups. Previous study by [34] have reported this conclusion which said that pre-treatment of cisplatin-injected rats with DBZ significantly reduced cisplatin-induced alterations in nephrotoxicity indicators and the histological architecture of the kidney, another research by [36] revealed that the Notch inhibitor DBZ can ameliorate the expression of fibrotic markers and severity of renal Fibrosis caused by unilateral ureter blockage in mice. Fibrosis caused by unilateral ureter blockage in mice. Moreover, the Notch regulated gene overexpression is responsible for fibrosis of kidney and disease progression, the dysfunctional mitochondria leading to cell death and dedifferentiation [37]. The analysis of results revealed that the Dibenazepin pre-treated group showed significantly lower level of Notch gene expression in renal tissue level and this result is in agreement with the previous study by [38] which demonstrated that ischemic mice were treated with the γ -secretase inhibitor DBZ, which inhibited Notch signalling and particularly decreased expression of Notch 1 ligands Jagged-1. Duan and Qin said that the blocking of Notch signalling pathway by using γ -secretase inhibitors decreases the amounts of Notch signalling components in podocytes and nephrons [39].

CONCLUSIONS

DBZ shown a nephroprotective effect due to ischemia/reperfusion (I/R). DBZ (γ -secretase inhibitor) exhibited anti-apoptotic, antioxidant, and anti-inflammatory characteristics by significant decreased in inflammatory, oxidative apoptotic markers and Notch1 and Jagged-1 expression in kidney tissues after renal ischemia/reperfusion damage. Therefore it may be a promising treatment people with RIRI.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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Shortening antibiotic duration in the treatment of acute cholangitis due to choledocholithiasis with successful biliary duct drainage

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ABSTRACT

Aim: To evaluate the efficacy and safety of short duration vs 14-day antibiotic therapy in acute cholangitis associated with common bile duct stone.

Materials and Methods: We divided 278 patients with all stages of acute cholangitis who underwent technically successful ERCP from January 2019 to June 2023 and had positive bile cultures into two groups: antibiotic therapy within two days of ERCP (short-course therapy, SCT; n = 48), and for >3 days (long-course therapy, LTC; n = 230).

Results: Three recurrence occurred in SCT and one recurrence occurred in LTC (p = 0.19). Clinical outcomes were similar between the two groups. The fever duration was similar between mild and moderate cholangitis within the same group and between the 2 groups. Duration of antibiotic therapy in LTC was 4.22 ± 1.37 days with 39 (81.3%) needing treatment for < 5 days.

Conclusions: Short duration antibiotic treatment based on fever resolution of 72 hours was effective and safe in patients with mild to moderate cholangitis due to common bile duct stone.

KEY WORDS: treatment, antibiotic, endoscopic retrograde cholangiopancreatography, acute cholangitis

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INTRODUCTION

Acute cholangitis is a severe complication of diseases hepatobiliary system. Despite advances in diagnosis and treatment diseases of the biliary tract, acute cholangitis remains quite complex diagnostic and far unresolved therapeutic and diagnostic problem. The unflagging interest of clinicians of any profile in this. The problem is due to a number of reasons. Acute purulent cholangitis (ACH) is one of the frequent complications of benign diseases of the extrahepatic biliary tract and large papilla of the duodenum.

It is connected not only with high mortality, which is 15-60%, but also, first of all, from it key role in the development of biliary sepsis [1,2]. Obstruction of the biliary tract, which develops in 87.1% of cases with choledocholithiasis [3], in 6-21% with stenosis of the large papilla of the duodenum [4]. Violation of the outflow of bile is facilitated by the attachment and reproduction of microorganisms, and hypertension leads to cholangiovenous reflux with a massive entry into the systemic

bloodstream of bacterial endotoxins, which reliably correlates with the progression of endogenous intoxication, the development of systemic inflammation and syndrome of multiple organ failure.

In the complex treatment of AC, certainly the leading role belongs to surgical intervention, aimed at decompression and recovery outflow of bile. Variants of treatment tactics at AC has gradually undergone some changes in recent years. Wide implementation in clinical practice the practice of radiodiagnostic methods, endoscopic and laparoscopic interventions, additional active remediation of the biliary tract allowed reduce mortality, reduce the number of complications, increase the effectiveness of treatment [5,6]. However, the wide implementation of decompression requires minimally invasive surgical technologies clear justification of indications, duration, sequence of application, criteria of their effectiveness, as well as terms of operative interventions. All that determines the expediency of further research aimed at increasing the effectiveness of

Table 1. Clinical characteristics of the patients receiving short- and long-course antibiotics

	SCT, n = 48 (17.3%)	LCT, n = 230 (82.7%)	P-value
Age	78.3±8.32	85.4 ± 7.91	0.33
Sex			
male	29	125	0.63
female	19	105	0.58
severity			
Mild	15	92	
Moderate	25	104	
severe	8	34	
Morbidity, n			
Diabetes mellitus, n (%)	9	43	0.48
Heart disease, n (%)	26	147	0.69
Chronic renal failure , n (%)	5	21	0.73
Hepato-pancreato-biliary cancers	8	19	0.63
Laboratory parameters			
WBC at diagnosis, /mm3 (mean)	10936	11127	0.86
Temperature >38°C, n (%)	37.6	37.9	0.38
Bilirubin at diagnosis mg/dl	2.71	3.04	0.51
ALT, mean (SD), U/L	206	142	0.317
ALP, mean (SD), U/L	314	332	0.817
AST, mean (SD), U/L	371	208	0.269

WBC – white blood cells, ALT- alanine transaminase, ALP - alkaline phosphatase, AST - aspartate aminotransferase

Source: compiled by the authors of this study

traditional, as well as the development of new methods of treatment patients with AC [7].

AIM

To evaluate the efficacy and safety of short duration vs 14-day antibiotic therapy in acute cholangitis associated with common bile duct stone.

MATERIALS AND METHODS

Patients with acute cholangitis diagnosed by specialists based on findings such as fever, abdominal pain, liver function test abnormality, or imaging studies will be eligible for trial entry. Approval of the Ethics Committee of the institute was obtained before commencing the study (IEC no. 2020/SPL-1645). The study participants are enrolled in this trial if they meet all of the inclusion criteria and none of the exclusion criteria.

INCLUSION CRITERIA

- 1. Patients are 18years or older.
- 2. Diagnosed as having acute cholangitis by treating.
- 3. Biliary duct obstruction was removed by endoscopic retrograde cholangiopancreatography (ERCP).

EXCLUSION CRITERIA

- 1. Antibiotic hypersensitivity.
- 2. Inadequate drainage due to remaining biliary strictures.
- 3. Biliary stent occlusion.
- 4. Sclerosing cholangitis.
- 5. Previous choledochojejunostomy.
- 6. Previous heart valve replacement.

In the SCT group, intravenous antibiotics will be continued at least for 4 days, and can be discontinued if all of the following criteria are fulfilled: 1) maintenance of body temperature under 37.5 °C for more than 48 h, 2) systolic blood pressure above 90 mmHg, 3) heart rate below 100 beats/min. In the LCT group, intravenous antibiotics will be given for the duration of usual care, at least for 7 days, at the discretion of both the treating doctors.

STATISTICAL ANALYSIS

To analyse categorical variables, we used Fisher’s exact test when appropriate. For continuous variables, we used the Shapiro-Wilk normality test to check whether the parametric model was appropriate. We then used the Student’s test when appropriate. For the sensitivity analysis, we used a propensity score for SCT using variables likely to influence the outcomes, for both the

Table 2. Time from admission to ERCP

	SCT, n = 48 (17.3%)	LCT, n = 230 (82.7%)	p-value
Duration from admission to ERCP (hours), mean	17.5	21.9	0.387
ERCP within 24 h	36	127	
ERCP within 36 h	10	71	
ERCP within 48 h	2	32	0.159

STC- short-course therapy, LTC - long-course therapy, ERCP - endoscopic retrograde cholangiopancreatography

Source: compiled by the authors of this study

Table 3. The laboratory findings of microbial cultures

	SCT, n = 48 (17.3%)	LCT, n = 230 (82.7%)	p-value
Blood culture			
Positive rate	15/28 (53.6%)	93/175 (53.2%)	0.134
Escherichia coli	5 (10.4%)	43 (18.7%)	0.351
Klebsiella sp	4 (8.4%)	31 (13.5%)	0.129
Enterococcus sp.	0	10 (4.4%)	0.228
Enterobacter sp.	1 (2%)	9 (3.9%)	>0.99
Citrobacter sp.	0	2 (0.9%)	0.483
Staphylococcus sp.	1 (2%)	9 (3.9%)	>0.99
Streptococcus sp.	1 (2%)	5 (2.2%)	0.478
Pseudomonas sp.	1 (2%)	2 (0.9%)	0.344
Aeromonas sp.	1 (2%)	15 (6.5%)	0.619
Others	1 (2%)	6 (2.6%)	>0.99
Negative	13 (27%)	101 (43.9%)	0.059
No culture	20 (41.6%)	55 (23.9%)	<0.001
Bile culture			
Positive rate	46/48 (95.9%)	230/230 (100%)	>0.99
Escherichia coli	22 (45.8%)	103 (44.8%)	0.287
Klebsiella sp	18 (37.5%)	98 (42.6%)	0.258
Enterococcus sp.	24 (50%)	101 (43.9%)	0.103
Enterobacter sp.	4 (8.3%)	35 (15.2%)	>0.99
Citrobacter sp.	6 (12.5%)	18 (7.8%)	0.108
Staphylococcus sp.	0	6 (2.6%)	0.401
Streptococcus sp.	5 (10.4%)	33 (14.3%)	>0.99
Pseudomonas sp.	1 (2%)	30 (13%)	0.598
Aeromonas sp.	2 (4.2%)	24 (10.5%)	0.798
Others	5 (10.4%)	19 (8.3%)	0.287
Negative	1 (2%)	2 (0.9%)	>0.99
No culture	1 (2%)	3 (1.3%)	>0.99

Source: compiled by the authors of this study

primary outcomes and the composite outcomes. A p-value of <0,05 was considered to indicate statistical significance.

ETHICS

This work complies with the principles of the Declaration of Helsinki.

RESULTS

During the study period 278 patients with acute cholangitis due to choledocholithiasis who had undergone ERCP were identified. Among the analyzed patients, 48 (17.3%) received SCT, while 230 (82.7%) received LCT. Acute cholangitis was mild in 107 patients, moderate in 129 patients and severe in 42 patients. The characteristics of all patients included herein are summarized in Table 1.

Table 4. Empiric antibiotic therapy

	SCT, n = 48 (17.3%)	LCT, n = 230 (82.7%)	p-value
Ampicillin/sulbactam	6 (12.4%)	51 (22.2%)	0.461
Piperacillin/tazobactam	5 (10.4%)	41 (17.8%)	>0.99
Ceftriaxone	4 (8.4%)	23 (10%)	>0.99
Cefuroxime	33 (68.8%)	81 (35.2%)	0.319
Meropenem	0 (0.0%)	12 (5.2%)	0.613
Ciprofloxacin	0 (0.0%)	15 (6.6%)	0.366
Levofloxacin	0 (0.0%)	3 (1.2%)	>0.99
Vancomycin	0 (0.0%)	2 (0.9%)	>0.99
Others	0 (0.0%)	2 (0.9%)	>0.99

Source: compiled by the authors of this study

Table 5. Primary and secondary outcomes of patients receiving short- and long-course antibiotics

	SCT, n = 48 (17.3%)	LCT, n = 230 (82.7%)	p-value
30-days mortality, n (%)	0	3 (1.3%)	1
Reccurence cholangitis within 3 month, n (%)	3 (6.3%)	1 (0.5%)	0.19
Bacteremia, n(%)	5 (10.5%)	89 (39%)	0.08
Duration of hospitalization	7 days (3-30)	7 days (3-120)	0.09

Source: compiled by the authors of this study

In both groups, we examined the time from the patient's admission to the hospital to ERCP. We also conducted a study of the state after ERCP (Table 2).

The laboratory findings of microbial cultures from the patients are summarized in table 3. The positive rates of blood and bile cultures were 53.6% (15/28) and 95.9% (46/48) in the SCT group, and 53.2% (93/175) and 100% 230/230) in the LCT group, respectively. There were no significant differences in the positive rates of blood or bile cultures. A total of 198 patients (71.2%) had polymicrobial infections, for *E.coli*, *Klebsiella* sp., *Enterococcus* sp., and *Enterobacter* sp. were most common pathogens (Table 3).

Table 4 summarizes the findings related to antibiotics in the present study. Cefuroxime, ampicillin/sulbactam, and piperacillin/tazobactam were the most commonly used antibiotics. There were no significant differences in the rate of use of each antibiotic. Four patients in the SCT group (8.3 %) did not receive any antibiotics.

The primary and secondary outcomes are presented in Table 5.

Accordingly, no significant differences in 30-day mortality rates (0%, 0/22 and 2.7%, 2/74 for the SCT and LCT group, respectively) and 3-month recurrence rates were observed between both groups. Although the LCT group were hospitalized longer than the SCT group, no significant difference was found ($p = 0.75$). Moreover, the LCT group had a higher acute bacteremic cholangitis rate than the SCT group, albeit not significantly ($p = 0.08$).

DISCUSSION

There is limited information regarding the duration of treatment for acute cholangitis. In the observation by Van Lent et al., it was suggested that a short treatment duration (≤ 3 days) appeared to be sufficient after appropriate drainage was performed [8]. However, their study was a descriptive case series without proper statistical analysis, and only 26.7% of patients had concomitant bacteremia. Dooley et al. recommended 7-10 days of treatment, although their recommendation was also not based on clinical evidence [9]. Kogure et al. proposed a temperature-based approach, where antimicrobial use was discontinued two days after fever resolution following successful endoscopic drainage [10]. However, this relatively small study was a case series consisting of only 18 patients, without a control group for comparison. Another study compared shorter versus longer antibiotic treatment for acute cholangitis with gram-negative bacteremia [11].

After studying the duration of antibiotic therapy for acute cholangitis with gram-negative bacteremia, Uno et al. (2016) concluded that a treatment duration of less than 2 weeks may be sufficient [11]. However, the mortality rate (11%) and the recurrence rate (24%) in patients were higher than in other studies. Doi et al. (2018) showed that a shortened (six days) duration of antibiotic therapy for acute cholangitis with successful drainage of bile ducts might be a reasonable option, although the duration of antibiotic therapy was defined as the 'total' duration, rather than the duration conducted

after the successful drainage of the bile ducts. By studying the outcomes of antibiotic therapy duration after successful bile duct drainage, this study allowed for a better comparison of 30-day mortality and 3-month recurrence rates. Thus, we believe that this study was the first to report that SCT (≤ 3 days) is not inferior to LCT (≥ 4 days) among patients with mild to moderate acute cholangitis caused by choledocholithiasis [12].

Gomi et al. recommended in the Tokyo Guideline a duration of 4e7 days after source control for non-bacteraemic cholangitis, and recommended a minimum of 2 weeks duration in association with bacteraemia, especially when caused by Gram-positive cocci, provided that anatomical problems were resolved upon the presence of residual stones or obstructions of the bile duct. In fact, infectious diseases specialists tend to recommend a relatively long duration of antimicrobial therapy for bacteraemic cholangitis, reflected in the statistically significant tendency for those patients on LCT to have an infectious diseases consultation found in our study. However, this recommendation was based on the guideline for the management of complicated intra-abdominal infection, which does not specifically recommend treatment for acute cholangitis [13]. In addition, the recommendations were not based on relevant clinical evidence.

Recently, a randomized controlled trial studying treatment duration for appropriately drained intra-abdominal infections compared outcomes 4e5 days after drainage with a conventional duration of up to 10 days [14]. The short treatment duration did not have worse outcomes such as the occurrence of recurrent intraabdominal infection or mortality. However, only 10.8% of the participants had acute cholangitis and only a few had concurrent bacteraemia [14]. Likewise, short courses of antimicrobial therapy of 6e10 days had similar outcomes compared with longer courses for Enterobacteriaceae bacteraemia [15]. Our findings were more specific to those who have acute bacteraemic cholangitis, for which many experts have considered longer treatment necessary. Therefore, our findings are quite novel.

Treatment of acute cholangitis consisted primarily of decompression of the bile ducts and elimination of the cause obstruction of the bile ducts [16, 17]. At the same time, depending on the general condition of the patients, the severity of the manifestation of cholangiogenic infection, endotoxemia and hyperbilirubinemia were produced one- and two-stage treatment methods using modern technologies and traditional surgical interventions. Typically, after completing two-stage and one-stage endoscopic interventions, external removal of toxic bile was carried out through external drainage [18, 19].

Although there is still no clear timing of ERCP in patients with acute cholangitis [20], a study showed that ERCP performed >72 hours after hospitalization had higher rates of organ failure and mortality than those performed before <72 hours. [21]. In another study, patients who underwent ERCP >48 hours after hospitalization had a higher incidence of liver failure than patients who underwent ERCP before 48 hours [22,23]. Many studies have observed a recommendation for ERCP within 24 hours of hospitalization; since mortality as a result of organ failure practically does not occur. Therefore, we recommend ERCP within 24 hours of hospitalization in patients with acute cholangitis.

CONCLUSIONS

Our study shows that short-term ATB therapy using 48-hour relief of inflammatory symptoms as a safe time to stop ATB therapy for acute cholangitis with or without bacteremia after successful endoscopic biliary drainage is safe and effective. By reducing the duration of ATB use, the cost of treatment, the risk of developing microbial resistance, as well as the risk of complications associated with antibiotics and multiple organ failure will be reduced.

PROSPECTS FOR FURTHER RESEARCH

Prospects for further research are to study reducing the duration of antibiotic therapy for acute cholangitis, with the aim of clarify methods of possible therapeutic correction.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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Intellectual analysis of criteria that influence the choice of medicines for dispensing by electronic prescriptions

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ABSTRACT

Aim: Identifying of key criteria that influence the process of dispensing medicines by pharmacists by electronic prescriptions, and establish its' relationship with the frequency of problems while paying off prescriptions.

Materials and Methods: In our work we used the survey method involving questionnaires. To screen the obtained results, we used the method of intelligent data analysis depending on building the associative rules. The survey subjects were pharmaceutical clerks employed by pharmacies in the Western, Central, Eastern, Northern and Southern regions of Ukraine, who directly sold MPs to the population. Respondents included individuals of different sexes, ages, and professional experience. The questionnaire contained several blocks that were dedicated to the analysis and identification of the main difficulties that arise when dispensing drugs by e-prescriptions and the criteria that influence the choice of a drug for dispensing by trade name when dispensing by e-prescriptions. A total of 2233 specialists were surveyed.

Results: We analyzed the criteria that had influenced a choice of trade name of prescription medicines filled for patients by a pharmacist under e-prescription in the form of an international non-proprietary name.

Conclusions: We have determined the criteria for selecting medicinal products by pharmaceutical clerks while filling e-prescriptions, which can be used in the practical activities of pharmaceutical workers as an integral part of the process of optimizing pharmacy inventories. The most important criteria have been found the amount of co-payment for medicine, the country of manufacture/medicines manufacturer, and the history of medicine administration by the patient. The criteria of the presence or absence of evidence-based data on the efficacy of a particular medicine and the medicine's belonging to generics or original medicines have been found the least important. The inability of a pharmaceutical clerk/pharmacy worker to select a medicine based on one or more of the above selection criteria is considered one of the reasons for failure to dispense up to 20% of medicinal products under e-prescriptions.

KEY WORDS: pharmacy, pharmacists, e-prescriptions, data mining, associative rules

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INTRODUCTION

Providing the population with medicines is a priority task of the healthcare system in any country. To reach the goal, some countries have launched various governmental reimbursement programs focused on higher economic and physical accessibility of pharmaceutical care for population by securing state financing of this segment of the economy [1]. Similar programs have also been launched in Ukraine. Such programs include the *Affordable Medicines* program, which was launched in 2017 [2,3]. Starting from April 2019, the program has completely gone into an electronic format. At the same time, electronic prescriptions (e-prescriptions) have gained momentum along with the development of the *Affordable Medicines* program. One of the key criteria for assessing the effectiveness of the policy of dispensing prescription medicinal products (MP) to customers is

considered an event of prescription filling. The Order of the Ministry of Health of Ukraine "On approval of Schedules for medicinal products and medical items subject to reimbursement under the program of state guarantees of medical care for the population" (hereinafter referred to as the Schedule) covers MPs and medical items (MI), manufactured both by domestic and foreign entities, indicating such indicators as international non-proprietary name (INN), trade name (TN), pharmaceutical form and presentation, dosage, number of units in a package, ATC code, manufacturer's name, country of manufacture, MP registration certificate number, MP registration certificate expiration date, wholesale price per package, retail price per package, daily dose of MP, reimbursement amount for daily dose of MP, reimbursement amount for MP consumer package, and co-payment amount for the consumer

package) [4]. A pharmaceutical clerk uses the Schedule to pick the required MP by TN and INN mentioned in a prescription, dispenses the medicine and fills the prescription. E-prescriptions have significantly improved management of circulation of prescription MPs, simplified the monitoring over MPs dispensing, and made it possible to effectively assess the projected needs for MPs based on the collected statistical data. At the same time, despite all advantages of e-prescriptions, the circulation difficulties are still pending, which, in turn, may lead to failure to fill some e-prescriptions [5].

AIM

Identifying of key criteria that influence the process of dispensing medicines by pharmacists by electronic prescriptions, and establish its' relationship with the frequency of problems while paying off prescriptions.

MATERIALS AND METHODS

The survey method involving questionnaires was used in the work. For this purpose, we used the *Questionnaire for pharmacists* developed by us. The survey subjects were pharmaceutical clerks employed by pharmacies in the Western (12.13% of surveyed respondents), Central (42.63% of surveyed respondents), Eastern (6.50% of surveyed respondents), Northern (25.65% of surveyed respondents) and Southern (13.12% of surveyed respondents) regions of Ukraine, who directly sold MPs to the population. Respondents included individuals of different sexes, ages, and professional experience. The questionnaire contained several blocks that were dedicated to the analysis and identification of the main difficulties that arise when dispensing drugs by e-prescriptions and the criteria that influence the choice of a drug for dispensing by trade name when dispensing by e-prescriptions. A total of 2233 specialists were surveyed. The results of the detailed analysis of the location of the pharmacy, the age and experience of pharmaceutical workers involved in dispensing drugs using e-prescriptions are presented in our work [6].

To evaluate the criteria and their combinations influencing the choice of medicines for dispensing by electronic prescription by pharmacists to patients under trade name and to establish relationships between them and the conditional number of e-prescriptions for which difficulties arise or which cannot be paid off 2003 questionnaires have been selected. 220 questionnaires were excluded from the study based on the criterion of the absence of respondents ranking the criteria that influence their choice of drug when dispensing it.

To evaluate the criteria influencing the choice of medicines for dispensing by pharmacists to patients under trade name, we used an intelligent data analysis method depending on the establishment of associative rules [7,8].

The use of data association analysis in pharmaceutical research is quite common thing [9]. But the literature review suggests that researchers are often limited to a fairly small number of criteria for verification of statistical significance of identified associative patterns.

For example, the work [10] counts only 2 criteria (support and confidence) out of 5 possible ones, the calculation of which is supported, for example, by the Python library mixtend package [7]:

"Support": The "support" metric reflects how frequently a certain combination of items (A and C together) appears in a dataset, relative to all transactions. It helps identify which itemsets are common or significant in the data, with higher support values indicating that the combination occurs more often.

support ($A \rightarrow C$) = support($A \cup C$), range: [0,1];

"Confidence": The "confidence" metric shows how likely it is to see the consequent (C) in a transaction if the antecedent (A) is present. It reflects the strength of the relationship between A and C, with higher confidence meaning the rule is more reliable for predicting C when A occurs. Note that the antecedent is the "if" part of the rule, meaning the item or set of items that must be present for the rule to apply. For example, in the rule $A \rightarrow C$, the antecedent is A, which represents the condition or starting point for predicting the consequent (C).

confidence($A \rightarrow C$) = $\frac{\text{support}(A \rightarrow C)}{\text{support}(A)}$, range: [0,1];

"Lift": The "lift" metric shows how much more likely A and C occur together compared to if they were unrelated or happened by random. A lift greater than 1 means A and C are related. And a lift of 1 suggests no association.

lift($A \rightarrow C$) = $\frac{\text{confidence}(A \rightarrow C)}{\text{support}(C)}$, range: [0,∞];

"Leverage": The "leverage" metric measures the difference between how often A and C actually occur together and how often we'd expect them to occur together if they were unrelated. A leverage value of 0 means there is no relationship between A and C.

leverage($A \rightarrow C$) = support($A \rightarrow C$) - support(A) × support(C), range: [-1,1];

"Conviction": The "conviction" metric shows how strongly the presence of the antecedent (A) predicts the absence of the consequent (C). A conviction of 1

indicates no dependency. And higher values mean a stronger dependence, with “inf” indicating a perfect rule where A always leads to C.

$$\text{conviction}(A \rightarrow C) = \frac{(1 - \text{support}(C))}{(1 - \text{confidence}(A \rightarrow C))}, \text{ range: } [0, \infty];$$

“Zhangs_metric”: Zhang’s metric measures whether A and C are associated or opposed, with values ranging from -1 to 1. Positive values (>0) mean A and C are related. And negative values (<0) indicate they are opposed.

$$\text{zhangs_metric}(A \rightarrow C) = \frac{(\text{confidence}(A \rightarrow C) - \text{confidence}(A' \rightarrow C))}{(\max[\text{confidence}(A \rightarrow C), \text{confidence}(A' \rightarrow C)])}, \text{ range: } [-1, 1].$$

Therefore, we made a search for associative rules using all available criteria for verification of statistical significance, and tested the above-mentioned methodology on the basis of bulk data obtained from the survey of pharmaceutical specialists [11].

DATA DESCRIPTION

The choice of MPs under TN is influenced by various criteria. In general, we have identified six main criteria that a pharmaceutical clerk uses when choosing MPs for filling e-prescriptions, which we suggested respondents to rank, assigning the first rank to the criterion they consider to be the most important, and accordingly assigning subsequent ranks to criteria that they consider to be less important in terms of their influence on the indicator under study up to rank 6, which corresponds to the least important criterion in their opinion. At the same time, it was allowed to assign the same rank to several criteria at the same time, if respondents considered them to be of equal value.

The selected criteria influencing the choice of a pharmacist included such options as the “amount of co-payment” (for MPs covered by the “Affordable Medicines” program, or the price of medicine for MPs not covered by the cost reimbursement programs) (ACP as mentioned in the article), “Manufacturer/ MP’s country of origin” (MCO), “History of medicine administration by the patient” (PEU), “Belonging of MP to original or generic one” (OGD), “Availability of the MP’s evidence-based efficacy data” (EBD), “Request of a doctor and/or a patient to dispense a medicine of a certain brand” (DPR). Picking such a criterion as the “Amount of co-payment” is justified by reckoning that the Schedule is inclusive of MPs, some of which are dispensed fully reimbursed and do not require patients to pay costs, while the rest are reimbursed only in part and the patients pay the balance. The co-payment sum may be different for different MPs, and accordingly, the price of

medicines not covered by the reimbursement program may also differ significantly, which is an important criterion for choosing medicines by patients. The criterion “History of medicine administration by the patient” is associated with the consumer preferences of patients in administration of MPs over a certain period of time. The choice of the criterion “Request of a doctor and/or a patient to dispense a medicine of a certain brand” is driven by the fact that, although the doctor indicates only INN in the prescription, patients quite often contact the pharmacy with an oral or written request from the medical specialist to dispense an MP of a certain TN under e-prescription. The criteria “Availability of the MP’s evidence-based efficacy data”, “Manufacturer/ MP’s country of origin”, and “Belonging of MP to original or generic one” are considered important as more and more attention is paid to the expediency of administering medicines with proven efficacy.

ANALYSIS METHOD

The association analysis was performed in 3 stages:

1. The most frequent combinations of elements (Frequent Itemsets).
2. Building associative patterns from item 1 elements.
3. Selection of the most statistically significant rules from item 2 elements under various criteria, their visualization and analysis.

On stage 2, we identified the most frequent value combinations among all rows, setting a minimum threshold “min_support” to screen the least frequent combinations.

ANALYSIS ALGORITHM AND PARAMETERS

We analyzed the data using the mixtend Python library for associative analysis and other auxiliary Python libraries for processing data tables (numpy, pandas), data reprocessing (sklearn), and for data visualization (matplotlib, networkx) according to the following algorithm:

1. All categorical features, including those with textual values, were assigned scores with values ranging from 0 to 6.
2. All categorical features were reassigned binary numbers and each column took the value 0 or 1, which meant whether the patient had the corresponding score for certain feature or not. For example, the question “Do you have difficulties in dispensing MPs according to e-prescription” (PED) with values 0, 1, ... 6 is encoded in columns PED_0, PED_1, ... PED_6, respectively. Each PED_X column of the table is filled 1 if PED=X, and 0 if not. The result is a binary table with values 0 or 1.

	PED	NDE	ACP	MCO	PEU	OGD	EBD	DPR
0	1	1	3	3	3	2	2	4
1	1	1	1	1	1	1	1	2
2	1	1	1	1	1	1	1	2
3	0	2	1	1	1	1	1	3
4	1	0	1	1	1	1	1	3
...	...	...	...	...	...	...	...	...
1999	1	0	6	6	6	6	6	1
2000	1	1	6	6	6	6	6	1
2001	1	3	6	6	6	6	6	2
2002	0	1	6	6	6	6	6	4
2003	2	1	6	6	6	6	6	5

Fig. 1. Data encoded in points
Picture taken by the authors

	PED_0	PED_1	PED_2	PED_3	PED_4	NDE_0	NDE_1	NDE_2	NDE_3	NDE_4	NDE_5	ACP_1	ACP_2	ACP_3
0	0	1	0	0	0	0	1	0	0	0	0	0	0	1
1	0	1	0	0	0	0	1	0	0	0	0	1	0	0
2	0	1	0	0	0	0	1	0	0	0	0	1	0	0
3	1	0	0	0	0	0	0	1	0	0	0	1	0	0
4	0	1	0	0	0	1	0	0	0	0	0	1	0	0
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1999	0	1	0	0	0	1	0	0	0	0	0	0	0	0
2000	0	1	0	0	0	0	1	0	0	0	0	0	0	0
2001	0	1	0	0	0	0	0	0	1	0	0	0	0	0
2002	1	0	0	0	0	0	1	0	0	0	0	0	0	0
2003	0	0	1	0	0	0	1	0	0	0	0	0	0	0

Fig. 2. Dataset encoded in binary system
Picture taken by the authors

- The most frequent combinations of elements in a binary table are calculated using the Apriori method, as well as sorted in descending order of the support criterion value. The condition "min_support" = 0.1 was chosen as the minimum threshold for selecting combinations.
- Calculation of associative rules from the most frequent combinations of elements selected in Item 3 and characteristics thereof. We set "confidence" as a metric for assessing the strength of associative rules, that is, a measure of how often the rule turns out to be correct. However, this may lead to ignoring some interesting patterns, so we set min_threshold=0 as the minimum threshold for this metric, which means the patterns will not be screened under this indicator. Screening will be performed further on according to more complex criteria.
- The most statistically significant associative rules were selected according to various criteria regarding the characteristics of these rules:

support	itemsets
0.69	(NDE_1)
0.42	(PED_1)
0.36	(ACP_1)
0.33	(NDE_1, PED_1)
0.29	(PED_2)
0.27	(PEU_1)
0.26	(EBD_6)
0.25	(DPR_1)
0.25	(NDE_1, ACP_1)
0.24	(PED_0)
0.24	(MCO_3)
0.23	(OGD_3)
0.20	(MCO_2)
0.20	(PED_2, NDE_1)

Fig. 3. Support value of the most frequent combinations of elements
Picture taken by the authors

	antecedents	consequents	antecedent support	consequent support	support	confidence	lift	leverage	conviction	zhangs_metric
0	(NDE_1)	(PED_0)	0.69	0.24	0.15	0.22	0.88	-0.02	0.96	-0.30
1	(PED_0)	(NDE_1)	0.24	0.69	0.15	0.61	0.88	-0.02	0.79	-0.15
2	(NDE_1)	(PED_1)	0.69	0.42	0.33	0.48	1.14	0.04	1.11	0.41
3	(PED_1)	(NDE_1)	0.42	0.69	0.33	0.79	1.14	0.04	1.48	0.21
4	(ACP_1)	(PED_1)	0.36	0.42	0.16	0.45	1.08	0.01	1.06	0.11
5	(PED_1)	(ACP_1)	0.42	0.36	0.16	0.38	1.08	0.01	1.05	0.13
6	(PED_1)	(MCO_3)	0.42	0.24	0.10	0.24	1.02	0.00	1.01	0.04
7	(MCO_3)	(PED_1)	0.24	0.42	0.10	0.43	1.02	0.00	1.02	0.03
8	(PED_1)	(PEU_1)	0.42	0.27	0.12	0.28	1.04	0.00	1.02	0.07
9	(PEU_1)	(PED_1)	0.27	0.42	0.12	0.44	1.04	0.00	1.03	0.05

Fig. 4. The first of the associative rules and characteristics
Picture taken by the authors

antecedents	consequents	antecedent support	consequent support	support	confidence	lift	leverage	conviction	zhangs_metric
(ACP_1, PED_1)	(NDE_1)	0.16	0.69	0.13	0.81	1.16	0.02	1.60	0.17
(PED_1)	(NDE_1)	0.42	0.69	0.33	0.79	1.14	0.04	1.48	0.21
(OGD_6)	(EBD_6)	0.16	0.26	0.13	0.78	2.94	0.08	3.31	0.79
(PEU_3)	(NDE_1)	0.19	0.69	0.14	0.73	1.06	0.01	1.15	0.07
(MCO_2)	(NDE_1)	0.20	0.69	0.15	0.72	1.04	0.01	1.11	0.05
(OGD_6)	(NDE_1)	0.16	0.69	0.12	0.72	1.04	0.00	1.10	0.04
(MCO_4)	(NDE_1)	0.18	0.69	0.13	0.71	1.03	0.00	1.07	0.04
(MCO_1)	(ACP_1)	0.17	0.36	0.12	0.71	2.00	0.06	2.24	0.60
(EBD_6)	(NDE_1)	0.26	0.69	0.19	0.71	1.03	0.00	1.06	0.03
(EBD_6, ACP_1)	(NDE_1)	0.15	0.69	0.11	0.71	1.02	0.00	1.05	0.03
(DPR_6)	(NDE_1)	0.17	0.69	0.12	0.71	1.02	0.00	1.04	0.02
(OGD_4)	(NDE_1)	0.19	0.69	0.13	0.71	1.02	0.00	1.04	0.02
(NDE_1, PEU_1)	(ACP_1)	0.18	0.36	0.11	0.61	1.71	0.05	1.66	0.51
(PEU_1)	(ACP_1)	0.27	0.36	0.16	0.61	1.70	0.07	1.63	0.56
(EBD_6)	(ACP_1)	0.26	0.36	0.15	0.58	1.62	0.06	1.52	0.52
(NDE_1, EBD_6)	(ACP_1)	0.19	0.36	0.11	0.58	1.61	0.04	1.52	0.47
(NDE_1, ACP_1)	(PED_1)	0.25	0.42	0.13	0.53	1.26	0.03	1.24	0.28
(DPR_1)	(ACP_1)	0.25	0.36	0.13	0.52	1.46	0.04	1.34	0.42

Fig. 5. The best of the associative patterns which simultaneously satisfy the given criteria
Picture taken by the authors

- “Confidence” ≥ 0.5,
“Lift” > 1,
“Leverage” ≠ 0,
“Conviction” > 1,
“Zhangs_metric” > 0.
- (1)
6. Visualization in the form of a graph of the best combinations of associative patterns according to various criteria and its analysis.

RESULTS

- We performed the analysis of the dataset formed on the basis of the survey results in accordance with the above algorithm.
1. The data have been accordingly marked in points (ranks) (fig. 1).
2. Converting the data into a binary table (fig. 2).
3. The most frequent combinations of elements were

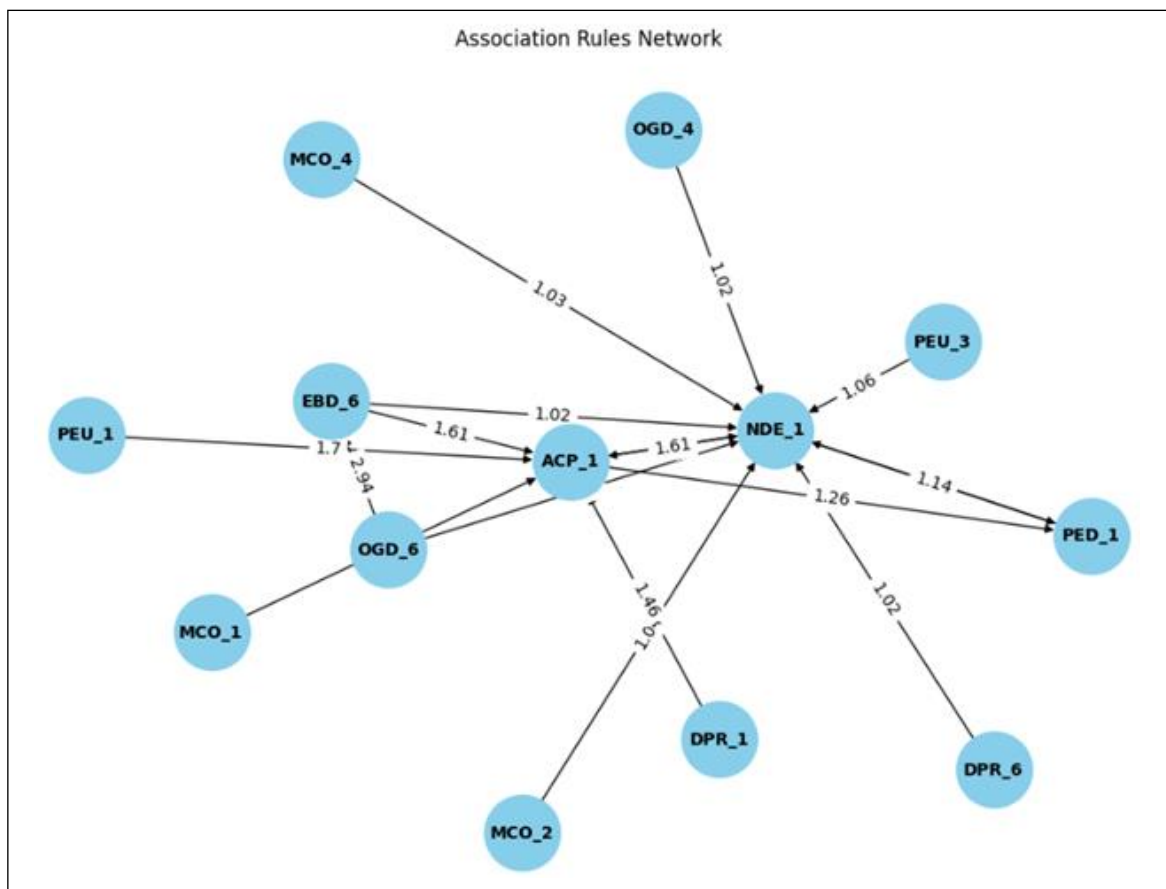


Fig. 6. The graph corresponding to the associative patterns which simultaneously satisfy the given criteria
Picture taken by the authors

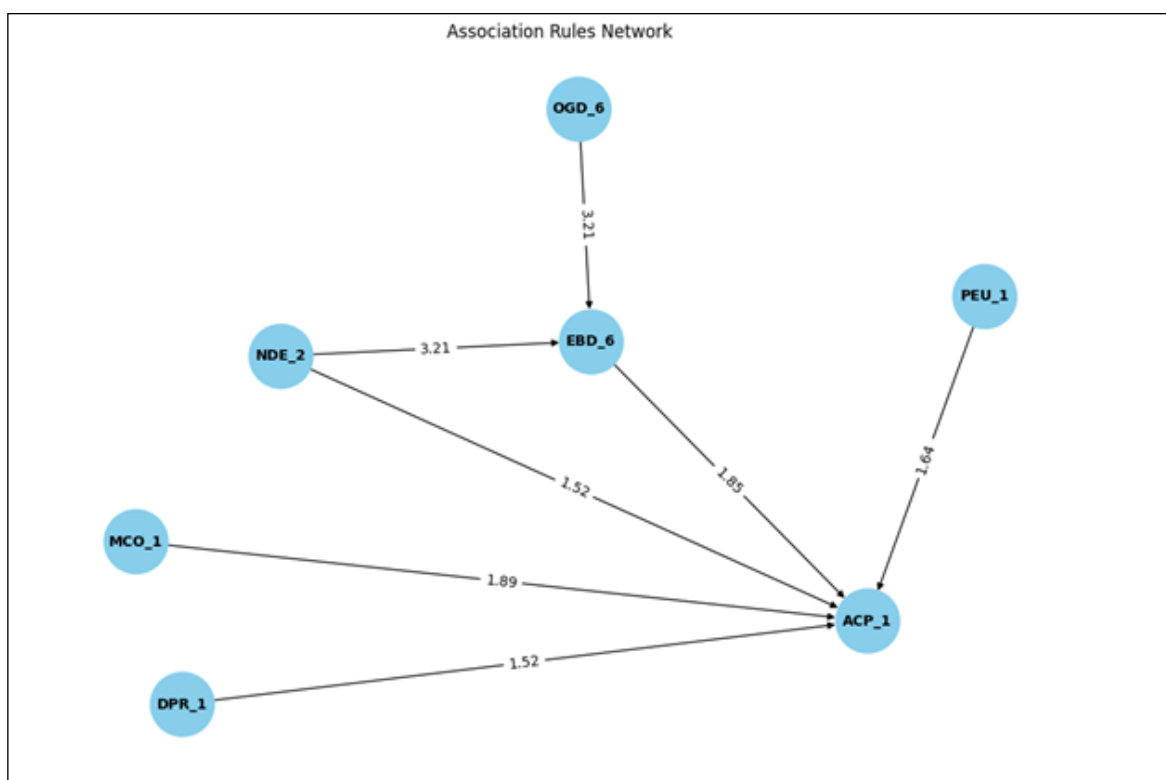


Fig. 7. The graph corresponding to the best associative patterns for the indicator "NDE_2"
Picture taken by the authors

defined under the Apriori method with $\text{min_support} = 0.1$ and sorted by support. (fig. 3) shows the best of these combinations that satisfy even greater constraint “ $\text{min_support} = 0.2$ ”.

4. The associative rules and characteristics were defined. (fig. 4) shows the first 9 of 83 patterns.
5. The best 18 out of 83 associative patterns which simultaneously satisfy the given criteria (1) were selected (fig. 5):
6. The associative patterns have been visualized in the form of a graph, where the vertices (nodes) represent elements from the left (antecedents) and right (consequents) parts of the pattern, while the edges (connections) between them show associations with a weight corresponding to the value of a certain metric (for example, “confidence” or “lift”). Fig. 6 shows a graph corresponding to the associative patterns of Fig. 5. The edges indicate the “lift” value of the metric.

An important criterion influencing the choice of a particular MP trade mark while filling e-prescription, is the amount of co-payment for medicines covered by the reimbursement program, or the price of prescription medicines not covered by governmental MP and MI reimbursement programs. At the same time, a significant factor is the “Conditional number of MPs failed to dispense” as a constant discrepancy between the number of issued and paid prescriptions persists. This indicator may fluctuate, depending on the influence of other factors, but always remains quite significant within the range from 10% to 20% [6]. The data of Fig. 6 suggests that the criterion “Amount of co-payment” (ACP) has a significant correlation with the factor “Conditional number of MPs failed to dispense” in 1 rank (ACP_1), and in turn corresponds to the conditional number of 10% undispensed prescriptions (NDE_1). The correlation of the ACP factor “Amount of co-payment” with DPR (Request of a doctor and/or a patient to dispense a medicine of a certain brand), MCO (Manufacturer/ MP’s country of origin) and PEU (History of medicine administration by the patient) in rank 1 indicates with a high probability that a pharmaceutical clerk considering the MP price/co-payment factor a significant one will be guided by the patient’s experience of administering a certain MP manufactured in a certain country, while the doctor’s request to dispense a medicine of a certain TN will also have a significant impact on the choice of medicine.

To make clearer conclusions under a similar algorithm [11], we have also picked the best associative patterns for the indicator “NDE_2” corresponding to a 20% of failed e-prescriptions (fig. 7).

As we can see from Fig.7, the NDE_2 criterion (failure to dispense MP under about 20% of e-prescriptions) is

also related to the amount of co-payment/price of the medicine (ACP), as in the previous case. At the same time, we have found a significant associative connection between the criteria “Amount of co-payment”, “Manufacturer/MP’s country of origin” (MCO_1), and “History of medicine administration by the patient” (PEU_1), as we also mentioned for the previous case. The relationship between the criteria ACP_1 and EBD_6, which is also related to OGD_6, suggests that the surveyed respondents consider the amount of co-payment to be the primary criterion for choosing MPs under TN, viewing the presence or absence of evidence-based data on the efficacy of a particular MP and the medicine’s belonging to generics or original MPs to be the least important at the same time.

DISCUSSION

Questions about factors that influence pharmacists’ decisions in relation to over-the-counter (OTC) medications to be recommended to clients have long been a concern for scientists. This multifaceted issue covers such aspects as professional competence of pharmacists, their interaction with patients and other actors of the healthcare system, and a footprint of regulatory and economic factors. Inter alia, the scientific article by Hanna L.A and Hughes C.M. [12], as well as the study conducted by Moritz K. et al. [13], present a detailed analysis of the factors that outline the algorithm of decision-making by pharmacists when dispensing over-the-counter medications. An important aspect of these works is also an assessment of the role and practical application of the principles of evidence-based approach in these processes. The studies were conducted in Northern Ireland and Japan, which allows us to admit accounting certain cultural and systemic features. The results of these studies suggest that a significant part of pharmaceutical professionals are generally aware of the importance of evidence-based approach in decision-making and meeting patient expectations, which means a reliance on information based on objective, unbiased scientific data and the results of carefully planned clinical trials, for sensible improvement of the quality of pharmacists’ daily counseling practice. However, a much smaller part of pharmacists has a thorough knowledge of the subject, makes deep insight into the issue, or critically evaluates the results of scientific research. The primary factors influencing a choice of over-the-counter medications appeared to be the ones related to safety of a medicine and a desire of patients (pharmacy clients) to administer a certain medicinal product. In our study, this factor corresponded to “Experience of using the medicine by a patient”

item, which also influenced the choice of medicines to be dispensed under e-prescription by a pharmaceutical clerk. The authors of the article [14] focused on studying the criteria based on the evidence-based approach and the incidence of using (or, conversely, not using) these criteria by pharmacists in routine professional counseling of patients regarding the over-the-counter medications dispensed from the pharmacy. We analyzed the pharmacists' communications with pharmacy clients related to evidence-based medicinal information, the patients' experience of using medicines and the pharmacists' experience of recommending them. The most significant criterion of medicine choice appeared to be the patient's experience with the medicine. The least mentioned were accounts of clinical studies of medicinal products and medicine recommendations by pharmaceutical clerks.

Thus, the important scientific information obtained by Moritz K. et al [13, 14] that was greatly consistent with the results of our study, confirmed the preference of some factors allegedly questioned in terms of evidence-based approach over seemed to be reliable evidence-based information that influenced clients' decision on purchasing OTC medicines.

The complex problem of insufficient communication and professional coordination between practicing physicians and pharmacists directly dispensing medicinal products from pharmacies is worth mentioning as a separate point. This issue was analyzed in detail in a profound study conducted by Grindrod K.A. and a large group of co-authors [15]. The authors of the above work revealed that the joint making decisions by practicing physicians and pharmacists was quite limited due to indirect communication and incomplete information exchange, especially regarding medicines' indications and patient's willingness to use them. Our work also did

not exclude the possibility of indirect communication between practicing physicians and pharmacists (via patients) regarding the choice of medicines dispensed under e-prescriptions.

The article [16] considers a vital role of pharmaceutical professionals in selecting generic drugs by patients. The study was conducted in the USA. It is emphasized that generic drugs cost is, as a rule, 85% less than that of brand medicines, which makes them much more affordable for patients with a limited budget. In our study, we also noticed the influence of belonging medicine to brand or generic name on its selection by a pharmaceutical clerk for dispensing under e-prescriptions. Perhaps, most patients made their choice depending on co-payment for a medicine which was much lower for generic drugs in most cases.

CONCLUSIONS

We have determined the criteria for selecting medicinal products by pharmaceutical clerks while filling e-prescriptions, which can be used in the practical activities of pharmaceutical workers as an integral part of the process of optimizing pharmacy inventories. The most important criteria have been found the amount of co-payment for MP, the country of manufacture/MP manufacturer, and the history of medicine administration by the patient. The criteria of the presence or absence of evidence-based data on the efficacy of a particular MP and the medicine's belonging to generics or original MPs have been found the least important. The inability of a pharmaceutical clerk/pharmacy worker to select a medicine based on one or more of the above selection criteria is considered one of the reasons for failure to dispense up to 20% of medicinal products under e-prescriptions.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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Normal serum bilirubin level is the risk factor for post-ERCP pancreatitis in Sphincter of Oddi dysfunction types I and II

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ABSTRACT

Aim: To evaluate the risk factors for PEP in SOD types I and II.

Materials and Methods: We retrospectively analyzed 107 cases of SOD type I, II with undergoing ERCP. Potential risk factors for PEP were investigated: age < 60 years, gender, history of cholecystectomy, history of EST, normal serum bilirubin level, common bile duct ≤ 10 mm, gallbladder stones, periamullary diverticulum, papilla size ≥ 15 mm, initial ERCP success, selective biliary cannulation, pancreatic cannulation/injection, Precut and EST. Factors both significant ($p < 0.05$) for univariate and multivariate analyses were identified as independent risk factor for PEP.

Results: The overall PEP rate was 14% (15/107). Univariate analysis (χ^2) showed that only bilirubin level was significantly ($p < 0.05$) associated with PEP. Multivariate analysis by multinomial regression showed that two factors were associated with PEP – normal bilirubin level (OR 9.574, 95% CI 1.869–49.034, $p = 0.007$) and Precut (OR 0.116, 95% CI 0.014–0.979, $p = 0.048$).

Conclusions: Normal serum bilirubin level is an independent risk factor for PEP in patients with Type I and Type II of SOD. In cases of suspected or confirmed Type I, II of SOD with normal bilirubin level, ERCP with EST should be avoided and replaced by medical treatment or, if ERCP had been chosen, advanced PEP prophylactic measures should be done.

KEY WORDS: Endoscopic retrograde cholangiopancreatography, sphincter of Oddi dysfunction, ERCP, post-ERCP pancreatitis, risk factor

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INTRODUCTION

Sphincter of Oddi dysfunction (SOD) is a benign motility disorder caused by either dyskinesia or stenosis of the sphincter of Oddi. The pathogenesis of the SOD is not well understood. It may have multiple clinical features often simultaneous: episodic pain in the epigastrium or the right upper quadrant, nausea, vomiting, jaundice and recurrent pancreatitis. Depending on clinical features and laboratory findings it has been categorized into biliary and pancreatic SOD [1,2].

According to the modified Milwaukee classification system, biliary SOD is classified into 3 types based on symptoms, biochemical abnormalities, and imaging results [3, 4]. Type I SOD is defined as biliary-type pain with both elevated liver enzymes and a dilated bile duct. Type II SOD presents with biliary-type pain with either elevated liver enzymes or a dilated bile duct. Type III SOD patients have biliary-type pain only without biochemical or imaging abnormalities [1].

Endoscopic retrograde cholangiopancreatography (ERCP) with endoscopic sphincterotomy (EST) plays an important role in SOD management. It was confirmed

by retrospective and prospective randomized trials that EST is an effective treatment for biliary SOD types I and II, as [5-7]. However, in SOD type III, EST has no advantage over placebo [7].

On the other hand, SOD is a well known risk factor for post-ERCP pancreatitis (PEP) [8-12]. But there is a lack of studies dedicated on analysis of PEP risk factors in a particular group of patients with SOD.

AIM

The aim of this study is to evaluate the risk factors for PEP in SOD type I, II.

MATERIALS AND METHODS

We retrospectively analyzed the medical data of 107 cases of biliary SOD type I, II with undergoing ERCP between January 2013 and December 2020 at Municipal non-profit enterprise city clinical hospital №2 named after prof. O.O. Shalimov of Kharkiv City Council.

Table 1. Baseline and Clinical Characteristics of the Patients with SOD

	Value
Age (range), yrs	63.7±13.8 (21-88)
Male/Female	21(19.6%)/86(80.4%)
Total bilirubin level (range), mg/dL	2.32±2.32 (0.47-14.5)
Common bile duct, mm	10.4±2.7
Cholecystectomy in history	26 (24.3%)
EST in in history	6 (5.6%)
Periampullary diverticulum	13 (12.2 %)
Initial ERCP success	103 (96.3 %)
Selective biliary cannulation	68 (63.5%)
Pancreatic cannulation/injection	21 (19.6%)
EST	86 (80.4 %)
Precut	38 (35.5 %)
PEP	15 (14 %)

Source: compiled by the authors of this study

ERCP

ERCP was performed by two experienced operators. Before the procedure all patients received diclofenac (100 mg) per rectum for PEP prevention. Premedicated with an injection of scopolamine butylbromide (10–20 mg) and local anesthesia of the pharynx with 8 % lidocaine were done.

Procedure usually was started with a guided sphincterotom or canula. Priority was given to obtain selective biliary cannulation which is defined as deep canulation of common bile duct (CBD) through naïve papilla followed by cholangiography without canulation of pancreatic duct or wirsungography. Needle-knife precut sphincterotomy (Precut) was used to achieve biliary access in case of failure of selective biliary cannulation after 5-10 attempts or approximately 5 min of trying.

We have never used transpancreatic precut as well as sphincter manometry. And we have not used prophylactic pancreatic stents placement in patients of this series.

After the procedure, the patient fasted until the next morning, received an intravenous infusion and ceftriaxone (2 g). Blood tests – hemoglobin, bilirubin and amylase levels were measured at baseline, 4-8 hours after the procedure, and next morning. ERCP-related adverse events were recorded; PEP was defined as upper abdominal pain with amylase levels more than three times the normal rate [13]. The severity of PEP was defined as mild (no organ failure, no local or systemic complications); moderate (transient organ failure, local or systemic complications without persistent organ failure); severe (persistent organ failure) [13].

The primary endpoint of this study was the occurrence of PEP.

STATISTICS

A number of potential risk factors for PEP were taken into analysis: age, gender, history of cholecystectomy, history of EST, serum bilirubin level, CBD size, gallbladder stones, parapapillary diverticulum, papilla size, initial ERCP success, selective biliary cannulation, pancreatic cannulation/injection, Precut and EST. All variables were made as categorical. For the univariate analysis, the Chi-square test (χ^2) was performed to identify differences in characteristics between patients with or without PEP. All variables were also taken for entry into multivariate analysis by multinomial logistic regression. Factors with $p<0.05$ both for univariate and multivariate analyses were identified as independent risk factors for PEP. All calculation were performed with SPSS® version 19 (IBM, USA).

ETHICS

This work complies with the principles of the Declaration of Helsinki.

RESULTS

Characteristics of the patients are shown in Table 1. The overall PEP rate was 14% (15/107). Among these 15 patient with PEP there was no severe case, 1 moderate and 14 mild pancreatitis. No procedure-related deaths, no hospital deaths occurred in any of the patients with PEP.

Univariate analysis showed that among 14 investigated factor only serum bilirubin level was significantly ($p<0.05$) associated with PEP (Table 2). Normal biliru-

Table 2. Univariate and multivariate analyses of risk factors for PEP in patients with SOD.

Variables	PEP (n=15)	Non -PEP (n=92)	Analysis				
			Univariate		Multivariate		
			χ^2	P	P	OR	CI (95%)
Female gender	14 (93.4%)	72 (78.2%)	1.857	0.173	0.069	0.735	0.073-7.366
Age < 60 years	5 (33.3%)	31 (33.6%)	0.001	0.979	0.620	1.481	0.313-7.010
Cholecystectomy in history	4 (26.6%)	22 (23.9%)	0.053	0.818	0.153	4.999	0.549-45.483
EST in in history	1 (6.6%)	5 (5.4%)	0.037	0.848	0.850	0.768	0.050-11.875
Normal Serum Bilirubin level	12 (80%)	39 (42.3%)	7.313	0.007	0.007	9.574	1.869-49.034
Common bile duct, mm ≤10	9 (60%)	52 (56.5%)	0.064	0.801	0.370	1.916	0.462-7.944
Gallbladder stones	9 (60%)	47 (51%)	0.411	0.522	0.113	4.709	0.692-32.049
Periapillary diverticulum	0 (0%)	13 (14.1%)	2.413	0.121	*	*	*
Papilla size, mm ≥15	1 (6.6%)	16 (17.3%)	1.110	0.293	0.543	.477	0.044-5.200
ERCP success	14 (6.6%)	89 (96.7%)	0.416	0.520	0.602	.438	0.020-9.702
Selective biliary cannulation	10 (66.6%)	58 (63%)	0.073	0.787	0.689	.633	0.067-5.951
Pancreatic cannulation/injection	5 (33.3%)	16 (17.3%)	2.078	0.150	0.318	2.800	0.372-5.951
Precut	3 (20%)	35 (38%)	1.833	0.176	0.048	0.116	0.014-0.979
EST	13 (80%)	73 (79.3%)	0.438	0.509	0.613	1.644	0.240-11.249

* Calculation is unfeasible because one of the comparable groups contains 0 cases

Source: compiled by the authors of this study

bin was in 80% (12/15) patient with PEP and in 42.3% (39/92) patients without PEP ($p=0.007$).

Multivariate analysis showed that two factors were associated with PEP – normal bilirubin level ($p=0.007$) and Precut ($p=0.048$).

Precut was used in 20% (3/15) patients with PEP while it was in 38% (35/92) patients without PEP ($p=0.048$).

As being significant both for uni- and multivariate analyses normal serum bilirubin level identified as the risk factor for PEP in patients with SOD.

DISCUSSION

PEP is the most common adverse event after ERCP and related endoscopic procedures [9, 10, 13, 14]. Being a serious complication in severe cases PEP may lead to mortality [9, 10, 15]. According clinical trials and studies the incidence of PEP ranges widely from 1% to 19.6% [8-10, 14-23]. SOD is a well known risk factor for PEP [8-10, 13, 15, 19]. Our previous study has also shown that SOD is an independent risk factor for PEP (OR 4.107; 95% CI, 1.726-9.771; $p=0.001$) [11]. But there are only a few studies describing risk factors for PEP in a particular group of patients with SOD [24]. That is why we dedicated our work to investigating PEP risk factors in such patients.

With implementing of CT, MRI, and endoscopic ultrasound the diagnostic role of ERCP has almost gone. And nowadays ERCP is used mainly as therapeutic procedure in malignant or benign biliary obstruction, CBD stones and other bilio-pancreatic pathology. Suspected cases of SOD requiring ERCP are gradually decreasing [24]. Nonetheless ERCP with EST is an effective treatment modality in biliary SOD types I and II while having no advantage in SOD type III [5-7]. That is why in present study we analysed only Type I and II of biliary SOD and have not included cases of Type III of biliary SOD as well as pancreatic SOD.

We have evaluated 14 protential risk factors for PEP in SOD – both patient related (age < 60 years, gender, CBD size ≤10 mm, normal bilirubin level, history of cholecystectomy, history of EST, gallbladder stones, parapapillary diverticulum, papilla size ≥15 mm) and procedure related (initial ERCP success, selective biliary cannulation, pancreatic cannulation/injection, Precut, EST). We have carefully chosen these variables to be studied and intentionally have not taken such factors as smoking, drinking and comorbidities, which appear in other studies [25, 26]. We consider them irrelevant to PEP. We have not considered the factor of difficult cannulation as in our technique manner precut papillotomy was done in case of it. All the variables were taken both

into univariate analysis and into multivariate regression as there is an opinion that such a model may give more reliable results [16, 27].

The incidence of PEP in patients with SOD reported to be from 9.1 % to 37.9 % [19, 21, 28-31]. Even after prophylactic pancreatic stenting PEP reported to be high – up to 25 % [30]. In our study the PEP occurred in 14 % of patient. That is higher than PEP incidence in non-selected studies (3.6%-9.5%) [8-11, 14-16, 18, 20-23].

Our investigation showed that the only risk factor significant both for univariate and multivariate analysis was normal serum bilirubin level (OR 9.574, 95% CI 1.869-49.034, $p=0.007$).

In one of the first work with a prospective, multi-centre design by M. L. Freeman et al normal serum bilirubin was an independent risk factor for PEP (OR 1.89, 95% CI 1.22-2.93, $p=0.0023$) [28]. In a work from Japan including 1,273 patients with native papillae who underwent ERCP for bile duct stones normal serum bilirubin was also an independent risk factor for PEP (OR 1.9, CI 1.01–3.6, $p=0.047$) [20]. In others studies where serum bilirubin level was investigated it turned out not to be a risk factor [14, 19, 21, 22, 25, 29]. There is an interesting paper describing risk factors of PEP in high-risk patients and normal serum bilirubin did not prove to be an independent risk factor for PEP [19]. Most of these studies whether bilirubin level turned out to be a risk factor or not were unselected and so obtained data could not be totally implemented on patient with SOD [14, 19, 21, 22, 25, 29]. In many studies this factor was not analysed at all [8, 16, 17, 20, 24, 26, 28, 30, 31]. We find serum bilirubin level very important factor to be investigate as hyperbilirubinemia is one of the signs of biliary obstruction so serum bilirubin level in combination with biliary dilatation plays a significant role in determining the indication for ERCP. And so we suppose that in case of SOD heperbilirubinemia could be a reliable criterion for ERCP usage both for potentiational therapeutic benefit and for PEP safety.

Aggressive prophylaxis of PEP is particularly important in patient with risk factors [13, 14, 20, 31]. For patients with SOD being in group of risk prevention of PEP is extremally important [13, 24]. The best way to prevent PEP is to exclude unnecessary ERCP in SOD.

That is why a case with normal serum bilirubin should be carefully examined and medical treatment should be considered. In case when ERCP has been chosen several options are available for PEP prevention.

Pancreatic stent placement was reported to be effective in the reduction of PEP including patients with SOD [32-34]. In contrast to these papers, there are some reports that pancreatic stenting cannot reduce the incidence of PEP [35-36].

Rectal nonsteroid anti-inflammatory drugs (NSAIDs) showed the efficiency in reduction of PEP incidence in majority of randomised controlled trials (RCT) and meta-analyses [13, 37, 38]. That is why in different guidelines there is a recommendation to use routine rectal administration of 100 mg diclofenac or indomethacin immediately before ERCP in all patients without contraindications to NSAIDs [13].

Also aggressive hydration was reported to be effective in prevention of PEP [13]. In patient with contraindications to NSAIDs it may be an alternative prevention measure [13]. According to a network meta-analysis, the combination of aggressive hydration and rectal NSAIDs is the most effective PEP prevention strategy. Its preventive efficacy was observed to be 70% to 99% higher than that of single prophylactic measures [39]. Therefore, aggressive prophylaxis for PEP with these strategies should be considered in patients with SOD especially with normal serum bilirubin.

This study has some limitations: first, this was a retrospective and single-center study; second, the number of cases is relatively small. The last one may lead to a bias to insignificance of certain factors which might be significant in lager sampling. That is why a large multicenter study is needed to confirm and clear-up the risk factors for PEP in Type I and II SOD.

CONCLUSIONS

In conclusion, normal bilirubin level is an independent risk factor for PEP in patients with Type I and Type II of biliary SOD. So in cases of suspected or confirmed Type I, II of biliary SOD with normal bilirubin level, ERCP with EST should be avoided and replaced by medical treatment or, if ERCP had been chosen, advanced PEP prophylactic measures should be done.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ICON infiltration technique as a modern approach to non-invasive caries treatment – A review of the 2018–2024 literature

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ABSTRACT

Non-invasive management of early caries lesions has become a cornerstone of contemporary minimally invasive dentistry. The ICON resin infiltration technique allows for the arrest of non-cavitated carious lesions and significant esthetic improvement, particularly in cases of white spot lesions. This review aims to present current scientific evidence on the effectiveness, indications, limitations, and clinical perspectives of ICON for the treatment of initial enamel caries. Recent systematic reviews, meta-analyses, and clinical studies from 2021–2024 were analyzed. The findings confirm that ICON is highly effective in arresting the progression of early carious lesions, especially on proximal surfaces, and in masking esthetic defects following orthodontic treatment and mild fluorosis. Proper case selection, strict adherence to the clinical protocol, and operator experience are crucial for therapeutic success. Limitations include reduced effectiveness in deep or inactive lesions, high technique sensitivity, and the cost of the procedure. Recent research focuses on combination therapies, novel infiltrant materials, and the application of digital diagnostics and artificial intelligence. ICON has the potential to become the standard for microinvasive treatment of early caries in the future, contributing to patient comfort, esthetic outcomes, and tooth preservation within the framework of preventive dentistry.

KEY WORDS: resin infiltration; early caries; microinvasive treatment; white spot lesions; enamel demineralization

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INTRODUCTION

Dental caries remains one of the most prevalent chronic diseases worldwide, affecting individuals of all age groups despite significant improvements in preventive dentistry [1]. Early enamel lesions are clinically visible as so-called *white spot lesions* (WSLs), which represent subsurface demineralization without cavitation. Their chalky, opaque appearance is an esthetic problem, particularly in anterior teeth, and also indicates the onset of caries progression [2].

Traditional management of these initial lesions includes topical fluoride application, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), hydroxyapatite-based pastes, bleaching, and microabrasion techniques [3–5]. However, these approaches show limited effectiveness in deeper subsurface lesions, depend on patient compliance, and often fail to completely restore enamel translucency [6].

In recent years, the development of resin infiltration technology (ICON®, DMG, Hamburg, Germany) has provided a micro-invasive alternative between preventive and

restorative strategies [7]. The principle of the technique is based on capillary penetration of a low-viscosity resin into the microporosities of demineralized enamel, thereby blocking diffusion pathways for acids and halting caries progression [8]. At the same time, the refractive index of the infiltrated enamel is modified, resulting in an immediate esthetic improvement through masking of WSLs [9].

Several systematic reviews and clinical studies confirm the efficacy of resin infiltration. Meta-analyses have shown that ICON provides superior esthetic masking compared to fluoride or CPP-ACP applications [2, 3]. Recent clinical and laboratory studies further support these findings. An *in vitro* study demonstrated that ICON resin infiltration increased enamel hardness and sealed prism structures, as evidenced by SEM and AFM analyses [10]. Another laboratory investigation using confocal laser scanning microscopy showed that the resin infiltrant penetrated up to 96% of the lesion depth [11]. In clinical settings, ICON proved effective in managing fluorosis, with a one-year follow-up confirming stable esthetic outcomes and high patient satisfaction [12]. Furthermore, randomized

controlled trials highlighted that ICON was significantly more effective than sodium fluoride varnish in reducing the severity of WSLs after six months [13].

AIM

This review aims to present current scientific evidence on the effectiveness, indications, limitations, and clinical perspectives of ICON for the treatment of initial enamel caries.

MATERIALS AND METHODS

For this narrative literature review, publications from 2018 to 2025 were analyzed. The search was performed in PubMed, Scopus, and Web of Science databases using the keywords *resin infiltration*, *ICON*, *white spot lesions*, *fluorosis*, and *minimally invasive dentistry*. Only articles in English were considered. Priority was given to systematic reviews, meta-analyses, randomized controlled clinical trials, and high-quality in vitro studies directly evaluating the ICON technique.

The inclusion criteria covered studies on early carious lesions, orthodontically induced white spot lesions, dental fluorosis, and molar-incisor hypomineralization. Case reports with long-term follow-up were also included if they provided relevant clinical insights. Studies older than 2018 were excluded unless they presented unique long-term results.

REVIEW AND DISCUSSION

MECHANISM OF ACTION

Resin infiltration works by penetrating the porous structure of early non-cavitated caries lesions with a low-viscosity, light-cured resin. The process begins with gentle etching of the enamel surface, which opens the microporosities and removes the hypermineralized surface layer. After drying with ethanol, the infiltrant resin is applied, allowing capillary action to draw the resin deep into the demineralized enamel. Upon polymerization, the infiltrant occludes the lesion body, blocking further acid diffusion, arresting caries progression, and reinforcing the weakened enamel structure [1, 2].

Recent morphological and in vitro studies confirm that ICON can infiltrate almost the entire body of active white spot lesions, providing a physical barrier to further demineralization and bacterial invasion [5,10]. Confocal laser scanning microscopy investigations demonstrated that the resin infiltrant can penetrate up to 96% of the lesion depth, underlining its potential to seal subsurface demineralization [11]. The success of infiltration depends largely on lesion activity and depth;

active, shallow lesions are most effectively treated, while inactive or deeper lesions (such as brown spots) may show incomplete penetration due to surface remineralization or subsurface lesion extension [5,6,11].

Importantly, resin infiltration also leads to optical blending of the lesion with healthy enamel, as the refractive index of the infiltrant closely matches that of sound tooth tissue. This not only halts the disease process but also provides immediate esthetic improvement [2,3].

CLINICAL PROTOCOL FOR ICON RESIN INFILTRATION

Recent guidelines and clinical studies (2018–2024) recommend the following stepwise protocol for the use of ICON resin infiltration in non-cavitated enamel lesions [2,3,5]:

- 1. Diagnosis and Case Selection:** Confirm the lesion is a non-cavitated, active (white spot) or early inactive (brown spot) carious lesion, confined to enamel or outermost dentin. Radiographic and clinical assessment are essential for appropriate case selection.
- 2. Isolation:** Apply a rubber dam or other isolation technique to prevent moisture contamination during the procedure.
- 3. Etching:** Apply 15% hydrochloric acid gel for 2 minutes to remove the hypermineralized surface layer and open enamel microporosities.
- 4. Rinsing and Drying:** Thoroughly rinse the etched surface with water, then dry with ethanol (Icon Dry) to achieve optimal dehydration and visual inspection of lesion penetration.
- 5. Resin Application:** Apply the low-viscosity resin infiltrant (ICON) onto the lesion surface for at least 3 minutes to ensure deep penetration. Remove excess resin and gently air-dry.
- 6. Light-Curing:** Polymerize the infiltrant using an appropriate curing light for 40 seconds.
- 7. Repeat (if necessary):** In deeper or more resistant lesions, the infiltration step can be repeated to maximize resin penetration.
- 8. Finishing and polishing:** Remove surface excess and polish the treated area to optimize smoothness and esthetics.

Studies confirm that following this protocol yields high rates of lesion arrest and esthetic improvement, particularly for active white spot lesions [2, 5, 10].

INDICATIONS FOR ICON RESIN INFILTRATION

ICON resin infiltration is primarily indicated for the management of non-cavitated enamel lesions, including

early carious lesions (ICDAS 1–2) and certain developmental enamel defects. The main clinical indications supported by recent evidence are:

INITIAL PROXIMAL (CONTACT) CARIES LESIONS

ICON is especially effective for non-cavitated carious lesions located on proximal surfaces of both permanent and primary teeth. A 2021 meta-analysis and subsequent randomized trials confirm that resin infiltration significantly reduces the risk of lesion progression compared to observation or fluoride varnish, with long-term studies reporting 60–80% reduction in progression risk [1, 6, 7, 13]. ICON is therefore recommended as the first-choice micro-invasive approach for radiographically detected early proximal lesions (E1, E2, outer D1), particularly in children, adolescents, and patients at increased caries risk.

WHITE SPOT LESIONS AFTER ORTHODONTIC TREATMENT

Resin infiltration is widely used for esthetic improvement of white spot lesions following multibracket orthodontic therapy. Systematic reviews and meta-analyses show a significant advantage of ICON over remineralizing agents and fluoride varnish, both in esthetic outcomes and lesion stabilization [2, 3].

MILD TO MODERATE DENTAL FLUOROSIS

ICON can successfully mask mild to moderate fluorosis, blending the appearance of affected enamel with healthy tissue. A prospective one-year clinical study demonstrated complete resolution of fluorotic opacities with stable esthetic results and high patient satisfaction [12]. ICON is therefore a valuable micro-invasive alternative to microabrasion or composite restorations in carefully selected cases.

DEVELOPMENTAL ENAMEL HYPOMINERALIZATION (E.G., MIH)

Infiltration has shown esthetic benefit in selected cases of molar-incisor hypomineralization, especially on incisors. However, outcomes remain less predictable for severely affected molars or teeth with extensive structural breakdown [4].

KEY SELECTION CRITERIA

Lesions must be non-cavitated, preferably active or early-inactive, accessible for isolation, and without

pulpal or periapical pathology. Proper diagnosis using radiographs and clinical examination is essential [3, 5, 7].

OFF-LABEL AND EMERGING APPLICATIONS

Although ICON resin infiltration is officially indicated for early carious lesions and white spot management, recent studies describe several promising off-label and emerging uses. These include:

- Masking of developmental enamel opacities (e.g., Turner's tooth, traumatic hypomineralization)

- Camouflage of mild enamel hypoplasia or post-traumatic defects

- Management of esthetic disturbances in MIH in anterior teeth not meeting classic inclusion criteria

- Blending of localized post-bleaching white marks or superficial post-traumatic stains.

Case reports and pilot clinical trials have demonstrated successful masking of such enamel defects, though current evidence is limited and outcomes less predictable than in classic indications [2, 5, 14]. Patients must be informed that off-label applications may not yield permanent or fully satisfactory esthetic results and may require retreatment. Long-term follow-up, such as the four-year case report presented by Cazzolla et al., shows that infiltration effects may persist, though periodic re-evaluation is recommended [14].

Future research and well-designed clinical trials are needed to clearly establish the safety and long-term efficacy of ICON in these off-label scenarios [2,6].

EFFECTIVENESS AND ESTHETIC OUTCOMES

Recent systematic reviews and clinical studies confirm that ICON resin infiltration is highly effective in arresting the progression of non-cavitated enamel caries lesions. In proximal lesions, multiple meta-analyses demonstrate that resin infiltration reduces the risk of lesion progression by 60–80% compared to no treatment or fluoride varnish, with follow-up extending up to seven years [1,6,7]. ICON is particularly beneficial in children and adolescents, as it helps avoid restorative interventions and preserves sound tooth structure [15].

A key advantage of resin infiltration is its esthetic benefit. ICON provides immediate masking of white spot lesions, post-orthodontic demineralization, and mild dental fluorosis. A 2023 network meta-analysis showed that resin infiltration delivers superior visual improvement compared to remineralizing agents and fluoride varnish, with consistently high patient satisfaction scores [2, 3]. A one-year clinical study further confirmed complete disappearance of fluorotic lesions and stable results [12], while long-term case reports documented

lasting esthetic improvement up to four years [14].

For selected cases of molar-incisor hypomineralization (MIH), particularly in anterior teeth, ICON infiltration results in significant color improvement and cosmetic blending, although the outcomes are less predictable in severely hypomineralized or structurally compromised teeth [4].

LIMITATIONS

While ICON resin infiltration has revolutionized micro-invasive caries management, several limitations and challenges remain. The technique is most effective in early, non-cavitated, and active white spot lesions. Inactive or deeper lesions (e.g., brown spots or those extending into outer dentin) frequently show incomplete resin penetration due to a hypermineralized surface or lesion depth [5,6,11]. Systematic reviews highlight that effectiveness significantly decreases when lesions extend beyond the outer third of dentin or present with a thick, remineralized surface layer [1,6,7].

Esthetic outcomes may also vary. Although ICON achieves impressive masking of white spot lesions and mild fluorosis, results are less predictable in severe developmental opacities, deep post-traumatic defects, or extensive MIH [2,3]. The degree of color blending and long-term stability can vary, and in some cases repeated infiltration or adjunctive procedures are necessary [14].

The procedure is also technique-sensitive and requires strict isolation, careful surface conditioning, and adherence to the protocol. Inadequate etching or incomplete drying may limit resin penetration, while operator experience has been shown to significantly affect both clinical and esthetic results [7].

ECONOMIC ASPECTS

An additional limitation of ICON therapy is its cost. The average price per tooth in European private practice ranges from 300–600 PLN, which is considerably higher than topical fluoride varnish (<100 PLN) or remineralizing agents (50–150 PLN). Nonetheless, ICON is less invasive and less costly compared to esthetic alternatives such as veneers or extensive microabrasion procedures. From a long-term perspective, successful infiltration can reduce the need for restorative interventions, potentially lowering future treatment costs [13].

ADVERSE EFFECTS

Although ICON resin infiltration is generally considered a safe and well-tolerated microinvasive technique, some side effects have been reported. The use of 15% hydro-

chloric acid during the etching step may cause transient gingival irritation if gingival tissues are not adequately protected [10]. Laboratory studies also indicate that infiltrated enamel surfaces may become rougher, which could theoretically favor plaque accumulation [10]. Furthermore, long-term clinical observations and case reports suggest that repeated applications may be required to maintain esthetic results in certain lesions, particularly in fluorotic or hypomineralized teeth [14].

FUTURE DIRECTIONS AND RESEARCH

The field of resin infiltration is evolving rapidly, with several promising developments on the horizon. Combination therapies are being explored, such as infiltration following partial microabrasion or in conjunction with remineralizing agents, to further enhance both caries arrest and esthetic outcomes [9]. Advances in diagnostic technologies, including digital caries detection and artificial intelligence-based imaging, may improve lesion selection, monitoring, and treatment planning, potentially increasing the success rate of ICON therapy [10].

Another area of research focuses on next-generation infiltrants, incorporating bioactive components such as hydroxyapatite nanoparticles or fluoride-releasing matrices. These modified formulations aim not only to seal enamel porosities but also to provide antibacterial properties and promote remineralization [15]. Preliminary laboratory studies suggest that such infiltrants may enhance long-term durability and broaden clinical indications.

Finally, further high-quality randomized controlled trials are needed to confirm the long-term stability of esthetic outcomes, assess cost-effectiveness in broader populations, and validate ICON use in off-label applications, such as developmental enamel defects and severe MIH [2,6,12].

CONCLUSIONS

Resin infiltration with ICON represents a micro-invasive and effective approach for managing early carious lesions and enamel opacities. Current evidence confirms its ability to arrest lesion progression and provide immediate esthetic improvement, particularly for orthodontic white spot lesions and mild fluorosis. Randomized clinical trials demonstrate superior outcomes compared to fluoride varnish, while long-term reports highlight durability of esthetic results.

Despite these advantages, ICON is not universally applicable. Its effectiveness is limited in deep or inactive lesions, and costs may restrict accessibility. Minor adverse effects, such as transient gingival irritation

or the need for repeated applications, should also be considered.

Overall, ICON remains a valuable treatment option within minimally invasive dentistry, bridging the gap

between preventive and restorative strategies. With ongoing research and development, resin infiltration may gain an even broader role in contemporary caries management.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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Prediction accuracy of tooth movement in digital treatment planning with aligners: A systematic review

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ABSTRACT

Aim: To evaluate the accuracy of common tooth movements performed with clear aligners and to summarize patient-centered outcomes.

Materials and Methods: A comprehensive search of PubMed, PubMed Central (PMC), Scopus, MDPI, and BMC Oral Health was conducted to identify English-language articles published between 1 January 2020 and 31 March 2025. The search strategy combined keywords and Medical Subject Headings (MeSH) terms, including “clear aligner”, “Invisalign”, “aligner accuracy”, “tooth movement predictability”, “distalisation”, “rotation”, “expansion”, “intrusion”, “torque”, “overbite correction”, “space closure”, “quality of life”, and “patient satisfaction”. Reference lists of the included articles were screened manually to identify additional eligible studies.

Conclusions: Distalization and rotation are predictably expressed with clear aligners, whereas transverse expansion, tipping/torque, overbite reduction, and particularly intrusion remain less reliable. Treatment planning should anticipate under-expression, incorporate overcorrections, use attachments and auxiliaries judiciously, and ensure robust patient education to enhance compliance and satisfaction.

KEY WORDS: aligner therapy, tooth movement, orthodontic planning

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INTRODUCTION

Orthodontic treatment has undergone a paradigm shift with the emergence of clear aligner therapy (CAT). The first commercial aligner system, Invisalign®, was introduced in 1999, heralding a new era of discreet and removable appliances for adults and adolescents. Since then, numerous companies have developed aligner systems that rely on computer-aided design and three-dimensional printing to manufacture custom trays that move teeth incrementally. Surveys indicate that more than 90% of Australian orthodontists and about two-thirds of practitioners in North America now offer clear aligners as part of their armamentarium [1].

Despite these advantages, clinicians have observed that actual tooth movements often diverge from digital predictions. Unlike fixed appliances, aligners deliver forces primarily to the crowns of the teeth. This low point of force application produces moments favouring tipping over bodily translation; as a result, achieving root torque, transverse expansion, and vertical intrusion with aligners can be challenging [2]. Additional factors

complicate predictability, including material elasticity, stage sequencing, patient compliance, and the presence or absence of attachments. Because aligners are removable, success depends heavily on patients wearing them for 20–22 hours per day, following a prescribed change interval, and maintaining good oral hygiene. Failure to adhere can compromise outcomes and prolong treatment.

Randomized controlled trials and scoping reviews indicate that wear schedules, electronic reminders, and remote monitoring programmes significantly influence patient compliance and treatment efficiency [2–4]. Recent systematic reviews and cohort studies have underscored that the accuracy of digital treatment planning varies with the complexity of the intended movement, and those innovations such as direct 3D printing of aligners and advanced software algorithms continue to evolve the field [5].

Digital treatment planning is central to clear aligner therapy (CAT). After intraoral scanning or taking polyvinyl siloxane impressions, clinicians receive a virtual

three-dimensional model of the patient's dentition. The software allows them to simulate movements, sequence stages, place attachments or buttons, and plan interproximal reduction [6]. The planned movements can be visualised from different angles and shared with patients, facilitating informed consent and motivation. Over the past decade, aligner planning software has become increasingly sophisticated, incorporating root visualisation, occlusal collision detection, and artificial intelligence-driven suggestions. Some systems integrate computed tomography data to assess alveolar bone limits and root positions, thereby minimising the risk of iatrogenic root resorption or periodontal compromise [7].

Despite these advances, the accuracy of digital planning remains limited by the ability of aligners to generate sufficient forces and moments. An important concept is the moment-to-force ratio: high ratios favour bodily movement, while low ratios result in tipping. Aligners, being thin plastic shells, typically produce relatively low moments because the distance between the point of force application and the centre of resistance is small. Attachments—small composite protrusions bonded to teeth—are designed to increase this distance and augment the mechanical coupling between the aligner and the tooth. Optimised attachment shapes (rectangular or ellipsoidal) and pressure points can improve control of rotations and angulations [8].

Clear aligners offer several advantages over conventional braces: improved aesthetics, enhanced comfort, reduced mucosal irritation, easier maintenance of oral hygiene, and fewer emergency visits for broken brackets. Their removability allows patients to eat without restrictions and simplifies brushing and flossing, potentially reducing the risk of decalcification and periodontal problems. The digital workflow offers precise visualisation of treatment progress, facilitating patient engagement and enabling remote monitoring. Studies suggest that aligners may produce less pain and psychosocial impact than fixed appliances, particularly during the initial adjustment period [9].

However, clear aligner therapy (CAT) has certain limitations. Its removability means that compliance is crucial; a systematic review found that only about one-third of patients wear their aligners as prescribed [10]. Movement control is less predictable in complex cases involving rotations, torque, vertical corrections, or extraction space closure. Aligners require careful staging to avoid conflicts between adjacent teeth, and overcorrection is often necessary to compensate for under-expression. Refinement stages, involving additional sets of aligners, are commonly required to achieve the desired outcome. Some patients may also

find the constant need to remove aligners before eating and to clean them inconvenient.

This systematic review aims to evaluate the accuracy of tooth movements planned digitally and executed with clear aligners between 2020 and 2025. Previous reviews have focused on earlier periods or on specific movements; this analysis includes more recent studies and synthesises data on distalisation, rotation, expansion, angulation, vertical corrections, and space closure. By calculating weighted mean accuracies and identifying factors influencing success, we aim to provide clinicians with realistic expectations and to guide treatment planning. Furthermore, we summarise patient-centred outcomes to contextualise mechanical findings and highlight the importance of compliance and communication.

AIM

To evaluate the accuracy of common tooth movements performed with clear aligners and to summarize patient-centered outcomes.

MATERIALS AND METHODS

A comprehensive search of PubMed, PubMed Central (PMC), Scopus, MDPI, and BMC Oral Health was conducted to identify English-language articles published between 1 January 2020 and 31 March 2025. The search strategy combined keywords and Medical Subject Headings (MeSH) terms, including “clear aligner”, “Invisalign”, “aligner accuracy”, “tooth movement predictability”, “distalisation”, “rotation”, “expansion”, “intrusion”, “torque”, “overbite correction”, “space closure”, “quality of life”, and “patient satisfaction”. Reference lists of the included articles were screened manually to identify additional eligible studies [11–13].

Inclusion criteria comprised prospective or retrospective clinical studies evaluating patients undergoing clear aligner therapy (CAT), in which planned tooth movements were compared with achieved outcomes. Studies were required to report numerical discrepancies (in millimetres or degrees) or percentage accuracy for at least one movement category. Eligible movements included sagittal (molar distalisation), rotational, transverse (arch expansion), angular (tipping and torque), vertical (overbite reduction and intrusion), and space-closure movements. Studies combining aligners with fixed appliances or temporary anchorage devices were included only if data specific to aligner-induced movements were separately analysable.

Exclusion criteria were case reports, in-vitro or finite-element simulations, non-English publications without accessible full text, and studies focusing ex-

clusively on fixed appliances or direct-to-consumer aligners lacking professional supervision.

Two reviewers independently screened titles and abstracts for eligibility. Full texts of potentially relevant articles were examined to confirm inclusion criteria. Discrepancies in study selection were resolved by discussion. Data were extracted into a standardised spreadsheet capturing study design, sample size, demographic characteristics (age, sex), aligner brand, movement category, planned and achieved movement magnitude, calculated accuracy, presence and type of attachments, use of auxiliaries (elastics, mini-implants, power arms), wear schedule (7- versus 14-day aligner change), and measurement method.

Measurement techniques included digital model superimposition on stable teeth or palatal rugae, cone-beam computed tomography (CBCT) overlays, and occlusal photographs. Where necessary, study authors were contacted for clarification.

ETHICS

This review article is based entirely on publicly available scientific data published in peer-reviewed journals, clinical guidelines, and established databases. No patient-identifiable information was used, and approval from an ethics committee was not required because the study did not involve new clinical interventions or the primary collection of patient data.

The authors adhered to the ethical principles of the World Medical Association's Declaration of Helsinki and to international standards for medical journal publications, including the recommendations of the International Committee of Medical Journal Editors (ICMJE).

No part of this work contains plagiarism or data fabrication. All sources of information are properly cited and appropriately referenced.

REVIEW AND DISCUSSION

Nine studies met the inclusion criteria, comprising five prospective cohorts and four retrospective analyses, with a total of 451 patients and approximately 940 jaws treated using clear aligner systems such as Invisalign®, Ordoline, Angel Aligners, Spark, and HeySmile. Patient ages ranged from 8 years (mixed dentition) to 55 years (adult orthodontics); most samples included adolescents and young adults. Wear schedules varied from weekly to biweekly aligner changes, and treatment duration ranged from three to 24 months. Attachments were used in most cases, although their type and number varied. Some studies employed inter-arch elastics, mini-implants, or power arms for anchorage or additional force vectors. Measurement methods included

digital model superimposition (six studies) and CBCT overlays (three studies).

Molar distalisation aims to correct Class II malocclusion or to provide space for anterior alignment. Among the included studies, distalisation was evaluated in three cohorts. One prospective study of 28 patients using the Ordoline system reported that first and second maxillary molars achieved 69,4% and 75,2%, respectively, of the planned 2 mm distal movement, corresponding to approximately 1,4–1,5 mm of clinical displacement [14]. A small retrospective series of 15 patients treated with Invisalign® found a mean accuracy of 87% for first molar distalisation, suggesting that patient-specific factors and attachment design influence outcomes [15]. However, a 2024 study involving 30 patients found significant discrepancies between planned and achieved distalisation and reported that Class II elastics did not improve distalisation or prevent mesial drift of lower incisors [16–17]. The pooled weighted mean accuracy across studies was 75,6%, with a 95% confidence interval of approximately 68–84%. Factors affecting distalisation included the amount of crowding, presence of second molars, attachment type, and patient compliance. Overcorrections of 20–30% were recommended to compensate for under-expression.

Rotational movements, particularly of premolars and maxillary canines, were evaluated in four studies. One prospective study reported a mean accuracy of 77,9% for rotations of canines and premolars using optimised attachments [18]. Another investigation found that rotations of mandibular incisors were less predictable than those of maxillary canines, with accuracy around 60%. A retrospective analysis of extraction cases revealed that large planned rotations (>20°) significantly increased the likelihood of requiring refinements [19–20]. Attachments improved rotational control but were associated with increased refinement rates for certain teeth (e.g., tooth 12) [21]. The overall pooled accuracy for rotations was approximately 78%, indicating that aligners can achieve moderate to high rotational control when attachments and staging are properly applied.

Transverse (dentoalveolar) expansion was assessed in three studies encompassing 120 patients. Weighted mean accuracy was 59,1%, with a tendency for under-expansion in the maxilla and occasional over-expansion in the mandible. One study found that the accuracy of expansion was influenced by the amount of planned expansion, with movements beyond 3 mm rarely achieved without significant overcorrection or auxiliary anchorage [22]. Another analysis comparing four aligner systems reported that expansion was more predictable in the lower arch, possibly due to lower resistance from the cortical plates [23]. Sex, crossbite

presence and initial crowding also affected outcomes. Overcorrection (planning for an additional 0,5-1,0 mm of expansion) and the use of attachments or auxiliary wires were recommended to improve outcomes.

Angular movements such as tipping and torque are challenging with aligners. Several studies have reported that maxillary incisor torque achieved about 53 % of the planned values, while lower incisor tipping achieved only 35% [10-13]. One prospective study assessing the mesiodistal angulation of maxillary central incisors found an accuracy of 53 % and noted that the use of optimised attachments improved control [10-11]. In extraction cases, controlling root angulation during space closure was particularly difficult; large planned movements significantly increased the need for refinements [9]. Some studies reported that attachments increased refinement probability on certain teeth, suggesting that the shape and placement of attachments must be tailored for each tooth type. Auxiliaries such as power arms, elastics or mini-implants may be required to achieve bodily movement and root parallelism, especially in extraction cases [14]. Overall, tipping and torque movements remain moderately predictable ($\approx$ 53-60%), and clinicians should anticipate under-expression [13].

Vertical corrections were the least predictable category. Two studies evaluated overbite reduction and curve of Spee levelling in adults treated with Invisalign®. They reported an average accuracy of approximately 52,7%, with improvement achieved mainly through anterior extrusion rather than true posterior intrusion [15]. Another study focusing on deep bite correction found that mandibular incisor intrusion achieved only 9,5% of the planned value [18]. These findings underscore the difficulty of generating vertical forces with aligners. Biological constraints, such as limitations in alveolar bone height and the influence of muscular function, further complicate vertical movements. Skeletal anchorage (mini-implants) or hybrid treatment with fixed appliances may be necessary to achieve significant intrusion or open-bite closure. Overcorrection strategies and the use of aligner materials with higher stiffness may also enhance vertical control.

A scoping review of 37 studies on patient experiences with clear aligner therapy (CAT) found that most patients appreciated the discreet appearance and removability of aligners [15]. Pain was generally less intense than with fixed appliances, peaking on the first day of each aligner change and subsiding thereafter. Patients reported transient speech disturbances, especially lisping, which typically resolved within one month. Eating was minimally affected because aligners can be removed; however, the need to brush after meals before reinserting aligners was often considered inconvenient. Oral-health-related quality of life declined temporarily due to discomfort and

difficulties in removing aligners, but improved aesthetics largely offset these drawbacks. Satisfaction depended on perceived progress, comfort, and the quality of communication with the treating orthodontist. Only 36% of patients fully adhered to prescribed wear schedules, highlighting the importance of compliance monitoring and digital reminders [16]. Direct-to-consumer aligners offered cost and convenience advantages but were associated with a 6,6% complication rate and limited professional oversight, raising concerns about safety and treatment success [17].

Recent literature published between 2020 and 2025 has expanded the understanding of CAT beyond the traditional focus on tooth movements and biomechanics. A comprehensive review of aligner fabrication and direct three-dimensional printing technologies described advances in materials science and additive manufacturing that enable custom thickness profiles and shape-memory polymers [19-20]. Structural analyses of commercially available aligners showed that differences in polymer chemistry and manufacturing processes affect mechanical properties and fit [21-23]. Systematic and scoping reviews evaluated the biocompatibility of aligner materials and reported that, while current thermoplastics release minimal cytotoxic by-products, future research should monitor potential endocrine disruptors [24].

Clinical practice surveys and consensus guidelines emphasised the importance of patient selection, clinician training, and remote monitoring to optimise outcomes [25-27]. Randomised trials investigated wear protocols, electronic reminders, and remote monitoring apps, demonstrating improved compliance and treatment efficiency [28-30]. Expert panels published modified Delphi and consensus statements outlining the indications and limitations of CAT, highlighting areas where fixed appliances remain superior and where hybrid approaches may be warranted [29-31].

Collectively, these contributions contextualize the present findings and underscore that the success of CAT depends not only on biomechanics but also on material science, digital workflows, patient behavior, and consensus-based treatment planning [32, 33].

This systematic review demonstrates that distalisation and rotational movements with clear aligners achieve approximately three-quarters of the planned displacement, whereas transverse expansion, tipping/torque, and overbite reduction achieve only moderate success, and vertical intrusion remains largely unreliable. These findings reflect the inherent biomechanics of aligners. Because aligners envelop the crowns of teeth, they generate forces near the occlusal surfaces. The moment-to-force ratio is therefore low, producing tipping rather than bodily translation.

The material properties of aligners — typically polyurethane or PET-G — influence force decay over time; research indicates that force levels can decline by 50% within 24 hours, potentially reducing movement efficiency. The addition of attachments increases the distance between the point of force application and the centre of resistance, thereby raising the moment-to-force ratio and improving control. However, attachments may also increase aligner visibility and patient discomfort, and do not fully overcome the mechanical limitations of the system.

Several factors affect predictability:

Tooth type and arch. Maxillary teeth typically move more predictably than mandibular teeth due to differences in bone density and arch form. Canines and premolars often rotate better than incisors or molars because of their geometry and available space [4];

Magnitude of planned movement. Larger planned movements (>3 mm of translation or >20° of rotation) are associated with lower accuracy and higher refinement rates [9]. Staging movements in smaller increments and planning overcorrections can improve outcomes;

Attachments and auxiliaries. Properly designed attachments (rectangular, ellipsoidal) and strategic placement enhance the coupling between aligner and tooth, improving rotation and tipping control. Interarch elastics and mini-implants can provide additional anchorage for distalisation and vertical movements [17];

Patient compliance. Aligners are effective only when worn for 20–22 hours per day. Non-compliance reduces force delivery and prolongs treatment. Digital monitoring tools, such as Bluetooth sensors and smartphone reminders, may enhance compliance by providing feedback to patients and clinicians;

Material properties and change interval. Force levels depend on aligner stiffness and thickness. Shorter change intervals (7 days) may reduce force decay and aligner deformation, whereas longer intervals (14 days) may allow more time for biological response but risk aligner distortion. Evidence regarding the optimal interval is mixed, and clinicians should individualise based on patient response.

The moderate predictability of transverse expansion, tipping/torque and overbite reduction suggests that clinicians should plan overcorrections of 20–30% and anticipate the need for refinement. Regular monitoring using digital models or intraoral scans allows early detection of under-expression and timely implementation of auxiliary techniques. For complex cases requiring significant intrusion, torque control or space closure, hybrid approaches combining aligners with fixed appliances or skeletal anchorage may be advisable. AI-driven treatment planning, high-frequency

vibration devices and adjunctive procedures such as micro-osteoperforations hold promise for enhancing movement efficiency, though more clinical evidence is needed.

Patient-centered outcomes highlight the importance of clear communication and expectation management. Patients should understand that aligners may not perfectly replicate virtual treatment plans and that refinement stages are common. Educating patients about the necessity of wearing aligners for most of the day and maintaining oral hygiene will improve outcomes. Clinicians should discuss potential discomfort, speech changes and the need to remove aligners before eating. Remote monitoring and teleorthodontics can reduce in-office visits and help maintain engagement. Aligners' discreet appearance and comfort make them attractive to adults who are concerned about aesthetics, but cost and the need for high compliance must be considered.

This review is limited by the small number of eligible studies, heterogeneity in design, measurement methods and outcome reporting, and the inclusion of only open-access articles. Sample sizes were modest, and prospective randomised controlled trials were scarce. Weighted mean calculations provide approximate estimates but cannot account for within-study variability. Additionally, most studies evaluated early treatment phases and did not assess long-term stability or post-treatment relapse. Future research should focus on large-scale, multicentre trials with standardised measurement protocols, including CBCT or 3D photogrammetry, to evaluate aligner efficiency across different movement categories. Investigations into new materials, such as shape-memory polymers or multi-layer laminates, could improve force decay characteristics. The integration of machine learning algorithms into treatment planning software may optimise staging and predict outcomes based on patient-specific variables.

CONCLUSIONS

1. Distalization and rotational movements with clear aligners achieve approximately 75–78% of planned movement and can be considered reasonably predictable when attachments and careful staging are used.
2. Transverse expansion, tipping/torque, and overbite reduction exhibit moderate predictability (≈53–62%), necessitating overcorrection and vigilant monitoring.
3. Root torque, space closure, and vertical intrusion remain unreliable; mandibular incisor intrusion achieves only ≈9.5% of planned values, suggesting that hybrid treatment or skeletal anchorage may be required for significant vertical corrections.

4. Patient education and digital monitoring enhance adherence and satisfaction, mitigating transient pain and psychosocial impacts.
5. Further randomized controlled trials with larger cohorts and standardized protocols are needed to refine accuracy estimates and evaluate adjunctive techniques.

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Problems and prospects of conducting molecular genetic research for the identification of persons who went missing or died during the hostilities in the occupied territories of Ukraine

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ABSTRACT

Aim: To present modern conceptual, methodological, technical, and regulatory advancements in the identification of persons missing or killed in hostilities and propose strategies for improvement.

Materials and Methods: Standard forensic methods can be used to identify individuals in forensic science. For example, medical and dental records of victims (forensic odontology), analysis of protrusions and swirls on human fingers (fingerprinting), identification of implant serial numbers.

Conclusions: It is proposed to supplement Part 2 of Article 84 of the Criminal Procedure Code of Ukraine as a new source of evidence – “information from DNA databases”.

KEY WORDS: hostilities, molecular genetic research, identification of persons, genomic information, forensic molecular genetic examination

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INTRODUCTION

The birth of molecular genetics is associated with a new stage in the development of biology, when a revolutionary breakthrough in the understanding of living nature occurred as a result of the joint work of scientists - physicists, chemists, microbiologists, and mathematicians. In 1953, two molecular biologists, J. Watson (USA) and F. Crick (UK), summarized the data of X-ray analysis and deciphered the structure of the DNA double helix. This was followed by an avalanche of discoveries (1956 - discovery of matrix RNA, 1958 - formulation of the “central dogma of molecular biology”, the basic principles of molecular genetics concerning the matrix principles of transcription, translation and replication)[1]. In the next two decades, the prerequisites for the possibility of reading (sequencing) the nucleotide sequence were created. In 2000, a large group of scientists (more than 200) decoded the *Drosophila* genome. And the following year, scientists from Celera Genomics (USA) reported the decoding of the human genome [1].

Molecular genetics examines hereditary mechanisms at the molecular level, finding applications in medicine (early diagnosis, gene therapy), agriculture (GMOs), ecology (genetic toxicology), and forensics. DNA analysis, first employed in forensic science over 20 years ago in the UK, has since become a crucial tool for solving crimes, identifying missing persons, and recognizing unidentified corpses [2]. The ongoing war in Ukraine has highlighted the importance of forensic genetics in identifying victims and missing individuals [3-5].

Today, there are certain fragmentary developments in the identification of unidentified human remains after numerous military actions of the last two centuries on all continents, persons who have long been missing, victims of natural and technogenic disasters, and various options for material collection, processing, testing, and interpretation are proposed [3, 4].

The Ukrainian Commissioner for Persons Missing in Special Circumstances reports that as of July 2023, the Ukrainian register of missing persons contains data on

more than 24 thousand people, and there is no information about 7 thousand of them yet [5]. According to him, more than 8,000 people have been found, of whom 60% are alive and are or have been in captivity. Also, the possible whereabouts or places of death of more than 8 thousand Ukrainians are known. Since the beginning of the Russian-Ukrainian war, search groups have been set up and the bodies of more than 1,500 fallen soldiers have been returned.

It is not always possible to identify deceased persons returned from the aggressor country and extracted from the combat zone. It is also worth recalling the constant enemy attacks on the civilian objects with civilian casualties and the problems with identifying the remains. The identification of unknown victims is one of the most difficult tasks of forensic medicine. Most of the human remains found are heavily decomposed or completely skeletonized, making direct identification difficult. In many cases, human remains are dismembered (e.g., victims of mass accidents or terrorist attacks) or decomposed in a way that prevents anthropological analysis. Soft tissue decomposition is also connected with the degradation of genetic material inside cells, which is broken down into smaller fragments by released enzymes.

AIM

To present modern conceptual, methodological, technical, and regulatory advancements in the identification of persons missing or killed in hostilities and propose strategies for improvement.

MATERIALS AND METHODS

Forensic identification employs various standard methods: forensic odontology [7], fingerprint analysis, and implant serial number identification [8]. DNA profiling, introduced in 1984 and first successfully applied to human remains in 1991 [7], remains a gold standard in forensic genetics.

DNA profiling involves comparing genetic material from unknown samples with reference databases or relatives' samples. Various factors affect identification accuracy, including excavation conditions, sample collection, transportation, storage, and degradation due to environmental exposure [9]. Technological advancements have automated DNA extraction and comparison processes, facilitating international cooperation [10].

Interpol's guidelines recognize DNA analysis as one of the most reliable forensic methods [11]. The UN has acknowledged its humanitarian significance in Resolutions A/HRC/RES/10/26 and A/HRC/RES/15/5. To ensure accuracy, forensic DNA analysis requires high-quality,

uncontaminated samples from both victims and their relatives.

ETHICS

All sources used in this literature review are publicly available.

REVIEW AND DISCUSSION

For the purposes of identifying disaster victims, the comparative DNA analysis must fulfill two basic pre-conditions: a high-quality, non-contaminated DNA tissue sample from the victim's body or a separate body part (postmortem sample) is required, as well as a non-contaminated DNA reference sample from the presumed victim or his/her genetic relative(s) (ante mortem sample).

In the future, the issues of storage of postmortem samples, accreditation of the laboratory that performed the test may be necessary to determine whether the evidence can be admissible in court.

The next step in identifying a person is to compare the results with the data from the genomic information database. For the purpose of identification, databases containing physical anthropological characteristics and DNA profiles of victims have been created in different countries. The missing persons databases include a reference database containing mitochondrial DNA profiles of maternal relatives of missing persons, along with a separate database containing mitochondrial DNA profiles of unidentified human remains [7, 11]. These databases are also linked to relevant organizations (Polish Genetic Database of Victims of Totalitarianism, Institute of Genetic Engineering and Biotechnology in Sarajevo, Canadian Forces Victim Identification Program, Finnish Association for War Memorials, US Army Central Identification Laboratory in Hawaii, National Missing and Victims System of Australia, etc.) [11].

The development of DNA databases has contributed to the identification of missing persons and the development of investigative leads to assist law enforcement. Forensic DNA databases consist of DNA profiles of convicted individuals (and in some jurisdictions, arrested individuals), forensic DNA evidence, human remains of unidentified missing persons, and direct family DNA samples of missing persons. Long-term storage of tissue samples (including DNA) through biobanks of umbilical cord blood, sperm, eggs, etc. can be useful for future assessment of the deceased person and family members of the deceased.

In Ukraine, the Unified Register of Missing Persons was launched in May 2023 [12]. It contains all the data about

the missing military personnel. Since the launch of the register, more than 5,000 requests for extracts have been registered. The registry is a secure database with a clearly defined circle of officials who have access: representatives of the Office of the Commissioner for Missing Persons, the National Police and the Department of Informatization of the Ministry of Internal Affairs of Ukraine. Upon application to the National Police by relatives of missing persons, the register cards are formed, and the Office of the Commissioner for Missing Persons checks each person. The Armed Forces of Ukraine themselves must launch a search and submit information to the registry if they have reason to believe that a military person is missing or captured. For each case of disappearance, the police initiate a case, which is registered in the Unified Register of Pre-trial Investigations. If it is established that a person is in captivity or there is evidence of his or her death, the National Police must transfer the case to the Security Service of Ukraine to continue the pre-trial investigation.

The National Police and the Office of the Commissioner for Persons Missing in Special Circumstances are directly involved in the search for missing defenders. These two bodies use a joint electronic register of missing persons, where all identified information is collected. In addition, they receive data from the Ministry of Defense, which has its own groups to search for the dead, and from services involved in the return of the prisoners of war.

The International Committee of the Red Cross maintains its own register and can sometimes visit people in captivity (it must inform relatives of such visits).

On July 09, 2022, the Verkhovna Rada of Ukraine adopted the Law "On State Registration of Human Genomic Information" [12-15]. The law regulates the procedures for creating, recording and operating a register of human genomic information, improves the efficiency of law enforcement agencies by investigating crimes and identifying the perpetrators, systematizes and improves the search for missing persons and the identification of unidentified persons. The law provides for two options for registering human genomic information - mandatory state and voluntary. It is determined that persons who are prosecuted (or convicted, sentenced and serving a sentence of imprisonment or have an unexpunged or outstanding criminal record) for committing grave or especially grave crimes, as well as all categories of crimes against sexual freedom and sexual inviolability of a person, are subject to mandatory registration, and unidentified corpses and missing persons are also included in the register. Close relatives of missing persons are also subject to mandatory registration.

Taking into account the experience and difficulties in identifying those killed since the beginning of the

Russian-Ukrainian war, a mechanism was proposed for obtaining biological samples from persons mobilized, conscripted or voluntarily enrolled in military service, and military personnel, in order to ensure their identification if necessary. Other citizens of Ukraine are entered into the register of genomic data on a voluntary and paid basis.

Since the beginning of the Russian-Ukrainian war, there has been an urgent need for molecular genetic research related to the war crimes of the occupiers. It is known that more than 6,000 DNA examinations have already been ordered for the purpose of identifying individuals, and almost half of them have been completed. Obviously, there is a need to create a certain tool to systematize the information received and give it legal status for use as evidence in the processes of proving and presenting crimes against Ukraine in court. The generalized international experience (in the USA, Great Britain, Poland, Germany, Italy, Spain, France) convinces of the need to create a single DNA database and to determine the single holder of the DNA database information, where genomic anonymized information of persons specified in the law is collected [9]. For this reason, the law provides for the creation of a single state database with very limited access and the right of inspection in accordance with the established procedure for certain representatives of law enforcement and intelligence agencies.

Currently, the genomic database contains more than 45,000 DNA profiles, most of which are material obtained at crime scenes and less than a quarter of which are materials of the suspects. This Law opens up new opportunities for forensic physicians and forensic experts to identify individuals, which will not only make the work of law enforcement agencies much more efficient, but also allow them to coordinate efforts in the fight against crime.

In the context of full-scale aggression, the identification of persons who have gone missing or died is of high relevance, but the Law will be needed further, in peacetime. First of all, the adoption of this Law is necessary to regulate the provisions of a number of international documents. For example, Article 37 of the Law of Ukraine "On Ratification of the Council of Europe Convention on the Protection of Children against Sexual Exploitation and Sexual Abuse" requires the collection and storage of genomic information of persons convicted of offenses in order to prevent the commission of a crime and the possibility of criminal prosecution [13]. Article 6 of the Convention for the Protection of Individuals with regard to Automatic Processing of Personal Data requires that automated data processing be prohibited if the country's legislation is unable to provide appropriate security.

Since the adoption of the Law, domestic legislation has already met the requirements.

Secondly, the accumulated international experience convinces of the effectiveness of the procedure for collecting, accumulating and storing DNA profiles for the purpose of identifying criminals and effective investigation of crimes.

The Law also simplifies and speeds up the identification of missing military personnel. In 2014, the Armed Forces of Ukraine did not have a range of resources (specialized units, search specialists) to identify missing military personnel, so they relied on the assistance of the International Committee of the Red Cross Delegation in Ukraine. In 2016, the procurement of the necessary equipment for comprehensive genetic examinations began. Since the identification of a missing person requires the examination of samples of close relatives or personal belongings to conduct a kinship search, the question arose of obtaining permission from these relatives to process their personal data, as this is a prerequisite for placing genomic data in the DNA database. In the absence of close relatives, identification of the deceased becomes virtually impossible. In the world practice of identification of military personnel, the procedure of taking samples in advance, with their placement in repositories, is widely used, and after completion of military service and no need to use them, the samples are destroyed without being examined [7].

The new law provides that during martial law, biological material is subject to mandatory sampling from police officers, military personnel, and members of the territorial defense, but the sampling of material in peacetime from these persons should be carried out only with the person's consent.

The adopted Law will contribute to both crime prevention and to increasing the efficiency and speeding up the investigation of crimes (especially if a person has committed a crime repeatedly), as well as to minimizing crime in general and to the search for missing persons and identification of unidentified persons. According to the Law, persons who have been notified of suspicion of committing intentional grave or especially grave crimes against the foundations of national security of Ukraine, against peace, security of humanity and international law and order, health, life, dignity, honor, freedom, public safety sexual freedom and sexual inviolability of a person, property, in the field of trafficking in narcotic drugs, psychotropic substances, their analogues or precursors, a person must be registered in the Electronic Register of Human Genomic Information (as of the beginning of 2023, there are more than 50 thousand registered).

Despite the high relevance of the adopted law, we should focus on some of its drawbacks and risks that were not taken into account. For example, the opinions of legal scholars and human rights defenders about the

risks of violation of the rights and privacy of individuals were not taken into account. The final version of the Law stipulates that the Electronic Register is state property, and the Ministry of Internal Affairs of Ukraine is the holder of this property. The role of the administrator is performed by a legal entity appointed by the Ministry of Internal Affairs and managed by it or its subordinates. Therefore, the registry will be operated and regulated by the Ministry of Internal Affairs. However, there is no guarantee of transparent and independent work of the registry databases, which not only creates potential corruption risks, but also creates preconditions for misuse of information (transfer to other persons, other bodies).

According to the Law, the procedure for access to the Electronic Register and use of its information is determined by the holder of the Electronic Register (the Ministry of Internal Affairs). The Ukrainian Parliament Commissioner for Human Rights is supposed to monitor the observance of human rights during the state registration of the registry information. It was expected that this should be a certain preventive measure, as well as the possibility of appealing certain actions or decisions on state registration of genomic information in court. However, the Commissioner has no direct influence on the holder of the Electronic Registry. It is considered reasonable to entrust such supervision to a separate established body for periodic or permanent inspections and consultations.

The law does not detail/clarify a number of issues: minimizing the risks of access to personal data of relatives of a missing person after the death of that person is established by destroying genomic information; ensuring and finalizing procedures for monitoring compliance with the law in the operation of the register; developing a procedure for access to the register by other authorities to prevent misuse of this data.

In modern legal proceedings, DNA testing is considered the new "queen of evidence" or "gold standard". However, courts and judges do not consider the results of DNA genetic testing to be conclusive and compulsory. In their opinion and according to the case law, DNA analysis is one of the types of evidence, and according to Part 2 of Article 94 of the Criminal Procedure Code of Ukraine, none of the evidence has a pre-established force [16]. Therefore, there is a proposal to supplement Part 2 of Article 84 of the Criminal Procedure Code of Ukraine with this source of evidence - "information from DNA databases". This would be the basis for the development of the necessary acts at the departmental level for the use of genomic data for identification of persons, and one of the directions of implementation of such a project is the proposal to create a centralized (common) database of genomic data in Ukraine with the formation of a single coordination center [17].

CONCLUSIONS

Identification of unknown victims is one of the most difficult tasks in forensic medicine. DNA analysis is one of the “golden standards” by which an identity can be confirmed by comparing the profile of an unknown person with both a DNA profile taken during life (from genomic information databases) and biological relatives. The development of DNA databases has contributed to the identification of missing persons and the development of investigative leads to assist law enforcement agencies.

In May 2023, the Unified Register of Missing Persons was launched in Ukraine, which includes all data on missing military personnel. Previously, the Law “On State Registration of Human Genomic Information” was adopted, which regulates the procedures for creating, recording and maintaining a register of human genomic information, improves the efficiency of law enforcement agencies and provides for two options for registering human genomic information - mandatory state and voluntary. The Law also significantly simplifies the process of identifying missing military personnel.

The Law does not ensure transparent and independent work of the registry databases, which creates potential corruption risks and creates the possibility of misuse of information, in particular, its transfer to other persons or

bodies for other purposes. It is proposed to entrust control over the observance of human and civil rights when entering data into the registry to a separately created regulatory structure (body) for periodic or permanent inspections and consultations. The Law does not detail/clarify a number of issues: minimizing the risks of access to personal data of relatives of a missing person after the death of this person is established by destroying genomic information; it is necessary to ensure and finalize procedures for monitoring compliance with the law in the work of the registry; develop a procedure for access to the registry by other authorities to prevent misuse of this data.

It is proposed to supplement part 2 of Article 84 of the Criminal Procedure Code of Ukraine with a new source of evidence - “information from DNA databases».

FURTHER PROSPECTS

Use of existing and newly created databases, methodologies and development of a regulatory framework for the identification of children abducted by the Russian Federation for the purpose of confirming their identity and restoring their citizenship and rights, as well as in cases of illegal separation and abduction of children from their parents.

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The impact of delivery mode on neonatal microbiota development and long-term health outcomes: A literature review

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ABSTRACT

Early microbial colonization plays a foundational role in shaping the infant's immune, metabolic, and neurological development. The mode of delivery significantly influences this process. Vaginal birth enables physiological exposure to maternal microbiota, while elective cesarean section disrupts this transfer and results in delayed colonization and reduced microbial diversity. This review examines how different delivery modes affect neonatal gut microbiota composition and evaluates their long-term implications for health, with a focus on immune regulation, metabolic programming, allergic conditions, and neurodevelopmental outcomes. Relevant literature from 2010 to 2024 was reviewed using PubMed, Scopus, and Web of Science databases. Human studies comparing elective cesarean section and vaginal delivery were included if they analyzed microbiota profiles and at least one long-term clinical parameter. Out of 245 initially screened articles, 36 were selected based on methodological quality and relevance. Evidence indicates that cesarean section is associated with altered microbial succession patterns that may increase the risk of autoimmune diseases, allergies, obesity, and cognitive or behavioral disturbances. Proposed mechanisms include impaired development of regulatory T cells, reduced production of short-chain fatty acids, and dysregulated immune responses. Although a few studies suggest transient protective effects in specific clinical scenarios, these findings are inconsistent and population-dependent. Supportive interventions such as exclusive breastfeeding and microbiota-based therapies may help restore microbial balance and mitigate adverse outcomes. Future research should focus on identifying biomarkers of dysbiosis and developing personalized strategies to promote microbiota-driven health from birth.

KEY WORDS: cesarean section, intestinal microbiota, microbial transmission, immune tolerance, chronic disease

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INTRODUCTION

The human microbiota - understood as a complex ecosystem of microorganisms inhabiting body surfaces, including the gastrointestinal tract, skin, and mucous membranes - plays a fundamental role in maintaining homeostasis. It contributes to digestion, vitamin synthesis, protection against pathogens, and immune regulation. Early microbial colonization is essential for the proper maturation of the immune and metabolic systems, with long-term consequences for health throughout childhood and adulthood.

One of the key factors influencing the composition and developmental trajectory of the neonatal microbiota is the mode of delivery. Vaginal birth allows for exposure to the maternal vaginal, perineal, and anal microbiome, initiating gut colonization by symbiotic bacteria. In contrast, infants delivered by elective ce-

sarean section do not pass through the birth canal and are instead first exposed to skin-associated flora from medical personnel and the hospital environment. This results in reduced microbial diversity and delayed microbiota maturation, which have been correlated with various adverse health outcomes and developmental alterations across multiple organ systems [1, 2].

AIM

This literature review aims to assess how the mode of delivery affects the development and composition of the neonatal gut microbiota and to identify potential long-term health consequences arising from disruptions in its colonization. The focus is placed on immune, metabolic, and neurological outcomes linked to early-life microbial patterns.

MATERIALS AND METHODS

This narrative literature review was conducted using structured searches in three major databases: PubMed, Scopus, and Web of Science. The search covered publications from 2000 to 2025 and employed standardized MeSH terms and English-language keywords, including: *cesarean section*, *vaginal delivery*, *mode of delivery*, *gut microbiota*, *infant microbiome*, *immune development*, and *long-term health outcomes*. Logical operators (AND, OR) were applied to refine the search strategy.

Inclusion criteria comprised original human studies (cohort, prospective, or randomized) and systematic reviews comparing elective cesarean section (ECS) - performed before the onset of labor - with physiological vaginal birth without surgical or pharmacological intervention. Eligible studies were required to assess neonatal gut microbiota composition and include at least one indicator of long-term health outcomes, such as autoimmune, allergic, metabolic, or neurodevelopmental conditions.

Exclusion criteria included:

- Preterm births (<37 weeks of gestation),
- Urgent or emergency cesarean sections,
- Neonates with congenital anomalies or those admitted to neonatal intensive care units.

An initial pool of 245 publications was screened. After title and abstract screening, 48 articles met preliminary criteria. Full-text assessment and evaluation of methodological rigor led to the final inclusion of 36 studies in the synthesis. While this review does not follow PRISMA guidelines in full, elements of the GRADE framework were used to qualitatively rank the strength of evidence.

OPERATIONAL DEFINITIONS

For the purposes of this review, the following definitions of key terms have been adopted:

Vaginal birth (physiological delivery) – a spontaneous, non-instrumental birth initiated by natural uterine contractions and proceeding without surgical or pharmacological interventions (e.g., without oxytocin stimulation). This type of delivery enables the newborn to come into contact with the maternal vaginal, perineal, and anal microbiota, providing the first crucial stimulus for intestinal colonization.

Elective cesarean section (ECS) – a surgical delivery performed prior to the onset of spontaneous labor, based on predefined medical or non-medical indications. In this case, the newborn's initial microbial exposure is restricted mainly to maternal skin flora, medical personnel, and the hospital environment (including sterile surfaces and conditioned air), leading to a distinct and often delayed pattern of microbial

colonization. Importantly, recent studies have shown that the length of postpartum hospital stay may further modulate this process, particularly in cesarean-born infants who experience prolonged exposure to sterile clinical settings and reduced physical contact with maternal microbiota [3, 4].

Microbiota – the entire community of microorganisms (including bacteria, archaea, fungi, and viruses) that inhabit the surfaces and internal spaces of the human body, forming a complex ecosystem closely associated with immunological, metabolic, and neurological processes. In this review, particular emphasis is placed on the gut microbiota, which during the neonatal period shapes mucosal immunity and influences the maturation of T and B lymphocytes.

EMERGING DATA: PLACENTAL AND RESPIRATORY TRACT MICROBIOTA

In recent years, the concept of the “sterile womb paradigm” has been challenged [5] detected bacterial DNA in placental tissue using 16S rRNA sequencing, suggesting the possibility of microbial “programming” in utero. However, the interpretation of these findings remains controversial due to methodological concerns - there is a risk of sample contamination, and the presence of DNA does not necessarily imply the existence of viable microorganisms [6].

Additionally, the mode of delivery also influences the composition of the respiratory and oral microbiota, which may affect infection risk and predisposition to atopic diseases in early life [2]. Cohort studies have shown that infants born via cesarean section are more frequently colonized by opportunistic bacteria in the upper respiratory tract, such as *Staphylococcus aureus* and *Klebsiella pneumoniae*, whereas beneficial strains such as *Corynebacterium* and *Dolosigranulum* are less commonly observed.

REVIEW AND DISCUSSION

NEONATAL MICROBIOTA: BIOLOGICAL FOUNDATIONS OF COLONIZATION

Colonization of the gastrointestinal tract and other body surfaces in the newborn begins immediately after birth and represents a crucial stage in the establishment of a homeostatic microbiome. During the first days of life, the microbiota undergoes dynamic shifts, with relative stabilization of its composition occurring within several months and full microbial maturity typically reached between two and three years of age. This process is shaped by numerous environmental

and perinatal factors, including exposure to maternal microorganisms, mode of delivery, feeding method, use of perinatal antibiotics, and contact with home or hospital environments [1, 2, 7].

In vaginal births, gastrointestinal colonization begins during passage through the birth canal, primarily involving maternal vaginal and intestinal microbiota. The dominant bacterial genera include:

Lactobacillus spp. – responsible for maintaining acidic pH and limiting pathogen proliferation,

Bifidobacterium spp. – producers of short-chain fatty acids (SCFAs), which strengthen the intestinal barrier and promote Treg lymphocyte development,

Bacteroides spp. – involved in modulating immune responses through interactions with TLR receptors and dendritic cells,

Prevotella spp. and non-pathogenic *Escherichia coli* strains – participating in the development of aerobic and anaerobic flora.

Additionally, skin-to-skin contact with the mother immediately after birth and breastfeeding further enhance symbiotic colonization by providing access to human milk oligosaccharides (HMOs), which selectively promote the growth of *Bifidobacterium longum* and *B. breve* [8].

In contrast, infants delivered via elective cesarean section acquire their initial microbiota primarily through contact with maternal skin, medical personnel, and the operating room environment. Their microbiota is typically dominated by:

Skin-associated bacteria: *Staphylococcus epidermidis*, *Corynebacterium spp.*, *Propionibacterium spp.*,

Environmental bacteria: *Enterobacter spp.*, *Klebsiella spp.*, *Clostridium difficile* – often associated with delayed gut microbiota maturation and an increased risk of opportunistic infections.

Studies employing 16S rRNA sequencing have demonstrated that differences in microbial composition may persist for 6-12 months or longer, particularly among formula-fed infants who are not breastfed [3]. Early colonization disturbances include reduced taxonomic diversity, delayed establishment of anaerobic communities, and decreased metabolic activity within the microbiome. Such early-life disruptions in microbial assembly are believed to influence the trajectory of immune, metabolic, and neurodevelopmental maturation [2]. For microbiota composition details, see Table 1 (Section 4.1).

LONG-TERM HEALTH CONSEQUENCES AND MICROBIOTA-DEPENDENT IMMUNOLOGICAL MECHANISMS

In the early months, something remarkable happens - the immune system learns to tell friend from foe. Gut microbes

are key to this education process. In this pivotal period, mechanisms of immune response, the balance between effector and regulatory T cells, and tolerance toward environmental and dietary antigens are established. Disruptions in this process - particularly those caused by altered colonization patterns following elective cesarean delivery - may increase the risk of immunological, metabolic, and allergic diseases, even years after birth [9-11].

IMMUNOLOGICAL MECHANISMS DEPENDENT ON THE MICROBIOTA

One of the key functions of the early microbiota is to support the development of immune tolerance, defined as the immune system's ability to distinguish harmless antigens (e.g., dietary components, commensal microbiota) from threats (e.g., pathogens). A properly colonized neonatal gut provides numerous molecular signals that stimulate the maturation of dendritic cells, regulatory T cells (Treg), anti-inflammatory cytokine production, and intestinal barrier function.

Key microbiota-driven mechanisms supporting immune tolerance include:

Polysaccharide A (PSA) produced by *Bacteroides fragilis* binds to the TLR2 receptor on dendritic cells, leading to the induction of IL-10 and differentiation of naive CD4+ T cells into Tregs. These Tregs suppress the activity of Th1, Th2, and Th17 cells, thereby preventing inflammatory responses.

Butyrate, one of the main short-chain fatty acids (SCFAs), produced by *Faecalibacterium prausnitzii*, *Roseburia spp.*, and *Anaerostipes spp.*, enhances tight junctions between enterocytes and promotes the expression of the Foxp3 gene essential for Treg development. Butyrate also modulates the NF- κ B pathway, reducing transcription of pro-inflammatory genes (e.g., IL-6, TNF- α).

Lactobacillus spp. stimulate the production of IgA in mucosal tissues and activate ILC3 cells to secrete IL-22, which supports epithelial integrity and resistance to infection.

Bifidobacterium spp. compete with pathogens for adhesion sites, produce lactic acid and acetate (lowering pH), and stimulate Paneth cells to secrete defensins - natural antimicrobial peptides.

The absence of these strains - common in infants delivered by cesarean section - results in:

↓ Treg numbers → ↑ Th17 dominance → ↑ IL-17 and IL-6 → chronic inflammation,

↓ IL-10 and TGF- β production → weakened suppressive mechanisms,

↑ exposure of enterocytes to PAMPs (e.g., LPS) → excessive activation of TLR4 → ↑ TNF- α and intestinal barrier damage [12].

Figure 1 shows the role of the microbiota in the development of immune tolerance.

Table 1. Comparison of dominant gut bacterial genera in newborns by mode of delivery

Bacterial genus	Vaginal delivery	Elective cesarean section
<i>Bacteroides fragilis</i>	Present, high abundance	Absent or low abundance
<i>Bifidobacterium spp.</i>	Present, dominant	Present, but significantly reduced
<i>Lactobacillus spp.</i>	Present	Rare or absent
<i>Clostridium difficile</i>	Rare	Present with high frequency
<i>Staphylococcus spp.</i>	Present in trace amounts	Dominant in the first days

Source: Adapted from [1,3,7]

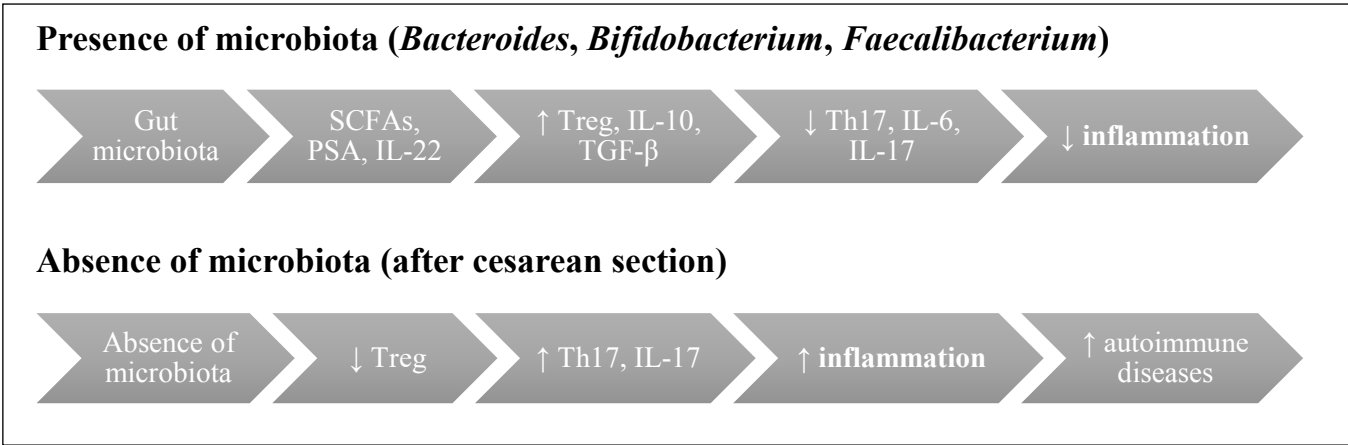


Fig. 1. The role of the microbiota in the development of immune tolerance
Source: Author's compilation based on: [9-13].

ALLERGIC AND ATOPIC DISEASES

Population studies have demonstrated that children born via cesarean section are at a higher risk of developing atopic conditions such as bronchial asthma, atopic dermatitis (AD), and food allergies. A meta-analysis by Rutayisire [14] estimated that this risk increases by 30% to 50% compared to vaginally delivered children.

A key role is played by the deficiency of *Bifidobacterium infantis* and *Bacteroides spp.*, resulting in Th2-dominant responses - overproduction of IL-4, IL-5, and IL-13, along with elevated serum IgE levels. Dysbiosis also impairs SCFA production and impairs gut barrier function, facilitating allergen translocation and initiation of inflammatory responses [11].

METABOLIC DISEASES

Data from the CHILD Cohort Study (2024) [15] indicated that children delivered via cesarean section exhibited higher BMI as early as age five. This correlated with an increased abundance of fermentative *Firmicutes* bacteria and reduced numbers of *Bacteroidetes*, leading to more efficient energy extraction from food and increased fat storage - the so-called "obesogenic microbiome."

Furthermore, reduced SCFA production weakens activation of GPR41/43 receptors in adipose tissue and the intestines, affecting glucose metabolism, appetite regulation, and low-grade inflammation. These

disturbances may predispose individuals to childhood obesity, insulin resistance, and type 2 diabetes [16].

AUTOIMMUNE AND INFLAMMATORY BOWEL DISEASES

Disruptions in early microbial colonization may initiate processes leading to autoimmune and inflammatory disorders. Children born by cesarean section have limited exposure to *Bacteroides fragilis* and *Faecalibacterium prausnitzii* - bacteria crucial for Treg induction and the IL-10/TGF- β cytokine balance.

- Lack of these microbial signals may contribute to:
 - Type 1 diabetes – due to Th1-skewed responses and destruction of pancreatic β -cells,
 - Inflammatory bowel diseases (e.g., Crohn's disease, ulcerative colitis) – *via* compromised gut barrier, LPS translocation, and NF- κ B activation,
 - Other autoimmune disorders (e.g., celiac disease, psoriasis) – where microbiota influence antigen presentation and activation of CD8+ effector T cells [10].

LONG-TERM HEALTH CONSEQUENCES OF MICROBIOTA DISTURBANCES DEPENDENT ON MODE OF DELIVERY

The influence of gut microbiota on host health extends far beyond the digestive system, playing a key

role in the development of the immune, metabolic, and nervous systems, as well as in the regulation of inflammatory responses and the functioning of the body's protective barriers. The first months of life are particularly critical, as this is the period during which the intestinal colonization profile is established. Disruptions in this process - typical for infants born via elective cesarean section - can initiate a cascade of abnormal microbial and immunological signals, resulting in lasting phenotypic changes and an increased risk of many chronic diseases.

AUTOIMMUNE DISEASES

Disrupted immune education resulting from neonatal dysbiosis can lead to the loss of tolerance to self-antigens and, consequently, to the development of autoimmune diseases.

Type 1 Diabetes – In genetically predisposed children (HLA-DQ2/DQ8), the absence of *Bifidobacterium longum* and *Bacteroides fragilis* correlates with reduced induction of Treg lymphocytes and excessive activation of the Th1 response. Reduced production of PSA and SCFAs limits the secretion of IL-10 and TGF- β , facilitating the destruction of pancreatic β cells by cytotoxic CD8+ lymphocytes.

Crohn's Disease and Ulcerative Colitis (IBD) – A decrease in the presence of *Faecalibacterium prausnitzii*, a key butyrate producer, leads to increased intestinal epithelial permeability, LPS translocation, and NF- κ B activation. The outcome is chronic inflammation, monocyte recruitment, and Th17 response induction.

Multiple Sclerosis (MS) – Meta-analyses have shown that alterations in the microbiota (reduced *Akkermansia muciniphila*, *Prevotella*, and increased *Methanobrevibacter*) occur already in the prodromal phase of MS. Dysbiosis affects microglial cells and the blood-brain barrier through SCFA and IL-6 signaling [17].

Celiac Disease – An increased number of *Escherichia coli* and decreased *Bifidobacterium breve* promote pro-inflammatory presentation of gluten peptides. IL-15 and IFN- γ support the activation of cytotoxic CD8+ lymphocytes and enterocyte apoptosis.

ALLERGIC AND ATOPIC DISEASES

Neonatal microbiota shapes the immune response profile, influencing the Th1/Th2 balance and the activation of mucosal tolerance mechanisms. Dysbiosis may predispose to atopic phenotypes.

Bronchial Asthma – Reduced abundance of *Bacteroides* and *Bifidobacterium* in C-section infants increases Th2 pathway activation, resulting in overproduction of IL-4, IL-5, IL-13, and elevated serum IgE. Diminished stimulation of TLR2/TLR4 further limits Treg activation.

Atopic Dermatitis (AD) – Reduced presence of *Lactobacillus rhamnosus* and *Bifidobacterium longum* weakens the skin barrier and lowers IL-10 and SCFA production, promoting colonization by *Staphylococcus aureus* and allergen penetration.

Food Allergies – The absence of colonization by bacteria that stimulate oral tolerance results in limited TGF- β and IL-10 production and a dominance of IgE-dependent mechanisms [11].

METABOLIC DISEASES

Gut microbiota plays a crucial role in regulating lipid and carbohydrate metabolism as well as hormonal signaling within the gut-brain axis. Alterations in early microbial composition following cesarean section have been linked to disturbances in metabolic programming and energy balance.

Childhood Obesity – An altered *Firmicutes/Bacteroidetes* ratio in C-section infants is associated with enhanced polysaccharide fermentation, excess acetate, and activation of GPR41/43 receptors. This promotes lipogenesis, increased energy harvest from the diet, and accelerated weight gain [15].

Type 2 Diabetes and Insulin Resistance – Bacterial LPS activates TLR4 on adipose tissue macrophages and pancreatic β cells. The resulting pro-inflammatory cytokines (IL-1 β , TNF- α) impair insulin signaling and contribute to glucotoxicity.

Metabolic Syndrome – Reduced production of short-chain fatty acids (SCFAs), particularly butyrate and acetate, weakens GLP-1 and PYY signaling, leading to hyperphagia, insulin resistance, and low-grade chronic inflammation [18, 19].

Notably, longitudinal studies confirm that cesarean-delivered infants exhibit delayed SCFA maturation and distinct microbial succession patterns throughout the first year of life, suggesting a mechanistic link between early dysbiosis and later metabolic alterations [1].

NEUROLOGICAL AND PSYCHIATRIC DISORDERS

The gut-brain axis represents a bidirectional neuro-immune communication pathway through which the intestinal microbiota influences neurogenesis, neurotransmitter homeostasis, and the development of social and emotional behaviors [20,21]. Experimental and clinical studies increasingly support the hypothesis that early-life dysbiosis, particularly after cesarean delivery, can disrupt neural signaling and alter stress response systems, predisposing to neurodevelopmental and behavioral disorders [21].

Autism Spectrum Disorder (ASD) – Deficiencies in bacteria responsible for producing γ -aminobutyric acid (GABA), such as *Lactobacillus reuteri*, and for regulating serotonin synthesis via tryptophan metabolism (*Bifidobacterium* spp.), together with elevated concentrations of propionic acid produced by *Clostridium* spp., may impair CNS maturation and synaptic plasticity [21,22].

Attention Deficit Hyperactivity Disorder (ADHD) – The microbiota modulates dopamine and norepinephrine availability in the prefrontal cortex and nucleus accumbens, thereby influencing impulsivity and attention. Dysbiosis is associated with synaptic instability and reduced neuroplasticity.

Depression and Anxiety Disorders – An overrepresentation of proinflammatory bacteria such as *Desulfovibrio* and *Enterobacteriaceae* correlates with elevated IL-6 and TNF- α levels and decreased BDNF concentration, which limits hippocampal neurogenesis and resilience to stress [20].

OTHER CONDITIONS

Irritable Bowel Syndrome (IBS) – Dysbiosis with a dominance of *Proteobacteria* and reduced *Lactobacillus* results in loosened tight junctions, increased intestinal permeability, and mast cell activation, which are responsible for abdominal pain and visceral hypersensitivity.

Skin Diseases (Acne, Psoriasis) – Microbiota disturbances modulate the skin cytokine profile (IL-17, IL-23), affecting keratinocyte proliferation and angiogenesis.

Increased Susceptibility to Infections – A deficiency in bacteria that stimulate IgA secretion (e.g., *Bifidobacterium breve*) and IL-22 impairs mucosal defense in the respiratory and gastrointestinal tracts, increasing susceptibility to viral and bacterial infections.

CRITICAL CONSIDERATIONS ON THE LITERATURE BASE

Despite a growing body of literature linking mode of delivery to microbiota development and long-term health, many existing studies are subject to significant methodological limitations. Observational and cohort designs often preclude causal inference and may be affected by uncontrolled confounding factors such as perinatal antibiotic use, environmental exposures, breastfeeding practices, or socioeconomic status. Additionally, regional variations in microbiota composition and differing definitions of dysbiosis complicate the generalizability of findings across populations. Future research should strive for standardized microbiota profiling methods, longitudinal follow-up, and more rigorous control of potential confounders.

POTENTIAL BENEFICIAL ASPECTS OF COLONIZATION FOLLOWING ELECTIVE CESAREAN SECTION

While the majority of literature emphasizes the adverse effects of altered gut colonization in infants born via elective cesarean section, some studies report potential short-term or context-specific protective phenomena. These observations remain preliminary, often population-dependent, and require further confirmation through large-scale prospective studies.

POTENTIAL PROTECTION AGAINST SELECTED INFECTIONS

The microbiota of cesarean-born neonates is frequently enriched with aerobic skin- and environment-associated bacteria, including *Staphylococcus epidermidis*, *Corynebacterium* spp., and *Micrococcus* spp. These organisms are capable of producing antimicrobial compounds - such as lysozyme, hydrogen peroxide, and bacteriocins - which may transiently inhibit the growth of Gram-negative pathogens like *Klebsiella pneumoniae* and *Enterobacter* spp. [22]. Although such effects are limited in scope and do not constitute a functionally mature microbiota, they may provide early, nonspecific microbial defense under certain conditions.

Additionally, some data suggest reduced colonization by enteropathogens such as *Campylobacter jejuni* and *Salmonella* spp. in cesarean-born infants, which could be beneficial in regions with limited sanitation and high rates of gastrointestinal infections [23].

Shao et al. [3], in their 2020 cohort study involving over 100 neonates from eastern China, reported *C. difficile* colonization in 72% of infants delivered by cesarean section - significantly higher than the 41% observed in vaginally born peers. This was particularly evident among formula-fed infants who did not attend daycare. While this finding may appear concerning, it likely reflects delayed microbial succession rather than true pathogenic colonization.

HYPOTHETICAL REDUCTION IN IMMUNE HYPERACTIVATION

In rare clinical scenarios, such as prematurity or congenital immunodeficiency, the delayed colonization associated with cesarean birth has been speculated to offer protection by limiting early immune overstimulation. Reduced exposure to microbial ligands may attenuate neonatal Th1/Th17 activation and decrease proinflammatory cytokine responses. Some studies report lower TLR4 and TLR9 expression levels in cesarean-born infants, which could reduce PAMP-induced inflammation

[19,24]. These hypotheses remain largely unconfirmed and warrant further mechanistic exploration.

INCONCLUSIVE ASSOCIATIONS WITH CELIAC DISEASE RISK

A cohort study by Sevelsted et al. [25] indicated a lower incidence of celiac disease among cesarean-born children carrying HLA-DQ2/8 alleles. The authors proposed that reduced colonization by *Bacteroides* spp. and lower early LPS exposure might modulate TLR4 activity, reducing autoimmune activation [10]. However, other studies have found contrary trends - linking cesarean birth to a higher risk of celiac disease - likely due to dysbiosis and impaired tolerance development [10,25]. Current evidence remains contradictory and insufficient to draw firm conclusions. More longitudinal studies are needed before we can confirm whether these trends are biologically meaningful or simply statistical noise.

CRITICAL PERSPECTIVE AND LIMITATIONS

Despite the above observations, most findings originate from observational studies with limited sample sizes, population heterogeneity, and incomplete control of confounding variables such as antibiotic use, breastfeeding, or hospital length of stay. Moreover, several seemingly beneficial effects — such as reduced pathogen carriage or dampened inflammation — may in fact reflect microbiota immaturity rather than a physiologically adaptive state.

It is therefore crucial to interpret these findings within context. The current evidence for protective aspects of cesarean-associated colonization is limited in duration, highly variable across populations, and does not offset the well-documented immunological, metabolic, and neurodevelopmental risks associated with disrupted early-life microbial exposure due to bypassing the birth canal. These interpretations align with the *hygiene hypothesis*, which proposes that reduced early microbial contact contributes to the rising prevalence of allergic and immune-mediated diseases in modern societies [13].

FACTORS MODIFYING MICROBIOTA COLONIZATION

Although this review focuses on the influence of the mode of delivery on microbiota development, it is essential to acknowledge factors that may modify the trajectory of microbial colonization in newborns - either by supporting or disrupting this process. Particularly significant are breastfeeding, antibiotic therapy, envi-

ronmental exposure, and interpersonal contact during the first weeks of life.

BREASTFEEDING

One of the most well-documented factors that can compensate for microbiota colonization disturbances in infants born via cesarean section is breastfeeding. Human milk contains over 200 types of human milk oligosaccharides (HMOs), which are indigestible by the infant but selectively stimulate the growth of symbiotic strains of the genus *Bifidobacterium* - especially *B. longum* subsp. *infantis*.

Research by Bogaert et al. (2023) [8] demonstrated that cesarean-delivered newborns who were exclusively breastfed regained a microbiota composition similar to that of vaginally delivered infants within the first 6 weeks of life. Furthermore, HMOs promote the production of short-chain fatty acids (SCFAs), support the maturation of the intestinal barrier, and inhibit the growth of pathogenic bacteria.

ANTIBIOTIC THERAPY

Perinatal antibiotic use - both in mothers during the peripartum period and in newborns - significantly impacts microbiota composition, particularly by reducing anaerobic bacteria. In combination with cesarean delivery, this often leads to persistent dysbiosis lasting several months. Even a single prophylactic dose of antibiotics can inhibit colonization by *Bacteroides* and *Bifidobacterium* species.

SOCIAL AND ENVIRONMENTAL CONTACT

Exposure to microorganisms through contact with siblings, caregivers, domestic animals, as well as through environmental hygiene and place of residence, also plays an important role in shaping microbiota development. Children raised in rural settings or exposed to soil and animal microbiota more frequently exhibit greater microbial diversity and a lower risk of atopic diseases (a phenomenon known as the “old friends hypothesis” or the expanded hygiene hypothesis).

POTENTIAL THERAPEUTIC INTERVENTIONS

In light of the risk of disrupted microbiota colonization in newborns, increasing interest is being directed toward microbiota-targeted interventions aimed at restoring a healthy microbial profile in cesarean-born infants. Pilot studies on vaginal microbiota transplantation (vaginal seeding) suggest that transferring the

maternal microbiome to the newborn immediately after birth may partially recreate the physiological colonization pattern. Additionally, third-generation probiotic formulations - containing specific strains of *Bifidobacterium* and *Faecalibacterium* - are being developed, with demonstrated potential to modulate immune responses and promote SCFA production. However, further research is required to assess the safety and efficacy of these interventions in diverse population groups.

LIMITATIONS OF THE REVIEW

This review has several limitations that should be acknowledged. First, although a systematic search strategy was employed, the review may be subject to selection bias, as only English-language publications and human studies were included. Second, heterogeneity in study design, microbiota assessment methods (e.g., 16S rRNA vs. metagenomics), and outcome definitions limits direct comparability and meta-analysis. Third, the long-term outcomes reported in the literature are often based on self-reported or registry-based data, which may not fully capture subtle neurodevelopmental or immunological phenotypes. Lastly, potential publication bias may lead to an overrepresentation of studies reporting significant associations. These limitations - including potential publication bias - should be considered when interpreting the findings of this review.

CONCLUSIONS

The evidence gathered in this review demonstrates that the mode of delivery exerts a profound influence on the early development of the neonatal microbiota, which in turn shapes the maturation of immune, metabolic, neurological, and barrier functions. Vaginal delivery provides the newborn with physiological exposure to maternal vaginal, perineal, and fecal microbiota, thereby ensuring balanced colonization and supporting the proper development of biological systems. In contrast, elective cesarean section consistently leads to delayed microbial succession, reduced taxonomic diversity, and altered colonization patterns, which have been linked

to an increased risk of allergic, autoimmune, metabolic, and neuroinflammatory conditions. These associations are underpinned by well-documented mechanisms, including impaired differentiation of regulatory T cells, reduced signaling mediated by short-chain fatty acids, enhanced activation of TLR4 receptors, and the maintenance of low-grade inflammation. Although a small number of studies report potential protective effects of alternative colonization trajectories, such as reduced carriage of selected pathogens, these findings appear transient, context-dependent, and of limited clinical relevance.

From a clinical perspective, the implications of these findings highlight the importance of promoting delivery modes and neonatal practices that favor healthy microbiota development. Whenever medically feasible, vaginal birth should be supported as the optimal route of delivery. In addition, early initiation and continuation of breastfeeding play a crucial compensatory role, while the avoidance of unnecessary perinatal antibiotic exposure remains essential. New approaches, such as microbiota-targeted therapies and vaginal seeding, are emerging as potentially valuable interventions, but their safety and efficacy must be rigorously confirmed before widespread adoption. At the population level, educational initiatives aimed at prospective parents and the integration of microbiota health into public health frameworks may further contribute to reducing long-term disease risks associated with disrupted neonatal colonization.

Future research should focus on identifying reliable biomarkers of neonatal dysbiosis, as these could allow early recognition and intervention. In parallel, there is a pressing need for the development of restorative strategies, including advanced probiotic formulations and personalized therapeutic approaches tailored to individual microbiota profiles. Moreover, the design of large-scale, longitudinal cohort studies integrating microbiological, immunological, and clinical outcomes will be essential to clarify causal relationships and strengthen the evidence base. Only through such efforts will it be possible to transform current knowledge into effective, individualized strategies that protect and promote microbiota-driven health from the very beginning of life.

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DATA AVAILABILITY

Data supporting the findings are available from the corresponding author upon request

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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Words from the wards: Paronyms in medical article titles

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ABSTRACT

Aim: To examine the presence of paronyms in medical article titles, analyze their stylistic features and pragmatic potential, and provide relevant guidelines for EAP students and early career writers.

Materials and Methods: We examined the occurrence of paronyms in medical article titles sourced from the PubMed database using its Advanced Search Builder ("search term[Title]" queries). The study relied on the methods of quantitative examination, stylistic, structural, etymological, and contextual analyses, using The Oxford Dictionary of Literary Terms (Baldick, 2015), Writing with Clarity and Style: A Guide to Rhetorical Devices for Contemporary Writers (Harris, 2016), Latin-Ukrainian Thesaurus of Clinical Terms (Bieliaieva, 2016), and Latin-Ukrainian Medical Encyclopedic Dictionary (Bieliaieva, Zhdan & Tsisyk, 2020). The study identified 43 paronymic pairs, 6 paronymic triads, and 1 paronymic tetrad across 207 medical article titles.

Conclusions: By examining how paronyms are used in article titles, EAP students and early career writers can develop a deeper understanding of the subtle distinctions between paronymic lexemes and subsequently use them correctly, recognize the rhetorical devices and employ the pragmatic strategies in academic writing, thus enhancing their language proficiency and developing critical thinking skills. This will ultimately promote mastery of the skills needed to create engaging and compelling titles, which can increase the visibility and impact of research articles.

KEY WORDS: paronyms, article titles, medical discourse, English for Academic Purposes, rhetorical devices, pragmatic functions

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INTRODUCTION

In the fields of linguistics and language teaching, the focus on article titles presents an abundant area for exploration and analysis [1-3]. Titles serve as the initial point of contact between the reader and the research, shaping the reader's expectations and influencing their decision to delve further into the study. Scholars have extensively explored the role of titles in scientific discourse, emphasizing their impact on article visibility, citation rates, and even acceptance rates [4, 5]. Indeed, in the age of digital information overload, effective titles play a particularly crucial role in making research more discoverable and attracting potential readers [2]. Hence, our interest in article titles stems from the considerable pragmatic potential of this aspect of academic writing. The study of article titles not only enriches our understanding of language use but also promotes effective pedagogical practices aimed at educating proficient writers, which renders this research relevant.

In designing article titles, various strategies can be employed to effectively capture the essence of the research while attracting readers' attention [2]. Each of

these strategies serves to enhance the communicative impact of the title, contributing to its effectiveness in conveying the significance of the research. One approach involves using noun phrases, which succinctly encapsulate the main topic and key findings of the study, providing a clear and informative preview of the content. Another strategy in designing article titles involves the use of semicolons, particularly in titles where multiple concepts or aspects need to be distinguished, providing clarity and facilitating comprehension for the reader (e.g., "Devic's opticomyelitis: a case report from the authors' clinical practice" [6]; "Alarmism vs eschatology: a conceptual analysis" [7]).

Sentence-based titles, on the other hand, incorporate verbs and present the research in a narrative style. The increasing use of interrogative titles is one more notable tendency [1]. In particular, there is a recurring trend of using popular culture references such as song titles, to stand out in the competitive publishing setting, as exemplified by the title "Should I Stay or Should I Go?" [8].

Meanwhile, titles with stylistically marked expressions, such as puns, rhymes, alliteration, etc., can inject

Table 1. Paronymic “noun + noun” pairs in article titles from PubMed

No.	Paronymic pairs	Examples from PubMed
1.	Access (n) – excess (n)	• “Access without excess” (J Med Philos, 1992) • “We need access, not excess” (Kans Med, 1992) • “Access versus excess: value-based cost sharing for prescription drugs” (Health Aff, 2004) • “Access, excess, and ethics--towards a sustainable distribution model for antibiotics” (Ups J Med Sci, 2014) • “Antibiotics: achieving the balance between access and excess” (Lancet, 2016) • “Access to excess” (JAAPA, 2017)
2.	Apposition (n) – opposition (n)	• “Nursing philosophy and nursing research: in apposition or opposition?” (Nurs Res, 1982)
3.	Bite (n) – byte (n)	• “Byte by bite: Use of a mobile bite counter and weekly behavioral challenges to promote weight loss” (Smart Health (Amst), 2017) • “From bite to byte: Dental structures resolved at a single-cell resolution” (J Dent Res, 2021) • “Can a byte improve our bite? An analysis of digital twins in the food industry” (Sensors (Basel), 2021) • “Bite by byte: Can fitness wearables help bariatric patients lose more weight after surgery?” (Surg Endosc, 2023)
4.	Break (n) – brake (n)	• “A break in the brake mechanism in diabetes: a cause of postprandial hyperglycemia” (Diabetes Care, 2008) • “Fast break on the fat brake: mechanism of peroxisome proliferator-activated receptor-delta regulation of lipid accumulation in hepatocytes” (Hepatology, 2008) • “ATP puts the brake on DNA double-strand break repair: a new study shows that ATP switches the Mre11-Rad50-Nbs1 repair factor between signaling and processing of DNA ends” (Bioessays, 2014) • “Editorial overview: Cardiovascular and renal: Putting the brake on heart-breaks: emerging targets and treatment strategies for cardiovascular and renal disorders” (Curr Opin Pharmacol, 2017)
5.	Flower (n) – flour (n)	• “From flour to flower: how Polycomb group proteins influence multiple aspects of plant development” (Trends Plant Sci, 2003)
6.	Patience (n) – patients (n)	• “Patients, patience and provocations” (RN, 1950) • “Perthes’ disease: stretching the patients’ patience” (Nurs Mirror, 1978) • “Pain in chronic pancreatitis. Patients, patience, and the impatient surgeon” (Gastroenterology, 1984) • “It’s just me: patients, patience, and parents” (Can Fam Physician, 2008) • “Patients, patience, and the publication process” (Proc Natl Acad Sci USA, 2010) • “Testing patients’ patience” (Nurs Manag (Harrow), 2012) • “Incorporating musculoskeletal ultrasound into your radiology practice: patients, patience, and why your department cannot do without it” (Skeletal Radiol, 2019)
7.	Plague (n) – plaque (n)	• “Letter: A plague of plaque” (Br Dent J, 1975) • “Plague (tuberculosis) and plaque (atherosclerosis)” (N Engl J Med, 1977) • “Genes for tight adherence of Actinobacillus actinomycetemcomitans: from plaque to plague to pond scum” (Trends Microbiol, 2001)
8.	Waist (n) – waste (n)	• “Research quarterly for exercise and sport lecture. Statewide physical fitness testing: A big waist or a big waste?” (Res Q Exerc Sport, 2009) • “Waist circumference: A waste of time?” (Heart, 2010) • “Less waste on waist measurements: Determination of optimal waist circumference measurement site to predict visceral adipose tissue in postmenopausal women with obesity” (Nutrients, 2018)
9.	Weight (n) – wait (n)	• “Intermittent fasting: Is the wait worth the weight?” (Curr Obes Rep, 2018) • “From REM to orexin: Clinical progress in narcolepsy – was it worth the wait? Was it worth the weight?” (Arq Neuropsiquiatr, 2021) • “Worth the wait? Preconception weight reduction in women and men with obesity and infertility: A narrative review” (Fertil Steril, 2022)

Source: compiled by the authors of this study

creativity and humor into the title, potentially increasing its appeal [9]. The latter strategy can be particularly effectively implemented through the use of paronyms, i.e., words with similar morphemic composition but

different meanings [10]. In our previous studies, we have explored the prevalence and functions of medical paronyms in healthcare professional settings [10, 11].

Table 2. Paronymic “noun + adjective” pairs in article titles from PubMed

No.	Paronymic pairs	Examples from PubMed
1.	Board (n) – bored (adj)	• “Is your secretary on board – or just bored? (Tex Hosp, 1978) • “A busy board is never bored” (Health Serv Manager, 1979) • “On board or bored stiff? How to run effective board meetings” (MGMA Connex, 2003) • “Bored of the board?” (J Calif Dent Assoc, 2005) • “Bored in board-and-care and other settings: Perspectives of Latinos with schizophrenia” (Psychiatr Serv, 2016) • “Don’t push your luck! Educational family board (not bored) game for school-age children living with chronic conditions” (J Pediatr Nurs, 2017) • “From bored games to board games: Student-driven game design in the virtual classroom” (J Microbiol Biol Educ, 2021)
2.	Course (n) – coarse (adj)	• “Time course of visual perception: coarse-to-fine processing and beyond” (Prog Neurobiol, 2008) • “The last course of coarse coding: Hemispheric similarities in associative and categorical semantic processing” (Brain Lang, 2022)
3.	Deer (n) – dear (adj)	• “Dear deer? Maybe for now. People’s perception on red deer (Cervus elaphus) populations in Portugal” (Sci Total Environ, 2020) • “Oh dear, SARS-CoV-2 in deer mice” (Lab Anim (NY), 2021)
4.	Duel (n) – dual (adj)	• “Top pop(ulation)s: dual, duo or duel?” (Nat Biotechnol, 2007) • “The duel between dual antiplatelet therapies” (N Engl J Med, 2013) • “Bringing back targets to “IMPROVE” atherosclerotic cardiovascular disease outcomes: the duel for dual goals; are two targets better than one?” (Circulation, 2015) • “Dual organ duel: The hepatorenal axis” (Adv Chronic Kidney Dis, 2017) • “Dual RNA-sequencing to elucidate the plant-pathogen duel” (Curr Issues Mol Biol, 2018) • “Fungal duel between <i>Penicillium brasilianum</i> and <i>Aspergillus nomius</i> results in dual induction of MiktoSpiromide A and Kitrinomycin A” (J Nat Prod, 2023)
5.	Fare (n) – fair (adj)	• “Fair-weather fare: Tips for smart eating during outdoor events (Diabetes Forecast, 2012)
6.	Hole (n) – whole (adj)	• “A whole new way to make a hole – or a whole new wheel?” (Catheter Cardiovasc Interv, 2005) • “Whole not hole: Expert face recognition requires holistic perception” (Neuropsychologia, 2010) • “The hole and the whole” (Br Dent J, 2013) • “Treating the whole not the hole: Necessary coupling of technologies for diabetic foot ulcer treatment” (Trends Mol Med, 2014) • “Whole-hearted for the hole-hearted” (Mil Med, 2015) • “The hole and the whole: Lessons from manipulation of Nipbl deficiency” (PLoS Biol, 2016) • “Strategies for achieving health equity: Concern about the whole plus concern about the hole” (Ethn Dis, 2019) • “Management of caustic injury: Better to evaluate patient as a “whole” rather than only through a “hole”! (Endoscopy, 2021) • “Whole versus hole: Enabling community nurses to implement holistic wound care” (J Wound Care, 2023)
7.	Principle (n) – principal (adj)	• “Changing Principals, Keeping Principles” (N Engl J Med, 2019); • “States of ignorance and ignorance of states: Examining the Quantum Principal Principle” (Stud Hist Philos Sci, 2021)
8.	Reel (n) – real (adj)	• “Real life and reel life” (Perspect Psychiatr Care, 2003) • “From reel time to real time: patient simulation for chaplain interns” (J Pastoral Care Counsel, 2008) • “Genetics in Hollywood: from real to reel” (Clin Genet, 2010) • “Social attention with real versus reel stimuli: toward an empirical approach to concerns about ecological validity” (Front Hum Neurosci, 2012) • “Bollywood’s cancer: disconnect between reel and real oncology” (Lancet Oncol, 2015) • “Jack Nicholson: A reel and real-life contribution to neurosciences” (World Neurosurg, 2017) • “Involuntary arm movements post-pacemaker insertion - real or reel syndrome?” (Acta Cardiol, 2023) • “Social attention patterns of autistic and non-autistic adults when viewing real versus reel people” (Autism, 2023)

Table 2. Cont.

9.	Vein (n) – vain (adj)	• “Serotonin (5-HT) in veins: not all in vain” (J Pharmacol Exp Ther, 2007) • “In the vein but in vain” (Paediatr Anaesth, 2008) • “DES in saphenous bypass grafts: A treatment in vain?” (Catheter Cardiovasc Interv, 2008) • “Examining the jugular vein is never in vain” (Circ Heart Fail, 2010) • “Congenital extrahepatic portocaval shunt: growth in vain” (J Pediatr, 2013) • “Evaluation of the diagnostic value of a venous phase in CT angiography of the extremities in the setting of trauma: is vein imaging in vain?” (Emerg Radiol, 2017) • “Function of TGFbeta (transforming growth factor-beta) receptor in the vein is not in vain” (Arterioscler Thromb Vasc Biol, 2022) • “Commentary: Operating on fewer veins in vain” (JTCVS Open, 2022)
10.	Week (n) – weak (adj)	• “Variation in intensive care unit outcomes by day of week: no weak-end” (Intensive Care Med, 2004) • “A week seems to be weak: tailoring duration of antibiotic treatment in Gram-negative ventilator-associated pneumonia” (Crit Care, 2013) • “No weak days? Impact of day in the week on surgical mortality” (ANZ J Surg, 2016) • “Do gastroenterologists have a “weak” day? Impact of day of the week on the quality of outpatient screening colonoscopies” (J Clin Gastroenterol, 2022)

Source: compiled by the authors of this study

AIM

This paper aims to examine the presence of paronyms in medical article titles, analyze their stylistic features and pragmatic potential, and provide relevant guidelines for EAP students and early career writers.

MATERIALS AND METHODS

We examined the occurrence of paronyms in medical article titles sourced from the PubMed database using its Advanced Search Builder (“search term[Title]” queries). The study relied on the methods of quantitative examination, stylistic, structural, etymological, and contextual analyses, using The Oxford Dictionary of Literary Terms [12], Writing with Clarity and Style: A Guide to Rhetorical Devices for Contemporary Writers (Harris, 2016), Latin-Ukrainian Thesaurus of Clinical Terms [13], and Latin-Ukrainian Medical Encyclopedic Dictionary [14]. The study identified 43 paronymic pairs, 6 paronymic triads, and 1 paronymic tetrad across 207 medical article titles.

ETHICS

This review article is based on an analysis of publicly available scientific data published in peer-reviewed journals, clinical guidelines and databases. No patient-identifying data was used during the work, nor was there a need to obtain approval from an ethics committee, as the study did not include new clinical interventions or initial collection of patient information.

REVIEW AND DISCUSSION

The study detected 43 paronymic pairs (Table 1 - 8), 6 paronymic triads (Table 9), and 1 paronymic tetrad

(Table 10) in 207 medical article titles. In this paper, we deliberately chose not to include proper medical paronyms such as “mucus – mucous”, “affect – effect”, etc., which we have examined in our previous studies [10, 11].

This intentional omission stems from the understanding that while these pairs of words have distinct medical meanings, their usage in titles might not provide the same stylistic nuance or playful ambiguity as other paronyms. In other words, the use of proper medical paronyms is dictated by the need to express accurate medical information in titles, rather than to form stylistically marked expressions, given the specific and precise nature of the medical context. Therefore, in this study, we chose to focus specifically on neutral (general English) paronymic lexemes that can effectively leverage wordplay with their counterparts from medicine to enhance the stylistic features and pragmatic implications of medical article titles, thus providing valuable insights for EAP instructional settings.

The detected paronymic pairs have been categorized by their grammatical properties, which revealed several notable patterns. Hence, the most common occurrences of paronyms are “noun + adjective” and “noun + verb” categories, represented by 10 pairs each. They are followed by 9 “noun + noun” pairs, 5 “verb + adjective” pairs, and 4 “verb + verb” pairs. Paronymic “noun + adverb” and “verb + adverb”, “noun + conjunction” and “verb + conjunction”, and “adjective + adjective” categories are the least common instances, with 1 pair in each case. It is necessary to point out that 7 paronymic lexemes (“waste”, “wait”, “break”, “brake”, “fare”, “wear”, and “weather”) exhibit dual functionality, serving both as nouns and verbs across various contexts. Consequently, lexemes “break” and “brake” form 3 paronymic pairs:

Table 3. Paronymic “noun + verb” pairs in article titles from PubMed

No.	Paronymic pairs	Examples from PubMed
1.	Brake (n) – break (v)	• “Activation of the JNK signaling pathway: breaking the brake on apoptosis” (Bioessays, 2003)
2.	Cell (n) – sell (v)	• “Stem cells – a hard sell to investors” (Nat Biotechnol, 2005) • “The stem cell hard sell: Report from a clinic’s patient recruitment seminar” (Stem Cells Transl Med, 2017)
3.	Dye (n) – die (v)	• “To die or not to dye. Poisoning from arsenical pigments in the 19th century” (Pharm Hist (Lond), 1996) • “What cells can do when they die (and how we can dye it)” (Cytometry A, 2010) • “It’s not the dye, but the “die” in dialysis: which coronary revascularization strategy really is best?” (Catheter Cardiovasc Interv, 2010)
4.	Flu (n) – flew (v)	• “Rapid action by Chandigarh: Flew away the threat of bird flu” (Indian J Med Microbiol, 2016)
5.	Heel (n) – heal (v)	• “Heel ulcers don’t heal in diabetes. Or do they?” (Diabet Med, 2005) • “Non-surgical way to heal severe heel pain. A new twist on an old exercise might bring relief from chronic plantar fasciitis” (Duke Med Health News, 2006) • “When a heel won’t heal: an important differential to consider” (J Am Podiatr Med Assoc, 2014) • “Heel ulcers do heal in patients with diabetes” (Int Wound J, 2017) • “Heal or no heel: Outcomes of ischaemic heel ulcers following lower limb revascularization from a multi-ethnic Asian Cohort in Singapore” (Int Wound J, 2020)
6.	Lead (n) – led (v)	• “Don’t be ‘mis-led’: few herbal products have been implicated in lead poisoning” (J Gen Intern Med, 2009) • “High-fat diet in mice led to increased severity of spermatogenesis impairment by lead exposure: perspective from gut microbiota and the efficacy of probiotics” (J Sci Food Agric, 2023) • “Lead and copper led to the dysregulation of bile acid homeostasis by impairing intestinal absorption in Bufo gargarizans larvae: An integrated metabolomics and transcriptomics approach” (Sci Total Environ, 2023)
7.	Meat (n) – meet (v)	• “Meet your meat: The argument for increasing education and public outreach regarding the regulation and safety of animal biotechnology” (Food Drug Law J, 2013) • “Meet meat: An explorative study on meat and cultured meat as seen by Chinese, Ethiopians and Dutch” (Appetite, 2017) • “Meet the meat alternatives: The value of alternative protein sources” (Curr Nutr Rep, 2020) • “Meet your meat: The effect of imagined intergroup contact on wanting and liking of meat” (Appetite, 2022) • “Tissue engineering challenges for cultivated meat to meet the real demand of a global market” (Int J Mol Sci, 2023) • “Meet the meatless: Demand for new generation plant-based meat alternatives” (Appl Econ Perspect Policy, 2023)
8.	Sea (n) – see (v)	• “Marine microbes see a sea of gradients” (Science, 2012) • “To see the sea and the shore” (Am Ann Deaf, 2021) • “What Darwin could not see: island formation and historical sea levels shape genetic divergence and island biogeography in a coastal marine species” (Heredity (Edinb), 2023)
9.	Waist (n) – waste (v)	• “Wait for weight or “waste” the waist: the benefits of early intervention in childhood obesity” (J Am Coll Cardiol, 2009) • “Waste the Waist”: The development of an intervention to promote changes in diet and physical activity for people with high cardiovascular risk” (Br J Health Psychol, 2012) • “Sleep doesn’t waste time, it’s good for the waist line” (Sleep, 2015) • “Waste the waist: A pilot randomised controlled trial of a primary care based intervention to support lifestyle change in people with high cardiovascular risk” (Int J Behav Nutr Phys Act, 2015)
10.	Weight (n) – wait (v)	• “Wait for weight or “waste” the waist: the benefits of early intervention in childhood obesity” (J Am Coll Cardiol, 2009) • “Don’t wait for the weight” (Resuscitation, 2017) • “Childhood obesity: How long should we wait to predict weight?” (J Pediatr Endocrinol Metab, 2018)

Source: compiled by the authors of this study

Table 4. Paronymic “verb + adjective” pairs in article titles from PubMed

No.	Paronymic pairs	Examples from PubMed
1.	Bear (v) – bare (adj)	“Can we bear another bare-metal stent study?” (Catheter Cardiovasc Interv, 2013)
2.	Blew (v) – blue (adj)	“Code Blue: how a hot medical stock blew up” (Fortune, 1998)
3.	Fare (v) – fair (adj)	“Giving medicine a fair trial. It needs to be established whether patients really fare better in trials” (BMJ, 2000) “The fat ones fare well – but is it fair to compare?” (Crit Care Med, 2006)
4.	Knew (v) – new (adj)	“Myocardial viability: what we knew and what is new” (Cardiol Res Pract, 2012) “Headache – what we knew and what is new: From a physician who is also a patient” (Neurol India, 2021) “Cancer and neurofibromatosis type 1 – confirming what we knew and telling us something new” (JAMA Netw Open, 2021)
5.	Lose (v) – loose (adj)	“Too loose to lose: an investigation into endoscopic anastomotic reduction to reestablish weight loss” (Gastrointest Endosc, 2018)

Source: compiled by the authors of this study

Table 5. Paronymic “verb + verb” pairs in article titles from PubMed

No.	Paronymic pairs	Examples from PubMed
1.	Appose (v) – oppose (v)	“Incomplete stent apposition: should we appose or oppose?” (JACC Cardiovasc Interv, 2010)
2.	Break (v) – brake (v)	“To break or to brake neuronal network accelerated by ammonium ions?” (PLoS One, 2015)
3.	Cease (v) – seize (v)	“Brain seizes, heart ceases: a case of ictal asystole” (J Neurol Neurosurg Psychiatry, 2000)
4.	Wring (v) – ring (v)	“Publication and citation in ophthalmology: glaucoma and the water-provocation test – wring out the old and ring in the new?” (Clin Exp Ophthalmol, 2008) “The dynamic vortex of a beating heart: wring out the old and ring in the new!” (J Am Coll Cardiol, 2014) “To ring or wring the bell?” (J Palliat Med, 2011)

Source: compiled by the authors of this study

“break” (n) – “brake” (n); break” (v) – “brake” (n); break” (v) – “brake” (v), whereas the remaining 5 words form 2 paronymic pairs each. The distribution of paronymic pairs across different parts of speech highlights the versatility of English in creating wordplay and puns, with certain structures being more prevalent than others.

Table 1 presents paronymic “noun + noun” pairs in the analyzed research articles.

As can be seen from Table 1, paronyms in medical article titles underlie deliberate wordplay, conveying contrasting concepts through juxtaposition. Through such linguistic devices, these titles not only convey the subject matter but also engage readers and evoke thought on relevant medical topics. The stylistic devices present in these titles include paronomasia, or puns (e.g., “Is the **wait** worth the **weight**?”; “Testing **patients’ patience**”; “The Consular **Corps(e)**”), antithesis (e.g., “A **corps**, not **corpse**”; “**Patients, patience**, and the

impatient surgeon”), rhyme (e.g., “Was it worth the **wait**? Was it worth the **weight**?”; “**Patients, patience** and provocations”), alliteration (e.g., “**Byte** by **bite**”; “From **bite** to **byte**”; “**Patients, patience**, and parents”), rhetorical questions (“A big **waist** or a big **waste**?”), and metaphorical language (e.g., “Putting the **brake** on heart-**breaks**”; “Stretching the **patients’ patience**”; “**Plague** (tuberculosis) and **plaque** (atherosclerosis)”, i.e., tuberculosis as the white “plague” of the 19th century vs. atherosclerosis as the white “plague” of the 20th century).

Table 2 presents paronymic “noun + adjective” pairs in the analyzed articles.

The stylistic devices present in these titles include paronomasia (“Variation in intensive care unit outcomes by day of **week**: no **weak**-end”; “A **week** seems to be **weak**”; “**Whole**-hearted for the **hole**-hearted”; “A busy **board** is never **bored**”; “From **bored** games to **board** games”), alliteration (“The last **course** of **coarse** coding”;

"**Dear deer?**"; "Top pop(ulation)s: **dual**, **duo** or **duel?**"; "**Dual** organ **duel?**", metaphors ("**Fair**-weather **fare?**"), antithesis (**Whole** versus **hole?**; "Changing **principals**, keeping **principles?**"; "**Real** life and **reel** life"), rhyme ("A busy **board** is never **bored?**"), and rhetorical questions ("**Bored** of the **board?**"; "On **board** or **bored** stiff?"; "A **whole** new way to make a **hole** – or a **whole** new wheel?"). These devices add creativity, humor, and depth to the titles, making them more engaging and memorable for the readers. One can observe several wordplays on the phrases "on board" and "board games", which create a sense of linguistic creativity, capturing the reader's attention and engaging them with the titles. Both "on board" and "board games" typically refer to being actively involved or participating, whereas the titles juxtapose these phrases to quite the opposite "**bored** stiff" and "just **bored**" expressions, which enhances the stylistic effect by introducing a playful and witty tone. These puns effectively attract readers' interest and make the titles stand out amidst a plethora of existing literature.

Table 3 presents paronymic "noun + verb" pairs in the analyzed research articles.

As one can observe from Table 3, paronyms underlie the following stylistic devices: paronomasia ("Don't be 'mis-**led**': few herbal products have been implicated in **lead** poisoning"; "Don't **wait** for the **weight**"), alliteration ("**Meet** the **meat** alternatives"; "**Meet** the **meatless**"; "To **die** or not to **dye**"; "**Heal** or no **heel**"), antithesis ("What cells can do when they **die** (and how we can **dye** it)"; "It's not the **dye**, but the '**die**' in dialysis"), metaphor ("Stem **cells** – a hard **sell** to investors"; "The stem **cell** hard **sell**"), and rhyme ("When a **heel** won't **heal**"; "**Wait** for **weight** or '**waste**' the **waist**"; "Don't **wait** for the **weight**"; "Sleep doesn't **waste** time, it's good for the **waist** line"). Table 3 also features several wordplays on the idiom "a hard sell", meaning something that is difficult to promote. Meanwhile, "To **die** or not to **dye**" is a wordplay on the famous Shakespearean phrase "to be or not to be" from *Hamlet*, and "**meet** your **meat**" is a play on the idiom "meet your match", which means to encounter someone or something that is as good as or better than oneself.

Table 4 presents paronymic "verb + adjective" pairs in the analyzed articles.

In the titles from Table 4, the stylistic devices relying on paronyms include paronomasia and alliteration ("Can we **bear** another **bare**-metal stent study?"; "Code **Blue**: how a hot medical stock **blew** up"; "Too **loose** to **lose**"), metaphor ("Giving medicine a **fair** trial"), rhyme ("What we **knew** and what is **new**"), and rhetorical questions ("The fat ones **fare** well – but is it **fair** to compare?"). It is necessary to observe that the title "Too

loose to **lose**" is a wordplay on the idiom "too good to lose/miss" implying that something is valuable and should not be lost.

Table 5 presents paronymic "verb + verb" pairs in the analyzed articles.

The examples in Table 5 employ paronomasia, alliteration, and rhetorical question ("Should we **appose** or **oppose?**"; "To **break** or to **brake** neuronal network accelerated by ammonium ions?"; "**Wring** out the old and **ring** in the new"), which prompt reflection and invite the readers to ponder the dilemma posed by the titles. Moreover, the titles with the phrase "**Wring** out the old and **ring** in the new" are a wordplay on the idiom "out with the old, in with the new", applied to emphasize the importance of a paradigm shift in the context of publication and citation practices in ophthalmology and in optimizing diagnostic and therapeutic interventions in cardiology, respectively. One can also observe rhyme and antithesis ("Brain **seizes**, heart **ceases**"), enhancing both the rhythmic pattern and subject matter of the title.

Table 6, Table 7, Table 8 present paronymic "noun + adverb" and "verb + adverb", "noun + conjunction" and "verb + conjunction", and "adjective + adjective" pairs, respectively.

The titles in Table 6 largely employ alliteration ("Who should **wear** **what**, **where**, and **when**" and "Tooth **wear** – **where** are **we** now?"). The repeated use of the "w" sound creates a catchy title, drawing attention to the subject matter of the paper, adding emphasis and rhythm to the phrase.

Rhetorical questions ("**Whether** **weather** affects arthritis"; "**Whether** the **weather** influences pain?"; "**Whether** **weather** matters") engage readers by prompting them to consider the topic and its implications. It is also necessary to point out that the "weather – whether" puns rely on wordplay on a popular tongue twister "We'll weather the weather whatever the weather", enhancing the memorability, and increasing the appeal of the article titles. Furthermore, wordplay and repetition ("**Whether** the **weather** will help us **weather** the COVID-19 pandemic") exploit the versatility of the word "weather", which can function both as a noun and a verb, thus enhancing the overall impact of the title.

These titles employ paronomasia ("**Complementary** medicines are not always **complimentary** in Down syndrome"), highlighting the discrepancy between the expectation of something being favorable and the reality that it may not always be beneficial, as well as rhetorical questions ("Should we be **complimentary** about **complementary** therapies?") to engage the reader and prompt consideration of the topic being discussed.

Table 6. Paronymic “noun + adverb” and “verb + adverb” pairs in article titles from PubMed

No.	Paronymic pairs	Example from PubMed
1.	Wear (n) – where (adv)	• “Tooth wear – where are we now?” (Br Dent J, 2023)
2.	Wear (v) – where (adv)	• “Medical guidance for respiratory protection: Who should wear what, where, and when?” (Ann Am Thorac Soc, 2023)

Source: compiled by the authors of this study

Table 7. Paronymic “noun + conjunction” and “verb + conjunction” pairs in article titles from PubMed

No.	Paronymic pairs	Examples from PubMed
1.	Weather (n) – whether (conj)	• “Whether weather affects arthritis” (J Rheumatol, 1985) • “Medical meteorology: Whether weather influences admissions” (Mayo Clin Proc, 2001) • “Whether the weather influences pain? Results from the EpiFunD study in North West England” (Rheumatology (Oxford), 2010) • “Whether subarachnoid hemorrhage depends on the weather?” (World Neurosurg, 2013) • “Whether the weather drives patterns of endemic amphibian chytridiomycosis: a pathogen proliferation approach” (PLoS One, 2013) • “Whether weather matters: Evidence of association between in utero meteorological exposures and foetal growth among Indigenous and non-Indigenous mothers in rural Uganda” (PLoS One, 2017) • “Whether conversion and weather matter to roundabout safety” (J Safety Res, 2018) • “Whether the weather will help us weather the COVID-19 pandemic: Using machine learning to measure twitter users’ perceptions” (Int J Med Inform, 2021) • “Whether weather matters with migraine” (Curr Pain Headache Rep, 2024)
2.	Weather (v) – whether (conj)	• “Whether the weather will help us weather the COVID-19 pandemic: Using machine learning to measure twitter users’ perceptions” (Int J Med Inform, 2021)

Source: compiled by the authors of this study

Table 8. Paronymic “adjective + adjective” pair in article titles from PubMed

No.	Paronymic pair	Examples from PubMed
1.	Complimentary (adj) – complementary (adj)	• “Complementary and complimentary neurology” (Arch Neurol, 2000) • “Perioperative nursing: complementary or complimentary” (AORN J, 2005) • “Academic and commercial genetic testing laboratories: complementary if not complimentary” (Per Med, 2007) • “Should we be complimentary about complementary therapies?” (J Clin Nurs, 2008) • “Complementary medicines are not always complimentary in Down syndrome” (J Pediatr, 2018)

Source: compiled by the authors of this study

Table 9 presents paronymic triads in the analyzed article titles.

As one can observe, the “ward – word – world” triad is one of the most productive paronymic combinations, with 20 examples in the analyzed article titles. The titles in Table 9 demonstrate a consistent use of puns, such as “**Peek a peak**”, “**plain** and **pain** talk”, “One **word** can mean a **world** of difference”, and “**Word** and **world** knowledge”. Rhetorical questions are utilized to engage the audience and stimulate curiosity, as seen in “Mesocolic **plane** surgery: just **plain** surgery?” and “Multiplanar reformations...is it **plain** which **plane** to use?”, prompting readers to consider the implications or uncertainties surrounding the topics addressed. Additionally, the titles often employ alliteration, as in

“**Rain, reign, rein**”, **peek-peak-pique**”, “Over-**sight** on op-**site**”, “From **words** to **wards**”, and “**Word** and **world** order”, which reinforces the memorability of the titles. Furthermore, some titles incorporate antithesis to juxtapose contrasts or emphasize unexpected connections, such as “Big **words**, halved brains and small **worlds**” and “Mediating **words**, mediating **worlds**”. One can also observe several metaphors (“The **world** is their **ward**”, “**Word** and **world** meet”, “to enter the labour **ward world**”) and rhymes (“Hard **words** from the **wards**”, “A novel **site** comes into **sight**”, “**Cite** with a **sight**”). These titles make strategic use of collocations and idiomatic expressions, such as “word order” and “world order”, “word knowledge” and “world knowledge”, “plain sight”, “hiding in plain sight”, and “sight unseen” to capture

the readers' attention, and highlight key points of the studies.

Table 10 presents a paronymic tetrad in the analyzed article titles.

The stylistic devices present in these titles include paronomasia largely based on the phrase "rite of passage" ("**Write right** – or *left*"; "Residency training: **rite** of passage, but is it **right** today?"; "Authorship: **rite, right**, or **write** of passage?"; "Freud's writing: his (**w**)**rite** of passage and its reverberations"), antithesis ("It's more than a computer can deliver: gross anatomy – a **rite** of passage and a **right** to learning"; "Screening pelvic examinations: **right**, wrong, or **rite**?"), alliteration ("**Wright** was **right**"; "**Write right!**"), rhyme ("Informed consent and patient's rights documents: a **right**, a **rite**, or a **rewrite**?"), repetitions ("Nurses: The **right** and **rites** to **write, right**?"), and rhetorical questions ("**Right** or **rite**?"; "Was **Wright right**?").

Moreover, the paronymic tetrad "Right – rite – write (v) – Write (eponym)" presents an interesting linguistic phenomenon. These paronyms are among the most prolific lexemes found in the *PubMed* article titles (23 examples). Furthermore, the inclusion of "Write" as an eponym, referring to Sewall Wright (1889 – 1988), an American geneticist celebrated for his contributions to the field of evolutionary theory, further enriches the semantic landscape, adding a layer of historical significance. Hence, the paronymic tetrad in Table 10 encapsulates the complexity and versatility of language, showcasing how a single word can transform in different contexts and interpretations.

In this context, it is necessary to emphasize that eponyms play a crucial role in medical discourse by honoring the contributions of individuals to medical knowledge and providing concise ways to reference diseases, conditions, anatomical structures, and medical procedures [15, 16]. However, their usage can pose challenges due to potential misspelling due to the lack of awareness, thus leading to confusion among healthcare professionals and students [15]. Therefore, additional instruction and guidance are necessary at medical universities to ensure proper understanding, spelling, and use of eponyms in clinical practice and academic discourse [15]. This instruction helps maintain clarity and accuracy in communication within the medical field while paying homage to the historical figures who have made significant contributions to medicine.

Furthermore, Tables 1-10 demonstrate several notable tendencies in terms of paronyms' etymological features. Out of the total number of detected paronyms, 40 lexemes can be traced back to classical and medieval Latin, – frequently through Old French and

sometimes after having undergone substantial spelling transformations in modern English [17]. Within this subgroup, 28 words with Latin roots form paronymic pairs and triads in English ("access"/"excess", "opposition/apposition", "oppose/appose", "complementary/complimentary", "dual/duel", "flour/flower", "patience/patients", "plague/plaque", "principal/principle", "cease/seize", "vain/vein", "core/corps/corpse", "pain/plain/plane"). For instance, the words "corps" and "corpse" originate from the Latin word "corpus", meaning "body", but have diverged in meaning over time, with "corps" taking on a figurative, collective sense, and "corpse" specifying the literal, deceased body [13]. Similarly, "apposition" and "opposition" stem from the Latin "positio", meaning "placing", yet they have branched off in meaning due to the prefixes attached to them ("ad-" (towards, in addition) and "ob-" (against), which alter the base meaning of the word they precede, with "apposition" denoting the addition of elements side by side, and "opposition" indicating contrast or conflict [13]. Hence, despite shared etymological roots in Latin, numerous words in modern English developed different meanings in the process of language evolution.

In addition, Latin-based and Old English words can also form paronymic pairs and triads due to coincidences in spelling, leading to potential confusion in understanding their meanings and usage. In our study, 12 Latin-based words ("reign", "rein", "real", "site", "cite", "fair", "rite", "waste (n,v)", "cell", "flu", and "course") form paronymic pairs and triads with lexical units stemming from Old English: "rain", "reel", "sight", "fare", "right/write", "waist", "sell", "flew", "coarse"). These linguistic convergences highlight the complexity and the rich history that has shaped the English language, with Latin contributing substantially to its vocabulary and evolution.

Indeed, the enduring significance of Latin, with its profound influence on English and other modern European languages cannot be underestimated [17]. The vast layer of English vocabulary is rooted in ancient Latin and Latinized Greek [17]. This underscores the importance of a solid foundation of Latin instruction for medical students and medical writers. It is a practical necessity for achieving clarity and precision in professional communication within the medical field, and for the effective exchange of knowledge. A thorough instruction in Latin helps medical students understand the meanings and origins of lexemes, avoid potential mistakes, and communicate with greater accuracy and specificity [17].

It is also necessary to observe that paronomasia (or pun) adds a significant layer of complexity and creativity to article titles. These linguistic devices can be

Table 9. Paronymic triads in article titles from PubMed

No.	Paronymic triads	Examples from PubMed
1.	Core (n) – corps (n) – corpse (n)	• “The core and corps of audiology” (Br J Audiol, 1989) • “The Consular Corps(e)” (Med Leg J, 1990) • “Disease intervention specialists as a corps, not corpse” (Sex Transm Dis, 2008) • “Heroism. A core value of the Army Nurse Corps” (Nurs Leadersh Forum, 2001)
2.	Peak (n) – peek (v) – pique (n)	• “Peek a peak: a glance at statistics for quantitative label-free proteomics” (Expert Rev Proteomics, 2010) • “Peek-peak-pique: Repeating motifs of subtle variance are targets for potent malaria antibodies” (Immunity, 2018)
3.	Pain (n) – plain (adj) – plane (n)	• “Mesocolic plane surgery: just plain surgery?” (Colorectal Dis, 2009) • “Multiplanar reformations in the measurement of renal length on CT: is it plain which plane to use?” (AJR Am J Roentgenol, 2015) • “Plain and pain talk in palliative and hospice care: multidirectional knowledge transfer and shared decision making” (Adv Skin Wound Care, 2015) • “Pain psychology: “Psychosomatic medicine, behavioral medicine, just plain medicine” (Pain Med, 2016) • “Plain radiographs of lumbar spine in patients with low back pain” (Arch Osteoporos, 2018) • “Fascial plane blocks in thoracic surgery: a new era or plain painful?” (Curr Opin Anaesthesiol, 2020) • “In plain sight: neurosyphilis presenting with back pain” (Intern Med J, 2023) • “Ambroxol for neuropathic pain: hiding in plain sight?” (Pain, 2023)
4.	Rain (n) – rein (n) – reign (n)	• “Rain, reign, rein – the tyranny of IT” (Endocr Pract, 2003)
5.	Sight (n) – site (n) – cite (v)	• “Over-sight on op-site” (J Dermatol Surg Oncol, 1981) • “Never cite sight unseen” (Am J Obstet Gynecol, 1998) • “A novel site comes into sight” (Elife, 2013) • “Cite with a sight” (J Phys Chem Lett, 2014) • “A new sight to explore site-specific N-glycosylation in donkey colostrum milk fat globule membrane proteins with glycoproteomics analysis” (Food Res Int, 2022)
6.	Ward (n) – word (n) – world (n)	• “From words to wards. 2. Communication or conflict?” (Lancet, 1961) • “From words to wards. 3. The planning process” (Lancet, 1961) • “From words to wards. 5. Effect of alterations” (Lancet, 1961) • “Air Force flight nurses. The world is their ward” (RN, 1969) • “Word and world order: semantic, phonological, and metrical determinants of serial position” (Cogn Psychol, 1993) • “Words in wards: language, health and place” (Health Place, 1999) • “Words and wards: a model of reflective writing and its uses in medical education” (J Med Humanit, 2006) • “Synthetic biology: discovering new worlds and new words” (EMBO Rep, 2008) • “Big words, halved brains and small worlds: complex brain networks of figurative language comprehension” (PLoS One, 2011) • “Waiting for permission to enter the labour ward world: first time parents’ experiences of the first encounter on a labour ward” (Sex Reprod Healthc, 2011) • “Mediating words, mediating worlds: Interpreting as hidden care work in a South African psychiatric institution” (Transcult Psychiatry, 2013) • “Observation “services” and observation “care” – one word can mean a world of difference” (Health Serv Res, 2014) • “Word and world knowledge among deaf learners with and without cochlear implants” (J Deaf Stud Deaf Educ, 2014) • “World atrial fibrillation awareness day – high time to spread the word” (J Atr Fibrillation, 2014) • “From a word to a world: the current situation in the interdisciplinary field of synthetic biology” (PeerJ, 2015) • “Hard words from the wards: Images of violence and violation in hospital poetry” (Perspect Biol Med, 2018) • “Single word reading in the real world: Effects of transposed-letters” (J Cogn. 2021) • “Mapping word to world in ASL: Evidence from a human simulation paradigm” (Cogn Sci, 2021) • “The pluriverse of intoxication: Words, lives, worlds in Islamic history” (Soc Hist Alcohol Drugs, 2022) • “Where word and world meet: Language and vision share an abstract representation of symmetry” (J Exp Psychol Gen. 2023)

Source: compiled by the authors of this study

humorous, thought-provoking, or used for rhetorical effect, making them a valuable aspect of language and communication. However, paronomasia can also pose challenges for non-native speakers of a language. Since puns often rely on wordplay and cultural nuances of language, they can be difficult to understand. As a result, incorporating the teaching of puns into language learning curricula can help non-native speakers develop a deeper understanding of the language, its nuances, and its cultural context. It not only enhances students' linguistic skills but also fosters creativity, critical thinking, and cultural awareness. By analyzing paronomasia, students gain insight into the multiple meanings of words and phrases, as well as the cultural references embedded within language. Moreover, puns can make language learning more engaging and enjoyable, motivating students to actively participate in the learning process. Hence, integrating the study of paronomasia into language curricula can enrich students' language proficiency and cultural literacy, empowering them to communicate effectively and fluently in diverse linguistic contexts.

In this context, the majority of detected paronyms formed a basis for homophonic puns (i.e., relying on words that sound the same but have entirely different meanings). Indeed, out of the total 43 paronymic pairs identified in the study, a significant majority (36 pairs) were found to be homophones, sharing identical pronunciation despite differing in spelling and meaning ("bite – byte"; "break (n,v) – brake (n,v)"; "flower – flour"; "waist – waste (n,v)"; "weight – wait (n,v)"; "complimentary – complementary"; "wring – ring"; "board – bored"; "course – coarse"; "deer – dear"; "duel – dual"; "fare (n,v) – fair"; "principle – principal"; "reel – real"; "vein – vain"; "week – weak"; "hole – whole"; "cell – sell"; "die – dye"; "flu – flew"; "heal – heel"; "lead – led"; "meet – meat"; "see – sea"; "bear – bare"; "blew – blue"; "knew – new"; "wear (n,v) – where"; "weather (n,v) – whether"). The remaining 6 paronymic pairs differ by one sound ("access – excess"; "appose – oppose"; "aposition – opposition"; "cease – seize"; "lose – loose"; "patience – patients"), and 1 pair ("plague – plaque") stands out by differing in two sounds. Among the 6 paronymic triads, 3 were homophonic ("peak" – "peek" – "pique"; "rain" – "rein" – "reign"; "sight" – "site" – "cite"). The paronymic tetrad also relies on the homophonic features of paronyms. Hence, as the provided examples demonstrate, authors extensively employ the rhetoric potential of homophones.

It is also necessary to observe that among the 43 paronymic pairs identified in the research, 25 pairs differ by just one letter ("access – excess"; "appose – oppose"; "aposition – opposition"; "bite – byte"; "cell

– sell"; "complimentary – complementary"; "coarse – course"; "deer – dear"; "duel – dual"; "heal – heel"; "hole – whole"; "knew – new"; "lead – led"; "lose – loose"; "meet – meat"; "plague – plaque"; "reel – real"; "see – sea"; "vein – vain"; "weather (n,v) – whether"; "week – weak"; "wear (n,v) – where"; "wring – ring"). At the same time, 18 paronymic pairs exhibited more pronounced variations, differing by two or more letters ("bear – bare"; "blew – blue"; "board – bored"; "break (n,v) – brake (n,v)"; "cease – seize"; "die – dye"; "fare (n,v) – fair"; "flower – flour"; "flu – flew"; "patience – patients"; "principle – principal"; "waist – waste (n,v)"; "wait (n,v) – weight").

Consequently, when dealing with paronyms, careful attention to spelling is crucial, as demonstrated by several examples found in *PubMed*. For instance, in the study "Evaluation of the effect of garlic tablet as a **complementary** treatment for patients with diabetic retinopathy" (*Randomized Controlled Trial J Diabetes Res*, 2022) the word "complementary" was misspelled in the article text: "Purpose: The aim of this study was to investigate the effectiveness of garlic (*Allium sativum* L.) tablets as a **complimentary complementary** herbal medication in diabetic macular edema" [18]. These misspelling instances emphasize the importance of meticulous attention from authors and editors to ensure accuracy, clarity, and precision in medical communication [19]. This is particularly relevant when using words that are similar in sound but differ in meaning to avoid unintended misinterpretations and maintain the integrity of scientific literature.

This potential confusion emphasizes the necessity for additional instruction on paronyms in educational settings. Such instruction could encompass strategies for distinguishing between closely related terms, highlighting their distinct meanings and contexts. In particular, targeted instruction on the spelling and use of paronyms in medical curricula is crucial to prevent a decrease in the overall quality of medical writing, where clarity and precision are paramount. Exploring paronyms not only enhances students' proficiency in medical language but also fosters their motivation and interest in learning a foreign language, as they engage with the intricacies of linguistic variation [20]. By engaging students in interactive learning experiences, educators can ensure that future healthcare professionals develop the necessary skills to communicate accurately and effectively in their medical writing [20, 21].

In curricular activities, instructors can incorporate dedicated lessons focusing on paronyms, providing students with theoretical knowledge, practical examples, and exercises to enhance their comprehension

Table 10. Paronymic tetrad in article titles from PubMed

No.	Paronymic tetrad	Examples from PubMed
1.	Right (n, adj) – rite (n) – write (v) – Write (eponym)	• “Write right” (Occup Health Nurs, 1970) • “Informed consent: right or rite?” (CA Cancer J Clin, 1979) • “Write right – or left: a practical approach to handwriting” (J Learn Disabil, 1983) • “Right or rite? Is “informed consent” really just a pipe dream?” (Can Doct, 1986) • “Nurses’ note. Six ways to write right” (Image J Nurs Sch, 1987) • “Write right” (Can Nurse, 1989) • “Write, right now: a guide for the novice nurse author” (Gastroenterol Nurs, 1989) • “Was Wright right?” (Science, 1991) • “Write right! (matching the manuscript with a journal)” (J Holist Nurs, 1994) • “Write right now: a commitment, an idea, a plan” (Nurs Staff Dev Insid, 1994) • “Informed consent and patient’s rights documents: a right, a rite, or a rewrite?” (Ethics Behav, 1999) • “Authorship: rite, right, or write of passage?” (J Nucl Med, 2000) • “Freud’s writing: his (w)rite of passage and its reverberations” (J Am Psychoanal Assoc, 2002) • “It’s more than a computer can deliver: gross anatomy – a rite of passage and a right to learning” (WMJ, 2003) • “Residency training: rite of passage, but is it right today?” (Obstet Gynecol Surv, 2004) • “Spatial differentiation for flower color in the desert annual <i>Linanthus parryae</i>: was Wright right?” (Evolution, 2007) • “Was Wright right? The canonical genetic code is an empirical example of an adaptive peak in nature; deviant genetic codes evolved using adaptive bridges” (J Mol Evol, 2010) • “Write right”: The GSAHS Clinical Documentation Project” (Health Inf Manag, 2010) • “Screening pelvic examinations: right, wrong, or rite?” (Ann Intern Med, 2014) • “Nurses: The right and rites to write, right?” (Creat Nurs, 2015) • “Write right, quite right: Orthography in Latin anatomical terms” (Clin Anat, 2018) • “Ceremonial purification: which rite is right in liver failure?” (Intensive Care Med, 2023) • “Wright was right: Leveraging old data and new methods to illustrate the critical role of epistasis in genetics and evolution” (Evolution, 2024)

Source: compiled by the authors of this study

and usage of these linguistic nuances in academic writing. Additionally, the inclusion of paronyms in language proficiency assessments and examinations can serve as a valuable tool for evaluating students’ understanding and mastery of these concepts. Educators can implement various tasks and activities to address this issue effectively, such as word matching exercises, where students pair paronyms with their correct definitions, contextual writing assignments to practice using paronyms accurately, and group discussions to explore the shades of meaning between similar terms [11]. By providing healthcare professionals with a deeper understanding of language subtleties, medical education can enhance clarity and precision in communication, which is indispensable for effective clinical practice and scholarly excellence within the field.

For EAP and medical writing instruction, educators may incorporate title analysis activities in which students examine published article titles for rhetorical and linguistic effects of paronym use. In addition, learners can engage in tasks that involve creating their own research titles using contextually appropriate paronyms, followed by peer feedback and justification of lexical choices. These exercises not only promote lexi-

cal awareness but also enhance students’ understanding of audience expectations and disciplinary tone. To deepen critical engagement, instructors might assign comparative exercises where students revise overly playful or ambiguous titles into more conventional forms – or vice versa – to explore the balance between creativity and clarity. Group discussions on the potential ethical or professional implications of humorous or pun-based titles can further prompt reflection on disciplinary norms. For assessment, instructors may use rubrics evaluating clarity, appropriateness, and communicative effectiveness of pun-based or paronymic titles. Such activities can encourage critical engagement with genre conventions in medical and academic writing.

Outside the classroom, extracurricular activities such as academic writing workshops, colloquia, or language clubs can offer additional opportunities for students to explore paronyms, exchange insights, and practice their skills in a collaborative and supportive environment. Furthermore, developing additional resources such as online glossaries specifically tailored to paronyms can facilitate self-directed learning and further reinforce students’ proficiency in this area. Tables 1-10 can serve as a valuable learning oppor-

tunity for language learners and writers to expand their vocabulary and deepen their understanding of word usage and context. Hence, by integrating this language aspect into both formal instruction and informal learning experiences, medical universities can empower future healthcare professionals with the linguistic competence necessary for effective communication in the medical field.

This study has several limitations. The dataset, drawn exclusively from PubMed-indexed articles, while comprehensive, may not fully represent the full spectrum of biomedical writing globally. Future studies could address this by incorporating additional databases or region-specific corpora to ensure a more diverse and representative sample. Additionally, the recognition and appreciation of paronyms and wordplay in medical titles may be influenced by cultural and linguistic background. Non-native English speakers may face greater difficulty in interpreting such titles, which could affect their comprehension or perception of the work's credibility. To mitigate this, future research might involve reader-response studies or cross-cultural surveys to better understand how different audiences interpret paronym-based titles. These steps would enhance the generalizability and applicability of the typology across broader linguistic and cultural contexts.

CONCLUSIONS

Thus, mastering the skills of creating engaging and compelling article titles is highly important for writers who aspire to publish internationally, as it is one of the factors that enhance the visibility and impact of their studies. The corpus of analyzed medical article titles demonstrated an extensive utilization of paronyms, serving as the foundation for various stylistic devices, such as paronomasia (puns), figurative language (metaphors), alliteration, antithesis, rhetorical questions, repetition, and rhyme. All these devices contribute to the dynamic tone and appealing rhythmic effect of the title structure, engage the reader's attention, evoke emotions and vivid imagery, highlight paradoxical issues, introduce ambiguity or multiple interpretations into the title, convey cultural connotations. The use of paronyms in medical article titles makes the reading more memorable, exerting an intriguing and thought-provoking impact and prompting the audience to think critically or interpret the title in different ways and to explore the article further.

Our findings have several implications for English language learners and early career writers, as they

emphasize the complexity of the English language, where subtle distinctions in pronunciation and spelling can have significant implications for communication and comprehension within specialized fields. First of all, the prevalence of homophones among the identified paronymic pairs demonstrates the importance of mastering this language phenomenon and understanding the context to avoid confusion in spoken communication. Secondly, our findings highlight the importance of precise spelling in distinguishing between paronyms. For language learners, this prioritizes the need for careful attention to linguistic variations to ensure clarity and precision of their writing.

Early career writers can adopt several strategies to avoid errors when using paronyms. Firstly, it is essential to conduct thorough proofreading to detect any instances of confusion between similar-sounding or -spelled words. Secondly, consulting reliable language references or dictionaries can provide clarity on the proper usage of specific paronyms, helping writers ensure accuracy in their discourse. Additionally, seeking feedback from peers or mentors can offer valuable insights into potential ambiguities or misunderstandings in the use of paronyms, allowing writers to make necessary adjustments for clarity.

The analysis of paronyms in article titles can be effectively integrated into EAP educational settings at universities and help language learners achieve several significant outcomes. By examining how paronyms are used in article titles, EAP students and early career writers can develop a deeper understanding of the subtle distinctions between paronymic lexemes and subsequently use them correctly, recognize the rhetorical devices and employ the pragmatic strategies in academic writing, thus enhancing their language proficiency and developing critical thinking skills.

Beyond its relevance to medical writers and educators, the typology and analysis presented in this study have potential interdisciplinary value. A range of fields, including pharmacy, nursing, public health, life sciences, psychology, and veterinary medicine, all of which are well represented in PubMed-indexed literature, share similar communicative goals and stylistic conventions. Researchers in these fields may benefit from a deeper understanding of how lexical choices, including paronym-based wordplay, affect audience engagement, clarity, and rhetorical impact. Thus, by offering a structured approach to evaluating and employing paronyms in article titles, this study provides a foundation for broader applications across the domain of biomedical communication.

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CONFLICT OF INTEREST

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Psychiatric examination of a person in criminal proceedings

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ABSTRACT

Aim: The purpose of the article is to determine the legal and tactical features of conducting a psychiatric examination of persons suspected of committing crimes.

Materials and Methods: In order to achieve the stated objective, the study employed a set of general scientific and specialized methods of cognition, including the system-structural method, the method of generalization, etc.

A survey was also conducted of 18 forensic psychiatric experts working in regional branches of state-specialized institutions of the Ministry of Health of Ukraine to clarify the features of the methods they use and the techniques of conducting examinations that are implemented during forensic psychiatric examinations.

Conclusions: In the context of enhancing the efficiency of law enforcement agencies and psychiatric institutions, it is essential to create an electronic register of expert opinions based on the results of psychiatric examinations.

KEY WORDS: forensic medical examination, crime, medical knowledge, criminal process, psychiatric knowledge

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INTRODUCTION

Situations in which individuals with mental illnesses and mental disorders commit criminal offenses are quite common in law enforcement practice [1-4]. This trend has emerged in many countries around the world because neurocognitive and mental disorders can lead to aggressive [5], inadequate [6], and sometimes even sadistic behavior in a person [7].

Various factors can contribute to the occurrence of behavioral and mental disorders. Recently, scientists have emphasized the need to study epilepsy as a phenomenon that may drive a person to commit socially dangerous acts [8, 9]. Scientists have also repeatedly highlighted the danger posed by elderly individuals, who often commit murders, attempted murders, and sexual crimes against children [10, 11].

At the same time, individuals with mental disorders often become victims of illegal actions. Scientists from Turkey note a relatively high rate of mental disorders among victims of sexual violence, which underscores the need for monitoring mental health in victims [12].

Thus, the presence of mental disorders is increasingly seen as a common characteristic of those involved in criminal proceedings. Our attention is drawn to the problem of crimes committed by individuals with mental disorders and illnesses, as these factors can

lead to legally significant consequences (depending on the person's procedural status). This issue will also influence the course of investigations and may shape certain aspects of the trial [13, 14]. Therefore, in this study, we will focus on the legal and tactical aspects of conducting psychiatric examinations of individuals who have committed crimes.

Achieving a balanced approach between the effectiveness of criminal procedural evidence and respect for human rights and freedoms requires clear guidelines, as our colleagues rightly point out. This should include considering the practices of the ECHR [15]. Consequently, to strengthen and modernize Ukraine's democratic legal system, it is important to deepen understanding of legislative shortcomings and practical approaches to appointing and conducting forensic psychiatric examinations in criminal cases. Given the topic of this study, the conclusions will be relevant for law enforcement practices in other countries.

AIM

The purpose of the article is to determine the legal and tactical features of conducting a psychiatric examination of persons suspected of committing crimes.

MATERIALS AND METHODS

The research methodology employs a systemic approach that aligns with the interdisciplinary nature of the phenomenon under investigation and facilitates the examination of the issue surrounding the use of psychiatric knowledge in criminal proceedings.

The historical method was applied to identify recent changes in mental health policy, the decline in the number of psychiatric beds, and the emergence of new trends in the regulation of criminal law.

The system-structural analysis was utilized to explore the connections between medical and psychiatric diagnoses of offenders and the forms of their procedural behavior. The logical method was employed to draw conclusions regarding the legal implications of failing to meet the criteria for appointing experts and to substantiate the need for legislative reforms.

The comparative legal method was used to examine forensic psychiatric practices across various countries (Ukraine, Iraq, Australia, Norway, France, the USA, and Bulgaria) to pinpoint shared issues and approaches concerning the assessment of offenders' sanity. An international comparison of practices related to registry maintenance, criteria for ordering expertise, and legal restrictions on methods such as hypnosis was conducted.

Content analysis of court decisions enabled the establishment of quantitative indicators for ordering psychiatric expertise and clarified problematic issues encountered during the stages of ordering and conducting such expertise.

Expert interviews with 18 forensic psychiatrists revealed issues in law enforcement, particularly the ordering of expertise without proper justification, the submission of incomplete materials, and legal inaccuracies in question formulation.

A statistical analysis was also performed using official data from the Ministry of Health of Ukraine regarding mental disorders in suspects who underwent expertise (including diagnoses such as alcoholism, post-traumatic stress disorder, emotionally unstable disorder, schizophrenia, etc.).

The dogmatic method was utilized to analyze the current criminal procedural legislation of Ukraine, specifically concerning the criteria for ordering expertise, the legal status of specialists and experts, and acceptable methods of evidence collection.

A survey conducted among specialists in the field of forensic psychiatry (through expert interviews) facilitated the identification of the actual reasons and challenges encountered during the appointment and execution of examinations and allowed for an evaluation of the practice of involving specialists in criminal proceedings.

The modeling and forecasting method was used to develop suggestions for enhancing current practices in conducting psychiatric examinations.

ETHICS

All sources used in this literature review are publicly available.

REVIEW AND DISCUSSION

International treaties guarantee the right of prosecutors, investigators, and judges to receive assistance from specialists in the fields of psychiatry and psychology to gather evidence [16]. At the same time, there are very different opportunities in the mental health field and psychiatric and psychological expertise throughout Europe [17]. Previous studies indicate an increase in criminal law measures and imprisonment in several Central and Eastern European countries [18]. Similar trends are observed in the Scandinavian countries [19]. At the same time, there has been a recent trend toward a decrease in the number of beds in psychiatric institutions; however, this is not due to a decline in the number of people with mental disorders and mental illnesses. Rather, it is a reorientation of mental health policies in European countries in connection with legal reforms, as indicated by 14 out of 18 psychiatrists we interviewed. However, it is unlikely that the number of socially dangerous acts committed by people with mental disorders and mental illnesses has significantly decreased.

In Australia, for example, 43 out of 435 people (9.9%) convicted between 1997 and 2005 in the state of Victoria were diagnosed with a mental illness. Scientists concluded that murderers with mental disorders were 3.19 times more likely to be motivated by revenge than offenders without disorders or those who did not have a diagnosis related to mental disorders. Scientists also found that the social danger of the latter lies in the fact that they were much more likely to kill members of their family and people they knew [20].

Researchers also indicate that 38.8% of people who committed murders showed a high level of problems with alcohol, and 30.8% had drug problems. At the same time, a large proportion of criminals who committed murders abused these substances during the year preceding the commission of the crime [21].

In Iraq, out of 70 cases of intentional murders analyzed, 38.6% of the murderers did not have mental illnesses, 17.1% had personality disorders, 32.9% had mental disorders, and 8.6% had neurotic disorders [22].

M. Bouthier and V. Mahé found that out of 79 French citizens who suffered from paranoia, which is a men-

tal disorder, and who were convicted of committing crimes, 30 people committed murders and attempted murders [23].

Lifetime psychotic symptoms were reported in 11% of US offenders, consistent with previous reports, including 18% of mass murderers who did not use firearms and 8% of those who did [24]. In Norwegian prisons, 60% of prisoners (2,857 prisoners as of 03.01.2024) had at least one form of mental disorder during the period from 2010 to 2019. There also appears to be an increase in the number of mentally ill offenders who commit serious crimes and are considered unaccountable. Similarly, in Bulgaria, the high number of people with severe mental disorders in prison is recognized as a challenge. Thus, cognitive disorders, mental illnesses, and signs of alcohol and substance abuse are typical characteristics of many individuals who commit violent crimes. The psychiatrists we interviewed also indicated such characteristics [25–28].

Therefore, the question arises regarding what problems occur during the conduct of psychiatric examinations of persons who have committed such crimes. According to our analysis, the following problems are the most legally significant:

1. The absence of a single, clearly defined criterion for the appointment of forensic psychiatric examinations, as indicated by 11 of the 18 psychiatrists we interviewed who are qualified as forensic experts. The criminal procedural legislation of Ukraine and many European countries provides that in cases where there is information raising doubts about a person's sanity or limited sanity, to determine their mental state, the investigator (or investigating judge) is obliged to order an examination. In Ukraine, such information is recognized as:

- 1) the presence of a mental disorder or mental illness in a person according to a medical document;

- 2) the person's behavior during or after the commission of a socially dangerous act was or is inadequate (clouding of consciousness, impaired perception, thinking, will, emotions, intelligence, memory, etc.) [29]. However, the psychiatrists we interviewed indicated that in 4 out of 10 cases, forensic psychiatric examinations are ordered by investigators for persons who do not have an officially established diagnosis related to mental illness or various types of addictions. This approach contradicts the norms of current legislation, as it violates the presumption of mental health. In addition, considering the first reason—the presence of a mental disorder or mental illness in a person according to a medical document—as a basis for ordering a forensic psychiatric examination, it should be noted that in Ukraine, there is no single register of mentally ill

patients, complicating the process of checking and obtaining data indicating that a person is registered with a psychiatrist. There are also no databases of forensic psychiatric examinations (registries), such as, for example, in Norway, which stores reports on almost all people accused of committing serious crimes in the country for whom psychiatric examinations have been conducted. Therefore, we believe that the practice mentioned above needs to be implemented in countries where it has not yet been initiated. This will allow the conclusions formulated to be used not only in scientific pursuits but also in law enforcement activities.

2. Failure to provide the psychiatrist with complete materials necessary for the examination. To simplify the process of establishing the presence of mental disorders or mental illnesses in a person, we analyzed the regulatory and directive documents of the Ministry of Health. We identified a list of those that may contain information raising doubts about the person's sanity. These include: a control card for the dispensary supervision of a patient with a mental disorder and a person who has mental disorders due to the use of psychoactive substances; a statistical coupon for a patient discharged from dispensary supervision of a psychoneurological/narcological institution; a statistical card for a patient who has left a psychiatric (narcological) hospital; a report of an inpatient, outpatient, in-person, and post-mortem forensic psychiatric examination; a report of a psychiatric examination of a convicted person; a registration journal of inpatient and outpatient forensic psychiatric examinations; a protocol of mandatory preliminary and periodic psychiatric examinations; a report of a psychiatric examination of a person who was in compulsory treatment; and a medical certificate for mandatory preliminary and periodic psychiatric examinations.

Particular attention should be paid to documents on the treatment of the suspect in connection with the suspect receiving head injuries, brain contusions, etc., as well as spinal injuries, and spinal cord lesions, which are currently being actively emphasized by scientists in their research [30]. This is explained by the fact that such injuries can subsequently provoke changes in anatomical and physiological processes in the human body and lead to disorders of mental activity or mental illness.

Another negative trend that has emerged relatively recently is the increase in the number of criminal offenses committed by individuals who use psychoactive substances. In 2022, 25,970 people were registered in dispensary groups for mental or behavioral disorders associated with the use of psychoactive substances. Of these, more than 75% (19,722 people) were treated on

an outpatient basis without hospitalization. From 2019 to 2022, there was a consistent trend toward an increase in the proportion of patients with opioid addiction among those undergoing treatment for disorders. In 2022, 74.4% of new patients in the dispensary group (i.e., those diagnosed with such a disorder for the first time) were addicted to opioids. This figure is the highest among all categories of psychoactive substances and shows a gradual increase from year to year. Meanwhile, the proportion of patients receiving assistance for polydrug addiction or cannabinoid use is gradually decreasing. This trend indicates a shift in the structure of drug addiction among psychiatric service patients. The data presented do not account for several regions currently under temporary occupation and pertain only to individuals who continue to permanently reside in the territory of Ukraine. These individuals are often referred for psychiatric examinations when they commit crimes. According to the Institute for Health Metrics and Evaluation at the University of Washington, Ukraine ranks 5th in the world for mortality from substance use disorders, 6th for disability from mental and behavioral disorders related to substance use, and 6th for disability from depressive disorders [31].

In addition, the methodological scheme of expert assessment of sources of information about the mental state of a person when conducting a forensic psychiatric examination should always be represented by the following documents:

1) Medical documentation: data from narcological, and psychiatric examinations, information about being on psychiatric/narcological registration; data from neurological examinations (signs of encephalopathy of intoxication, traumatic, vascular genesis, acute and chronic disorders of cerebral circulation, cerebral atherosclerosis with a description of intellectual-mnemonic and emotional disorders, state of consciousness, contact; signs of intoxication polyneuropathy, the presence of pathological reflexes, instability in the Romberg position, inaccuracy of the finger-nose test, etc.); data from somatic examinations, objective laboratory, and instrumental examination methods; in post-mortem psychiatric examinations - the cause of death from the conclusions of forensic medical experts, a medical certificate of death, a record of the cause of death.

2) Materials of criminal proceedings, as well as characteristics: official, working, from the place of residence, education; workbook, and other documents characterizing the sub-expert, statements, certificates, etc.

3) Testimony of victims, witnesses, suspects, including medical professionals; and uninterested persons in the

case, as well as interested participants in the case.

4) Results of a psychiatric/psychological/neurological examination of a person.

However, investigators do not usually follow such recommendations, so the expert must initiate a request for additional examination materials.

3. Raising legal issues before the expert. Most often, this concerns issues of sanity or limited sanity of a person and others.

4. Lack of an established approach to the need to order forensic psychiatric examinations for suspects in murders, terrorist acts, animal cruelty, and other sadistic and violent crimes.

There are several problematic issues, the cause of which is precisely the mistakes of psychiatrists who conduct psychiatric examinations. Such errors, in particular, include:

1. Conducting experiments and tests not provided for by the methods of conducting forensic psychiatric examinations. For example, the legislation of Ukraine does not regulate the participation of a psychotherapist in collecting evidence by immersing a participant in the criminal process—a patient—into a hypnotic sleep; however, such a practice is quite often implemented in the activities of doctors. Such facts actualize the task of making some changes to the criminal procedural legislation not only of Ukraine but also of the countries of the European Union, such as Poland, Slovakia, Hungary, and the Czech Republic. These medical and legal «amendments» should be aimed at more clearly formulating the definitions of the basic concepts and terms involved in or prohibiting the participation of a hypnotist during examinations aimed at obtaining evidence. It is important that, for example, the legislation of Georgia enshrines a ban on the use of hypnosis as a method of collecting evidence in criminal proceedings. Thus, the field of criminal justice requires constant updating, taking into account modern achievements in medicine and psychiatry.

2. The archaic nature of certain provisions that determine the procedure for conducting psychiatric examinations. The methods of conducting examinations should reflect only scientifically sound practices, the reliability of which can be verified and the application of which is not limited by the norms of national and international legislative acts.

3. The lack of practice in treating persons who, although not recognized as insane according to the results of the court case, were found to have mental disorders and mental illnesses. The results of the survey of psychiatrists emphasize the importance of early identification and treatment of people with

mental disorders in the general population, as this can reduce the possibility of criminal behavior. That is, the outlined need is to prevent recidivism among such persons. Both our and other studies evidence the fact that it is present. For example, in Finland, the recidivism rate 2015 was per 100,000 person-years. A total of 48 patients (9.6%) committed a violent crime. The median time to violent recidivism was 4.2 years. Factors associated with higher recidivism rates were male gender, the presence of a comorbid substance use disorder, and younger age at discharge [32].

Empirical court practice analysis demonstrates the lack of a comprehensive understanding by investigative bodies regarding the importance of conducting psychiatric examinations and the significance of the conclusions formulated by experts. First, this relates to the importance of clarifying the need for treatment of a person to prevent relapse among such individuals.

The grounds for ordering forensic psychiatric examinations also need to be reviewed and unified, including in several separate categories of cases (regarding suspects in murders, terrorist acts, animal cruelty, and other sadistic and violent crimes). Improvement of the methodology and methods of conducting psychiatric research is required. After all, a person's refusal to answer an expert's questions today serves as a basis for compensating for the vast array of other expert methods used to determine whether an individual has a mental disorder. In expert practice, this refusal necessitates an inpatient forensic psychiatric examination. Given that Ukraine has only one facility for inpatient assessments and a limited number of beds, this situation negatively impacts the adherence to the principle of reasonableness regarding the timelines of pre-trial investigations. Moreover, it effectively acts as a compulsory isolation measure, which, by its legal nature, restricts an individual's right to freedom.

Going beyond the outlined subject of the study, we believe that this article has significant value for further research into legal gaps in the legal system of Ukraine and methods of criminal procedure. After all, as scholars rightly point out, comparative legal research is particularly interesting and can contribute to the improvement of both local and global legal frameworks and the achievement of justice in an increasingly

interconnected world [33, 34]. Such knowledge from legal and judicial practice will aid the current study aimed at helping Ukraine create a reliable legal system that functions according to human rights and European constitutional values.

CONCLUSIONS

The high rate of crimes committed by persons with mental disorders, mental illnesses, and addictions to alcohol, drugs, and psychoactive substances indicates the importance of competent use of psychiatric knowledge during investigations. The effectiveness of the tasks solved by experts is determined by compliance with several legal principles and the correctness of the applied methods and techniques. At the same time, certain factors negatively affect the objectivity of the results obtained.

We have identified some problems that arise during psychiatric examinations: 1) due to legal gaps or shortcomings in current legislation; and 2) those allowed by experts conducting psychiatric examinations; 3) lack of approved research methods in conditions where the subject refuses to communicate and interact with the expert (committee of experts). Each of the identified problematic factors is thoroughly characterized. In the context of improving the quality of psychiatric examinations, it is important for the international community of practicing psychiatrists and lawyers to systematically review the methodology and methods of conducting psychiatric examinations, as well as the list of disorders and diseases that affect the human psyche.

In the context of enhancing the efficiency of law enforcement agencies and psychiatric institutions, it is essential to create an electronic register of expert opinions based on the results of psychiatric examinations. A proven practice that needs implementation is the introduction of a Unified Register of Persons with Mental Disorders. The system of institutions authorized to conduct inpatient psychiatric examinations in Ukraine must be expanded, along with the introduction of a procedure for the forced involvement of an individual in a psychiatric institution. Furthermore, it is vital to ensure the enforced treatment of such individuals, as well as those who commit criminal offenses while in a morbid mental state.

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Diagnostic evaluation, risk stratification, and peri-surgical management strategies in thyroid disorders

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ABSTRACT

Aim: To discuss the indications for elective thyroid surgery and peri-surgical clinical strategy in patients with thyroid diseases.

Materials and Methods: A comprehensive literature review was conducted from October 2024 to April 2025, analyzing relevant publications published between June 2005 and January 2024, sourced from PubMed, Scopus, Web of Science, and the Cochrane Library.

Thyroid disorders encompass various conditions, including Graves' disease, hyperthyroidism, and thyrotoxicosis. Hypothyroidism manifests as subclinical or overt. Surgical indications for thyroidectomy include symptomatic nodules, indeterminate/Bethesda III-V cytology, malignancy (Bethesda VI), or compressive symptoms. Ultrasound-guided Fine-Needle Aspiration (FNA), based on nodule size and EU-TIRADS risk stratification, is pivotal for evaluation. Advanced aids in risk assessment. Surgical complications (hemorrhage, hypoparathyroidism, recurrence laryngeal nerve injury) require prompt management. Molecular testing refines indeterminate cytology diagnoses. Thyroidectomy is contraindicated in anaplastic cancer and/or high surgical risk cases. Postoperative monitoring for hypocalcemia, voice changes, and bleeding is essential.

Conclusions: Resource constraints hinder widespread adoption of advanced thyroid diagnostics, creating challenges in optimal nodule management.

KEY WORDS: risk stratification, EU-TIRADS, thyroid disorders, elective thyroid surgery

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INTRODUCTION

Thyroid nodules are among the most frequently encountered abnormalities of the thyroid gland, documented clinically in roughly 1% of men and 5% of women worldwide. Despite their high occurrence, the majority of nodules do not harbor malignant potential, and only a small fraction - close to 5% - are confirmed to represent cancer. High-resolution ultrasonography has dramatically increased recognition of such lesion, with studies demonstrating their presence in as many as 68% of individuals undergoing imaging, particularly women and older patients.

Surgical management of thyroid disease is considered in spectrum of settings, ranging from benign to malignant conditions. Indications include nodular thyroid disease, hyperthyroidism, large obstructive goiters with or without substernal extension, and the various histologic types of thyroid cancer - most notably papillary and follicular carcinoma, but also medullary and anaplastic subtypes. In addition, thyroidectomy may be undertaken for diagnostic purposes in rare cases

such as primary thyroid lymphoma or in situations where the gland is affected by secondary deposits, most commonly metastases originating from renal cell or lung carcinoma.

Why do we need elective thyroid surgery:

Patients may be considered for thyroidectomy in several clinical contexts, encompassing both malignant and benign thyroid disease. Indications include differentiated thyroid carcinoma, toxic multinodular goiter, autonomously functioning adenomas, and goiters that lead to compressive manifestations such as dysphagia or airway compromise. Thyroidectomy may also be warranted in patients with Graves' disease who do not achieve adequate control with antithyroid medication or in whom medical therapy is contraindicated - for instance, women planning pregnancy [1].

AIM

In this review, we aim to discuss the clinical indications for elective thyroidectomy and to outline perioperative

strategies in the management of patients with thyroid disease.

MATERIALS AND METHODS

The review was performed in the period from 10.2024 to 04.2025. The materials for the literature review were publications on research from June 2005 to January 2024. Relevant articles were identified by two authors (OG, JK) searching PubMed's, Scopus, Web of Science databases, and Cochrane Library.

REVIEW AND DISCUSSION

DEFINITIONS IN THYROID DISEASE

Graves' disease represents an autoimmune condition in which antibodies aberrantly activate the thyrotropin (TSH) receptor on thyroid cells, driving hormone overproduction [2].

Hyperthyroidism denotes hormone excess due to intrinsic overactivity of thyroid gland, whereas thyrotoxicosis refers to the broader clinical state resulting from elevated circulating thyroid hormones regardless of source. Thus, while all hyperthyroidism results in thyrotoxicosis, the syndrome may also occur through other mechanisms, such as exogenous thyroid hormone administration (factitious thyrotoxicosis) or release of hormone from glandular destruction, as in subacute thyroiditis - neither of which represent true hyperthyroidism. The ability to reliably differentiate between these entities underpins both diagnostic accuracy and therapeutic decisions [2].

Hypothyroidism is defined as a deficiency of circulating thyroid hormones. It may present as subclinical hypothyroidism, where TSH is elevated but free thyroxine (FT4) and triiodothyronine (T3) remain within the reference range, or as overt hypothyroidism, characterized by reduced FT4 or total thyroxine (TT4) levels in conjunction with elevated TSH. This review focuses primarily on overt hypothyroidism [3].

Hypoparathyroidism is an uncommon endocrine disorder marked by hypocalcemia in the setting of inappropriately low or undetectable parathyroid hormone (PTH) concentrations [4].

Primary hyperparathyroidism, characterized by hypercalcemia accompanied by elevated or inappropriately normal PTH levels [5].

Secondary hyperparathyroidism most often arises in the context of chronic kidney disease, where disturbances in calcium-phosphate balance drive compensatory PTH secretion. In advanced stages, surgical intervention may become necessary as the only defin-

itive therapy, but it is reserved for clearly established indications [6].

Tertiary hyperparathyroidism represents the progression of long-standing secondary disease, in which the parathyroid glands acquire autonomy and continue to oversecrete PTH even after successful renal transplantation [7].

Voice assessment is central in thyroid disease. Patients with hoarseness frequently describe their voice as weak, rough, unstable, or report difficulties with pitch control. In otolaryngology, the term "dysphonia" broadly refers to any abnormality in vocal quality. Persistent hoarseness lasting more than two weeks should be regarded as an alarm symptom and investigated further, as it may indicate significant underlying disease.

Voice changes following thyroid surgery, including hoarseness, are frequently the result of recurrent laryngeal nerve (RLN) injury. Such deficits may be transient, but if they persist for more than six months, they are regarded as permanent [9].

Intraoperative bleeding remains another critical risk during thyroid surgery. Blood loss can vary considerably, but volumes exceeding 200 mL are considered clinically significant. The accumulation of a cervical hematoma may lead to tracheal compression and potentially life-threatening airway obstruction, requiring urgent surgical intervention [10].

Tetany, most commonly resulting from postoperative hypocalcemia, represents a further complication. It is characterized by neuromuscular irritability, with patients often reporting tingling, "pins and needles," or current-like sensations in the fingers, toes, or perioral region. In more severe cases, intermittent muscle spasms may occur [11].

Radiologically, thyroid nodules appear as discrete lesions separated from the surrounding parenchyma. Regular evaluation of nodular thyroid disease is necessary due to possibility of malignancy, toxicity or compressive symptoms [12].

Goiter refers to an abnormal enlargement of the thyroid gland, typically defined as a volume exceeding >18-20 ml in female and >25 ml in male. It can be classified as diffuse or nodular, and further divided into toxic (associated with thyrotoxic symptoms) or non-toxic forms. Globally, the leading cause of goiter remains iodine deficiency and in endemic areas prevalence may approach 25%, particularly among older populations. In these regions, many goiters remain clinically silent and do not require operative management. By contrast, in iodine-sufficient settings, multinodular goiter is more commonly associated with autoimmune thyroid conditions, including Hashimoto's thyroiditis and Graves' disease [1].

Surgical removal also provides a definitive histopathological diagnosis, particularly in nodules with uncertain malignant potential. Where available, molecular testing can further refine risk assessment. The extent of resection depends on disease distribution: lobectomy is usually sufficient for unilateral nodules, whereas near-total thyroidectomy may be warranted in multinodular cases. Even cytologically benign lesions may require surgery when large (>4 cm), symptomatic, or substernal in location, while toxic nodules - whether solitary or multinodular - also represent clear indications for operative management [2].

The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) established levels of histopathological findings in Fine-Needle Aspiration biopsy (FNA) and supported making a decision with treatment (surgical or non-surgical). I grade of the TBSRTC inform that FNA is nondiagnostic or unsatisfactory, II grade - the lesion is benign, III grade - atypia of undetermined significance (AUS), IV - follicular neoplasm or suspicious for a follicular neoplasm, V grade - suspicious of malignancy and VI grade - malignant [13,14].

EVALUATION AND MANAGEMENT OF THYROID NODULES

Most thyroid nodules do not require surgical intervention. However, non-functioning nodules exceeding 1 cm or those exhibiting suspicious sonographic features should be further evaluated, most often by fine-needle aspiration (FNA).

The American Thyroid Association highlights that the decision to perform FNA should be based on two major criteria: the nodule's size and the level of sonographic suspicion [1].

As part of the initial assessment, TSH measurement is recommended; if suppression is observed, thyroid scintigraphy should follow. In this setting, nodules larger than 1 cm with increased radionuclide uptake may warrant aspiration. Subsequent ultrasound-based risk stratification then refines the evaluation by linking morphological features with the likelihood of malignancy, thereby informing FNA indications [15].

ULTRASOUND AND ADVANCED IMAGING IN THYROID EVALUATION

Ultrasound serve as the primary imaging tool in the assessment of thyroid nodules. Its clinical utility includes:

Characterizing nodules with respect to their number, anatomical location, dimensions, and internal echotexture [16, 17].

Evaluating substernal extension and cervical anatomy [16, 17].

Assigning risk categories using standardized systems such as the European Thyroid Imaging and Reporting Data System [16, 17].

Detecting lymph nodes with suspicious features and guiding the choice of nodules most appropriate for FNA [16, 17].

Doppler ultrasonography provides valuable information on nodule vascularity and is particularly helpful in distinguishing solid components from cystic or necrotic areas. In addition, it guides the selection of appropriate sites for FNA and contributes to overall risk characterization [17].

Elastography has been introduced as a complementary technique to evaluate tissue stiffness, given that malignant nodules - most notably classical papillary thyroid carcinoma (PTC) - tend to be firmer than benign lesions. Despite this association, elastographic findings are not universally reliable across all thyroid malignancies and are therefore not formally included in the current risk stratification frameworks [17].

Contrast-enhanced ultrasound (CEUS) has demonstrated encouraging diagnostic accuracy, with some meta-analyses reporting high positive predictive values for malignancy. Nevertheless, the literature is inconsistent and susceptible to publication bias, and regulatory approval remains restricted to a limited number of countries. Where available, CEUS plays a particularly useful role in post-treatment monitoring, especially after thermal ablation, by enabling precise visualization of ablation margins and perfusion deficits [17].

Strain elastography assesses the relationship between applied compression and tissue deformation. By alternating probe pressure, elastographic maps can be generated and superimposed onto B-mode images, with stiffness represented through a color-coded scale [18].

Shear wave elastography (SWE), in contrast, provides quantitative data by creating elasticity maps and measuring stiffness in kilopascals. Nodules with stiffness values exceeding 65 kPa, or demonstrating a stiffness ratio above 3.7 when compared with adjacent normal thyroid tissue, should raise suspicion for malignancy [19].

Thyroidectomy, once regarded as the standard approach for goiter, has become a more selective intervention with the advent of advanced imaging techniques and effective non-surgical alternatives. In the setting of differentiated thyroid cancer, lobectomy is typically sufficient for microcarcinomas, whereas total thyroidectomy is considered in larger tumors (>1 cm), tall-cell histology, cases with extrathyroidal extension, bilateral disease, or lymphovascular invasion. Evaluation of suspicious cervical lymph nodes should include FNA [15].

Molecular analysis has emerged as an increasingly important adjunct in the evaluation of thyroid nodules

with indeterminate cytology, particularly those within Bethesda categories III to V. The most commonly investigated alterations involve mutations in BRAF and RAS, as well as rearrangements such as RET/PTC and PAX8/PPAR γ . The 2023 Bethesda update introduced a separate AUS category with nuclear features due to their higher malignancy risk. Given the false-negative rate of FNA (~4.36%), combining cytology with molecular testing and repeat aspiration enhances diagnostic accuracy.

Absolute contraindications to thyroidectomy are rare in clinical practice. Surgical eligibility should instead be weighed against factors such as patient age, comorbid conditions, and the generally indolent behavior of most thyroid cancers. In the setting of anaplastic carcinoma, surgery is usually discouraged unless a complete resection can be achieved with acceptable morbidity and in the absence of distant metastases. For outpatient thyroidectomy, certain scenarios are better regarded as relative limitations rather than strict contraindications - these include very large goiters, substernal extension, pronounced thyroiditis, or challenging surgical anatomy [15].

COMPLICATIONS OF THYROID SURGERY

Common complications following elective thyroidectomy include:

Hemorrhage: Although rare, it can be life-threatening due to airway compromise from hematoma. Immediate decompression is essential.

Hypoparathyroidism: Occurs in up to 33% of total thyroidectomies. Leads to hypocalcemia and may cause paresthesia, tetany, or seizures. Prophylactic calcium administration is often part of postoperative protocols.

Recurrent laryngeal nerve (RLN) injury: Leads to hoarseness, voice fatigue, or aspiration. Most cases are transient, but permanent paralysis occurs in less than 1%.

Superior laryngeal nerve injury: Affects voice pitch and quality. Incidence ranges widely (0–58%).

Infection: Reported in approximately 6% of cases.

Other complications: Esophageal or tracheal injury, Horner's syndrome, dysphagia, chyle leak [15].

Among the early complications of thyroidectomy, transient hypocalcemia is the most frequent, affecting roughly 20–30% of patients and recurrent laryngeal nerve (RLN) injury, which occurs in 5–11% of cases. Although bilateral RLN damage is exceedingly rare (<0.1%), its occurrence represents a critical event requiring immediate airway intervention.

Postoperative bleeding, reported in 0–6.5% of procedures, most often results from slipped ligatures or venous tears. Intraoperative hemostasis, maintenance of stable thyroid function, and the use of the Valsalva maneuver to identify occult bleeding are important preventive measures. Compressive hematomas need immediate surgical evacuation, in contrast to minor, non-compressive hematomas, which may be observed and treated conservatively.

Other postoperative issues include:

Dysphagia: Generally resolves with neck massage and conservative management.

Seroma or lymphatic leakage: Often resolve with aspiration.

Horner's syndrome: A rare complication due to sympathetic chain injury, usually self-limited.

Wound healing issues: More frequent in obese patients. Proper drainage and care improve outcomes.

Tracheal or esophageal injury: Rare, however may require stenting or conservative management.

Thyrotoxic storm: Rare today due to routine use of preoperative beta-blockers [20].

CONCLUSIONS

Regarding diagnostic approaches, advances in imaging techniques have greatly expanded diagnostic accuracy, yet their prohibitive cost can limit availability in developing countries and in healthcare systems with restricted resources. Likewise, while molecular testing may in the future refine management of Bethesda category III nodules and support decisions regarding elective thyroid surgery, the expense of such methods currently prevents their broad adoption in many settings. Diagnostic lobectomy offers the advantage of preserving thyroid function; however, it may still necessitate a second operation if malignancy is later identified, which increases patient risk - particularly in those with comorbidities that make repeated exposure to general anesthesia hazardous.

As for alternative management, watchful waiting combined with appropriate outpatient care can represent a reasonable strategy. Yet, systemic barriers, including procedural costs, shortages of trained personnel, and limited access to equipment, may hinder feasibility not only in low-income countries but also in some developed nations where healthcare systems are underfunded or inefficiently managed.

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Forced sterilization: the legal and historical aspects

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ABSTRACT

Aim: To analyze and summarize the legal, historical aspects of forced sterilization, to outline, systematize the issues related to violations, restrictions on the realization of the right to reproduction, assess their impact on human rights in modern conditions.

Materials and Methods: Theoretical basis for studying issue includes scientific publications and assessments by leading experts in the field, the conclusions of international non-governmental organizations. Practical basis for studying issue includes international regulations. The authors of the paper have also taken into account decisions of the European Court of Human Rights and analyzed Ukrainian judicial practice. The methodological basis of scientific research is general scientific, special scientific methods of cognition. Determinants in the study of this problem are the analysis, synthesis, generalization of the experience and legislative base regarding the legal regulation of human reproductive rights.

Conclusions: The prohibition of forced sterilization has come a long way, from a mere recommendation that states take measures to prevent forced sterilization to a legal obligation to criminalize such acts in national legislation. The prohibition of forced sterilization is an effective means of protecting reproductive rights, which are inalienable human rights. That is why for a person to realize his or her reproductive rights, they must be established not only at the international level but also at the level of national legislation.

KEY WORDS: human rights, reproductive rights, eugenics, right to health care, forced sterilization

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INTRODUCTION

Sterilization is a serious intervention in a person's reproductive health, as it affects one of the basic functions of the human body, affecting various aspects of a person's integrity, including their physical and mental well-being and emotional, spiritual and family life. Typically, such surgeries are based on cutting the ejaculatory duct (vasectomy) in men and tying the fallopian tubes in women, as they do not affect the sexual desire or personality of the operated persons (unlike, for example, castration).

Today, despite the growing recognition of negligent, abusive and degrading treatment of women during childbirth and other obstetric procedures in healthcare facilities, there is no global consensus on how these cases can be identified and measured [1].

The World Health Organization advocates the division of obstetric violence into seven distinct categories: physical interference (such as beating and slapping), non-consensual care (lack of informed consent to procedures), non-disclosure (sharing confidential information without consent), and undignified care (such as intentional humiliation, such as swearing and yelling at women), discrimination (usually based on

ethnicity, race, economic status, education, religion, or age), abandonment (leaving a woman alone during and/or after childbirth), and keeping in facilities (e.g., keeping a woman or baby in a clinic until the bill is paid) [2].

Various variations of non-consensual assistance, including during childbirth, include forced sterilization [3]. Such non-consensual actions are particularly common in low- and middle-income countries, where women are often not informed about the risks and reasons for interventions during childbirth and are not asked for consent for procedures to be performed during childbirth [4]. For example, Zishilo Dlodlu from South Africa was sterilized without her consent. It happened in 2011 in a hospital during her third delivery. Zishilo is one of many women who have been subjected to forced sterilization in the past 20 years in 38 countries around the world [5].

AIM

To analyze and summarize the legal and historical aspects of forced sterilization, to outline and systematize the issues related to violations and restrictions on the

realization of the right to reproduction, assess their impact on human rights in modern conditions.

MATERIALS AND METHODS

Theoretical basis for studying this issue includes scientific publications and assessments by leading experts in the field, the conclusions of international non-governmental organizations. Practical basis for studying this issue includes international regulations, including European Convention on Human Rights, Council of Europe Convention on preventing and combating violence against women and domestic violence and other. The authors of the paper have also taken into account decisions of the European Court of Human Rights and analyzed Ukrainian judicial practice. The methodological basis of scientific research is general scientific and special scientific methods of cognition. Determinants in the study of this problem are the analysis, synthesis and generalization of the experience and legislative base regarding the legal regulation of human reproductive rights.

The main methodology was the philosophical method, which includes a dialectical approach to scientific research. This made it possible to find the reasons for the existence of forced sterilization in historical retrospect, to determine their internal patterns. The transcendental method made it possible to determine the essence of human reproductive rights by revealing the subjective conditions of their formation. The hermeneutic method served as an auxiliary mechanism for explaining legal norms aimed at regulating the institution of human sterilization. The dialectical general scientific approach was applied in revealing the genesis of human reproductive rights and forced sterilization, in studying the legal position of the European Court of Human Rights on these issues, in understanding the peculiarities of the balance of interests of the individual and society in the implementation of human reproductive rights. The provisions of the current legislation regulating the sterilization process and the decisions of the European Court of Human Rights were analyzed using a systematic method, and the right to respect for the rights of the fourth generation in the human rights system was also considered. The method of ascending from the concrete to the abstract has determined the main objects of the legal protection of human reproductive rights. The sociological and legal group method of research has made it possible to clarify the social conditioning of the existence of forced sterilization in historical retrospect. Special scientific methods have acquired special importance in the research, in particular the method of interpretation of legal norms. It has been used to study

the content of regulatory legal acts regulating the issue of the institution of sterilization.

ETHICS

This review article is based on an analysis of publicly available scientific data published in peer-reviewed journals, clinical guidelines and databases. No patient-identifying data was used during the work, nor was there a need to obtain approval from an ethics committee, as the study did not include new clinical interventions or initial collection of patient information.

REVIEW AND DISCUSSION

The legal regulation of forced sterilization has gone through several stages of evolution: the legalization of forced sterilization at the national level, the formation of an international discourse and the gradual condemnation of this practice; its prohibition; and the enshrining of the prohibition in international legal acts.

Scientists consider forced sterilization from a medical, social, historical, political, gender, psychological, international and national legal perspective.

Meghan A. in her article highlights the problem of physical and psychological violence against women in medical institutions [1]. The report by Bowser D. provides evidence of violence, inadequate information, humiliation and physical abuse of women in healthcare facilities [2]. It's noted in the research of Castro R. that a significant proportion of women experience violence during childbirth in Mexico [3]. Martín-Badia et al. believe that obstetric violence contradicts basic bioethical principles [6-13]. These researches don't explicitly mention forced sterilization, but the violation of women's rights in these cases creates conditions for sterilizations without the consent of the patients.

A large group of articles and sources include analyses of the national experience of legal regulation of forced sterilization. In particular, in her article, Avila V. directly analyses cases of sterilization of women in California prisons [14]. Lira, N. in her article examined cases of forced sterilization of Mexican Americans in California and made a legal analysis of this [15-26]. Dyer, O. analyses the historical experience of legal regulation of sterilization in Japan [15]. The article by Zampas highlights the problem of forced and coerced sterilization of women in Europe as a violation of human rights guaranteed by international treaties [27]. The research conducted by the American University Washington College of Law analyses cases of forced sterilization in Peru as crimes against humanity and their legal consequences [28]. Reilly, P. R. in his work studied the legislation and court

decisions (Buck v. Bell case) that regulated forced sterilization in the United States [29]. Tsuji, Y. conducted a research of legislation and legal practice in Japan on forced sterilization and abortion [30].

The importance of international recognition of the fact that forced sterilization of women is a fundamental violation of the prohibition of discrimination is an important step in ensuring the cessation of this practice was argued in the work of Patel, P [31]. Nikolić, I. in his article analyses international experience in the legal regulation of forced sterilization of women, drawing attention to the need for reforms in this area [32].

Obstetric violence is still too invisible, and the word “violence” in this phrase and formulation is unacceptable, and difficult to define and describe, as it is a subjective experience. Obstetric violence has been analyzed from a legal, sociological and clinical perspective, but the bioethical aspect must also be considered as it violates basic bioethical principles (nonmaleficence, beneficence, autonomy, justice, vulnerability, dignity and fairness). In addition, whether it is called violence or not, it is ethically important that as long as women have such a negative experience during pregnancy and childbirth, obstetric care is not in line with the principle of humanism [6]. It has been repeatedly emphasized that in the modern conditions of the development of civilization, the introduction of the principle of humanism in all spheres of social life, restrictions on human rights must be reasonable and proportionate [7].

Although for a long time, the international community did not recognize the problem of forced sterilization, with the development of theoretical studies and recognition of the reproductive rights of individuals as part of the general right to health at the international level, the discussion of the prohibition of forced sterilization began.

Thus, in 1968, a proclamation was adopted in Tehran, which stated that parents have the basic human right ‘to determine freely and responsibly the number and spacing of their children’ [33]. At the same time, the document didn’t contain any norms that would regulate the mechanisms for protecting this right and an explicit prohibition on forced sterilization. This resulted in an ambiguous interpretation of this norm by countries and the introduction of forced sterilization at the state level [34]. This proves the need for further development of the international legal discourse and regulatory consolidation of the protection of this right.

In Cairo in 1994, the Programme of Action of the International Conference on Population and Development enshrined reproductive human rights [35]. According to the document, they include the human right to

decide freely and responsibly on the number, spacing and timing of children, as well as the right to access information and means to exercise this right. They also include the right of all people to make decisions about reproduction free from discrimination, coercion and violence. Although we do not see an explicit prohibition on forced sterilization in these provisions, the principles enshrined effectively preclude the practice. The programmer has influenced the policies of many states that have implemented the principles of voluntary and informed choice in contraception and sterilization in their legislation. For example, Albania, Benin and Chad have adopted laws guaranteeing the right to voluntary sterilization and other reproductive services in line with the ICPD principles [36].

In 1998, the Rome Statute of the International Criminal Court was supplemented by a new article that classified forced sterilization as a crime against humanity (Article 7) [37]. The act states that “Sterilization was forcibly committed as part of a widespread or systematic attack directed against any civilian population, with knowledge of the attack”. In 2021, the ICC found Dominic Ongwen guilty of crimes against humanity and war crimes, including forced sterilization. This was the first time that the ICC issued a judgment that included forced sterilization as a crime against humanity under Article 7(1)(g) of the Rome Statute [38].

In 2002, the Council of Europe adopted the Istanbul Convention (Council of Europe Convention on preventing and combating violence against women and domestic violence) [8]. This is the first legally binding document in Europe on the prohibition of violence against women and domestic violence, it prohibits forced sterilization in Article 39. This article criminalized the intentional conduct of a person to perform a surgical intervention, the purpose or effect of which is to terminate a woman’s ability to reproduce naturally without her prior and informed consent or understanding of the procedure. States Parties undertake to take the necessary legislative or other measures to ensure the criminalization of these intentional acts. To implement this, states must adapt their legislation by the requirements of the Convention, ensuring effective investigation and punishment of such crimes. The implementation of the Istanbul Convention, in particular Article 39, is monitored by the Group of Experts on the Elimination of Violence against Women and Domestic Violence (GREVIO). The body monitors the implementation of the Convention’s provisions in the national legislation of the States Parties [8].

In 2005, the Universal Declaration on Bioethics and Human Rights (UNESCO) was adopted, which stipulates that any preventive, diagnostic and therapeutic medical

intervention should be carried out only with the prior, free and informed consent of the person concerned, based on adequate information [35]. Although no article in the Convention explicitly prohibits forced sterilization, the enshrining of the above rights essentially prohibits forced sterilization.

In 2006, The UN Convention on the Rights of Persons with Disabilities (CRPD) enshrined provisions on the rights of persons with disabilities to bodily integrity, the right to parenting, the right to voluntary, and informed consent to medical procedures and reproductive autonomy [39]. A person's right to make decisions should not be overridden by the decisions of a third party. Although there is no article in the Convention that explicitly prohibits forced sterilization, the enshrining of the above rights essentially prohibits forced sterilization. The UN Committee on the Rights of Persons with Disabilities in its General Comments explicitly recognizes forced sterilization as a violation of the rights under the CRPD [40].

In 2017, significant changes were introduced to the legislation regulating the sterilization of individuals in Ukraine, specifically the criminalization of forced sterilization of incapacitated persons, even with the consent of their guardians.

According to the Law of Ukraine "Fundamentals of the Legislation of Ukraine on Health Care", informed consent is a prerequisite for any medical procedure, including sterilization" [40]. Part 4 of Article 134 of the Criminal Code of Ukraine [41] also provides for criminal liability in the form of restraint of liberty for up to five years with or without deprivation of the right to hold certain positions or engage in certain activities for up to three years for forcing sterilization without the voluntary consent of the victim, and part 5 provides for another five years of imprisonment for forcing sterilization without the voluntary consent of the victim if it caused the victim's death or other serious consequences. The provisions of this article are the implementation of Ukraine's obligations under the Istanbul Convention and the CRPD. However, the practical application of this provision in Ukraine is extremely rare, as evidenced by the small number of sentences passed under Article 134 of the CC [42]. This is due to the difficulty of proving coercion and the low level of awareness of victims of their rights.

An analysis of court decisions in Ukraine, particularly from the Unified State Register of Court Decisions, shows that there have been no charges or court rulings under Part 4 of Article 134. This indicates the absence of documented forced sterilization cases in Ukraine since the criminalization of such acts. In our view, this may be explained by two reasons.

Firstly, Part 4 of Article 134 of the Criminal Code of Ukraine (CCU) was introduced on 6 January 2017. Prior to that, sterilization of individuals had been qualified as grievous bodily harm, and the punishment had been determined under Articles 121, 128, or 140 of the CCU, depending on the form of guilt. The introduction of Part 4 of Article 134 led to legal conflicts with existing provisions, especially with Article 121, which carries a more severe sanction. This has complicated the legal classification of acts related to forced sterilization.

Secondly, the issue is rooted in the historical absence of legal regulation concerning forced sterilization in the Ukrainian SSR, as well as the official ban on eugenics and genetics in the 1930s. In the USSR, there was no centralized campaign similar to the American laws on forced sterilization between 1907 and the 1960s. Furthermore, no specific law prohibited or regulated forced sterilization.

Forced sterilization has historically been used as a tool of eugenics and associated with Nazi Germany [5]. According to the Law for the Prevention of Hereditarily Diseased Offspring of 1933 allowed for the forced sterilization of any citizen who, according to the opinion of the 'Court of Genetic Health', suffered from a list of genetic disorders.

However, Germany is not the only country that implemented such a policy through various social programs. During 1907 and 1931, countries such as Norway, Denmark, Finland, the United States, Estonia, the Free City of Danzig, Switzerland, Canada, and Japan passed laws allowing for forced sterilization. This practice was mainly implemented about people with mental illness and intellectual disabilities. In Europe, Denmark was the first country to adopt a law allowing forced sterilization of persons with mental disorders or hereditary diseases in 1925 [43].

Negative eugenics, which is aimed at preventing the reproduction of people with "undesirable" characteristics for a particular society at a certain stage of its existence, was part of the policy in the United States of America and Scandinavian countries.

In 1907, Indiana was the first state to legalize involuntary sterilization. Chapter 215 was intended to prevent the reproduction of persons who were 'proven felons, idiots, imbeciles, and rapists' [44]. The law empowered the governors of the institutions where such persons were held to appoint a committee of experts consisting of two physicians to examine the mental condition of such prisoners [45]. In 1909, the governor suspended the law, and in 1921, the law was overturned by the Indiana Supreme Court. And in 1927, a new law was passed that allowed forced sterilization. Before it was repealed in 1974, more than 2,300 citizens of the state

were forcibly sterilized [46]. According to the North Carolina law; people with an IQ below 70 were subjected to forced sterilization [47].

In 1924, the court case *Buck v. Bell* was heard to test the constitutionality of the new law on forced sterilization. As a result of the proceedings, the US Supreme Court upheld the law and recognized sterilization as legal with a court decision that is known for the quote: 'Three generations of imbeciles is enough [47]. This had immediate consequences: over the next few years, about ten states passed sterilization laws.

Sterilization never became widespread in American prisons, in part because of federal court rulings in Iowa and Nevada that found it to be a violation of the Eighth Amendment's prohibition against cruel and unusual punishment [48]. But between 1907 and 1939, some 30 states passed laws that gave the heads of state institutions for the mentally disabled the right to sterilize those they deemed unfit to be parents.

In 1942, the US Supreme Court struck down an Oklahoma law that allowed the forced sterilization of certain 'repeat offenders', but refused to revisit or limit the precedent of *Buck v. Bell* [49].

After World War II, most state eugenic sterilization programs in the United States gradually ceased to exist.

The province of Alberta in Canada passed a sterilization law in 1928. Over the next 60 years, 2834 people were sterilized in Canada [50].

For example, the eugenic policy of forced sterilization in Finland in 1935-1970 is described by M. Matila in the article "Regional Differences in Finland's Eugenic Forced Sterilization Policy and The Influence of Actors 1935-1970" [10]. We also find similar examples in the history of Sweden. In the 1930s, the country implemented the idea of racial biology, hygiene, and the "improvement" of society through forced sterilization. In 1934, the Riksdag passed the Law on Forced Sterilization [11], which was in effect until 1976. The state sterilized various groups of people, such as people with mental disabilities, women who applied for maternity benefits (child benefits) but were found to have too many children, and women in specialized institutions (places of captivity) [12]. After the repeal of the law that allowed this type of forced sterilization, in 2000 the government publicly apologized and paid financial compensation to those who had been forcibly sterilized.

This policy of the countries was aimed at building an "ideal" society, countries populated by "ideal" people, healthy, strong and economically profitable, and for this, it is necessary to "cleanse" the gene pool of nations, which is possible through forced sterilization.

The second stage of the forced sterilization procedure in Sweden was implemented in 1972 based on the

Act on the Determination of Human Sex in Special Cases, which defined the conditions under which trans people have the right to change their sex. Until 2013, sterilization was the main requirement for transition operations and change of "official" sex. Transgender people were also not allowed to retain any germ cells, so they had to choose between taking on a new gender or becoming a biological parent [13]. Since January 2013, forced sterilization can no longer be used for sex reassignment in Sweden, as it is contrary to the Swedish Constitution and the European Convention and is discriminatory. After the Riksdag abolished the requirement for forced sterilization in the legal text, about 160 people filed a claim for compensation for forced sterilization to the Chancellor of Justice.

The article "Controlling Reproduction and Disrupting Family Formation: California women's Prisons and the Violent Legacy of Eugenics" by A. Vrindevani and J. Ellis [14] is devoted to forced sterilization in California prisons over the past two decades. The authors emphasize that prisons in the United States serve as a place and embodiment of gender and racial state violence. The US incarcerates more people than any other country, both in number and per capita. Women's prisons account for 10% of the total prison population, but women's prisons remain under-researched, and the violence that occurs in women's prisons is rampant, widespread, and racially and gender-specific. The article emphasizes that eugenics policies were popularized and promoted in the United States and around the world in the 20th century. The height of the eugenics era was marked by the forced sterilization of "deviant" individuals, but such policies sometimes go beyond surgical interventions. The hierarchical, racialized social stratification of "deviant" individuals in prisons is driven by eugenic logic, policy, and practice and is inconsistent with reproductive justice.

Under the Eugenics Protection Act of 1948, about 16,500 people were forcibly sterilized in Japan, some as young as 9 years old. Another approximately 8,500 were sterilized after they or their parents gave their consent, which was obtained under heavy pressure from the state. About 70% were girls. The purpose of forced sterilization and the adoption of such a law is to prevent the birth of defective offspring from a eugenic point of view [15].

Recent studies show that the procedure of forced sterilization is not exclusively in history books and is widely used in the 21st century. For example, a report by Global Public Health describes the case of four Indigenous women in Canada who claimed to have been forced to undergo sterilization between 2005 and 2010. In

Japan, sterilization is one of the mandatory conditions of sex reassignment surgery for transgender people. If they refuse sterilization, they are refused surgery [5].

"I would never have agreed to sterilization. What they did to me was very cruel. Now I feel like a useless thing. I am dead," says a woman who was sterilized in a South African hospital. Hospital records show that she signed her consent for sterilization [16]. However, the woman herself denies that she gave consent and believes that she was forcibly sterilized, in particular because she is and HIV-positive. The United Nations Special Rapporteur on the right to health, Dr. Tlaleng Mofokeng, notes that women are simply forced to sign consent forms for sterilization during childbirth, when they are particularly vulnerable, without explaining what exactly they are signing [5].

In general, there is evidence of a widespread lack of processes for informing patients or obtaining their consent for sterilization procedures during childbirth. Respondents from Latin America, Africa and, Eastern Europe confirmed the absence of routine patient information and obtaining consent for obstetric intervention [17].

Therefore, access to information is crucial for patients to give their consent to medical procedures and treatments. Numerous cases have been documented around the world where women have been routinely or systematically denied their right to informed consent before sterilization [18]. To be considered legal and valid, consent to sterilization must be given freely and voluntarily, without threats or inducements, after the patient has been informed about the risks and benefits of the procedure, and after alternative counseling about other forms of contraception [19].

Forced sterilization often targets vulnerable populations, especially women from marginalized sections of society, based on discriminatory beliefs and policies about who should have children and who should not. Racial and ethnic minorities, indigenous women, women with intellectual or mental disabilities, transgender individuals, and women living with HIV are particularly vulnerable to coercion of sterilization [20].

This is why there are calls today for states to reform informed consent laws to protect the autonomy of decision-making regarding sterilization procedures, especially for marginalized groups, including persons with disabilities and adolescents, and to ensure that laws do not inherently coerce individuals to undergo forced sterilization.

Forced sterilization has also been the subject of consideration by the European Court of Human Rights. The factual circumstances of the case concerned the testimony of Gypsy women in the Czech Republic,

Hungary and Slovakia who, according to their testimony, were forced to agree to sterilization during childbirth.

In the case "V.C. v. Slovakia" [21], the applicant, a Roma woman of ethnic origin, was sterilized immediately after the birth of her second child. She signed a consent to sterilization during childbirth after being informed that if she had a third child, she or the child would die. After the sterilization, she was ostracized from Roma society and soon divorced her husband. Infertility was the main reason for the divorce. In its decision, the European Court of Human Rights stated that the applicant must have experienced humiliation, suffering and a sense of inferiority in connection with the sterilization.

The European Court of Human Rights recognized a violation of Article 8 of the Convention for the Protection of Human Rights and Fundamental Freedoms [22] (the right to respect for private and family life) due to the existence of insufficient legal guarantees that could ensure special attention to reproductive health of women from the Roma community. In general, the forced sterilization of Roma women is a large-scale problem. The European Court of Human Rights has repeatedly considered cases in which this issue was raised, in particular, in "K.H. and others v. Slovakia" [23], and "V.C. v. Slovakia" [24]. In all these cases, the Court stated a violation of Article 8 of the Convention on the Protection of Human Rights and Fundamental Freedoms. We see that the decisions of the European Court of Human Rights play an important role in the process of enshrining reproductive rights in national legislation. The perfection of legislation, which regulates any sphere of activity, is an important condition for the effective performance by the state of its functions [25].

As for the domestic investigation of cases of forced sterilization of Roma in Slovakia, the investigation was launched in 2003, suspended three times, but reopened after complaints to the Constitutional Court, which found the investigation ineffective. The UN Committee on the Elimination of Discrimination against Women recommended that Slovakia take all necessary measures to ensure fair consideration of the complaints submitted by Roma women regarding their forced sterilization and to provide victims of such practices with effective legal remedies [26].

The Czech authorities also acknowledged the fact that there had been cases of sterilization of Roma women in the past, but they do not believe that such actions were carried out "for reasons of racial or national prejudice." For example, Iveta Cherveniakova was illegally sterilized without her consent in 1997 after the birth of her second daughter by cesarean section. In November, the Supreme Court overturned a 2007

decision by the Regional Court, according to which the city hospital had to pay €20,460 in compensation and apologize for violating her rights. The decision was overturned because the three-year statute of limitations had expired, and only an apology was required from the hospital [26].

The British TV channel BBC One broadcasted secret footage of the use of forced sterilization of young people with severe mental and physical disabilities, disabled people in several social care homes. The National Human Rights Defender (Ombudsman) made unannounced visits to psychiatric institutions. During the visits, the facts of forced sterilization in geriatric psychiatric departments were confirmed [26].

In general, the forced sterilization of certain categories of people often became part of population policy not only in totalitarian or authoritarian regimes. In India, women were offered sterilization to overcome population growth. Poor illiterate women were rushed through the formal consent procedure. They were asked to leave a fingerprint without reading the contents of the form or fully explaining the procedure [27]. In connection with this situation, some scholars point to the need to recognize sterilization as forced not only when this procedure is carried out without the person's consent, but also when the person has not been explained the nature and consequences of the sterilization. Failure to provide individuals with complete information

about sterilization didn't formally indicate physical or psychological coercion, but essentially limited freedom of choice [28].

CONCLUSIONS

The prohibition of forced sterilization has come a long way, from a mere recommendation that states take measures to prevent forced sterilization to a legal obligation to criminalize such acts in national legislation. Although there are now states where there are cases of forced sterilization, and in some even as part of demographic policy, the world community has defined these actions as a crime against humanity. Forced sterilization interferes with the very essence of human reproductive rights, the right to choose how to dispose of one's body. The ratification of the Istanbul Convention by modern countries is a step towards solving the problem of ensuring the reproductive rights of citizens of modern countries.

The prohibition of forced sterilization is an effective means of protecting reproductive rights, which are inalienable human rights. That is why for a person to realize his or her reproductive rights, they must be established not only at the international level but also at the level of national legislation. By enshrining a direct prohibition in the legislation of a particular state, its citizens can be sure that their rights will not be violated.

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A Full list of literature sources is available from the author

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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Postgraduate medical education in Ukraine: Addressing modern challenges

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ABSTRACT

Aim: To synthesize the principles of distance learning for medical professionals based on a review of the literature and the authors' own experience, and to identify optimal algorithms and tools for the online training of physicians during the war in Ukraine.

Materials and Methods: The authors' experience in delivering distance education to physicians was systematized and summarized. The literature analysis was conducted using PubMed, Google Scholar, Web of Science, and Scopus databases, regulatory legal acts and practical experience of training physicians in wartime conditions. The newest publications up to 5 years old or the most thorough publications that vividly described the essence of the topic were chose. When searching for information on the features of the current educational platforms used in PGME we used the following combinations of keywords: "distance education", "postgraduate medical education", "modern challenges", "war in Ukraine". 32 sources were selected that fully corresponded to the results of the request.

Conclusions: Modern technologies have significantly transformed the system of postgraduate medical education (PGME). The challenges of war, pandemic, and other global crises have underscored the necessity for the widespread and effective use of digital tools. Analysis indicates that distance learning technologies represent a vital solution for maintaining the quality of PGME. However, their successful implementation requires addressing technical, organizational, and methodological challenges. A comprehensive approach is essential, involving the development of new standards for distance learning, upgrades to technical infrastructure, and the active collaboration of all stakeholders. Utilizing widely adopted, reliable, and accessible information and communication technologies (ICT) in the pedagogical process enables the successful overcoming of contemporary challenges in medical education. The rapid adaptation of both instructors and medical trainees to new modes of knowledge acquisition helps maintain high teaching standards, ensures stability and continuity of training, and facilitates effective feedback.

KEY WORDS: distance education, war in Ukraine, postgraduate medical education, modern challenges

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INTRODUCTION

The relevance of this study is driven by profound changes in Ukraine's healthcare system, resulting from ongoing reforms, the COVID-19 pandemic, and, most significantly, the war. These factors necessitate corresponding adaptations in postgraduate medical education (PGME). Current realities require medical professionals to rapidly adapt to new conditions and acquire essential knowledge and skills, including emergency response, patient care in conflict zones, and clinical management in resource-limited settings.

PGME is crucial for ensuring the professional development of healthcare workers, enhancing the quality of medical services, and enabling adaptation to the rapidly evolving healthcare landscape, especially during crises [1,2]. The challenges facing PGME in Ukraine stem from both systemic issues within the healthcare sector and external factors, including global advancements in medical science and educational technology [3].

Moreover, the military conflict has profoundly shaped the evolution of PGME in Ukraine. Wartime conditions introduce distinct challenges, such as a shortage of qualified instructors in affected areas, disrupted access to educational materials, and the critical need for flexible training solutions. In response, the expansion of distance learning technologies has become imperative [4].

This situation underscores the urgent need to modernize the PGME system by integrating new educational standards that leverage digital technologies and flexible learning formats [5].

AIM

To synthesize the principles of distance learning for medical professionals based on a review of the literature and the authors' own experience, and to identify optimal algorithms and tools for the online training of physicians during the war in Ukraine.

MATERIALS AND METHODS

The authors' experience in delivering distance education to physicians was systematized and summarized. The literature analysis was conducted using PubMed, Google Scholar, Web of Science, and Scopus databases, regulatory legal acts and practical experience of training physicians in wartime conditions. The newest publications up to 5 years old or the most thorough publications that vividly described the essence of the topic were chose. When searching for information on the features of the current educational platforms used in PGME we used the following combinations of keywords: "distance education", "postgraduate medical education", "modern challenges", "war in Ukraine". 32 sources were selected that fully corresponded to the results of the request.

ETHICS

This review article is based on an analysis of publicly available scientific data published in peer-reviewed journals, clinical guidelines and databases. No patient-identifying data was used during the work, nor was there a need to obtain approval from an ethics committee, as the study did not include new clinical interventions or initial collection of patient information.

REVIEW AND DISCUSSION

Postgraduate medical education in contemporary Ukraine faces multiple challenges driven by socio-economic, political, and technological changes. These challenges necessitate the adaptation of the medical training system to meet new demands [6, 7].

The key challenges are as follows:

TRANSFORMATIONS IN HEALTHCARE

Healthcare Reform: The transition to a new model of medical service financing, exemplified by the National Health Service of Ukraine, demands that medical professionals acquire new competencies in management, communication, and strategic planning [8].

Workforce Shortage: The emigration of physicians to EU countries has led to a significant deficit of qualified specialists in Ukraine [9].

Rising Burden of Chronic Diseases: There is a growing need to train specialists in managing aging populations and the increasing prevalence of non-communicable diseases [10].

ACCESS TO MODERN KNOWLEDGE

Outdated Educational Standards: Curricula often lag behind international benchmarks, necessitating

alignment with current global recommendations and clinical protocols [11].

Limited Access to Up-to-Date Resources: Many physicians lack access to the latest scientific publications, clinical guidelines, and modern educational platforms.

DIGITALIZATION OF EDUCATION

Online Learning: While the COVID-19 pandemic accelerated the adoption of distance education, limitations in technical infrastructure and insufficient faculty training often hinder the effective implementation of digital technologies [12].

Technological Literacy: Physicians must be trained to work with electronic medical records, telemedicine systems, and other digital tools essential for modern healthcare.

FINANCIAL BARRIERS

High Cost of Education: Many advanced training courses, particularly those aligned with international standards, are financially inaccessible for a significant number of physicians.

Insufficient Funding for Educational Initiatives: Public investment in medical education remains limited, restricting opportunities for professional development.

ETHICAL AND PROFESSIONAL CHALLENGES

Communication Skills: Healthcare reforms have heightened the need for medical professionals to develop strong communication skills for effective interaction with patients and colleagues.

Evidence-Based Medicine: Integrating evidence-based practices into daily clinical work remains a challenge.

Medical Errors: Legal and ethical aspects of medical errors receive insufficient attention, necessitating a greater focus on accountability and patient safety.

METHODOLOGICAL ASPECTS

Inadequate adaptation of training materials for distance learning, with limited use of interactive teaching methods.

A need for new approaches to assessing the knowledge and skills of medical professionals.

Challenges in organizing practical training in an online environment.

IMPACT OF THE WAR

Military operations in Ukraine present severe additional challenges to the healthcare system:

Staffing Shortages: The migration of healthcare workers to safer regions or abroad, coupled with combat casualties, has drastically reduced the availability of qualified specialists.

Increased Healthcare Workload: A surge in patients with combat trauma, stress-related disorders, and complications from chronic illnesses demands a highly skilled healthcare workforce.

Adapting to New Work Environments: There is an urgent need to rapidly adopt care protocols suited for resource-limited and war-torn settings.

Infrastructure Damage: The destruction of medical and educational facilities necessitates the development of alternative learning methods, such as distance and mobile learning platforms.

Security Threats: Students and faculty in conflict zones face heightened security risks.

Need for Specialized Training: Doctors, rehabilitation specialists [6], and psychologists require additional training to meet the specific demands of wartime healthcare.

Psychological Pressure: Continuous stress and the high risk of professional burnout among healthcare workers necessitate the inclusion of psychological support programs in PGME. This psychological burden on both educators and learners reduces training effectiveness.

Training in Resource-Limited Conditions: There is a critical need for training that is applicable in environments where access to resources is severely restricted.

The ongoing military conflict remains the most significant factor impacting postgraduate medical education in Ukraine [6,7]. These challenges require a comprehensive adaptation of the medical education and training system to meet new realities [13,14].

POSSIBLE SOLUTIONS

Develop a national strategy for PGME that addresses the specific challenges of wartime.

Implement hybrid training formats that integrate online learning with in-person practical sessions.

Create digital simulators and virtual laboratories to enhance hands-on training opportunities.

Enhance digital literacy among instructors and trainees through targeted educational programs.

Strengthen cybersecurity measures for online education platforms to protect sensitive data.

Support the psychological well-being of educators and learners through specialized online resources and mental health programs.

Establish financial support mechanisms for both educational institutions and learners to ensure accessibility and continuity of learning.

Improve coordination among government agencies, educational institutions, and international partners to enhance the sustainability and effectiveness of the educational process.

EDUCATIONAL PROCESS IN WARTIME CONDITIONS

The ongoing hostilities have created profound challenges for higher education, with ensuring the stability and continuity of the educational process being paramount.

Evidence indicates that the specific circumstances of conflict-affected regions have become a decisive factor for both educators and trainees [15]. The full-scale invasion, including the occupation of territories and widespread aerial bombardments, has led to the mass displacement of the population internally and abroad. Consequently, many learners, educators, and staff have been forced to relocate.

In areas under Ukrainian control, the educational process is recommended to be conducted in one of three formats: in-person, hybrid, or distance learning. The choice of format is directly linked to the ability to ensure the safety of all participants. As a result, distance learning has emerged as the predominant mode [12,16].

The introduction of martial law has led to significant adjustments across all sectors, including higher education. The substantial autonomy granted to educational institutions by the Law of Ukraine "On Higher Education" has enabled a timely and dynamic adaptation of the educational process. This flexibility was previously demonstrated during the COVID-19 pandemic, when institutions rapidly transitioned to distance or blended learning [17].

Thanks to advancements in recent years, educational institutions can now implement curricula effectively despite the challenging circumstances. Modern technology has brought substantial changes to the PGME system. The war, pandemic, and other global challenges have underscored the urgent need for the widespread and effective use of digital tools in education [18]. Under martial law, distance and blended learning have become a necessity rather than an alternative [19,20].

The IT industry responded swiftly to the needs of educators and learners by expanding network capacity and developing a wide range of digital tools. Numerous blogs offering training recommendations appeared on social media, and courses on creating educational resources and organizing online learning were launched [21].

Considering the key functions of multimedia, O. Bondarenko proposes the following classification:

Presentation: Supporting the delivery of new educational materials.

Simulation: Training learners through scenario analysis and decision-making tasks.

Control: Assessing the assimilation of educational materials.

Information and Reference: Providing essential theoretical resources for independent work [22].

Continuing Medical Education

Continuing education is essential for maintaining the professional competence of healthcare professionals. The integration of digital technologies has made continuing medical education more flexible and accessible [23,24].

ADVANTAGES OF DIGITAL EDUCATION

Flexibility and Accessibility: Healthcare professionals can learn at their convenience, integrating education with their clinical work. This is particularly crucial given unpredictable work schedules during wartime.

Continuity of Learning: Remote platforms allow education to continue safely, regardless of geographic location, which is essential during evacuations or when working in conflict zones.

Access to Educational Resources: Online platforms provide access to international databases, up-to-date clinical guidelines, and protocols, which are crucial for professional growth.

Risk Minimization: Online learning eliminates the need for in-person attendance, reducing potential threats to life and health.

Content Relevance: Digital platforms allow for the timely updating of materials to reflect the latest clinical guidelines.

Interactivity: The use of video and multimedia enhances the learning experience, improving comprehension and knowledge retention.

EXAMPLES OF INITIATIVES

Online webinars and conferences organized by international associations and leading medical universities.

Continuing education courses on platforms such as Coursera, Udemy, and specialized medical portals.

Mobile applications for regular updates to medical knowledge (e.g., Medscape, UpToDate).

Participation in online conferences and congresses to stay abreast of global medical developments.

PROBLEMS AND CHALLENGES

Technical Barriers: A stable internet connection and modern equipment are essential prerequisites.

Motivation: Self-directed learning demands a high level of discipline and personal responsibility.

Financial Constraints: The cost of many platforms and courses can be prohibitive, limiting accessibility.

Thus, while digital technologies offer significant opportunities, their effective implementation requires careful planning and support at the state level [25-27].

Despite its advantages, distance education cannot fully guarantee stability and continuity under wartime conditions.

The main risks include:

Limited internet access due to infrastructure damage.

Frequent power outages.

Interruptions from air raids requiring movement to shelters.

Lack of computer access for some learners.

Urgent evacuations from active combat zones.

In these circumstances, the feasibility of remote learning depends primarily on internet availability, which remains stable in most areas not directly affected by active hostilities. This approach helps preserve human resources, maintain communication, and, most importantly, ensure the safety of all participants [15].

TOOLS FOR ORGANIZING DISTANCE AND ONLINE LEARNING

The most popular educational platforms include:

Moodle

Advantages: Flexible course customization, support for various learning formats, robust assessment tools.

Disadvantages: Steep learning curve for non-technical users; requires a stable internet connection.

Google Classroom

Advantages: User-friendly interface, seamless integration with Google services, mobile compatibility.

Disadvantages: Limited functionality for organizing complex medical training and practical sessions.

Microsoft Teams

Advantages: Supports video conferencing, document collaboration, and interactive tools for group work.

Disadvantages: Requires a license for full functionality; can be resource-intensive.

Zoom

Advantages: Stable connection, accessible interface, supports large groups, includes recording and polling features.

Disadvantages: Limited functionality in the free version; potential data security concerns; requires a stable connection.

Coursera and EdX

Advantages: Access to courses from leading global universities; certification options; extensive course selection.

Disadvantages: High costs for individual users; limited adaptation to Ukrainian educational standards [28,29].

Webinar-oriented platforms have become essential. The authors selected Zoom for its simplicity, accessibility, and reliability. Its widespread use within the medical community also reduces the learning curve for participants. During sessions, instructors can pause every 20-30 minutes to gather feedback via the chat function, allowing them to adjust the pace and structure of the presentation to maintain audience engagement.

PRESENTATION AS A TOOL FOR VISUALIZATION IN ONLINE EDUCATION

In time-limited educational sessions, the primary goal is to explain key concepts clearly. Effective visualization of learning content is essential for enhancing the quality and efficiency of education [30]. Educators therefore require a versatile tool to organize, present, and easily edit teaching materials. Computer presentations effectively meet these needs, facilitating dynamic and adaptable teaching [31].

TOOLS FOR CREATING PRESENTATIONS

Microsoft PowerPoint

Advantages: Intuitive interface, wide range of templates, support for multimedia and animations, seamless integration with other Microsoft products.

Disadvantages: High licensing cost; can be resource-intensive; collaboration can be difficult without cloud storage.

Google Slides

Advantages: Free to use, facilitates real-time collaboration, accessible via web browser, integrates with Google Drive.

Disadvantages: Fewer advanced features compared to PowerPoint; dependent on an internet connection.

Canva

Advantages: User-friendly for creating visually appealing presentations; extensive library of templates.

Disadvantages: Limited animation features; advanced tools require a subscription.

Microsoft PowerPoint is widely regarded as the most convenient and powerful tool for educators. Its acces-

sible interface allows users to create well-structured presentations incorporating text, tables, charts, images, video, and audio. The ease of editing and its flexibility make lesson preparation efficient. In general, PowerPoint enhances online educational sessions through effective visualization, significantly improving learners' comprehension [32]. The authors' experience confirms its exceptional efficiency, as teaching is fundamentally an act of presenting information [31].

For communication outside of formal sessions, popular messaging apps such as Viber, WhatsApp, or Telegram can be used. The authors' department uses Viber due to its widespread adoption and accessibility in Ukraine.

CONCLUSIONS

Modern technologies have significantly transformed postgraduate medical education. The challenges posed by war, pandemics, and global crises have underscored the necessity of effectively leveraging digital tools. These shifts in the healthcare system must be reflected in the PGME system through the adaptation of educational processes to evolving realities.

Our analysis shows that remote technologies are an essential solution for maintaining the quality of PGME. However, their successful implementation requires addressing technical, organizational, and methodological challenges. A holistic approach is needed, including the development of new standards for remote learning, upgrades to technical infrastructure, and active collaboration among all stakeholders.

The use of widely accepted, reliable, and accessible information and communication technologies offers a practical way to overcome current challenges in medical education. Based on practical experience, a recommended approach is to utilize PowerPoint for content presentation and Zoom for online delivery, as both are accessible, reliable, and widely adopted.

As instructors and medical trainees adapt to new methods of knowledge acquisition, it becomes possible to maintain high teaching standards, ensure stability, support uninterrupted learning, and provide effective feedback.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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Technologization of iatrogenic crime investigation process

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ABSTRACT

Aim: To identify and analyze the factors that mandate the technologization of iatrogenic crime investigation process and to propose actionable strategies for integrating modern technologies and algorithms into investigative practices for this class of criminal proceedings.

Materials and Methods: This research is based on an analysis of official WHO documents, 31 relevant judgments from the European Court of Human Rights (ECHR) concerning adverse medical outcomes, 19 verdicts and decisions from Ukrainian courts on medical malpractice, and statistical data from the Prosecutor General's Office of Ukraine for the period 2016-2021. The primary analysis is supplemented by a review of recent scientific articles from leading academic databases.

Conclusions: It has been proven that although patient harm resulting from the provision of low-quality medical care is very widespread worldwide and often becomes the subject of criminal investigation, Ukraine demonstrates a very low level of investigation and detection of iatrogenic crimes. It is proposed that these shortcomings be corrected through technologization, which includes the algorithmization of investigative actions and the application of the latest technologies.

KEY WORDS: iatrogenic crimes, investigation technologization, digital technologies, iatrogenic crime investigation algorithm

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INTRODUCTION

Patient harm, defined as the impairment of bodily structure or function or any detrimental effect resulting from actions taken during healthcare provision rather than the underlying disease [1], represents a major global issue. According to WHO estimates, approximately one in ten patients experiences harm while receiving medical care, contributing to over 3 million deaths annually worldwide from unsafe practices. This positions patient harm as the 14th leading cause of global morbidity and mortality [2] and often prompts criminal investigations.

A primary contributor to preventable patient harm is diagnostic error, the risk of which is particularly acute in outpatient and primary care settings. In the United States, nearly 800,000 individuals die or suffer permanent disability annually due to diagnostic failures [3]. Another study reveals that 12 million patients in the U.S. are affected by diagnostic errors, a third of which result in patient injury [4]. These errors lead to adverse events in approximately 5% of outpatients and 17% of hospitalized patients [5] and are a leading cause of medical malpractice litigation [6].

Regarding personnel, an analysis of 1,500 patient complaints in China found that physicians were the subject of the majority (53%), followed by logistics

staff (19%), nurses (16%), technicians (4%), and administrators (2%) [7]. Nurse fatigue, stress, and burnout are known to cause frequent errors that compromise patient safety [8].

Surgical errors carry the highest risk of severe patient injuries and deaths. It is estimated that intraoperative errors are the main problem in 75% of cases of surgeons' negligent actions [9].

The harm caused to patients can be not only physical, causing adverse consequences or disability, but also psychological, manifesting in anger, persistent feelings of injustice even long after the event. This forces patients to seek compensation through the judicial system [10].

Ukraine demonstrates a very low level of investigation and detection of iatrogenic crimes, referral of indictments to court, and proving the guilt of specific medical workers. Thus, according to the analysis of statistical data from pre-trial investigation bodies, it was established that under Article 140 of the Criminal Code of Ukraine (Improper performance of professional duties by medical or pharmaceutical workers) in Ukraine from January 2016 to December 2021, 4,042 such criminal offenses were registered. At the same time, only in 13 (0.32%) criminal proceedings were medical

workers served with notifications of suspicion, and only 9 (0.22%) proceedings were referred to court with indictments. Moreover, a significant portion of such cases (1,577 or 39.01%) are subsequently closed by pre-trial investigation bodies, primarily because insufficient evidence was established to prove the person's guilt in court [11]. This indicates problems in the investigation of iatrogenic crimes, which can be prevented by optimizing the investigation process by using the latest technological solutions to accelerate the methods of collecting, analyzing, and evaluating evidence.

Given this, investigating iatrogenic crimes requires technologization, including algorithmizing investigative actions and applying the latest technologies.

AIM

To identify and analyze the factors that mandate the technologization of iatrogenic crime investigation process and to propose actionable strategies for integrating modern technologies and algorithms into investigative practices for this class of criminal proceedings.

MATERIALS AND METHODS

To achieve the research objectives, a range of materials was analyzed, including: the WHO Global Patient Safety Action Plan 2021-2030 [12]; the WHO Global Monitoring Report on tracking universal health coverage (2023) [13]; the WHO Global Patient Safety Report (2024) [2]; the WHO World Health Statistics (2025) [14]; and a WHO technical report on patient safety event reporting systems [15]. The analysis also encompassed 31 judgments from the European Court of Human Rights (ECHR) on adverse medical outcomes, 19 decisions from Ukrainian courts concerning medical malpractice, and statistical data from the Prosecutor General's Office of Ukraine (2016-2021). This was supplemented by a review of scientific literature from the past five years sourced from databases such as Web of Science, Scopus, Google Scholar, PubMed, Directory of Open Access Journals (DOAJ), and the Open Ukrainian Citation Index (OUCI).

To achieve the set goal, the following general scientific research methods were used: empirical (observation, description), theoretical (analysis, synthesis, abstraction, generalization, induction, deduction, explanation, classification, etc.), systemic, functional, formal-logical, and others.

The formal-logical method was used to typify investigative actions during iatrogenic crime investigations. The functional method was applied to analyze the causes and consequences of medical care defects. Analysis and synthesis were used to summarize existing scien-

tific perspectives and develop original conclusions.

ETHICS

All sources used in this literature review are publicly available.

REVIEW AND DISCUSSION

A comprehensive review of existing literature and legal precedents reveals that the imperative for technologizing the iatrogenic crime investigation process is predicated on several intersecting challenges: 1) the high and increasing incidence of improper medical care, both globally [5], and in Ukraine [16], with 50% of such cases linked to procedural violations [17]; 2) the absence in many nations of a unified, standardized [15], and transparent system for reporting adverse events and medical errors [12]; 3) the lack of electronic health records in 10% of countries and their poor integration into national healthcare systems in 75% of countries [2]; 4) the tendency for official inquiries to produce cursory reports that attribute adverse outcomes to the patient's underlying condition or classify them as accidents, rather than assessing the quality of care provided [15]; 5) the inherent difficulty for non-specialists to analyze voluminous and complex medical documentation; 6) the challenge of establishing a direct causal relationship between a provider's actions (or omissions) and patient harm or death; 7) the demonstrably low rates of successful investigation, prosecution, and conviction in iatrogenic crime cases. Analysis of Ukrainian court verdicts under Art. 140 of the Criminal Code confirms that acquittals often result from the prosecution's failure to prove guilt definitively [18] or submitting evidence deemed inadmissible or insufficient [19].

For investigators, establishing causality is further complicated by a lack of forensic literature detailing the common causes of adverse medical outcomes and the consequences of different care defects. An analysis of ECHR jurisprudence in this domain has identified several distinct categories of violations at the treatment stage. These include treatment-tactical errors, such as failing to screen for allergic reactions [20, 21], violating safety protocols for injections or blood transfusions [22, 23], making poorly justified decisions on the timing of medical interventions [24], selecting a suboptimal patient management strategy (e.g., observation instead of immediate surgery) [25], and providing inadequate patient monitoring [26]. Beyond these, our analysis also identified organizational and deontological violations. Organizational failings include a lack of care continuity [27], poor coordination between emergency physicians

and hospital administration [25], or other departments [26], inappropriate patient hospitalization [25], and breaches in asepsis and sterilization protocols [26]. Deontological violations encompass failures to adequately consult with the patient or family about their medical history [21], inform them of treatment risks [21], obtain informed consent [21], and uphold patient rights to information and choice of provider [28]. Violations of donor rights, particularly the right to informed consent for organ removal, have also been noted [29, 30]. Importantly, a single adverse event may stem from multiple, compounding violations [21, 26].

Investigators spend much time during the investigation of iatrogenic crimes processing large arrays of documents and establishing a significant number of circumstances (fact of violation of medical care provision standard, type and stage of medical care, patient's physiological state, their behavioral characteristics, professional and personal qualities of the medical worker, etc.) and appointing forensic examinations (forensic medical, pharmaceutical, psychological, criminalistic, etc.). In some cases, they do not manage to qualitatively form the evidence base within the procedurally defined investigation timeframes, which emphasizes the urgent need for algorithmization of both individual procedural actions of the investigator and the entire crime investigation process. This necessity is based on several key aspects highlighted in the works of various authors.

First, despite its complexity, criminal investigation can be broken down into logical, sequential, and repeatable steps to achieve desired results. Forming a mental map (algorithm) of the investigation process allows the investigator to describe the course of their conclusions at any stage of criminal proceedings [31].

Second, investigators during the investigation of iatrogenic crimes mainly perform typical actions, as they solve similar tasks in each investigation. This provides grounds for identifying the optimal sequence of actions in typical investigative situations, which can be presented through criminalistic algorithms and investigative programs [32].

Third, technologization of investigative activity is impossible without its formalization, which consists in ordering and systematizing investigation procedures based on clearly defined rules, methods, and algorithms. This aims to program investigative activity and ensure its effectiveness, objectivity, and legality [33].

Based on this, we propose the following algorithm for investigating iatrogenic crimes: 1) acceptance of a statement or notification about a committed iatrogenic crime; 2) initiation of criminal proceedings and interrogation of the applicant (if present); 3) examination of protocols or reports of internal audit of healthcare

institutions (HCI), acts of medical-control commissions, conclusions based on results of clinical-expert assessment of quality and volumes of medical care by clinical-expert commissions and medical councils of HCI; 4) examination of medical documentation (medical record of inpatient, protocol of pathological-anatomical examination, discharge from inpatient care, medical record of outpatient, journals of operative interventions registration in inpatient care and registration of patient admission to inpatient care or refusals of hospitalization, journal of outpatient registration, informed voluntary patient consent for medical procedures, etc.); 5) appointment and conduct of forensic medical examination of medical care quality provision; 6) examination of electronic medical record of the patient - electronic medical records, prescriptions, results of laboratory and instrumental studies, graphic files, scanned images, digital photographs; 7) interrogation of witnesses; 8) appointment and conduct of forensic examinations (computer-technical, pharmaceutical, handwriting, etc.); 9) review of audio and video recordings (if available) that record the time, place (according to file metadata) and nature of actions of the doctor and patient; 10) notification of the medical worker about suspicion and their interrogation.

Implementing modern technologies in investigating iatrogenic crimes, which significantly simplify the collection, storage, and analysis of digital evidence, will allow for faster and more efficient obtaining digital information from electronic devices [34]. In investigating iatrogenic crimes, specifically, investigators use existing digital databases and create their own for storing and quick access to vast volumes of information. This simplifies cross-analysis of information and prevents loss of evidence. Documentation technologies provide increased speed and accuracy in researching large volumes of data in an automated manner, improving their visualization and accessibility for researchers and more effective classification [35].

The integrity and security of digital evidence from the moment of their collection to presentation in court, transparent storage, and protection from falsification and unauthorized access are of great importance. Blockchain technology can ensure such conditions [36], which are mainly associated with cryptocurrencies but can be applied in criminal investigations to provide reliable storage, verification, and protection of digital evidence from unauthorized changes.

Computers in medicine are used as a means of creating and storing medical documentation, as well as elements of diagnostic and therapeutic hardware. Digital forensics tools allow establishing the fact of changing the content of a medical document or the date of its

creation, recovering previously deleted or hidden data (emails, text messages, web browsing history, individual files) from computers, smartphones, and cloud storage (Dropbox Plus, Google Drive Premium, or iCloud Drive). Individual technologies allow searching for computers and other digital information carriers and quickly processing large volumes of data [37].

Smartwatches, fitness trackers, and other medical workers' and patients' gadgets are sources of personal information about their owners, which can serve as evidence of criminal acts when investigating iatrogenic crimes. Search for information about contacts of the suspect (accused) and victim, as well as other substantial evidence, can be carried out in information-telecommunication systems.

Within computer-technical examination, research of computer equipment used in medical equipment can also be conducted. Such research will allow confirming or refuting testimonies of medical workers about malfunctions of diagnostic, therapeutic, surgical, or rehabilitation devices.

Artificial intelligence (AI) is actively used in law enforcement to reduce subjectivity and prevent errors in data interpretation, improving the quality of evidence in court [34]. During the investigation of iatrogenic crimes, the investigator can independently consult one of the AI systems with a request to build an algorithm of their future actions (investigation plan) based on the characteristics of a specific investigative situation.

Language models operating based on artificial intelligence (for example, ChatGPT (Generative Pre-trained Transformer) or Llama (Large Language Model Meta AI)) allow analyzing large volumes of evidence information in the form of text data, extracting essential facts from them, and identifying inconsistencies in testimonies and other evidence information [38]. Some scientists even note that many machine learning algorithms are used in crime investigation, including logistic regression to search for relationships between certain formalized features and the probability of a specific result [32].

The ability of AI to visualize large data arrays (represent them in the form of diagrams, graphs, maps, and other visual forms) [32], allows the investigator to understand and interpret information and analyze it easily. Such tools include Domo, Microsoft Power BI, Tableau, Polymer, Qlik, and IBM Cognos Analytics [39]. The investigator also needs to manage quality and evidential significance of data extracted from large information arrays [40].

During forensic medical examination of corpses, an alternative to traditional autopsy is virtual autopsy (virtopsy) - diagnostic technology based on digital visualization methods (for example, computed and

magnetic resonance tomography) for detecting and analyzing changes in the body without conducting traditional autopsy [41]. Its advantages include greater accuracy, evidence integrity, possibility of their review at any stage of criminal proceedings without the need to repeat the procedure, minimization of invasiveness, efficiency, and safety. Virtopsy provides detailed three-dimensional visualization of anatomical structures, which facilitates injury detection and more accurate determination of cause of death [34].

The development of 3D printing technology has led to significant progress in reproducing physical evidence [40]. 3D copies of medical instruments, bodily injuries, internal human organs, bones, and other objects related to iatrogenic crimes can be used in the courtroom for their visual presentation. 3D printing can also be applied when creating anatomical models during preparation for corpse autopsy.

CONCLUSIONS

Our research allows us to assert that patient harm resulting from the provision of low-quality medical care in the form of morbidity and mortality is widespread worldwide and often becomes the subject of criminal investigation. However, Ukraine shows a very low level of investigation and detection of iatrogenic crimes. These shortcomings can be corrected through technologization, which includes algorithmization of investigative actions and application of the latest technologies.

An algorithm of investigative actions for investigating iatrogenic crimes is proposed, consisting of the following stages: 1) acceptance of a statement or notification about a committed iatrogenic crime; 2) initiation of criminal proceedings and interrogation of the applicant (if present); 3) examination and analysis of departmental (special) investigation documents and medical documentation on the patient; 4) appointment and conduct of forensic medical examination of medical care quality provision and (if necessary) other forensic examinations (computer-technical, pharmaceutical, handwriting, psychological, etc.); 5) examination of the patient's electronic medical record; 6) interrogation of witnesses and the victim (in cases where such status is granted); 7) research of audio and video recordings (if available) that record the time, place (according to file metadata) and nature of actions of the doctor and patient; 8) notification of the medical worker about suspicion and their interrogation.

To optimize the process of investigating iatrogenic crimes, we propose using several digital technologies, making it faster, more efficient, and objective, ultimately contributing to protecting patient rights.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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A case of providing a surgical treatment for patient with silent sinus syndrome

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ABSTRACT

This article presents a case report of patient with silent sinus syndrome. This syndrome is characterized by reduction in size of sinus with accumulation of pathological content inside it due to obstruction of osteomeatal complex, development of enophthalmos and resorption of bone walls. Such process occurs because of hypoventilation of maxillary sinus and followed by development of chronic maxillary sinusitis. Except generally accepted clinical observations, computer tomography scan of paranasal sinuses and endoscopic endonasal examination are essential for the verification of this syndrome in patients. We believe that in order to prevent sinus collapse it is necessary to provide a surgical treatment which involves making a wide antrostoma through endoscopic approach and correction of middle nasal turbinate and other intra-nasal structures especially those who are located near to osteomeatal complex. This will provide normal conditions for ventilation of middle nasal meatus. Appropriate postoperative examination and care is also required for successful recovery.

KEY WORDS: silent sinus syndrome, middle nasal turbinate, endoscopic antrostomy

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INTRODUCTION

Silent sinus syndrome (SSS) is a pathological condition which occurs due to obstruction of osteomeatal complex and results in development of enophthalmos. This disease is characterized by reduction in size of sinus with accumulation of pathological content inside it. First it was described by Montgomery W.W. in 1964 [1]. He analyzed two clinical cases of enophthalmos and diplopia which were associated with maxillary sinus collapse. Later this disease was described by other authors. Term "Silent sinus syndrome" was first applied by Soparker C.N. et al. in 1994 after examining 19 patients with similar symptoms [2].

The etiology and pathogenesis of this syndrome are still not fully understood. The most widespread explanation for its development is the theory of obstruction of the osteomeatal complex (OMC) [3]. The ostia of the maxillary, ethmoid, and frontal sinuses; middle meatus; infundibulum; uncinate process; and anterior ethmoid cells are the anatomical structures that form the OMC [4]. Its main function is to provide aeration and mucus clearance for the paranasal sinuses. Different anatomical variations of the OMC and its obstruction can influence the volume of the maxillary sinus. Collapse of the middle nasal meatus, altered anatomical structure of the middle

nasal turbinate and lateralization of the uncinate process are leading to occlusion. As a result, secretions accumulate inside the maxillary sinus (MS) and ciliated epithelium cannot transport them toward the sinus opening [5]. These secretions are resorbed, causing a vacuum effect that leads to hypoventilation of the MS. Under such conditions, negative pressure is formed, causing the walls of the MS to migrate inward. These circumstances also contribute to bone thinning and remodeling due to increased osteoclast activity. Hypoxoxygenation inside the MS creates an anaerobic environment favorable for bacterial growth and the development of bacterial sinusitis. Second theory of SSS pathogenesis is erosion which occurs due to chronic inflammation. Cytokines inhibit the replication of osteoblasts and collagen synthesis which is facilitating the osteopenia process. The third theory concerns maxillary sinus hypoplasia [6].

Also there are publications of SSS, which occurred in frontal [7], and ethmoid sinuses [8]. The last was the result of traumatizing during nasal swab test. According to Annino DJ Jr and Goguen LA (2008), the diagnosis of SSS requires such criteria as: no history of previous chronic rhinosinusitis, enophthalmos or trauma, during last 6 months no episodes of acute rhinosinusitis, computer tomography (CT) scan which proves maxillary



Fig. 1. CT scan of patient in frontal projection of paranasal sinuses. Left silent sinus syndrome. Deviation of nasal septum and lateratization of middle nasal turbinate

Picture taken by the authors

roof remodeling, and no documented congenital deformity or anatomic anomalies of the sinus of orbital cavity [9]. Deviation of nasal septum, lateralization of middle nasal turbinate, narrowed infundibular passage, and abnormal nasal anatomy are factors that facilitate the development of this disease [10]. Magnetic resonance imaging (MRI) is not mandatory for establishing diagnosis, but may also be helpful. The displacement of eyeball inferiorly and the inferior rectus muscle descent along with the globe are signs of SSS [11].

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At medical center "LORIMED" LLC were treated 32 patients with unilateral SSS. The criteria for establishing

this diagnosis are radiological and ophthalmological [12]. All patients, except generally accepted clinical observations, were performed a CT scan of paranasal sinuses and endoscopic endonasal examination. Patient's complaints: deformation of the face (sagging of the cheek on the affected side), periodic pain in projection of sinus or teeth, rhinorrhea mainly from the same half of the nose. Some patients complained of asymmetry of the eyeballs.

CT scans showed increased radiolucency of the MS cavity, retraction of its walls with decreased volume, deepening of the molars, retraction of all sinus walls toward the lumen, expansion of the middle nasal meatus, deformation of the uncinate process, and demineralization of the sinus walls. In some patients, compensatory enlargement of the orbit, thickening of its lower wall, and deviation of the nasal septum toward the affected sinus were observed.

Endoscopic examination showed discharges of different consistency from middle nasal turbinate and its abnormal localization, distorted uncinate process and in some patients deviation of nasal septum in direction of affected sinus were determined.

Endoscopic endonasal antrostomy with widening of the middle meatus and exclusion of pathological content were performed in all patients. In order to prevent the collapse of the middle nasal meatus and closing of the antrostoma in some patients was performed fixation of the middle nasal turbinate by stitching it to the nasal septum. Except endonasal antrostomy it was also performed septoplasty and vasotomy of the inferior nasal turbinate in patients who



Fig. 2. Endoscopic endonasal examination of patient on the 14th day after surgery. Antrostoma and middle nasal turbinate which is stitched to nasal septum

Picture taken by the authors

had problems with nasal breathing or deviated nasal septum obstructed the way to affected sinus. Postoperative examination and care lasted during 3 month. Based on our case report we will describe our methods of surgical treatment in patients with this pathology.

CASE REPORT

In July 2024 a 30-year-old female visited an otolaryngologist with complaints of headache, bilateral nasal

obstruction, purulent rhinorrhea, and fever over 37.8 °C. Considers herself sick for two weeks and had been using decongestants. She denied trauma, congenital anomalies, and previous episodes of acute or chronic rhinosinusitis. Conservative treatment was prescribed: amoxicillin 1 g twice daily for 7 days, mometasone 2 sprays in each nostril twice daily for 7 days, and ibuprofen 400 mg twice daily for 5 days. No drug allergies were identified. After days after treatment patient felt better, but still had complaints on nasal obstruction and slight pains in the area of left MS. On CT scan increased radiolucency of left MS cavity and retraction of its walls with a decreasing of its volume were observed. Patient was referred on surgical treatment. A diagnosis was established: Deviation of nasal septum to left with disorders of nasal breathing. Left silent sinus syndrome (Fig. 1).

Endoscopic endonasal antrostomy with septoplasty was performed, and the middle nasal turbinate was fixed to the nasal septum. Surgery was performed under general anesthesia. Patient had no complaints on the 14th day after surgical treatment. During the endoscopic examination – mucus-lining pale pink, pure, antrostoma is functional, middle nasal meatus is vide and nasal breathing is free (Fig. 2).

On the 30th day after surgery a control CT scan was performed (Fig. 3).

The patient's general condition was good, with no complaints and no need for further follow-up.

The pathology of SSS is widely discussed but remains insufficiently understood due to its rarity. Proper classification, definition, differential diagnosis, and accurate treatment remain important clinical issues. Other pathologies such as paranasal sinus hypoplasia, facial deformities, trauma-related cases, surgery-related



Fig. 3. CT scan of patient on the 30th day after surgery in frontal projection of paranasal sinuses. Vide middle nasal meatus, functional antrostoma and pneumatized maxillary sinus

Picture taken by the authors

changes, and others must be considered in the differential diagnosis [13].

CT is essential for establishing the final diagnosis. Reduction of maxillary antrum volume with wall retraction, complete opacification of the affected sinus, and lateralized uncinate process are typical signs.

Because of its clinical variability, SSS patients may initially present to specialists in other fields. Multidisciplinary collaboration between ophthalmologists, maxillofacial surgeons, and otolaryngologists is essential for proper management [14].

The main surgical goal is to restore proper MS ventilation and OMC patency. Endoscopic antrostomy and correction of intranasal structures, when necessary, are sufficient to restore MS drainage and reduce sinus-related symptoms. Older methods such as the Caldwell–Luc procedure are now rarely used.

CONCLUSIONS

Basic conditions that necessary for prevention of sinus collapse are surgical treatment which involves making an antrostoma through endoscopic approach and providing normal conditions for ventilation of middle nasal meatus. In order to achieve that, we need to perform resection of uncinate process with reverse punch or shaver to minimize the damage of mucosal membrane of antrostoma. Correction of middle nasal turbinate or stitching it to nasal septum prevents collapse middle nasal meatus. Septoplasty or correction of other intra-nasal structures especially those who are located near to osteomeatal complex are important requirements for ventilation of all nasal cavity and especially affected sinus. Appropriate postoperative examination and care make a significant contribution in success of surgical treatment.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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